The Effect of Water Conditions on Decomposition Rate: A Comparative Study of the Estimation of Post-mortem Submersion Interval

by

Ibukunoluwa Olowe
BSc (Hons), MSc

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Central Lancashire

July 2023

RESEARCH STUDENT DECLARATION FORM

Type of Award: Postgraduate Research Degree (PhD)

School: Natural Sciences

1. Concurrent registration for two or more academic awards

I declare that while registered as a candidate for the research degree, I have not been a registered candidate or enrolled student for another award of the University or other academic or professional institution.

2. Material submitted for another award

I declare that no material contained in the thesis has been used in any other submission for an academic award and is solely my own work.

3. Collaboration

Where a candidate's research programme is part of a collaborative project, the thesis must indicate in addition clearly the candidate's individual contribution and the extent of the collaboration. Please state below:

N/A

4. Use of a Proof-reader

No proof-reading service was used in the compilation of this thesis.

Signature of Candidate: 

Print name: Ibukunoluwa Olowe

ABSTRACT

Many factors affect aquatic decomposition and the accuracy of Post-mortem Submersion Interval (PMSI) estimations but have not been researched thoroughly. The extent of decomposition in bodies recovered from water is measured using visual decomposition scoring systems (DSS). The applicability of the Heaton, van Daalen and Reh DSS is limited because they were developed with predominantly pale-skinned individuals in temperate climates. This PhD aimed to (1) determine the effect of water temperature, hardness, salinity, and current on decomposition rate in human analogues, (2) compare the effectiveness and suitability of these DSS for assessing decomposition changes in human analogues/human remains and estimating PMSI in dark-skinned individuals.

Laboratory studies, where 660 mice decomposed for 35 days, were conducted to investigate the effect of salinity, hardness and current on decomposition rate. To augment the laboratory results with larger human analogues, 35 pigs were decomposed for 70 days in water of varied salinity. To evaluate the accuracy of the DSS in PMSI estimation of human cases, the Heaton, van Daalen and Reh DSS to assess the extent of decomposition visible in pictures of drowning victims from the Netherlands, U.K., and South Africa and compared with known PMSI. A 'drowning database' of data from global drowning cases cited in scientific literature and media was collated to understand worldwide drowning demographics.

This research demonstrated that temperature, salinity, hardness, and current significantly affected the decomposition rate of human analogues ($p<0.05$) and has shown for the first time that discolouration changes can be misleading in dark skin-toned individuals when using the DSS, and these DSS are inaccurate for estimating the PMSI of dark and pale-skinned bodies in advanced decomposition states or recovered in winter. Recommendations for applying the DSS to dark-skinned individuals have been made to improve the estimation of PMSI. Therefore, temperature, salinity, current, and hardness levels should be considered when estimating the PMSI of bodies recovered from marine environments, and more global studies should be conducted to improve the suitability of these DSS in assessing decomposition and estimating PMSI in darker-skin-toned individuals.

TABLE OF CONTENTS

RESEARCH STUDENT DECLARATION FORM II

ABSTRACT III

TABLE OF CONTENTS IV

ACKNOWLEDGEMENTS X

LIST OF FIGURES XI

LIST OF TABLES XIX

LIST OF ABBREVIATIONS XXIII

DISCRETION WARNING XXV

1 INTRODUCTION 1

1.1 Drowning, Aquatic Decomposition and Decomposition Scoring Systems 1

1.2 Research Questions 3

1.3 Research Outline 3

1.3.1 Laboratory Studies on Water Conditions 3

1.3.2 Field Studies on Salinity Concentration 4

1.3.3 Comparative Human Image Analysis 4

1.3.4 Drowning Case Database 4

1.4 Aims and Objectives 5

1.4.1 Aims 5

1.4.2 Objectives 6

2 EXISTING RESEARCH INTO AQUATIC DECOMPOSITION 7

2.1 Taphonomy 7

2.2 Terrestrial Decomposition 7

2.2.1 Stages and Chemistry of Terrestrial Decomposition 8

2.2.2 Accumulated Degree Days 20

2.2.3 Estimating Post-mortem Interval 20

2.3 Drowning 30

2.3.1 Physiology of Drowning 30

2.3.2 Diagnosis of Drowning and its Difficulties 32

2.3.3 Body Displacement in Water 40
2.3.4 Detection, Recovery, and Identification of Drowned Bodies 42
2.4 Aquatic Decomposition 45
2.4.1 Process and Chemistry of Aquatic Decomposition 45
2.4.2 Stages of Aquatic Decomposition 51
2.5 Water Parameters Affecting Aquatic Decomposition 53
2.5.1 Temperature 53
2.5.2 Salinity 54
2.5.3 Depth 55
2.5.4 Water Flow 55
2.5.5 Aquatic Organisms 56
2.5.6 Body Coverings 60
2.6 Water Profile of Water Bodies in the U.K. 62
3 PMSI AND DECOMPOSITION SCORING SYSTEMS 63
3.1 Methods of Estimation of PMSI 63
3.1.1 Weighing Decomposition 63
3.1.2 Microbial Community Assembly (MCA) 63
3.1.3 Entomological Methods 64
3.1.4 Decomposition Scoring Systems 65
3.2 Megyesi *et al*. (2005) TBS 65
3.2.1 Description 65
3.2.2 Limitations 68
3.3 Heaton *et al*. (2010) DSS 70
3.3.1 Description 70
3.3.2 Limitations 72
3.4 van Daalen *et al*. (2017) DSS 73
3.4.1 Description 73
3.4.2 Limitations 74
3.5 Reh DSS 75

3.5.1 Description 75
3.5.2 Limitations 76
3.6 Heaton vs van Daalen DSS 76
3.7 Limitations of Scoring Systems in General 77
4 SKIN COLOUR AND DECOMPOSITION 78
4.1 Physical Structure of the Skin 78
4.2 Skin Colour and Tone 81
4.2.1 Determinants of Skin Colour 81
4.2.2 Measuring Skin Colour 83
4.2.3 Importance of Skin Colour in Forensic Science 87
4.3 Lack of Representation of Skin Tones 89
5 PART ONE: LABORATORY STUDIES ON WATER CONDITIONS 91
5.1 Introduction 91
5.1.1 Aims 93
5.1.2 Objectives 94
5.2 Methodology 95
5.2.1 Pilot Study 95
5.2.2 Laboratory Salinity Study 95
5.2.3 Laboratory Hardness Study 99
5.2.4 Laboratory Current Study 100
5.2.5 Laboratory Skin Tone Study 102
5.2.6 Measuring Decomposition 103
5.2.7 Statistical analysis 107
5.2.8 Risk Assessment 107
5.3 Results and Discussion 108
5.3.1 Laboratory Salinity Study 108
5.3.2 Laboratory Hardness Study 128
5.3.3 Laboratory Current Study 136
5.3.4 Laboratory Skin Tone Study 147

5.4 Limitations of the Laboratory Studies 158
5.5 Conclusions of the Laboratory Studies 160
6 PART TWO: FIELD STUDIES ON SALINITY CONCENTRATION 162
6.1 Introduction 162
6.1.1 Aims 163
6.1.2 Objectives 163
6.2 Methodology 164
6.2.1 Breakdown of Field Studies 164
6.2.2 Initial Salinity Study 164
6.2.3 Changing Salinity Study 168
6.2.4 Statistical Analysis 170
6.3 Results 171
6.3.1 Initial-Salinity Study 171
6.3.2 Changing-Salinity Study 182
6.4 Discussion 196
6.4.1 Initial-Salinity Study Discussion 196
6.4.2 Changing-Salinity Discussion 199
6.4.3 Study Comparisons 203
6.5 Limitations of the Field Studies 203
6.6 Conclusions of the Field Studies 205
7 PART THREE: COMPARATIVE HUMAN IMAGE ANALYSIS 206
7.1 Introduction 206
7.1.1 Aims 207
7.1.2 Objective 208
7.2 Methodology 209
7.2.1 Collation of Images and Case Notes 209
7.2.2 Exclusion Criteria 210
7.2.3 Collated Information 210
7.2.4 Measuring Decomposition 215

7.2.5 Temperature Data 215
7.2.6 PMSI Estimations 216
7.2.7 Evaluating the Sufficiency of FADS, BADS and LADS 219
7.2.8 Statistical and Further Analysis 219
7.3 Results 220
7.3.1 Demographic Profiling 220
7.3.2 TADS 220
7.3.3 DSS Comparison 221
7.3.4 PMSI Estimates 222
7.3.5 PMSI Estimations with Missing Body Parts 228
7.4 Discussions 230
7.4.1 Demographic Profile 230
7.4.2 PMSI Estimation 231
7.4.3 Validating the Sufficiency of a Complete TADS 234
7.4.4 Skin Tone, Scoring Observations, and Recommendations on the DSS 234
7.5 Limitations 236
7.6 Conclusion 237
8 PART FOUR: DROWNING CASE DATABASE 238
8.1 Introduction 238
8.1.1 Aim 239
8.1.2 Objectives 239
8.2 Methodology 240
8.3 Results and Discussion 241
8.3.1 Breakdown of Data 241
8.3.2 Drowning Prevention Strategies 246
8.3.3 Limitations 251
8.4 Conclusion 252
9 DISCUSSION 253
9.1 Decomposition Patterns in Humans and Human Analogues 253

9.2 Effect of Water Conditions on Decomposition Rate 255
9.2.1 Temperature 256
9.2.2 Salinity 256
9.2.3 Hardness 257
9.2.4 TDS 258
9.2.5 Current 258
9.2.6 pH 259
9.3 Decomposition Scoring Systems 260
9.3.1 Differences between the Heaton and van Daalen DSS 260
9.3.2 Skin tone, Decomposition and DSS 261
9.3.3 DSS Limitations 262
9.3.4 DSS Recommendations 263
9.4 Study Limitations 265
9.5 Further Research 266
10 CONCLUSIONS 267
10.1 Key Results 267
10.2 Implications 268
REFERENCES 270
APPENDIX 309

ACKNOWLEDGEMENTS

I want to start by saying I am incredibly grateful to God for keeping me safe and allowing me to complete this PhD. I would also like to extend my deepest gratitude to my family, especially my parents, Prof V.I Olowe and Mrs R.O Olowe, my brothers, Ayo and Anjola, and other family members, Taiwo, Idowu, and Mr and Mrs Akindele, for their support, encouragement, and prayers all through the duration of this PhD.

I am deeply indebted to my supervisor, Prof Anna Williams, without whom completing this PhD would not have been possible. I cannot begin to express all the ways and the times I have appreciated you during this PhD, but thank you so much for your valuable advice, constant support, kindness, constructive feedback, and care, most especially at the points when this PhD research seemed unachievable to me.

Special thanks to all the major collaborators from other institutions for their unwavering support and invaluable contribution to this PhD research, especially in the Human Image Comparison Analysis, in which they extensively combed through the different drowning databases and selected the cases used in this PhD research. Various contributors include Prof Wilma Duijst, Deborah Spin, and Melanie Need from Maastricht University, Dr Laura Heathfield, Dr Calvin Mole, Dr Victoria Gibbon, and Lisa from the University of Cape Town, and Alan Wilson, the Blackpool and Fylde Coroner. I gratefully acknowledge the help and effort each of them put into this PhD research, especially Dr Laura Heathfield.

I would also like to use this opportunity to extend my sincere thanks to Dr Peter Cross and Irene van Zwieten for their unwavering and unparalleled support throughout this PhD, most especially during the field studies conducted at the TRACES facility and their input or words of advice for the structuring and designing of the field studies.

I would like to recognise the assistance from the Learning and Information Services support technicians, especially Natalie Valentine, Aaron McKean, Teresa Oughton and Kane Fox. I also gratefully acknowledge the assistance of other UCLan staff members, such as Dr Chandrashekhar Kulkarni and Dr Rick Peterson, for their various helpful contributions to this PhD as examiners, supervisory team members and other essential roles they played.

Many thanks to my fellow PhD researchers, Caley Mein, Catherine Maidment, Gabriela Mroz and Michael Parsons, for their invaluable insights and unwavering guidance throughout this PhD research. I hope and pray each of us gets to this point with the continued support we give one another. I would also like to recognise the assistance received from my dear friends such as Mojisola, JonP, Kolly, Udochi, Bogzy, Ifeanyi, Deji, DamolaBond, Lumy, Victoria, Haja, Veco, Oyinda, Dayo Dee, Bolaji, Dayo, Lekan, Daniel, Sanmi, Bukola, Adeoti, Seun, Judith, Bisola, Gerry, David and many more.

LIST OF FIGURES

Figure 2.1: Livor Mortis in a cadaver (Goff, 2009). 9

Figure 2.2: Presence of rigor mortis in the upper limbs of a cadaver (Goff, 2009). 10

Figure 2.3: Pictorial representation of Tache Noire in a body because of drying (Goff, 2009). 10

Figure 2.4: Blowflies trying to enter the body orifices (Rodriguez & Bass, 1985). 11

Figure 2.5: Fresh stage of decomposition in a human cadaver (Rodriguez & Bass, 1985). 11

Figure 2.6: Evidence of marbling on a cadaver (Goff, 2009). 13

Figure 2.7: Bloat stage of decomposition in a human cadaver (Rodriguez & Bass, 1985). 14

Figure 2.8: Picture of a mummified body (Rodriguez & Bass, 1985). 15

Figure 2.9: A body with a caved abdomen during the active decay stage (Rodriguez & Bass, 1985). 16

Figure 2.10: Advanced decay stage of decomposition in a human cadaver (Rodriguez & Bass, 1985). 16

Figure 2.11: A cadaver's skull that has reached the skeletonization stage of decomposition (Goff, 2009). 17

Figure 2.12: The first four stages of decomposition, as observed in the carrion of a pig (Dekeirsschieter *et al*., 2012). 18

Figure 2.13: Overview of the chemistry of terrestrial decomposition (Dent *et al*., 2004). 19

Figure 2.14: Mechanical stimulation with a steel chisel and the contraction response (Warther *et al*., 2012). 22

Figure 2.15: Schematic of the Nomogram developed for PMI estimation in early post-mortem (Henssge, 1988). 24

Figure 2.16: An application of the nomogram method (Henssge & Madea, 2004). 26

Figure 2.17: Autonomic changes leading to arrhythmias (Shattock & Tipton, 2012). 32

Figure 2.18: A-Washerwoman's Foot; B-Skin and nail slough off in the toes (Stephenson *et al*., 2019). 33

Figure 2.19: Foam released from nasal airways due to pulmonary oedema (Stephenson *et al*., 2019).34

Figure 2.20: Picture of emphysema aquosum of the lungs in drowning (Stephenson *et al*., 2019).....35

Figure 2.21: Computed tomography scans of A-sphenoidal sinus; B-lungs; C-trachea; D-bronchi; E-stomach (Wang *et al*., 2020). 36

Figure 2.22: Beaker, hot plate, centrifuge, and microscope used in acid digestion (Zhou *et al*., 2020). 38

Figure 2.23: Different diatom under 400x magnification light microscopy (Zhou *et al*., 2019). 39

Figure 2.24: Schematics of the forces during horizontal and vertical displacement. a- specific gravity; b- forces acting on the body; c-phases of movement (Mateus *et al*., 2013).....41

Figure 2.25: A Remotely Operated Vehicle in use (Ellingham *et al*., 2017). 43

Figure 2.26: ROV equipped with a robotic arm (Ellingham *et al*., 2017). 43

Figure 2.27: A metal cage used to recover submerged remains (Ellingham *et al*., 2017). 44

Figure 2.28: Pictorial examples of washerwoman's hand and degloving (van Daalen *et al*., 2017). ...45

Figure 2.29: Example of vascular marbling on a body (Gelderman *et al*., 2018).....47

Figure 2.30: Pictorial illustration of discolouration of the limbs of a body (Gelderman *et al*., 2018)..47

Figure 2.31: The process of adipocere formation (Forbes *et al*., 2005).....48

Figure 2.32: An illustration of a hydrolysis reaction during adipocere formation (Moses, 2012).....49

Figure 2.33: Presence of adipocere on recovered remains (Karhunen *et al*., 2008). 50

Figure 2.34: Bloated face of a body recovered from water (Kahana *et al*., 1999).....52

Figure 2.35: Exposure of the skeleton during the Advanced floating decay stage (Van Daalen *et al*., 2017). 52

Figure 2.36: Example of the disintegration of soft tissue during the Early floating decay stage (Kahana *et al*., 1999). 53

Figure 2.37: Picture of a Squat lobster feeding on a pig underwater (Anderson & Bell, 2014). 57

Figure 2.38: Various shapes and sizes of extensive lesions [a-female victim b-male victim] (Ribéreau-Gayon *et al*., 2016)..... 58

Figure 2.39: Examples of the Galatheae scavenging marks left on bones (Ellingham *et al*., 2017)..... 58

Figure 2.40: Crab feeding on a bone (Ellingham *et al*., 2017)..... 59

Figure 2.41: Evidence of predation by Shark [Selachimorpha] (Ellingham *et al*., 2017)..... 59

Figure 2.42: Bodies exhibiting extensive decomposition in the cervico-cephalic and mandible regions, but the trunk looks preserved due to the prior presence of clothing (Ellingham *et al*., 2017)..... 60

Figure 3.1: Photograph of Barnacles attached to the shoe and garment on a recovered body (Magni *et al*., 2015)..... 64

Figure 3.2: Pictorial depictions of a TBS of 16 (Pictures from Palazzo *et al*., 2021; van Daalen *et al*., 2017)..... 67

Figure 3.3: Pictorial depiction of a TAD score of 13 (Pictures from Caruso, 2016; Palazzo *et al*., 2021). 72

Figure 3.4: Decomposition changes and estimated monthly PMSI figures for the Reh DSS (Madea, 2015)..... 75

Figure 4.1: Skin Structure. (a) Schematic representation (b) Characteristics of the skin layers (Moniz *et al*., 2020)..... 79

Figure 4.2: Schematic view of the cross-section of the skin layers (Venus *et al*., 2010)..... 81

Figure 4.3: MGC tool being used to assess a baby's skin and wound colour (August *et al*., 2019). 84

Figure 4.4: The CIELAB colour space diagram (Ly *et al*., 2020). 85

Figure 4.5: Operating principle of a colourimeter and spectrophotometer (Ly *et al*., 2020)..... 86

Figure 4.6: A colourimeter in use (Ly *et al*., 2020). 86

Figure 4.7: A spectrophotometer in use (Ly *et al*., 2020)..... 86

Figure 4.8: Examples of various livor mortis colours (Tsokos & Byard, 2016)..... 88

Figure 4.9: The Massey-Martin (2003) skin colour guide (Louie & Wilkes, 2018)..... 89

Figure 5.1: Boxes placed indoors in a laboratory setting. 96

Figure 5.2: Cable ties and fishing weights attached to mice. 96

Figure 5.3: Data Logger placed in a transparent container. 98

Figure 5.4: Pictures of mice completely submerged with fishing weights. 100

Figure 5.5: Rotating turbine at the bottom of the box. 101

Figure 5.6: Mice immersed in the water at a depth about three-quarters down the box. 102

Figure 5.7: Dark and Pale mice before they were immersed in water. 103

Figure 5.8: Example of how a mouse in the fresh stage of decomposition was scored. 106

Figure 5.9: Example of how a mouse in the advanced stage of decomposition was scored. 107

Figure 5.10: Graph of the Average TADS (using Heaton DSS) of the mice against the PMSI of each group. 109

Figure 5.11: Graph of the Average TADS (using van Daalen DSS) of the mice against the PMSI of each group. 110

Figure 5.12: Graph showing temperature fluctuation in the water groups across the 35 days. 111

Figure 5.13: Graph of the Average TADS (using Heaton DSS) of the mice against the Log ADD of each group. 112

Figure 5.14: Graph of the Average TADS (using van Daalen DSS) of the mice against the Log ADD of each group. 113

Figure 5.15: Physical decomposition changes in each salinity group across the 35 days. 115

Figure 5.16: Graph of the Average TADS (using Heaton DSS) of the mice against the PMSI of each group. 117

Figure 5.17: Graph of the Average TADS (using van Daalen DSS) of the mice against the PMSI of each group. 118

Figure 5.18: Graph showing temperature fluctuation in the water groups across the 35 days. 119

Figure 5.19: Graph of the Average TADS (using Heaton DSS) of the mice against the Log ADD of each group. 120

Figure 5.20: Graph of the Average TADS (using van Daalen DSS) of the mice against the Log ADD of each group. 120

Figure 5.21: Physical decomposition changes in each group across the 35 days. 123

Figure 5.22: Unknown white patches observed on a mouse in the Sea group. 124

Figure 5.23: Graph of the Average TADS of the mice against the PMSI of each group. 129

Figure 5.24: Graph showing temperature fluctuation in the water groups across the 35 days. 130

Figure 5.25: Graph of the Average TADS of the mice against the Log ADD of each group. 131

Figure 5.26: Physical decomposition changes in each group across the 35 days. 133

Figure 5.27: Graph of the Average TADS (using Heaton DSS) of the mice against the PMSI of each group. 137

Figure 5.28: Graph of the Average TADS (using van Daalen DSS) of the mice against the PMSI of each group. 138

Figure 5.29: Graph showing temperature fluctuation in the water groups across the 35 days. 139

Figure 5.30: Graph of the Average TADS (using Heaton DSS) of the mice against the Log ADD of each group. 140

Figure 5.31: Graph of the Average TADS (using van Daalen DSS) of the mice against the Log ADD of each group. 141

Figure 5.32: Physical decomposition changes in each group across the 35 days. 144

Figure 5.33: Graph of the Average TADS (using Heaton DSS) of the mice against the PMSI of each group. 148

Figure 5.34: Graph of the Average TADS (using van Daalen DSS) of the mice against the PMSI of each group. 149

Figure 5.35: Graph showing temperature fluctuation in the water groups across the 35 days. 150

Figure 5.36: Graph of the Average TADS (using Heaton DSS) of the mice against the Log ADD of each group. 151

Figure 5.37: Graph of the Average TADS (using van Daalen DSS) of the mice against the Log ADD of each group. 152

Figure 5.38: Physical decomposition changes in each group across the 35 days. 155

Figure 5.39: Mushroom-like organism found in a freshwater box. 158

Figure 5.40: Pictures of the skin tone of the two types of mice used. a- pale mice; b- dark mice. 159

Figure 6.1: Argos boxes placed outdoors in a field setting for the Initial salinity study. 165

Figure 6.2: Pictures of the mesh, data logger and pig in the box for the Initial salinity. 165

Figure 6.3: Illustration of scoring a pig with a low extent of decomposition using the two adapted DSS. 166

Figure 6.4: Illustration of scoring a pig with an advanced extent of decomposition using the two adapted DSS. 167

Figure 6.5: 85 L Really Useful boxes placed outside on the pallets for the Changing salinity study. 168

Figure 6.6: Set-up procedures for the Changing salinity study. 169

Figure 6.7: Graph of the TADS (using Heaton DSS) of the pigs against the PMSI of each salinity group. 172

Figure 6.8: Graph of the pigs' TADS (using van Daalen DSS) against the PMSI of each salinity group. 173

Figure 6.9: Graph showing temperature fluctuation in the water groups across the 70 days. 174

Figure 6.10: Graph of the TADS (using Heaton DSS) of the pigs against the Log ADD of each salinity group. 175

Figure 6.11: Graph of the pigs' TADS (using van Daalen DSS) against the Log ADD of each salinity group. 176

Figure 6.12: Physical decomposition changes in the pigs of each group across the 70 days. 181

Figure 6.13: Graph of the TADS (using Heaton DSS) of the pigs against the PMSI of each salinity group. 183

Figure 6.14: Graph of the pigs' TADS (using van Daalen DSS) against the PMSI of each salinity group. 184

Figure 6.15: Graph showing temperature fluctuation in the water groups across the 70 days. 185

Figure 6.16: Graph of the TADS (using Heaton DSS) of the pigs against the Log ADD of each salinity group. 186

Figure 6.17: Graph of the pigs' TADS (using van Daalen DSS) against the Log ADD of each salinity group. 187

Figure 6.18: Physical decomposition changes in the pigs of each group across the 70 days..... 195

Figure 6.19: Presence of adipocere on the back and trunk of a pig as at day 21. 196

Figure 6.20: Pictures of the skulls of the pigs from the three groups. Control (a); Brackish (b); Sea (c). 197

Figure 6.21: Fresh lower limbs of the pig in the Sea group as of Day 56..... 198

Figure 6.22: Presence of insect activity on the pigs by day 14. 199

Figure 6.23: Maggots infestation by day 20. 200

Figure 6.24: Adipocere observed on pigs on days 27 (Sea-Constant) and 35 (Fresh-Changing). 201

Figure 6.25: Green algae build up in the boxes of the Fresh-Changing group. 202

Figure 6.26: Bones of pigs turned black because of the mesh bleaching..... 204

Figure 7.1: The Massey-Martin (2003) skin colour guide (Louie & Wilkes, 2018). 211

Figure 7.2: Pictures and the skin colour score assigned to them. 213

Figure 7.3: Collated information obtained from case notes. 214

Figure 7.4: Average daily temperatures in Zwolle, Netherlands, for the first week of March 2020. . 215

Figure 7.5: Graph used to develop a regression formula for the cases. 217

Figure 7.6: Breakdown of the TADS, PMSI estimates and demographic profile of each case. 224

Figure 7.7: Pale appearance of the skin of a dark-skinned cadaver during marbling and wrinkling of the limbs. Picture source: Office Autopsies database/Livelink. 235

Figure 8.1: A schematic of the drowning case database. 241

Figure 8.2: Proportion of drowning deaths by sex in the U.K. [2010-2013] (National Water Safety Forum, 2015)..... 243

Figure 8.3: Education guidelines for drowning prevention (Moran *et al*., 2011) 247

Figure 8.4: Flowchart of the steps in each Phase of the Peden *et al*. (2020) study..... 251

Figure 9.1: Decomposition changes and features observed in the human (images) and human analogues. 254

LIST OF TABLES

Table 2.1: The stages of decomposition along with the odour and insect activity (Verheggen *et al*., 2017). 12

Table 2.2: List of equations developed for PMI estimation through potassium ions in the vitreous (Zilg *et al*., 2015). 21

Table 2.3: Corrective factors in the nomogram method (Henssge, 1992). 25

Table 2.4: Stages of aquatic decomposition (Stuart & Ueland, 2017). 51

Table 2.5: Summary of conditions that affect the decomposition rate. 61

Table 2.6: Water profile of seas and rivers in the U.K. 62

Table 3.1: Descriptive stages of decomposition for the TBS system (Megyesi *et al*., 2005). 66

Table 3.2: Descriptive stages of decomposition for the Heaton *et al*. (2010) DSS. 70

Table 3.3: Descriptive stages of decomposition for the van Daalen *et al*. (2017) DSS. 73

Table 3.4: Differences between the Heaton *et al*. (2010) & van Daalen *et al*. (2017) DSS. 76

Table 4.1: The Fitzpatrick Skin Type scale (Sachdeva, 2009). 83

Table 5.1: Breakdown of the groups (30 mice per group) for each salinity study. 96

Table 5.2: Concentrations of the salts in the various groups. 97

Table 5.3: Water quality of the tap water used for the studies in this research (United Utilities, 2023). 97

Table 5.4: Breakdown of the groups (30 mice per group) for the Hardness study. 99

Table 5.5: Hardness concentrations of the various groups. 99

Table 5.6: Breakdown of the groups (30 mice per group) for the Current study. 100

Table 5.7: Breakdown of the groups (30 mice per group) for each water condition studied. 102

Table 5.8: Adapted Heaton DSS. 104

Table 5.9: Adapted van Daalen DSS. 105

Table 5.10: The average TADS of the salinity group mice on the sampling days. 108

Table 5.11: Post Hoc test results of both DSS. 109

Table 5.12: Mann-Whitney U test results. 110

Table 5.13: ADD and Log ADD of the salinity groups across the 35 days. 111

Table 5.14: Average TADS of the mice in each salinity group on the sampling days. 116

Table 5.15: Post Hoc results of the statistics for the Heaton DSS. 116

Table 5.16: Mann-Whitney U test results. 118

Table 5.17: ADD and Log ADD of the salinity groups across the 35 days. 119

Table 5.18: Average salinity concentrations of each group on the sampling days. 121

Table 5.19: Average pH levels of each group on the sampling days. 121

Table 5.20: Average TDS concentrations of each group on the sampling days. 122

Table 5.21: Average TADS of the mice in each hardness group on the sampling days. 128

Table 5.22: Post Hoc test results. 128

Table 5.23: ADD and Log ADD of the salinity groups across the 35 days. 130

Table 5.24: Average TADS of the mice in each group on the sampling days. 136

Table 5.25: Post Hoc test results of both DSS. 137

Table 5.26: Mann-Whitney U test results. 138

Table 5.27: ADD and Log ADD of the groups across the 35 days. 140

Table 5.28: Average salinity concentrations of each group on the sampling days. 141

Table 5.29: Average pH levels of each group on the sampling days. 142

Table 5.30: Average TDS concentrations of each group on the sampling days. 142

Table 5.31: Average TADS of the mice in each salinity group on the sampling days. 147

Table 5.32: Post Hoc test results of both DSS. 147

Table 5.33: Mann-Whitney U test results. 149

Table 5.34: Average ADD and Log ADD of the salinity groups across the 35 days. 150

Table 5.35: Average salinity concentrations of each group on the sampling days. 152

Table 5.36: Average pH levels of each group on the sampling days. 153

Table 5.37: Average TDS concentrations of each group on the sampling days. 153

Table 6.1: Breakdown of the groups (5 pigs per group) for the Initial salinity study. 164

Table 6.2: Breakdown of the groups (5 pigs per group) for the Changing salinity study. 168

Table 6.3: Average TADS of the pigs [Possible Maximum Score: Heaton=25, van Daalen=18]. 171

Table 6.4: ADD and Log ADD of the three salinity groups across the 70 days of decomposition. ... 175

Table 6.5: Average TADS of the pigs 182

Table 6.6: Post Hoc test results of both DSS. 183

Table 6.7: Mann-Whitney U test results comparing the normalised Heaton DSS scores with the normalised van Daalen DSS scores. 184

Table 6.8: ADD and Log ADD of the three salinity groups across the 70 days of decomposition. ... 186

Table 6.9: Average salinity concentrations of each group on the sampling days. 188

Table 6.10: Average pH levels of each group on the sampling days. 189

Table 6.11: Average TDS concentrations of each group on the sampling days. 190

Table 7.1: Identified keywords used to search the databases for cases. 209

Table 7.2: Breakdown of cases that met the study criteria. 210

Table 7.3: Collated characteristics of the drowned victims. 211

Table 7.4: TADS of all the cases used. S/N stands for sample number. 221

Table 7.5: Recorded rounded-up PMSI in the case notes and the Estimated PMSIs using the three DSS. S/N stands for sample number. 225

Table 7.6: Post Hoc test results of the four sets of PMSI. 227

Table 7.7: Estimated PMSI using Inc-TADS. S/N: sample number, H: Head, T: Trunk, L: Limbs. . 228

Table 7.8: Post Hoc test results of the three sets of PMSI. 229

Table 8.1: List of keywords and search terms indexed into search engines. 240

Table 8.2: Demographic profile and the number of cases in the Drowning Case Database. 242

Table 8.3: Age categories of the drowned victims (2012-2019) in the Water Incident Database (Hills *et al*., 2021). 244

Table 8.4: Consolidated list of the 11 strategies identified in Phase I (Peden *et al*., 2020). 248

Table 8.5: The final 13 strategies at the end of Phase IV (Peden *et al*., 2020). 249

LIST OF ABBREVIATIONS

ADD	Accumulated Degree Days
a-TADS	Adapted Total Aquatic Decomposition Score
ATP	Adenosine Triphosphate
AWERB	Animal Welfare Ethical Review Board
BADS	Body Aquatic Decompositional Score
CE	Capillary Electrophoresis
CHIRP	Compressed High Intensity Radar Pulse
CIE	Commission Internationale de l'Eclairage
DNA	Deoxyribonucleic Acid
DPRC	Drowning Prevention Research Centre
DSS	Decomposition Scoring Systems
FADS	Facial Aquatic Decompositional Score
FST	Fitzpatrick Skin Type
GPR	Ground Penetrating Radar
GWA	Genome-Wide Association
HTFs	Human Taphonomy Facilities
Inc-TADS	Incomplete TADS
LADS	Limb Aquatic Decompositional Score
LMICs	Low- and Middle-Income Countries
MCA	Microbial Community Assembly
MGC	Metric Graduated Colour
MoU	Memoranda of Understanding
NCIS	National Coronial Information System
NWSF	National Water Safety Forum
NZHIS	New Zealand Health Information Service
OLS	Ordinary Least Squares
PAI	Pre-appearance Interval
PCR	Polymerase Chain Reaction
PCT	Post-mortem Computed Tomography
PI	Presence Interval
PMCTA	Post-mortem Computed Tomography Angiography
PMI	Post-mortem Interval
PMMR	Post-mortem Magnetic Resonance Imaging
PMSI	Post-mortem Submersion Interval
PPT	Parts Per Thousand

ROV	Remotely Operated Vehicles
RoSPA	Royal Society for the Prevention of Accidents
SNPs	Single Nucleotide Polymorphisms
TADS	Total Aquatic Decomposition Score
TBS	Total Body Score
TDS	Total Dissolved Solids
TRACES	Taphonomic Research in Anthropology: Centre for Experimental Studies
UK	United Kingdom
USA	United States of America
VOCs	Volatile Organic Compounds
WAID	Water Incident Database (WAID
WHO	World Health Organization
WSNZ	Water Safety New Zealand

DISCRETION WARNING

The content of this thesis is intended for readers of a forensic background and contains graphic decomposition images of animals and humans that may be disturbing to readers. Viewer discretion is advised.

1 INTRODUCTION

1.1 Drowning, Aquatic Decomposition and Decomposition Scoring Systems

Drowning occurs when a body is immersed in water or other liquid until there is no access by air to the lungs due to the blockage of the airways by the fluid (Byard, 2017; Szpilman & Morgan, 2021). Drowning is a common cause of death; annually, over 200,000 people die by drowning worldwide (Reijnen *et al*., 2018; Yorulmaz *et al*., 2003). Due to this phenomenon, there is an increased need to understand the process of drowning and aquatic decomposition (Heaton *et al*., 2010). Therefore, more studies are being conducted to investigate the factors affecting aquatic decomposition and improve the estimation of Post-mortem Submersion Interval [PMSI] (Byard, 2018; Mateus & Vieira, 2014; Reijnen *et al*., 2018; van Daalen *et al*., 2017). Death by drowning in various water environments occurs because of recreational accidents, natural and transport disasters, suicide, and homicide (Byard, 2017; Papadodima *et al*., 2010).

In the United Kingdom, the Human Tissue Act (2004) was designed for the regulation of all activities involving the post-mortem examination and retention of human tissue, organs, or bodies, with consistent emphasis on the need for fully informed consent; this Tissue Act prevents the use of human remains for decomposition studies (Human Tissue Authority, 2004; Pringle *et al*., 2010). Due to these ethical restrictions on the use of human bodies for research, particularly in the U.K., these accidents, case studies, and the use of human analogues in empirical studies serve as a source of vital information for estimating PMSI for forensic sciences and help with the location of drowned victims (Mateus & Vieira, 2014).

Like decomposition in air and soil, the decomposition process and rate in water are complex, but the rate of aquatic decomposition differs from terrestrial decomposition due to a range of different factors; however, these factors have not been researched thoroughly (Reijnen *et al*., 2018; Saukko & Knight, 2015). These factors include the cooler temperatures experienced in aquatic environs, tidal fluctuations, the absence of insects, water currents, turbulence in the water, water salinity, and the presence of microorganisms and scavengers in water (Campobasso *et al*., 2001; Di Maio & Di Maio, 2001; Humphreys *et al*., 2013; Kahana *et al*., 1999; Rodriguez, 1997). There is little existing research on decomposition in water or PMSI estimation methods, especially compared to the amount of literature about decomposition and Post-mortem Interval (PMI) estimation on land or other terrestrial environments. Therefore, further research is required to understand better the water conditions and factors affecting the aquatic decomposition process to help improve the estimation methods of PMSI.

The interval between immersion/death and the recovery of human remains from a body of water is known as Post-mortem Submersion Interval (Humphreys *et al*., 2013). Accurately estimating the PMSI in drowning cases plays an essential role in reconstructing the events around death, excluding suspects, and identifying unknown human remains (Moffatt *et al*., 2016; van Daalen *et al*., 2017). Various

methods have been formulated to give estimations of the PMSI of bodies recovered from the water, and these methods have been adaptations of the techniques for estimating PMI on land/in terrestrial environments. One of the methods involves indicating the presence of microorganisms such as bacteria, protozoans, algae, and fungi on the bodies recovered from water (Benbow *et al*., 2015; Lang *et al*., 2016; Zimmerman & Wallace, 2008). Another set of methods involves adapting the Total Body Score (TBS) system developed by Megyesi *et al*. (2005) for bodies decomposing on land for those recovered from water.

Various researchers have modified this TBS scoring system into different Decomposition Scoring Systems (DSS), such as the Total Aquatic Decomposition Score (TADS), which scores the extent of decomposition in bodies recovered from water (Heaton *et al*., 2010; Van Daalen *et al*., 2017). These DSS are based on visual assessments of the external and internal observations of the decomposition changes visible on the head, neck, trunk, and limbs (Heaton *et al*., 2010; Reijnen *et al*., 2018; Van Daalen *et al*., 2017). These decomposition features assessed include anatomical changes such as disarticulation, bone exposure and skeletonization, but the majority of these observable changes are discolouration changes such as livor mortis (purple), marbling (red or pink), washerwoman's hand (white or pale) and the greenish colour observed during putrefaction stage of decomposition because of bacterial activities (Heaton *et al*., 2010; Van Daalen *et al*., 2017). These changes are assessed, scored, and recorded as TADS; this, along with the Accumulated Degree Days (ADD), is then used to estimate the PMSI (Mateus & Vieira, 2014). ADD represents the accumulation of heat energy needed for biological and chemical reactions in soft tissues for decomposition (Palazzo *et al*., 2020). ADD is calculated by adding the average daily temperatures exposed to the decomposing body (Humphreys *et al*., 2013).

Most studies on aquatic decomposition have been carried out in regions such as Europe, Australia, and the USA. The conventional methods for PMSI estimation and the available DSS were developed from these studies (Byard, 2017; Heaton *et al*., 2010; Humphreys *et al*., 2013; Reijnen *et al*., 2018). Some common DSS formulated from these aquatic decomposition studies include Heaton *et al.* (2010), van Daalen *et al.* (2017) DSS and the Reh (1967) table. These existing DSS are limited because they have been developed from studies carried out in countries with temperate climates and individuals with predominantly pale skin and are rarely implemented in cases with darker skin tone victims. This means that the existing DSS and PMSI estimation methods may apply only to cases with comparable temperate climates and waterways but most often apply to individuals with pale skin tones. This leaves a gap in knowledge and uncertainty on whether these methods could be helpful in cases of drowning or body deposition in water environments in regions such as Africa, Asia, the Caribbean, or with darker skin-toned individuals. Therefore, there is a potential for investigations and PMSI estimations of cases involving individuals with darker skin tones to be compromised.

1.2 Research Questions

The following are key questions that focus on the gaps in knowledge of this research and need to be answered to ensure the aims of this research will be met.

1. Will each water condition studied statistically significantly affect the decomposition rate and PMSI estimation of human analogues?
2. Will changing salinity concentration statistically significantly affect the decomposition rate and PMSI estimation of human analogues?
3. Will the adapted Heaton and van Daalen DSS be suitable and effective for assessing the extent of decomposition in human analogues such as mice and pigs?
4. Will there be a statistically significant difference in the TADS of the human analogues when comparing the adapted Heaton and van Daalen DSS scores of each analogue?
5. Will there be a statistically significant difference in the TADS of the human cases when comparing the Heaton and van Daalen DSS scores of each case?
6. Can this research improve the suitability of the existing DSS for assessing decomposition changes in individuals with darker skin tones other than the reference individuals?
7. Can the DSS be adapted regionally (regardless of the skin tone of the victim) because of the varying climates compared to Europe?
8. Can this research improve the accuracy and precision of the available PMSI estimation methods?

1.3 Research Outline

The research was divided into four broad parts: *Laboratory Studies on Water Conditions, Field Studies on Salinity Concentration, Comparative Human Image Analysis and Drowning Case Database*. Further details on these four broad parts are given below. The research was divided into these sections because each part of the study had a specific human analogue or data on which experiments and analyses were conducted (See explanations below). This thesis will present each sub-components with its introduction, methodology, results, and discussion sections.

1.3.1 Laboratory Studies on Water Conditions

Before the commencement of the laboratory studies, a pilot study[1] was conducted. The pilot study was designed to have two purposes. Firstly, to test if the Heaton and van Daalen DSS could be adapted (a-TADS) and be suitable for assessing the extent of decomposition in human analogues such as mice. Secondly, to help provide the experience, skills, and practice of procedures essential and necessary for further empirical aquatic decomposition studies. These procedures included assessing the extent of decomposition with the scoring systems, preparing a standard set-up for aquatic decomposition studies, cleaning or discarding biological wastes, and other aspects that would later help in the laboratory and field studies.

[1] Undertaken at the University of Huddersfield before transfer to the University of Central Lancashire in the first year of study.

The laboratory experiments were designed to investigate the effect of water temperature, salinity, hardness, current, total dissolved solids (TDS), pH and skin tone on the decomposition rate of human analogues (mice- *Mus musculus*) in a laboratory setting. Mice were chosen for these experiments because of their small size, cost-effectiveness in purchase and storage, and the potential to study large numbers at once. Therefore, large-scale mice experiments were carried out, giving the laboratory studies a high statistical power.

1.3.2 Field Studies on Salinity Concentration

These studies mirrored the laboratory experiments and investigated water salinity's effect on porcine human analogues' decomposition rate. Promising results were obtained from the laboratory studies investigating salinity's effect on mice's decomposition rate; hence, studies investigating the effect of salinity on larger human analogues' decomposition rates were needed for comparisons. The studies were conducted using pigs (*Sus scrofa domesticus*) at the TRACES Animal Taphonomy Facility at the University of Central Lancashire in an outdoor setting.

1.3.3 Comparative Human Image Analysis

Due to ethical restrictions on using human bodies in research in the U.K., a retrospective analysis was conducted on pictures of drowned victims from forensic cases obtained from the U.K., Netherlands, and South Africa. A retrospective study was chosen because of the time constraints of the study and solved closed cases were the best option for such a study. A prospective study would have taken longer than the allocated time for the PhD research, and primarily only open cases with many missing data that were still *sub judice* would have been available.

Where possible, the autopsy records and photographs of human drowning victims from the U.K., Netherlands, and South Africa were assessed to evaluate the extent of decomposition using Heaton *et al*. (2010), van Daalen *et al*. (2017) and Reh (1967) scoring systems against the known PMSI in a variety of water conditions. Temperature data from each location and the decomposition scores were used to estimate the PMSI. This estimated PMSI was compared to the stated PMSI in the case files to see if there was a significant difference between the estimated PMSI based on the DSS and the known PMSI values. This analysis also aimed to improve the suitability of the DSS for assessing decomposition changes in individuals with dark skin tones.

1.3.4 Drowning Case Database

In addition, data from drowning incidents and cases published in the literature and news media to date were collated into an Excel spreadsheet file and referred to as a "Drowning Case Database", comprising age, sex/gender, temperature (body, water and ambient), known PMSI, and other essential information about each drowning case. This database serves as a reference for drowning cases, can help determine if there is a correlation between any data obtained (especially the water conditions) and the PMSI, provide insights or establish any trends in the frequency of drowning, demographics, levels of risk,

make meaningful comparisons across various locations and where possible, suggest prevention and intervention steps for drowning occurrences.

1.4 Aims and Objectives

1.4.1 Aims

This research had three broad aims:

1. To investigate the impact of varying concentrations or levels of water temperature, salinity, hardness, pH, TDS, skin tone and current on the decomposition rate of human analogues (mice and pigs).

H_1 = There is a significant difference in the decomposition rate of a human analogue exposed to varying concentrations or levels of the water conditions listed above.
H_0 = There is no significant difference in the decomposition rate of a human analogue exposed to varying concentrations or levels of the water conditions listed above.

2. To investigate if the Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS could be adapted, suitable and effective for assessing the extent of decomposition in mice and pigs.

H_1 = There is a significant difference in the TADS of the pigs when comparing the Heaton and van Daalen DSS.
H_0 = There is no significant difference in the TADS of the pigs when comparing the Heaton and van Daalen DSS.

3. To investigate if the Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS are suitable to achieve accurate PMSI estimates for drowning cases from regions or cases involving individuals with dark skin tones.

H_1 = There is a significant difference between the estimated PMSIs and case note PMSIs when applying the Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS to drowning cases involving dark skin tone victims.
H_0 = There is no significant difference between the estimated PMSIs and case note PMSIs when applying the Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS to drowning cases involving dark skin tone victims.

1.4.2 Objectives

1. To carry out laboratory and field studies using mice and pigs to investigate the effect of varying levels of the following conditions: a) temperature, b) salinity, c) hardness, d) pH, e) TDS, f) skin tone, and g) current, on the rate of aquatic decomposition. By carrying out these experiments and investigating the impact of each condition, this study will further understand the processes of aquatic decomposition under these different factors and water conditions and improve and validate the current DSS and PMSI estimation methods.

2. To statistically compare if there is a significant difference in the TADS when using the adapted Heaton and van Daalen DSS for assessing the extent of decomposition in the human analogues for laboratory and field studies. This will validate whether the DSS are suitable for scoring decomposition changes in human analogues.

3. To compare case files, pictures and autopsy reports of human bodies recovered from water regions in the U.K., Netherlands, and South Africa. The comparative study was conducted by analysing the images obtained from these countries; Heaton and van Daalen's DSS were used to assess the degree of decomposition in these pictures. Temperature data of each country and the decomposition scores formulated were used to estimate the PMSI; this estimated PMSI were compared to the stated PMSI in the case files to see if there was a significant difference between both PMSI values. This will help determine if there are similarities between the animal analogues and the human cases and if the cases show the same variations with water conditions. This analysis also aimed to improve the suitability of the DSS for assessing decomposition changes in individuals with dark skin tones.

4. To create a "Drowning Case Database" that stores details such as age, sex, temperature (body, water and ambient), estimated PMSI, and other essential information about drowning cases published worldwide. It will serve as a reference for drowning cases and help determine if there is a correlation between any data obtained and the PMSI.

2 EXISTING RESEARCH INTO AQUATIC DECOMPOSITION

The process of aquatic decomposition is complex and is affected by various factors (Reijnen *et al*., 2018; Saukko & Knight, 2015). To investigate the process of aquatic decomposition and the influencing factors, it is necessary to understand the context of taphonomy, decomposition process in terrestrial environments, and the process of drowning and to look at existing research into aquatic decomposition.

2.1 Taphonomy

In scientific disciplines such as archaeology, palaeontology, palaeobiology, anthropology, zoology, geology, and forensic science, "Taphonomy" has different definitions (Lyman, 2010). Taphonomy, as a word, originated from two Greek words; "*tophos"*, meaning "burial", and "*nomos"*, meaning "law" (Schotsmans *et al*., 2017). In 1935, a Russian palaeontologist, Ivan Antonovich Efremov, defined Taphonomy as "the study of the transition of organisms from the biosphere to the lithosphere" (Turner *et al*., 2013). In a forensic context, Taphonomy can be broadly defined as the use of models, analyses, and approaches to study the diverse factors and conditions of an organism's decomposition (Haglund & Sorg, 1997; Verheggen *et al*., 2017).

Forensic taphonomy aims to provide accurate estimations of post-mortem interval (PMI), give vivid reconstructions of the events of death and decomposition, aid the judicial system in death cases, provide more understanding of the products of decomposition, aid in identifying unknown human remains, and to investigate the factors that affect human decomposition (Roberts *et al*., 2017; Schotsmans *et al*., 2017). Therefore, taphonomy plays an essential role in reconstructing the circumstances linked to decomposition, from the moment death occurs until after the point of skeletonization (Schotsmans *et al*., 2017).

2.2 Terrestrial Decomposition

Decomposition is a multidimensional and natural process associated with physical, biological, and chemical changes that involve the degradation of an organism's biological macromolecules into smaller organic units (Perrault *et al*., 2015). Terrestrial decomposition occurs on land or in the air, while aquatic decomposition occurs in bodies of water. The factors affecting the decomposition process in aquatic environments have not been researched thoroughly, but the limited knowledge of aquatic decomposition has been acquired through adaptations of factors related to terrestrial decomposition. For example, decomposition scoring systems such as the TADS system used to assess the extent of decomposition of bodies recovered from water was adapted from the TBS system created by Megyesi *et al*. (2005) for bodies decomposing on land (Heaton *et al*., 2010; van Daalen *et al*., 2017). Therefore, an in-depth understanding of the process and stages of terrestrial decomposition is essential for investigating the process of aquatic decomposition and the factors that influence it.

2.2.1 Stages and Chemistry of Terrestrial Decomposition

The physical processes of terrestrial decomposition are broadly understood and can even be categorised into ordinal parts, but there is limited knowledge about the underlying chemistry of the terrestrial decomposition process (Iqbal *et al*., 2017). Terrestrial decomposition is a continuous and intricate process; although it can be categorised into stages based on gross observations, classifying it into stages is subjective (Matuszewski *et al*., 2010). Depending on the geography, organism and weather conditions, the stages of terrestrial decomposition may vary from 1–9 stages (Goff, 2009); still, it is mainly classified into five distinct stages, which include Fresh, Bloat, Active decay, Advanced decay and Skeletonization (Dekeirsschieter *et al*., 2009).

2.2.1.1 *Fresh Stage*

The fresh stage usually begins immediately after death and lasts three or four days in a temperate climate (Shrestha *et al*., 2022). In the winter months, it can last for more than seven days (Shrestha *et al*., 2022). This stage is mainly initiated by the actions of biological factors such as reactive oxygen species, bacteria, enzymes, fungi, microorganisms, or scavengers (Boumba *et al*., 2008; Paczkowski & Schütz, 2011). The intrinsic activities of these biological factors lead to biochemical changes such as autolysis, putrefaction, and liquefaction (Forbes *et al*., 2017). The fresh stage includes a chemical reaction known as autolysis and includes physical changes such as *rigor mortis, algor mortis, livor mortis*, and tache noire (Goff, 2009; Shrestha *et al*., 2022).

The process through which body cells and organs undergo self-digestion and degradation by the activity of intracellular enzymes is known as autolysis (Di Maio & Di Maio, 2001). Autolysis begins about 4 minutes after death (Rosier *et al*., 2014). After death, a compromise in the integrity of the cell membrane occurs due to a lack of supply of oxygen, a decrease in cytoplasmic pH and the presence of aerobic microorganisms; this reduction in cell membrane integrity initiates the release of lysosomes and enzyme-containing organelles (Tsokos, 2005). Autolysis is said to first occur in the pancreas due to the release of proteolytic and hydrolytic enzymes that digest the organ (Hamilton & Green, 2017). Autolysis also occurs rapidly in other organs, such as the liver, spleen, lungs, kidneys, stomach, and brain, which are rich in hydrolytic enzymes. Proteins, carbohydrates, and fats are broken down by these organs' enzymes, like proteases, amylases, and lipases (Zhou & Byard, 2011). The products of autolysis serve as nutrients for microorganisms in the body and this initiates putrefaction (Mayer, 2012).

The onset of algor mortis occurs immediately after death. Algor mortis is the body's cooling process after death due to its inability to regulate its internal temperature through homeostatic regulation and heat loss to the environment (Goff, 2009; Shrestha *et al*., 2022). During algor mortis, the internal body temperature reduces until the ambient temperature is reached; this process can last up to 18 to 20 hours (Fisher, 2007). Exposure to sunlight, weather conditions, clothing, and body size can affect the rate of body cooling (Henssge & Madea, 2004; Shrestha *et al*., 2022).

Livor mortis is the gravitational pooling of blood in the lower dependent areas, both externally in the skin capillaries and venules and in the internal organs resulting in the red-blue-purple discolouration of the skin after death (Hayman & Oxenham, 2016; Simmons & Cross, 2013). The mechanism of livor mortis is complex; the onset usually occurs within 30 minutes to 3 hours after death, and the pooling patterns of the blood being formed in this process become evident and fixed at about 12 hours after death (Byard, 2020; Shrestha *et al*., 2022). An example of livor mortis in a body is illustrated in Figure 2.1 below. The red-purple discolouration in this body can be observed on the back, the part of the body pressed against the surface it is placed upon.

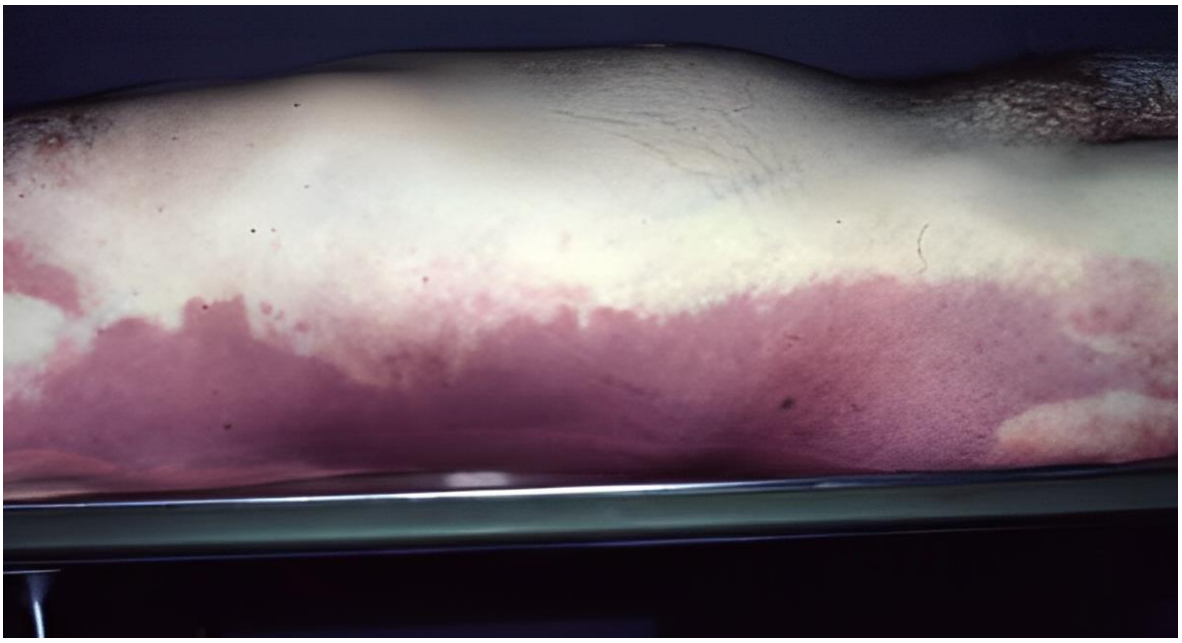


Figure 2.1: Livor Mortis in a cadaver (Goff, 2009).

Rigor mortis is a post-mortem chemical change characterised by the stiffening of muscles in the body due to the depletion and lack of production of Adenosine Triphosphate [ATP] (Shrestha *et al*., 2022). After death, oxygen supply stops and aerobic respiration in the muscle cells is halted; this leads to the depletion of ATP needed to break down the covalent bond formed between myosin and actin fibres (Anders *et al*., 2013). This bond and the build-up of lactic acid cause muscle contraction and rigour (Goff, 2009). The onset of rigor mortis occurs approximately 2 hours after death; it is initially observed in the facial muscles and spreads to other muscles in the body between 6 to 8 hours after death (Anders *et al*., 2013). The duration of rigor mortis can last up to 84 hours before the muscles start to relax (Goff, 2009). The covalent bonds formed between the myosin and actin fibres are broken down at this phase by proteolysis, and the rigor in the muscles is lost with relaxation starting in the facial muscles and spreading to the limbs and other body parts (Shrestha *et al*., 2022). An example of rigor mortis occurring in the limbs of a body can be seen in Figure 2.2, with the right arm pointing upwards and the left arm bent while the body is in a supine position.



Figure 2.2: Presence of rigor mortis in the upper limbs of a cadaver (Goff, 2009).

Tache noire is the drying and black discolouration of the cornea when the eye remains open and exposed after death (Goff, 2009). An example of a tache noire can be seen below in Figure 2.3.

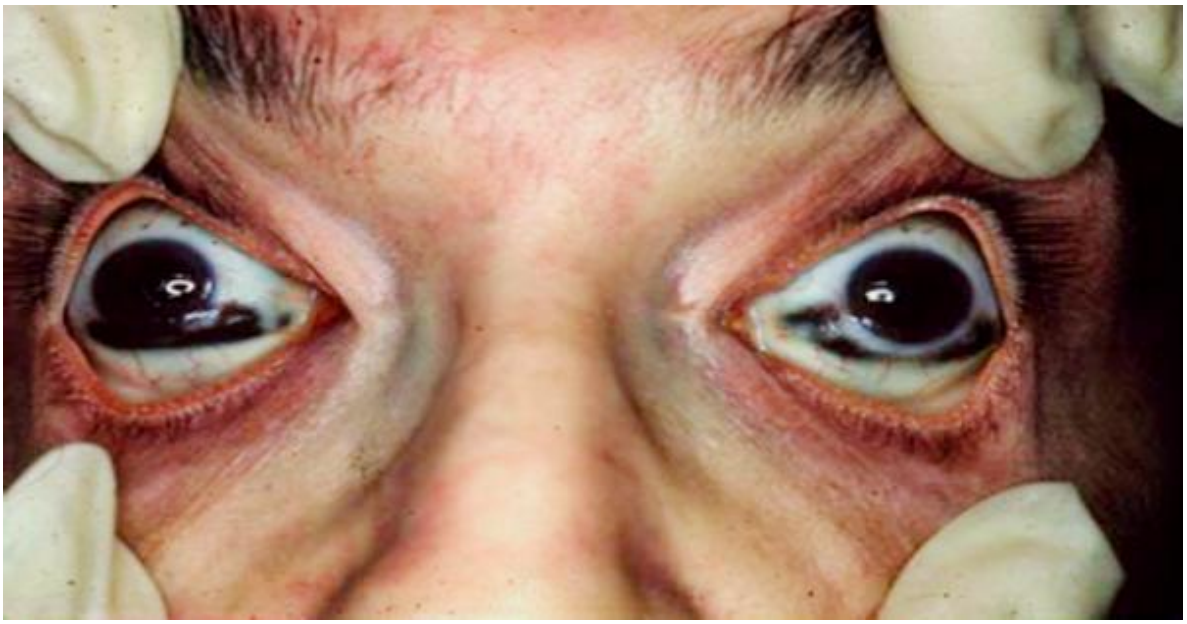


Figure 2.3: Pictorial representation of Tache Noire in a body because of drying (Goff, 2009).

Oviposition or the entry of insects into body orifices to deposit eggs can also occur during the fresh stage (Verheggen *et al*., 2017). An example of insects entering the body through the nasal openings of a corpse can be seen in Figure 2.4. These colonies of insects are attracted to a cadaver because of a wide range of compounds known as volatile organic compounds (VOCs); these compounds are responsible for the smell emanating from a decomposing body (Verheggen *et al*., 2017). Figure 2.5 is a picture of a

decomposing body in the fresh stage of decomposition, while Table 2.1 shows the breakdown of the characteristics of some of the insect activities and the type of smell for each stage of decomposition,



Figure 2.4: Blowflies trying to enter the body orifices (Rodriguez & Bass, 1985).

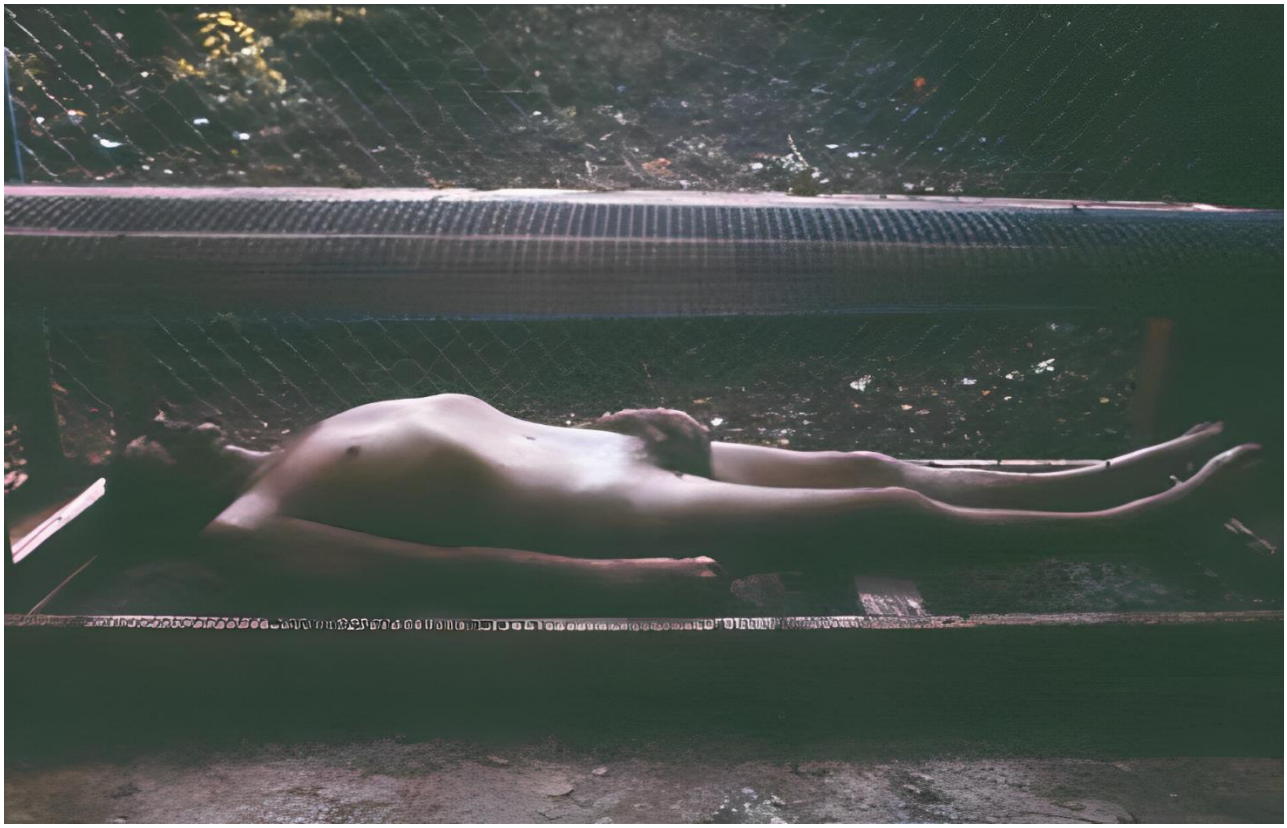


Figure 2.5: Fresh stage of decomposition in a human cadaver (Rodriguez & Bass, 1985).

Table 2.1: The stages of decomposition along with the odour and insect activity (Verheggen et al., 2017).

	Stage	Description	Insect Activity	Odour Evolution
Early Decomposition	Fresh	-First hours following death -Onset of autolysis -Rigor mortis, livor mortis, algor mortis	Initial attraction of flies to carcass and oviposition in body orifices	Minimal perceived odor
	Bloat	-Onset of putrefaction -Gas accumulation due to microorganisms and macromolecule breakdown	-Continued attraction and oviposition -Development of fly larvae and initial larval masses found	-Strong perceived odor -Odour typically perceived as containing sulfur compounds, methane, and ammonia
Late Decomposition	Active Decay	-Deflation of the body -High rate of tissue removal by microorganisms, insect activity, and macromolecule breakdown	Active consumption of tissue by matured insect larvae	Strong and complex odor comprising a wide array of compounds
	Advanced Decay	-Reduction in moisture -Some remaining tissue typically in torso region	-Reduced insect activity and migration of fly larvae -Colonization by beetles	Reduction in perceived odor but still complex and present
	Dry/Remains	-Hair, cartilage, and bone remain -Mineral degradation	-Minimal fly and beetle activity -Regular assemblage re-established	-Reduced odor -Perceived odor mostly from surrounding soil

2.2.1.2 *Bloat Stage*

The bloat stage begins approximately two days after death and can last up to 2 weeks (Shrestha *et al*., 2022). The onset of the bloat stage is characterised by putrefaction, which leads to the accumulation of gases in the body, primarily observed in the abdomen as slight inflation or bloat (Dekeirsschieter *et al*., 2009). Putrefaction begins approximately 48–72 hours after death (Rosier *et al*., 2014). Due to the reduction in oxygen levels in the body after death, the process of putrefaction is mainly stimulated by anaerobic bacteria that result in the breakdown and liquefaction of soft tissues (Janaway *et al*., 2009). The degradative action of anaerobic bacteria in the respiratory and digestive tracts leads to the build-up of gases; this causes bloating, which is observed mainly in the abdomen (Zhou & Byard, 2011).

Green discolouration of the abdomen is one of the first physical signs of putrefaction (Janaway *et al*., 2009). It is caused by the release of hydrogen sulphide from sulphur-containing amino acids, which then combine with haemoglobin to create sulfhaemoglobin (Goff, 2009; Rosier *et al*., 2014). The spread of the green sulfhaemoglobin through the capillaries creates a dark dendritic or mosaic pattern just under the skin surface, known as "marbling" (Goff, 2009). Marbling is typically observed on the abdominal region, neck, and limbs, and an example of this can be seen in Figure 2.6.



Figure 2.6: Evidence of marbling on a cadaver (Goff, 2009).

These putrefactive reactions involving anaerobic bacteria in the body lead to the breakdown of the soft tissues and the accumulation of gases that cause a foul smell [Table 2.1] (Swann *et al*., 2010). The smell produced by the decomposition process at this stage is said to be that of gases such as hydrogen sulphide, methane, carbon dioxide, and ammonia (Irish *et al*., 2019). The production and release of these gases from the body attract blowflies (*Calliphoridae*), leading to significant masses of maggots (Swann *et al*., 2010; Verheggen *et al*., 2017).

Due to the metabolic reactions occurring during the putrefaction process in this decomposition stage, the body temperature increases and can significantly rise above the ambient temperature (Goff, 2009). As gases accumulate, body fluids and the by-products of the putrefactive reactions get released through the body orifices and other openings because of the internal pressure built up in the body; this process is known as liquefaction (Goff, 2009; Swann *et al*., 2010). Liquefaction leads to a reduction in the bloat, and it marks the end of the bloat stage. An example of a body in the bloat stage can be seen in Figure 2.7; the abdominal region and genitals of the body are bloated due to the build-up of gases produced during putrefaction.



Figure 2.7: Bloat stage of decomposition in a human cadaver (Rodriguez & Bass, 1985).

2.2.1.3 *Active Decay and Advanced Decay Stage*

The decay stages can start about four days after death or as late as ten days after death and can last for up to 6 months (Shrestha *et al*., 2022). Splitting the Active decay and Advanced decay stages into start and end points is mainly subjective, but the liquefaction process and the feeding on the cadavers by the maggot masses can be referred to as the start points (Goff, 2009). Liquefaction reduces the body's bloated state and causes the appearance of a caved abdomen and a loose appearance of skin due to the release of accumulated gases in the bloat phase (Shrestha *et al*., 2022).

In these stages, the body decomposes further, with the skin sloughing off and the breakdown of soft tissues and internal organs, leaving the muscles, tendons, cartilage, and bones exposed (Goff, 2009; Shrestha *et al*., 2022). The skin sloughs off due to the release of hydrolytic enzymes (during autolysis) between the dermis and epidermis, resulting in the loosening and slippage of the epidermis; vesicles or blisters are initially observed, but over time the nails, hairs and large areas of the skin slough off (Simmons & Cross, 2013). Active and Advanced decay are the stages with the most pungent odour; the skin becomes dark, there is evidence of tissue decomposition, and reduced moisture content as the body deflates (Gennard, 2012; Vass *et al*., 2004; Verheggen *et al*., 2017). A reduction in the moisture content of the decomposing body, especially in dry climates or a location with high temperatures, can lead to mummification (Goff, 2009; Shrestha *et al*., 2022). Large body sections become desiccated with a leathery appearance, and bacterial and insect activities are inhibited, thus preserving the body (Goff, 2009). An example of a mummified body can be seen in Figure 2.8, the tissues have been desiccated, and the body looks preserved.



Figure 2.8: Picture of a mummified body (Rodriguez & Bass, 1985).

Figure 2.9 is an example of a body in the active decay stage; the presence of a caved abdomen due to the release of accumulated gases can be observed on the body.



Figure 2.9: A body with a caved abdomen during the active decay stage (Rodriguez & Bass, 1985).

Figure 2.10 is an example of a body in the advanced decay stage. There is evidence of bone exposure, a dark discolouration of the skin, and some soft tissue drying up.



Figure 2.10: Advanced decay stage of decomposition in a human cadaver (Rodriguez & Bass, 1985).

2.2.1.4 *Skeletonization*

This stage can start as early as two months after death or as late as nine months and last for years with no fixed endpoint (Shrestha *et al*., 2022). In this stage, disarticulation of bones occurs, and only the bones, teeth, cartilages, some soft tissues, and hairs remain (Goff, 2009; Verheggen *et al*., 2017). Skeletonization is tagged "complete" when all soft tissue has been decomposed and tagged "partial" when there is still the presence of soft tissue (Dent *et al*., 2004). Chemical factors such as insects, bacteria and acidic environments can cause the fracture and decalcification of bones leading to the disarticulation and wearing of bones (Pinheiro, 2006). Other factors contributing to the disarticulation and scattering of human remains during the skeletonization stage include scavengers and rain (Moraitis & Spiliopoulou, 2010). The sequence of disarticulation of the bones could vary for different cadavers, but most times, it occurs from the distal to the proximal parts of the body; the bones of the hands, wrists, feet, and ankles disarticulate first, followed by the knee joints, mandible, and cranium while the vertebrates tend to disarticulate last (Cameron & Oxenham, 2012; Ellingham *et al*., 2017; Haglund, 1993). Figure 2.11 is an example of a disarticulated skull.



Figure 2.11: A cadaver's skull that has reached the skeletonization stage of decomposition (Goff, 2009).

Figure 2.12 illustrates the first four stages of terrestrial decomposition as observed in a pig.



Figure 2.12: The first four stages of decomposition, as observed in the carrion of a pig (Dekeirsschieter et al., 2012).

Figure 2.13 is an illustration of the summary of the decomposition process; products of the self-digesting chemical process known as autolysis are further broken down by the process of putrefaction, leading to the production of gases. Putrefactive products are released from the body through liquefaction, further disintegration of the remains occurs, and the decomposition process continues till the skeletal stage is reached (Dent *et al*., 2004).

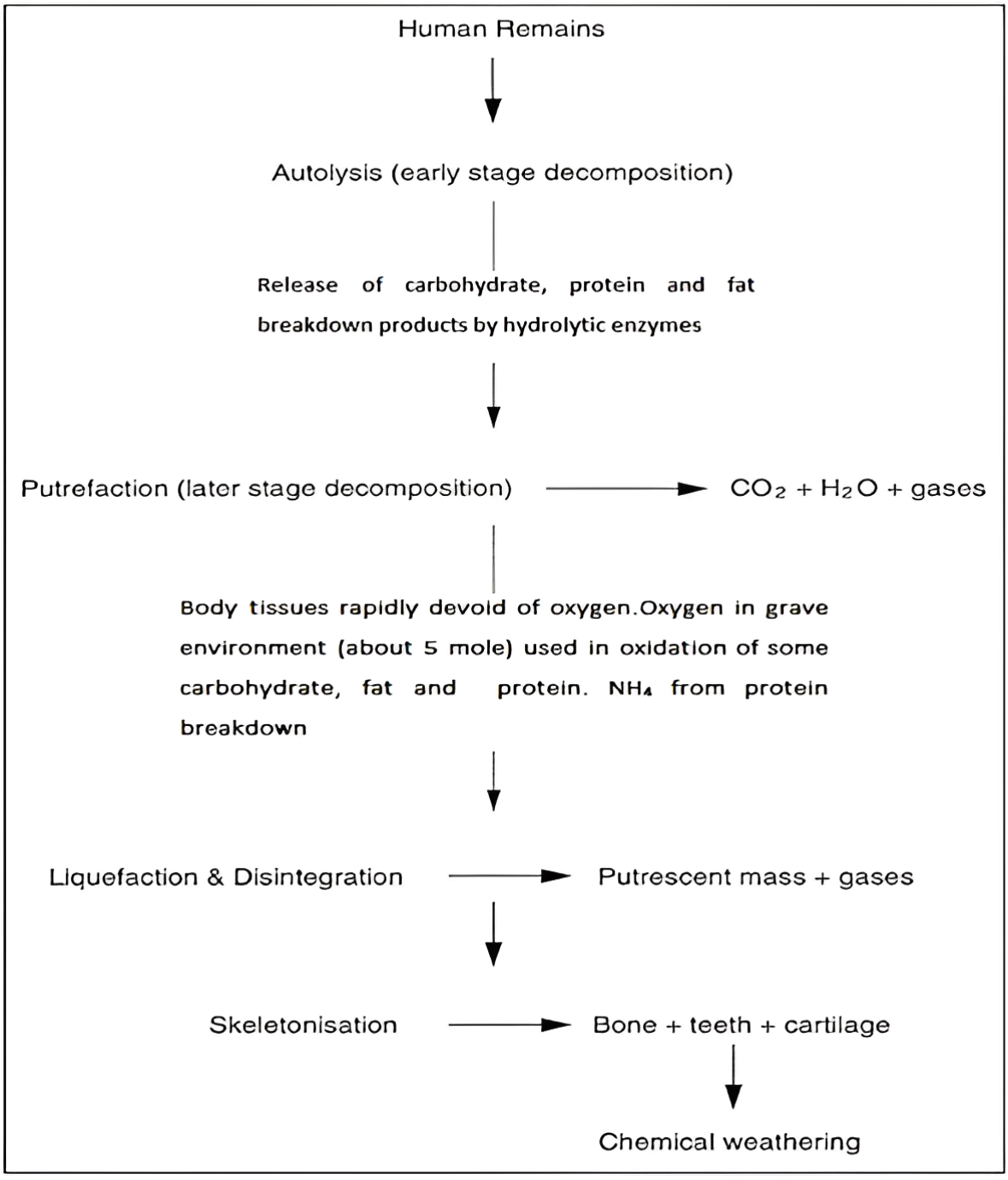


Figure 2.13: Overview of the chemistry of terrestrial decomposition (Dent et al., 2004).

2.2.2 Accumulated Degree Days

The rate of decomposition of a body is affected by the temperature of the surrounding environment; the higher the temperature, the faster the decomposition rate (Champaneri, 2006). Hence, the decomposition process depends on temperature and time (Megyesi *et al*., 2005). ADD represents the accumulation of heat energy needed for biological and chemical reactions in soft tissues for decomposition (Palazzo *et al*., 2020). ADD is calculated by adding the average daily temperatures exposed to the decomposing body (Humphreys *et al*., 2013). ADD is a construct of chronological time and temperature. There is no difference in the decomposition rate (temperature-time) when the ADD is compared against the extent of decomposition (Heaton *et al*., 2010). To put in context, a body exposed to a temperature of 30 ℃ for 4 days will have the same decomposition rate as a body exposed to 4 ℃ for 30 days; both bodies have an ADD of 120.

2.2.3 Estimating Post-mortem Interval

Estimating the PMI of a discovered body is an essential process in forensic science but is possibly the most challenging part of identifying human remains (Simmons, 2017; Wescott, 2018). PMI estimation aids in missing person cases, identification of human remains, reconstruction of the events around death and finding the cause (Cockle & Bell, 2015; Simmons, 2017). Shortly after death, physical observations such as *rigor mortis, livor mortis, algor mortis*, body temperature, the excitability of muscles and the concentration of potassium ions in the eyes can be used to predict the PMI of a body (Henssge & Madea, 2004; Madea *et al*., 2014; Warther *et al*., 2012; Zilg *et al*., 2015). These methods are only valid for short PMI durations and early stages of decomposition, approximately up to 72 hours after death (Gelderman *et al*., 2018). The use of entomology, determination of ATP levels in body organs, colonisation by bacteria and the presence of volatile fatty acids in soil samples around a cadaver are examples of PMI estimation methods that can be used for cases at later decomposition stages (Charabidze *et al*., 2013; Dash & Das, 2020; Fancher *et al*., 2017; Hauther *et al*., 2015; Mao *et al*., 2013). As the extent of terrestrial decomposition increases, the complexity of estimating the PMI increases; therefore, Henssge & Madea (2007) suggested that PMI estimation methods should be precise and quantitative and consider the factors affecting terrestrial decomposition.

2.2.3.1 *Mortis Triad*

Rigor mortis, livor mortis, and algor mortis are the three physical changes commonly used for PMI estimation in the early phase of the decomposition process (Saukko & Knight, 2015; Shrestha *et al*., 2022). The onset of rigor mortis is approximately two to four hours after death; it fully develops after about twelve hours and can be observed for about three days before it disappears (Bonzon *et al*., 2015; Madea *et al*., 2017). The PMI of bodies found with rigor mortis can be estimated to be in this range of time intervals. The onset of livor mortis usually occurs within 30 minutes to 3 hours after death, and the pooling patterns of the blood being formed in this process become evident and fixed at about 12

hours after death (Byard, 2020; Shrestha *et al*., 2022). A body found with livor mortis can be estimated to have been dead for a minimum of approximately 12 hours (Shrestha *et al*., 2022). During algor mortis, the internal body temperature reduces until the ambient temperature is reached; this process can last up to 18 to 20 hours (Fisher, 2007). When a body is found with its temperature equal to the ambient temperature, it can be assumed to have a PMI of approximately 20-24 hours; algor mortis is the most accurate method of estimating PMI in the early phases of the decomposition process, but it is an intensive and cumbersome process that requires knowledge and experience before being applied due to the temperature differences between the body surroundings at different times (Shrestha *et al*., 2022).

2.2.3.2 *Vitreous Potassium Concentration*

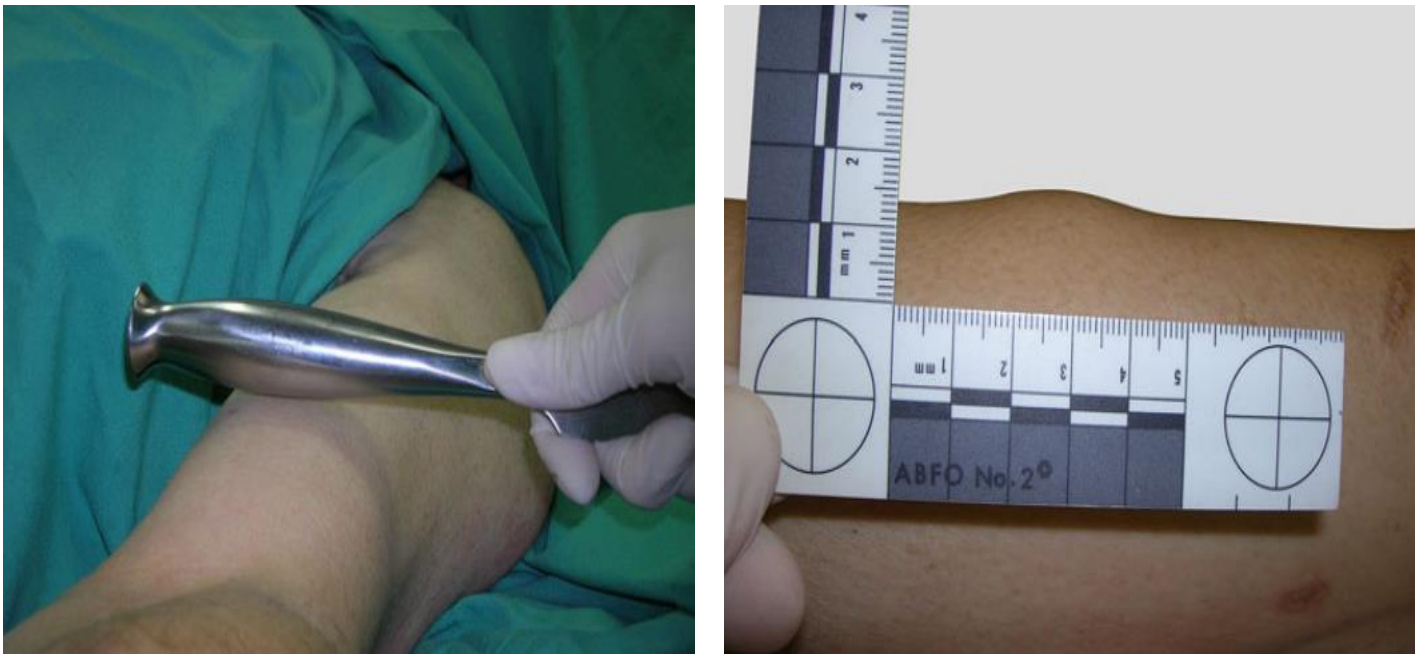
In antemortem states, there is a high concentration of potassium ions (~150 mmol/L) in the cells, but after death, potassium ions leak out of the cells into the extracellular fluid through diffusion (Dominiczak & Woodward, 2007). There are few cells in the vitreous of the eyes, and the potassium concentration of the vitreous and extracellular fluid are similar; therefore, there is a post-mortem rise in the potassium levels in the vitreous like that occurring in extracellular fluids (Tumram *et al*., 2011). This increase in potassium ions in the vitreous has been used as a method for PMI estimation over the past decade (Zilg *et al*., 2015). Various studies have proposed different numerical equations for PMI estimation with potassium concentration in the vitreous, which are listed in Table 2.2 below. Table 2.2 includes the authors, the proposed equations, the study's sample size and the maximum PMI estimate from each study.

Table 2.2: List of equations developed for PMI estimation through potassium ions in the vitreous (Zilg et al., 2015).

Authors (year)	Equation (h)	n	Max PMI (h)	Comments
Adelson *et al*. (1963) [4]	PMI = 5.88 [K^+]-31.53	209	21	-
Sturner and Gantner (1963) [3]	PMI = 7.14 [K^+]-39.1	125	104	-
Hanson *et al*. (1966) [5]	PMI = 5.88 [K^+]-47.1	203	310	-
Coe (1969) [29]	PMI = 6.15 [K^+]-38.1	145	100	A separate equation was provided for a PMI < 6h
Stephens and Richards (1987) [10]	PMI = 4.20 [K^+]-26.65	1427	35	Outliers, drownings, SIDS, electrolyte imbalances, and temperature extremes were excluded
Madea *et al*. (1989) [11]	PMI = 5.26 [K^+]-30.9	107	130	Cases involving elevated urea and prolonged agony were excluded
James *et al*. (1997) [14]	PMI = 4.32 [K^+]-18.35	100	80	Also included hypoxanthine
Munoz *et al*. (2001) [15]	PMI = 3.92 [K^+]-19.04	133	40	Only non-hospital cases were examined, there was a change in variables
Zhou *et al*. (2007) [18]	PMI = 5.88 [K^+]-32.71	62	27	-
Jashnani *et al*. (2010) [19]	PMI = 1.076 [K^+]-2.81	120	50	Mostly includes cases involving sepsis or tuberculosis
Bortolotti *et al*. (2011) [30]	PMI = 5.77 [K^+]-13.28	164	110	-
Mihailovic *et al*. (2012) [43]	PMI = 2.749 [K^+]-11.98	32	30	Repetitive sampling
Siddamsetty *et al*. (2013) [37]	PMI = 4.701 [K^+]-29.06	210	170	-
Present study	$PMI = \frac{\ln[(M - C_0)/(M - [K^+])]}{L_0 + m_A + m_T T}$	462	409	No cases were excluded. The proposed equation includes temperature and decedent age.

2.2.3.3 *Idiomuscular contraction*

After death, some muscles contract due to an exterior mechanical stimulation of the muscle; this local contraction of the muscle is known as idiomuscular contraction, and it has been used as a method for PMI estimation (Henssge & Madea, 2004). A limited number of studies have been published on this technique, and the available literature or studies are in German; therefore, it is challenging to estimate a maximum post-mortem time limit for idiomuscular contraction to occur though most studies estimate a maximum of 13 hours (Henssge & Madea, 2007; Warther *et al*., 2012). Warther *et al*. (2012) studied 270 corpses and confirmed that an estimate of 13 hours post-mortem is the maximum limit for idiomuscular contraction. Figure 2.14 shows the mechanical stimulation of the upper arm muscles and the contraction response experienced.



(a)- stimulation of the biceps. ***(b)- idiomuscular contraction response.***

Figure 2.14: Mechanical stimulation with a steel chisel and the contraction response (Warther et al., 2012).

2.2.3.4 *Entomology*

Entomological analysis of the presence of insects on human cadavers has been a highly useful practice for PMI estimation in the early and late post-mortem phase for over two decades (Lei *et al*., 2019; Villet & Amendt, 2011). Forensic entomologists use two approaches for estimating PMI; based on insect development and succession (Shrestha *et al*., 2022). The insect development approach utilises the presence and estimates of the ages or stages of immature insects retrieved from a cadaver and its surroundings, using this vital information as the minimum estimate of the PMI (Amendt *et al*., 2011). The succession approach mainly investigates different crucial occurrences such as the duration successive insect species colonise a cadaver, the stage of decomposition at which this colonisation occurs, the pre-appearance interval (PAI) and the presence interval (PI) of insect family succession on

a cadaver (Mañas-Jordá *et al*., 2018; Matuszewski, 2021). These data are essential for PMI estimation in the late decomposition phase as the PAI of the insect could be longer than the duration for the development of the insect; therefore, to estimate PMI accurately, it may be necessary to combine insect age with the PAI (Bajerlein *et al*., 2018). The PAI of insects can also validate estimates of maximum PMI in cases where insect evidence is absent on a cadaver (Wells, 2019). Due to the variation of insect development and succession across geographical locations, it is essential to cross-reference data from different locations when estimating PMI through entomology analysis (Owings *et al*., 2014). A variety of families of insects have been used in PMI estimation using the insect development and succession approaches; these include the Calliphoridae, Staphylinidae, Silphidae, Sarcophagidae, Dermestidae, Cleridae, Nitidulidae, Fanniidae, Muscidae, Histeridae, and Pteromalidae families (Matuszewski, 2021).

2.2.3.5 *Microbiome Analysis*

Microbes are comprised of a large community of single-celled bacteria, eukaryotes, viruses, and fungi; these microbes could be intrinsic to a cadaver or from the surrounding environment of the cadaver and are essential in PMI estimation because cadaver decomposition can be utilised for a predictable succession of microbes that reflects the different stages and duration of decomposition (Metcalf, 2019). DNA samples of these microbial communities are sequenced using various sequencing techniques to understand the succession and changes of these microbes over the course of the decomposition process (Iancu *et al*., 2018; Tozzo *et al*., 2022). Various studies have investigated the succession and changes of the microbial community associated with different human body parts such as the skin (Hyde *et al*., 2015), digestive tract (DeBruyn & Hauther, 2017; Hauther *et al*., 2015), oral, eyes, nose, and ear orifices (Johnson *et al*., 2016; Pechal *et al*., 2018), internal organs (Javan *et al*., 2016), bones (Damann *et al*., 2015), and soils around a cadaver (Cobaugh *et al*., 2015; Finley *et al*., 2016). The Adserias-Garriga *et al*. (2017) study sequenced DNA obtained from three human cadavers through oral swabs and found that Firmicutes and Actinobacteria were the predominant species in the fresh stage, Tenericutes were predominant in the bloat stage of decomposition while a different community of Firmicutes were the highest occurring phylum during the advanced decay stage.

2.2.3.6 *Nomograms*

The use of temperature and the cooling of a body, most especially in the fresh stage or early post-mortem, has been utilised as a PMI estimation method for many years, dating over a century (Henssge & Madea, 2004). Biological reactions and processes, such as post-mortem heat production and hypothermia or a fever occurring antemortem, have negligible effects on the cooling of a body (Henssge & Madea, 2004). Henssge (1988) developed a nomogram method to predict the PMI of bodies in early post-mortem with rectal temperature. This nomogram is easy and quick to use and can be used on the crime scene to estimate the PMI. This nomogram method considered the body weight, rectal temperature, ambient temperature, and corrective factors used to consider the presence and effect of

clothing on a body or when the body is found in water. An example of the nomogram can be seen in Figure 2.15 below. The left and right scales on the nomogram represent the rectal and ambient temperatures, respectively, while the scale at the bottom represents the body weight. The top arch represents the corrective factors, while the other arches represent the estimated PMI values in hours.

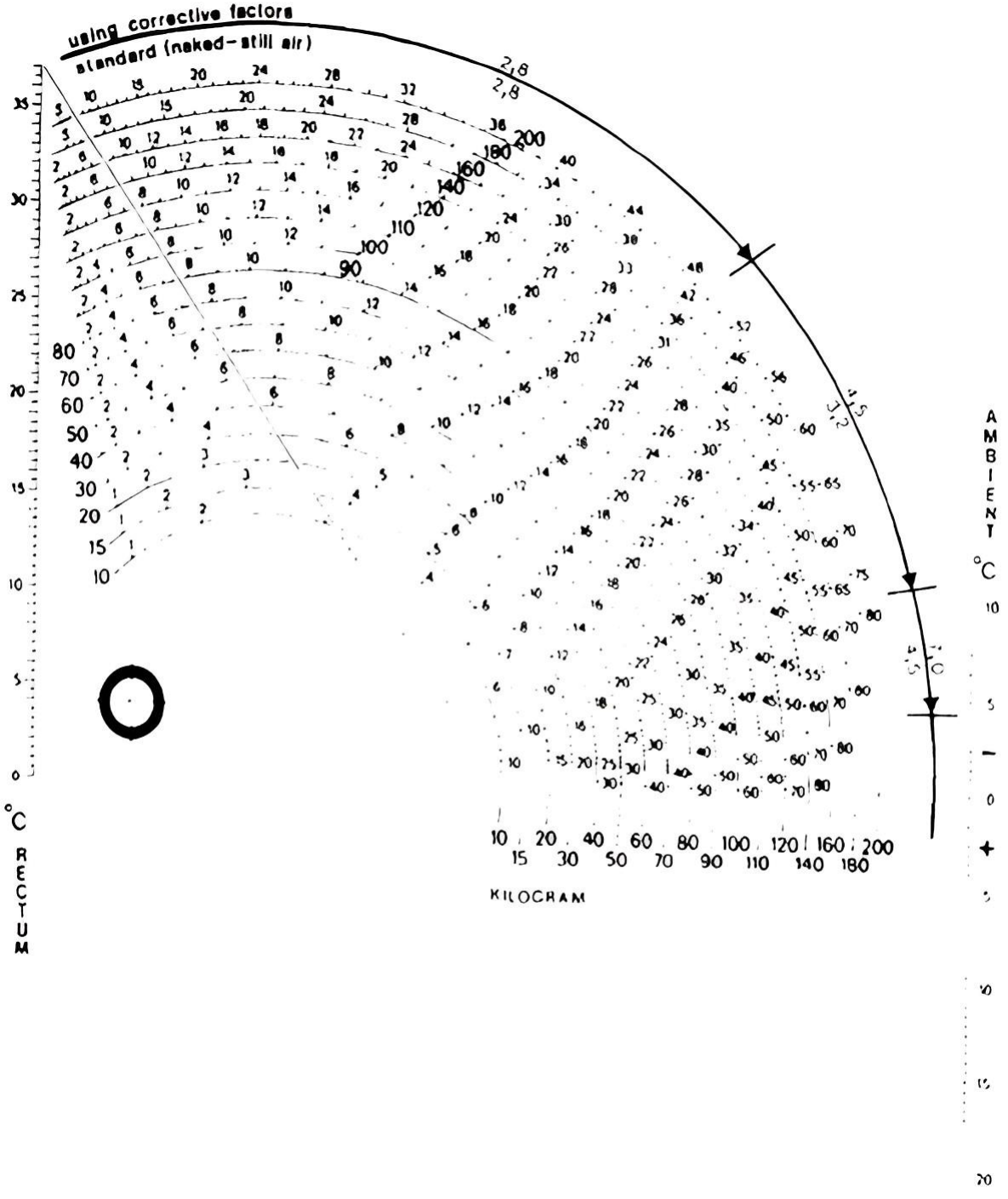


Figure 2.15: Schematic of the Nomogram developed for PMI estimation in early post-mortem (Henssge, 1988).

Table 2.3 lists the corrective factors used in the nomogram method. The corrective factors consider if a body is naked or clothed (thin or thick layers), still or moving, and submerged. The corrective factor is multiplied by the body weight to obtain a corrected weight.

Table 2.3: Corrective factors in the nomogram method (Henssge, 1992).

Dry Clothing/covering	***In air***	***Corrective factor***	***Wet through Clothing/covering Wet body surface***	***In air***	***In water***
		0.35	Naked		Flowing
		0.5	Naked		Still
		0.7	Naked	Moving	
		0.7	1-2 thin layers	Moving	
Naked	Moving	0.75			
1-2 thin layers	Moving	0.9	2 or more thicker	Moving	
Naked	Still	1.0			
1-2 thin layers	Still	1.1	2 thicker layers	Still	
2-3 thin layers		1.2	More than 2 thicker layers	Still	
1-2 thicker layers	Moving or	1.2			
3-4 thin layers	still	1.3			
More thin/thicker layers	Without influence	1.4			
		-			
Thick bed spread plus clothing combined		1.8			
		2.4			

Figure 2.16 is an application of the nomogram method. This nomogram was for a 70 kg body with a rectal temperature of 27 ℃, fully clothed in 3–4 thin layers and found on land with an ambient temperature of 15 ℃. The following steps were taken to estimate the PMI using the nomogram:

- The corrective factor of 1.3 for 3–4 thin layers of clothing (obtained from Table 2.3) was multiplied by the body weight of 70 kg to obtain a corrected weight of 90 kg.
- A line is drawn from 27 ℃ on the rectal temperature scale to 15 ℃ on the ambient temperature scale.
- This line drawn between the two scales intersects with a line that extends from the top of the rectal temperature scale.
- A second line is drawn from the middle of the circle (beside the bottom of the rectal temperature scale) to pass through the intersection and the arch for the corrective factors (at the 2.8 range).
- The corrected body weight of 90 kg is traced from the weight scale till it intersects with this second line drawn. A value of 17 hours was obtained at this intersection.
- Because the second line passes through the corrective factor arch at the 2.8 range, then the estimated PMI is: 17 hours ± 2.8 hours.

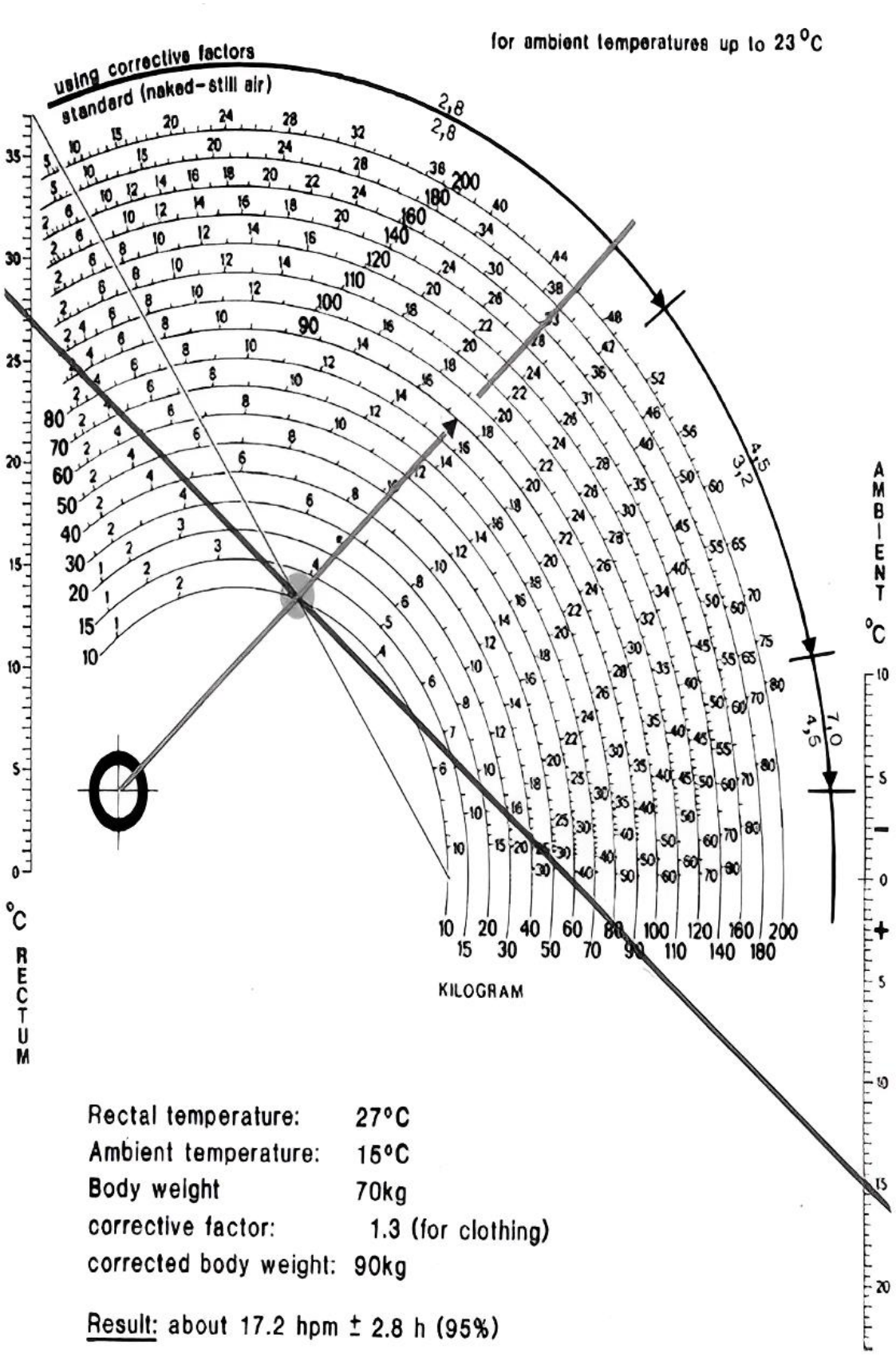


Figure 2.16: An application of the nomogram method (Henssge & Madea, 2004).

The application of this nomogram method to bodies recovered from water has some limitations. Firstly, it considers the weight and rectal temperature of a body and the ambient temperature, but other factors such as water flow, salinity, movement of surrounding air, water depth, humidity, insect activity when a body floats and scavenging by an aquatic organism are not considered in this method (Noor *et al*., 2018). These factors not considered affect the decomposition rate of a body; therefore, this method could give false PMI estimates. In addition, two bodies that have the same weight but different compositions, such as body fat, water content, body hair, and stature, will undergo body cooling at different rates (Byard, 2020). Considering just the body weight, the nomogram method implies that the two bodies will cool at the same rate; this limitation will lead to inaccurate PMI estimates with up to approximately 7-hour differences when using the nomogram method (Wilk *et al*., 2020). Finally, this nomogram method is invasive and requires entry into the rectum of the cadaver; this could lead to cross-contamination of the body or the destruction of other evidence that can be obtained internally (Wilk *et al*., 2020).

2.2.3.7 *Aerobic and Anaerobic Conditions*

To estimate the PMI of bodies decomposing in aerobic (bodies found above ground) and anaerobic (buried bodies) conditions, Vass (2011) developed two formulae that consider the extent of soft tissue decomposition, temperature, adipocere, an ADD constant, humidity and moisture, compared to the TBS system that only considered temperature. These formulae were developed using data obtained over a span of 20 years (~1991–2011) from the University of Tennessee's Anthropology Research Facility. This facility focuses on various decomposition experiments on human remains.

The formula for bodies found above ground is shown below.

$$PMI_{Aerobic} = \frac{1285 \times \left(\frac{decomposition}{100}\right)}{0.0103 \times temperature \times humidity}$$

Where:

1285: a constant representing ADD value at which the release of volatile fatty acids from soft tissue ends.

Decomposition: a percentage of the extent of decomposition that has occurred.

0.0103: a constant representing the moisture effect on decomposition rates.

Temperature: ambient temperature of the location of the body.

Humidity: average humidity value of the location of the body.

The formula for bodies found above ground is shown below.

$$PMI_{Anaerobic} = \frac{1285 \times (\frac{decomposition}{100}) \times 4.6 \times adipocere}{0.0103 \times temperature \times soil\ moisture}$$

Where:

1285: a constant representing ADD value at which the release of volatile fatty acids from soft tissue ends.

Decomposition: a percentage of the extent of decomposition that has occurred.

4.6: a constant representing a reduced decomposition rate because of reduced oxygen levels.

Adipocere: a multiplier constant for the contribution of the estimated percentage adipocere formed.

0.0103: a constant representing the moisture effect on decomposition rates.

Temperature: ambient temperature of the burial site of the body.

Soil moisture: a percentage representing the soil moisture of the burial site.

These formulae have been applied to real-life cases worldwide and have had success. The formula for bodies found above ground was applied to the case of a partly clothed 42-year-old man found outdoors and died of strangulation (Vass, 2011). Data on temperature, humidity, and the percentage of decomposition were obtained and substituted into the formula to calculate the PMI. A value of 63.8 days was obtained with the formula. After an extensive investigation, it was discovered that the man was killed 65 days before the day the corpse was found.

The formula for buried bodies was applied to the case of a fully clothed woman that was found in a shallow grave. Necessary data on soil moisture, temperature, and percentage of decomposition was obtained and substituted into the formula. A value of 6628 days which is approximately 18.2 years, was obtained. DNA tests indicated that the identified victim had gone missing 18 years ago. These cases demonstrate that these formulae have promising potential in accurately estimating the PMI of bodies found on land or buried, and more studies need to be conducted using these formulae. The Vass (2011) study suggests that the accuracy of these formulae can be evaluated and improved by applying these formulae to more cases from many locations worldwide that undergo different weather and environmental conditions.

2.2.3.8 *TBS System*

The most frequently used PMI estimation method for cases at later decomposition stages is the Total Body Score (TBS) system developed by Megyesi *et al*. (2005); it was adapted from the Galloway *et al*. (1989) and Vass *et al*. (1992) studies. Further details on the TBS system and its flaws are explained later in this thesis (See Section 3.2). Physical decomposition changes in the head, trunk and limbs are observed and scored to assign a TBS for the body. Megyesi *et al*. (2005) formulated a regression equation for the TBS system that can be used to extrapolate the ADD of the body with a standard error. The TBS is substituted into the regression equation formulated to calculate the ADD. The regression equation formulated by Megyesi *et al*. (2005) is displayed below:

$$Log_{10}ADD = 0.002\ (TBS*TBS) + 1.81 \pm 388.16$$

The average daily temperatures are then added retrospectively till the ADD value is reached to estimate the PMI (Simmons, 2017).

2.3 Drowning

Drowning is a process that occurs when a body is immersed in water or other liquid until there is no access by air to the lungs due to the blockage of the airways by the fluid (Byard, 2017; Szpilman & Morgan, 2021). Drowning has three possible outcomes: fatal, nonfatal, and nonfatal, with permanent injuries or neurological impairment (Beerman *et al*., 2018). One of the top ten causes of death for those under 25 years is drowning, which is especially common in low- and middle-income nations (Franklin *et al*., 2020). Globally, drowning results in about 1000 fatalities daily and many more cases with lifetime morbidity (Tipton & Montgomery, 2022).

Due to ethical restrictions on the use of human bodies for research, particularly in the U.K., drowning cases serve as a source of vital information for aquatic decomposition, estimating PMSI for forensic sciences and helping with the location of drowned victims (Mateus & Vieira, 2014). Therefore, reviewing the existing research and studies on drowning is essential. Tipton & Montgomery (2022) conducted an extensive review of reports, websites and literature that focused on the duration and subjective experience of drowning, along with inputs from two medical experts on their experiences with drowning casualties. From their study, they were able to divide the events of drowning into the following six sequences: (i) Struggle to keep the airway clear of the water, (ii) Initial submersion and breath-holding, (iii) Aspiration of water, (iv) Unconsciousness, (v) Cardio-respiratory arrest, and (vi) Death. To understand the drowning process and contribute to forensic investigations of drowning and aquatic decomposition, the physiological mechanisms associated with these events and sequences of drowning need to be explored.

2.3.1 Physiology of Drowning

The exact mechanism of the process and pathophysiology of drowning is complex and has only been speculatively addressed over the years (Bierens *et al*., 2002; Layon *et al*., 2009; Szpilman *et al*., 2012). Drowning physiology has been split into two broad distinct events: immersion; when the upper airway is above water, and submersion; when the upper airway is underwater (van der Ploeg *et al*., 2010). The physiology of immersion is stipulated to include processes such as integrated cardiorespiratory reactions to skin and body temperature changes, cold shock, physical incapacitation, and hypovolemia, while submersion includes fear of drowning, diving response, autonomic conflict, airway reflexes, water aspiration, swallowing, emesis, and body electrolyte disorders (Bierens *et al*., 2016).

2.3.1.1 *Immersion*

The phrase for water temperatures at which heat production and heat loss are equal is “thermoneutral”; water temperature thermoneutrality is approximately 35 °C (Bierens *et al*., 2016; Schmid *et al*., 2009). Asides from rare occurrences of hot water cases (Nishiyama *et al*., 2011; Suzuki *et al*., 2017; Suzuki *et al*., 2019), most drownings occur below this thermoneutral point, and the first process of drowning is

the “skin cooling” or cold shock response of the body (Bierens *et al*., 2016). Cold shock peaks in the first 30 seconds of immersion; it is initiated by nociceptors in the skin between water temperatures of 10–25 ℃ and diminishes during the next 2–3 minutes of immersion (Campero *et al*., 1996; McKemy, 2005; Tipton *et al*., 1991). At the onset of cold shock, the following conscious reaction is to prevent water from entering the mouth and airways by holding one’s breath and spitting water out, but this response lasts for approximately a minute until it is overcome by cold shock (Szpilman *et al*., 2012; Tipton & Golden, 2014).

2.3.1.2 *Submersion*

Towards the end of immersion, a person holds their breath or spits water out of their mouth to prevent water from entering the mouth and airways, but water gets aspirated into the airways, and mild irritation of the laryngeal mucosa leads to coughing, a protective reflex response known as laryngeal closure reflex to prevent the entry of water (Bierens *et al*., 2016; Szpilman *et al*., 2012). Other protective reflexes experienced by the airways to prevent water aspiration include the pharyngoglottal, esophagoglottal and aerodigestive reflexes (Dua *et al*., 2011). In some cases, laryngospasm occurs, but it is rapidly terminated by hypoxia in the brain, aspiration of water continues in the airways, vomiting could also occur, and hypoxemia rapidly leads to unconsciousness and apnoea (Ertekin, 2011; Jean, 2001; Lang, 2009; Matsuo & Palmer, 2008; Miller, 2008; Salomez & Vincent, 2004; Steele & Miller, 2010).

These autonomic changes in the respiratory system result in fatal arrhythmias such as tachycardia, bradycardia, pulseless electrical states, and asystole (Grmec *et al*., 2009). Figure 2.17 shows how the autonomic changes and responses during drowning cause arrhythmias. The immersion phase of drowning initiates two autonomic responses; the diving response, which is an oxygen-conserving response that occurs when a drowning victim tries to hold their breath, and the cold shock response initiated by the receptors in the skin (Bierens *et al*., 2016; Shattock & Tipton, 2012).

The diving and cold shock response activate the parasympathetic and sympathetic nervous systems, respectively; the parasympathetic stimulation leads to bradycardia, and the sympathetic stimulation leads to tachycardia (Shattock & Tipton, 2012; Tipton *et al*., 2010). In the presence of underlying health factors such as atherosclerosis, ischemic heart disease, channelopathies, and other cardiac diseases or conditions, bradycardia and tachycardia may be the actual cause of death in the drowning process (Shattock & Tipton, 2012). The fatal drowning process usually occurs in seconds to a few minutes; however, in rare circumstances, such as hypothermia or drowning in ice water, the drowning process can last for an hour (Tipton & Golden, 2011).

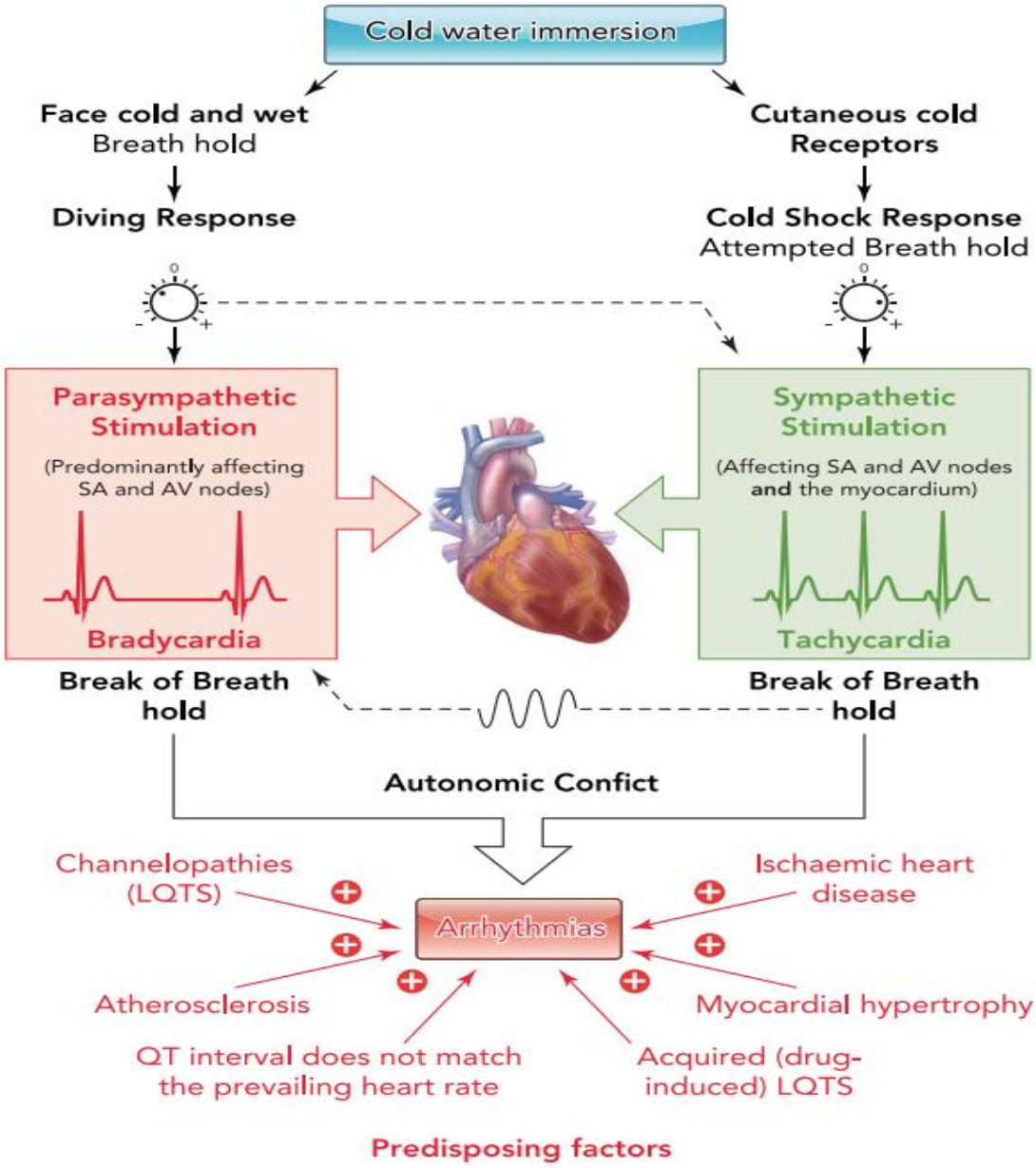


Figure 2.17: Autonomic changes leading to arrhythmias (Shattock & Tipton, 2012).

2.3.2 Diagnosis of Drowning and its Difficulties

The investigation and diagnosis of suspected drowning deaths, most especially unwitnessed cases, is a complex process that calls for ruling out other potential causes of death and is backed up by thorough autopsies, scene investigations, and detailed histories (Byard, 2015; Stephenson *et al*., 2019). Also, it is imperative to acknowledge that not every individual whose body is pulled from the water has drowned (Raux *et al*., 2014). Some might have been deposited in the water post-mortem. Many factors affect the diagnosis of drowning, particularly environmental factors, delayed body retrieval, comorbidities, animal predation and the lack of pathological findings (Stephenson *et al*., 2019). Various techniques for diagnosing drowning and the limitations of the techniques are discussed below.

2.3.2.1 *Presence of Pathological Features*

When a body drowns, some pathological changes may be observed on the skin; though these features are not established changes that ascertain drowning has occurred, they could act as a means of drowning diagnosis (Byard, 2013). These observable features include skin slippage of the hands and feet, also known as washerwoman's hands, tongue protrusion, scratches, and lacerations on the limbs from dragging and struggles to stay afloat, and mud, debris, and foam around the facial orifices (Alexander & Jentzen, 2011; Bernitz *et al*., 2019; Byard, 2017; Lunetta, 2016). Figure 2.18 shows examples of the "washerwoman's foot" feature occurring on a foot whose sole has a wrinkled surface. Figure 2.18 also shows the feature of skin and nails sloughing off from a toe.

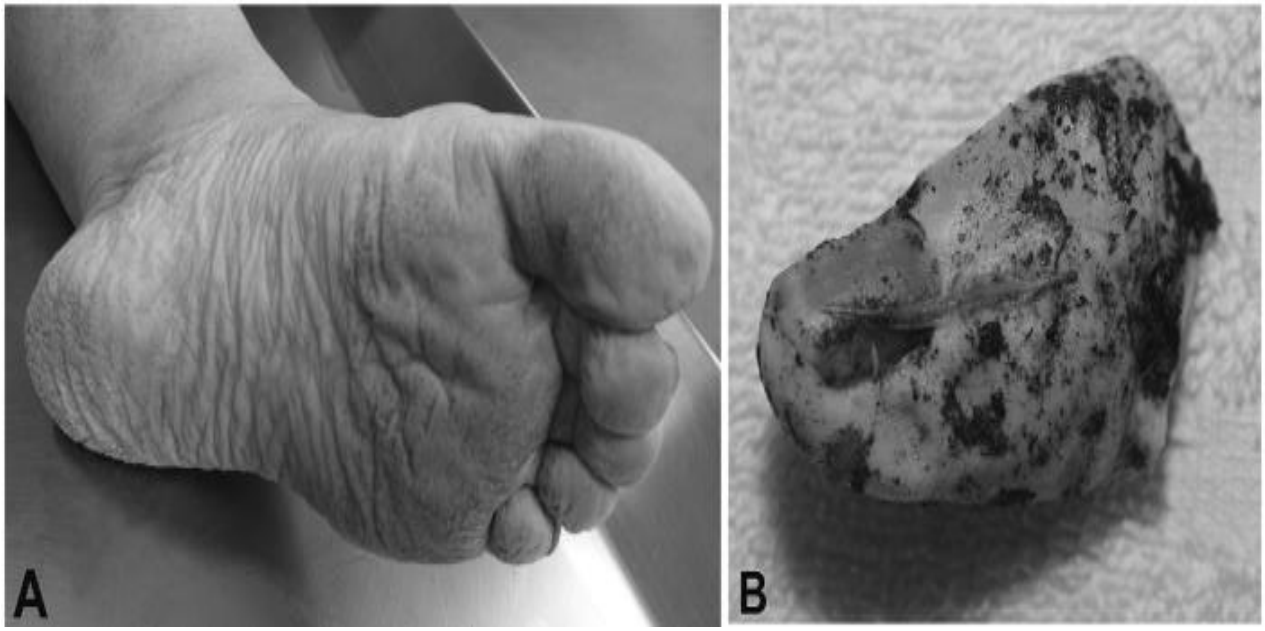


Figure 2.18: A-Washerwoman's Foot; B-Skin and nail slough off in the toes (Stephenson et al., 2019).

The presence of fluid or foam in the airway passages, paranasal sinuses, middle ear, nasal pharynx, oropharynx, trachea, and stomach (Wydler's sign) has been used as a standard diagnostic tool for drowning (Byard, 2017; Stephenson *et al*., 2019; Vander Plaetsen *et al*., 2015; Wardak *et al*., 2014). However, fluid or foam is neither always present nor unique to drowning. Reijnen *et al*. (2019) suggested that fluid could enter the airways post-mortem and cause foam formation. Therefore, the presence of foam is not always exclusive to drowning. Also, the foam may be present in the airways in head trauma or opiate overdose cases (Stephenson *et al*., 2019; Wardak *et al*., 2014).

Over time, the foam could get degraded because of environmental factors; therefore, in cases of longer PMSI, there will be a lesser chance of foam in the airways (Reijnen *et al*., 2017). In addition, causes of death such as poisoning, cardiovascular issues and burning could lead to fluids in the sinuses (Kawasumi *et al*., 2013). Therefore, fluids in the sinuses cannot be regarded as an exclusive fatal

drowning diagnosis (Lunetta, 2016; Vander Plaetsen *et al*., 2015). Figure 2.19 is an example of foam released outside the body through the nasal airway passage due to pulmonary oedema.



Figure 2.19: Foam released from nasal airways due to pulmonary oedema (Stephenson et al., 2019).

2.3.2.2 *Spleen Size*

A reduction in the size of the spleen has been used as a diagnostic tool for drowning over the years, but caution must be taken in this process because spleen size reduction could be caused by hypoxia and reduced blood pressure (Palmiere *et al*., 2019; Stephenson *et al*., 2019; Wardak *et al*., 2014).

2.3.2.3 *Weight of the Lungs*

Water aspiration could lead to pleural effusions, emphysema aquosum, and pulmonary oedema during drowning (Alexander & Jentzen, 2011; Byard, 2017; Wardak *et al*., 2014). Emphysema aquosum is the hyperinflated or hyperextended state of the lungs due to water logging; it makes the lungs meet in the midline of the chest region, thus covering the heart (Stephenson *et al*., 2019). Figure 2.20 is an example of lungs with emphysema aquosum; from the picture, it is difficult to see the heart because the

hyperextended lungs have covered it up. Therefore, increased weight and size of the lung have been adapted to diagnose drowning (Sugimura *et al*., 2010; Tse *et al*., 2018). The weight of the lungs of drowned victims has been compared to that of victims of other causes of death, and the lungs of drowned victims have been proven to weigh more significantly (Hadley & Fowler, 2003; Zhu *et al*., 2003). However, using lung weight as a diagnostic tool has its constraints because an increased lung weight can be observed in other conditions asides from drowning, and the weight of the lungs can reduce over a longer PMSI (Tse *et al*., 2018a; Tse *et al*., 2018b). Lunetta (2016) stated that the lung weight method of drowning diagnosis has no significant diagnostic value, but Tse *et al*. (2018a) suggested that using a lung-heart weight ratio will be of more significance in drowning diagnosis.

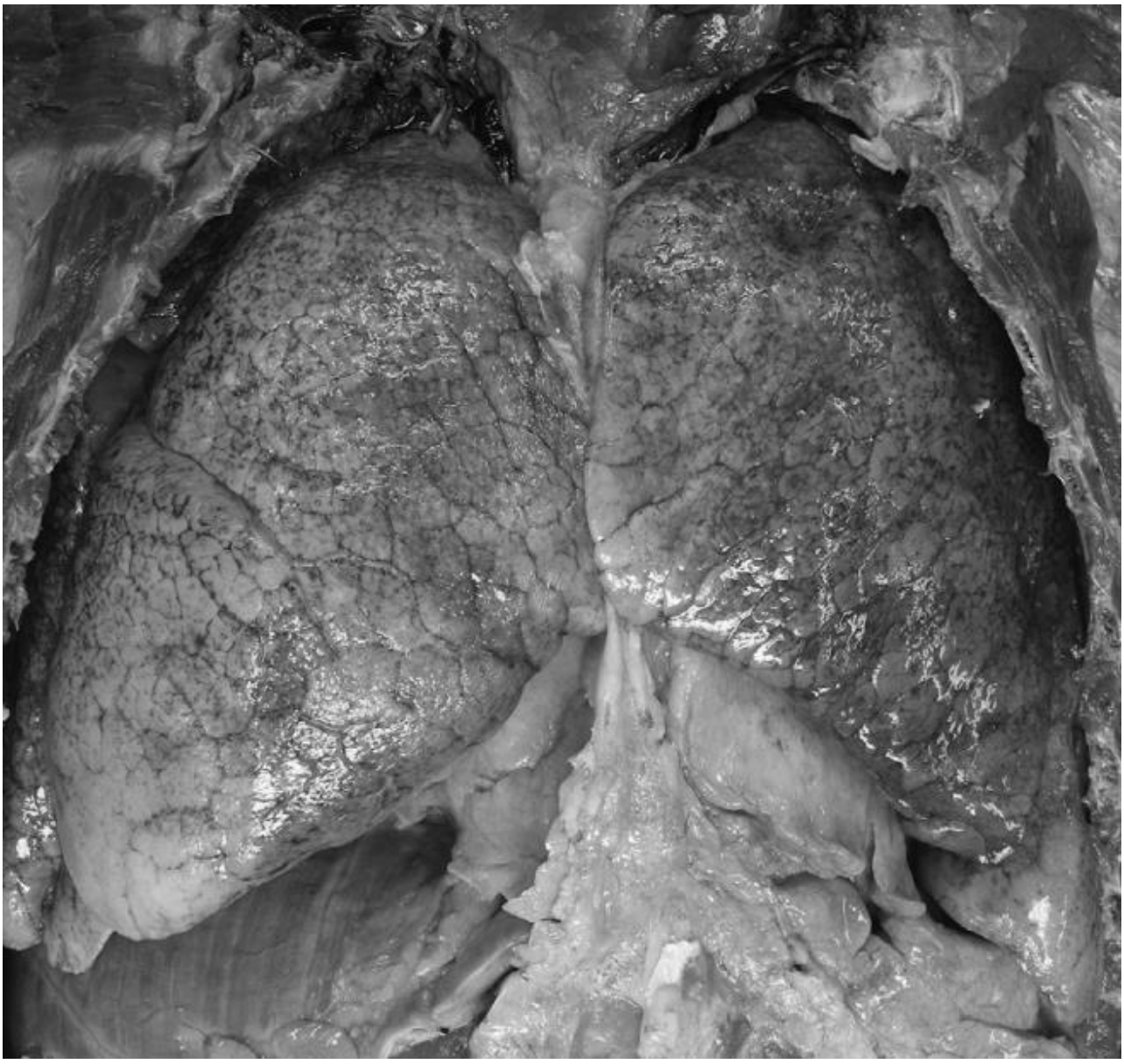


Figure 2.20: Picture of emphysema aquosum of the lungs in drowning (Stephenson et al., 2019).

2.3.2.4 *Post-mortem Computed Imaging*

Post-mortem forensic imaging devices such as Magnetic Resonance Imaging (MMR), Computed Tomography Angiography (CTA), and Computed Tomography (CT) have been widely used in the diagnosis of drowning by assessing morphologic features of internal organs that suggest drowning has occurred (Chen, 2017; Hyodoh *et al*., 2016; Raux *et al*., 2014; van Hoyweghen *et al*., 2014). Figure 2.21 is a CT scan of various organs of a 34-year-old woman who drowned and had her body submerged for approximately 24 hours; the arrowheads in the picture indicate the positions in the organs filled with fluid due to drowning.

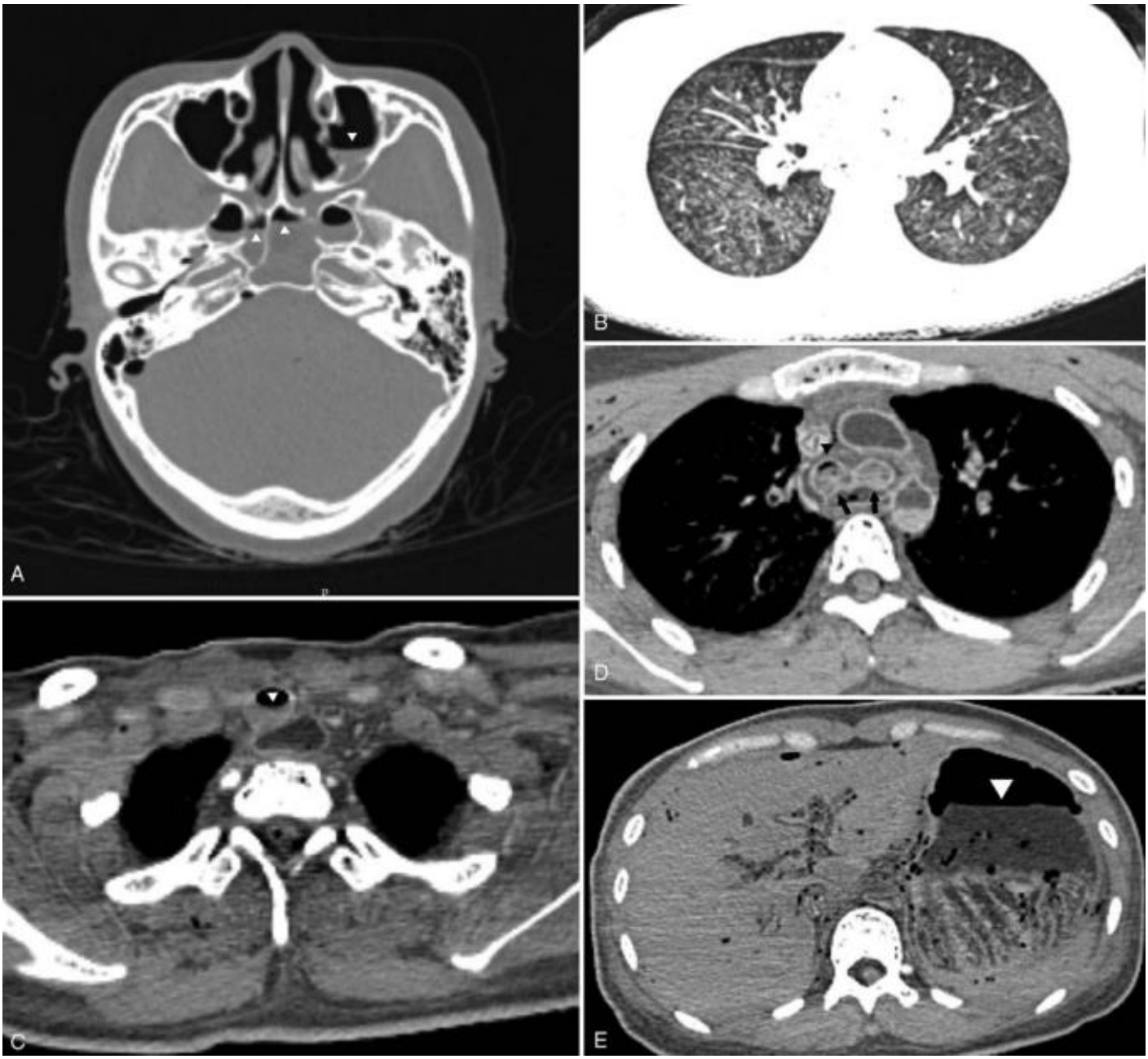


Figure 2.21: Computed tomography scans of A-sphenoidal sinus; B-lungs; C-trachea; D-bronchi; E-stomach (Wang et al., 2020).

These imaging devices are minimally invasive to non-invasive, and the CT has been useful for visualising anatomically difficult-to-access body parts like the paranasal sinuses (Kawasumi *et al*., 2012). A significant limitation of using CT imaging successfully is the need for an extensive understanding of the features of drowning pathology (Christe *et al*., 2008). Wang *et al*. (2020) suggested that the combination of CT and an endoscopic autopsy could be used in the successful diagnosis of drowning and yield more comprehensive morphological findings while retaining minimal invasiveness.

2.3.2.5 *Diatoms*

Diatoms are single-celled algae found in both fresh and seawater; during the process of drowning, they move into the lungs during antemortem violent aspiration of water and can diffuse from the lungs into other closed organs through the systemic circulation (Stephenson *et al*., 2019; Xiao *et al*., 2021). Detecting diatoms in the lungs or other closed organs in a body has been commonly used as a diagnostic tool for supporting evidence of drowning (Fucci *et al*., 2017; Ludes *et al*., 2013). Aside from the lungs, detecting diatoms in the bone marrow also suggests that aspiration of water occurred antemortem (Krstic *et al*., 2002). During the diagnosis of drowning, the presence of diatoms in the body could be a false positive case because diatoms have been found in the organs of humans who have died from other causes (Fucci *et al*., 2017). Diatoms could gain access to the body antemortem via gastroenteric absorption when diatom-containing food such as seafood are ingested or through inhalation of inert chemical materials, while the presence of diatoms in only other organs asides the lungs could be used to exclude drowning as a cause of death (Lunetta *et al*., 2013).

The use of diatoms has successfully pinpointed the point-of-entry or downing site of a body that has drifted in the water by comparing the diatoms found in the body with that of the water suspected to be the point-of-entry (Saukko & Knight, 2015; Stephenson *et al*., 2019). Determining a drowning site with diatom testing is possible because some diatom species are unique to specific water types or environments and can be presented as evidence to confirm a drowning location or link suspects to a crime scene because these diatoms are not easily concealed (Zhou *et al*., 2020). Diatoms cannot thrive in the human body and are therefore embedded in the water from the drowning site (Carballeira *et al*., 2018).

Ludes *et al*. (1999) analysed diatom profiles extracted from the lungs of 20 bodies recovered from known drowning sites and 20 from unknown (water where the bodies were found immersed at the time the body was recovered, which may not be the actual drowning site). Results indicated that there was a 70% correlation between the diatom types in the lungs and the unknown site, while there was a 100% correlation between the diatom types found in the lungs and the known drowning site; the 30% that showed no correlation between the lungs and the unknown drowning site could be a difference between the actual drowning site and the site of recovery (Ludes *et al*., 1999). Carballeira *et al*. (2018) studied rats submerged in four different freshwater sources for 15 days. Their study concluded that organs that

have open access to the outside of the body, such as the lungs and stomach, have a higher potential for identifying a drowning site through the diatoms found in them compared to the kidney, bone marrow, heart, and spleen; they were able to identify diatom species specific to each freshwater source on the rats submerged (Carballeira *et al*., 2018).

The traditional method for diatom detection is known as the acid digestion method (Zhou *et al*., 2020). A diatom cell wall structure comprises frustules, a hydrated silicon dioxide cell wall that is not easily destroyed (Rana & Manhas, 2018). To separate diatoms from organ tissues, the tissue is dissolved in a strong acid solution such as hydrogen peroxide, nitric acid, hydrochloric acid, Proteinase K, and sulfuric acid (Bortolotti *et al*., 2011; DiGiancamillo *et al*., 2011; Kakizaki *et al*., 2019; Ming *et al*., 2007; Scott *et al*., 2014). After digestion, the products are centrifuged until the components are separated, and a visual assessment of the diatoms is conducted using a microscope. Care must be taken when carrying out the acid digestion process because excessive digestion could lead to digested diatoms, and the visual examinations with the microscope will indicate false negative results (Zhou *et al*., 2020). Figure 2.22 illustrates the steps taken during the acid digestion method; digestion, centrifugation, and microscopy, while Figure 2.23 is an example of what diatoms look like under a 400x magnification of light microscopy.



Figure 2.22: Beaker, hot plate, centrifuge, and microscope used in acid digestion (Zhou et al., 2020).

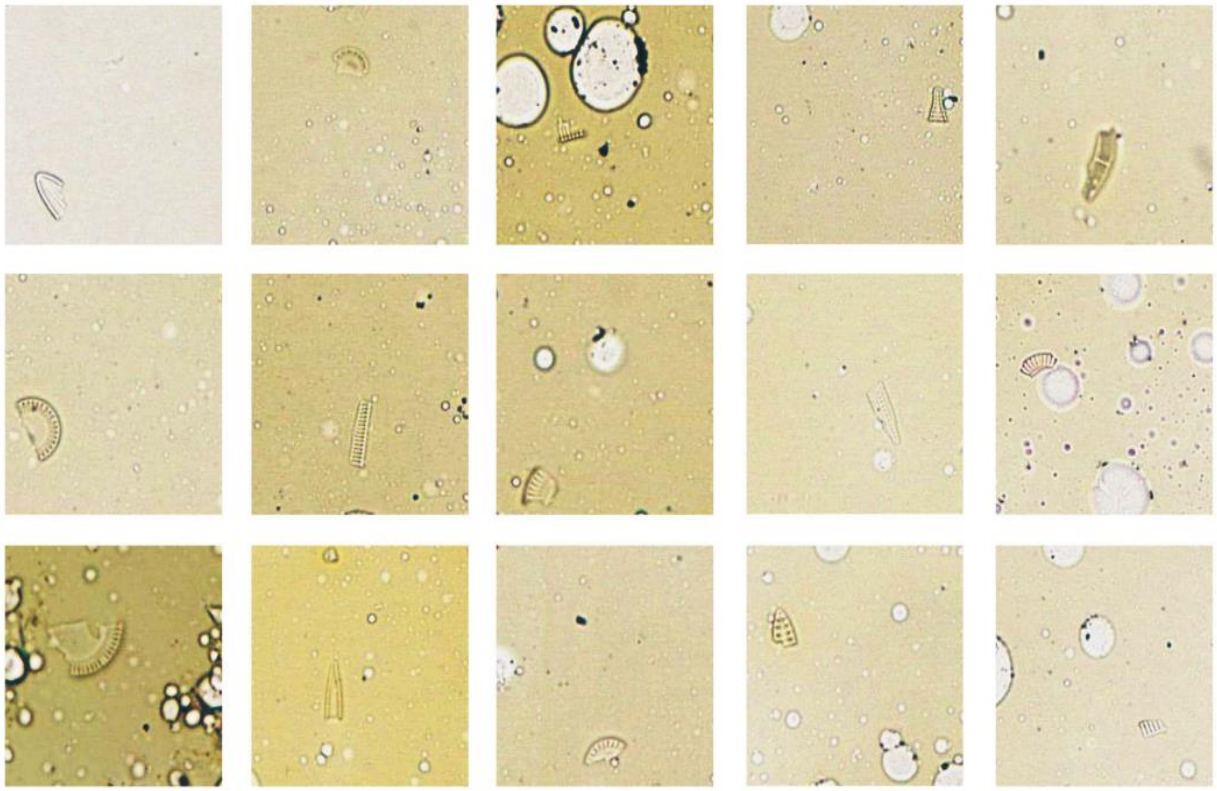


Figure 2.23: Different diatom under 400x magnification light microscopy (Zhou et al., 2019).

However, the use of conventional diatom testing in diagnosing forensic drowning cases has had its constraints and has been criticised since its introduction as a diagnostic tool (Byard, 2015). The diatom test is laborious, time intensive, and an air pollutant (Xiao *et al*., 2021). There have been arguments about the validity, sensitivity, and reliability of the test since diatoms may be present in the tissues of bodies that did not drown or may not be present in those that did (Hürlimann *et al*., 2000; Lunetta *et al*., 2013; Xiao *et al*., 2021). It has been noted that antemortem and post-mortem environmental contamination, which can happen during the submersion phase by diatom penetration through wounds, from resuscitation, or during the sampling procedure, has a detrimental impact on the usefulness of diatom testing (Di Giancamillo *et al*., 2010; Sonoda *et al*., 2022).

Zhao *et al*. (2013) created a novel diatom test technique utilising membrane filtration, microwave digestion, and scanning electron microscopy. Further studies have been conducted to improve the reliability and sensitivity of diatom testing (Kakizaki *et al*., 2015; Zhao *et al*., 2017). It has been suggested that to increase the reliability and accuracy of the diatom testing processes, diatom tests should be combined with polymerase chain reaction [PCR] (Rácz *et al*., 2016; Rutty *et al*., 2016). The Xiao *et al*. (2021) study attained high specificity for drowning diagnosis by combining a multiplex PCR and capillary electrophoresis (CE) to detect genes of drowning-related diatoms, cyanobacteria, and Aeromonas species.

2.3.3 Body Displacement in Water

In forensic analyses related to downing, due to the complex nature and reaction of the aquatic system with the body, difficulties may arise when trying to determine the point of entry and displacement trajectory, which in turn affect the estimation of PMSI for that body (Mateus *et al*., 2013). When a body drowns, it drifts away from its point of entry into the water horizontally or vertically; the direction of displacement depends on several factors (Dickson *et al*., 2011; Ellingham *et al*., 2017). In the Blanco Pampín & López-Abajo Rodríguez (2001) study, the death investigation records of nine bodies recovered from the sea indicated that the bodies drifted as far as 220–420 km within 3 to 26 days, with one of the bodies drifting as far as 380 km in 3 days.

The specific gravity, the density of a body and the density of the water it is submerged in influence the vertical displacement of the body; the average density of the human body is 0.98 $gcm^{-3,}$ while that of seawater is 1.024 gcm^{-3} (Mateus *et al*., 2013; Simmons & Heaton, 2013). This similarity in the densities of the human body and water indicates that a slight change in the density would considerably affect the body's buoyancy because less dense bodies have a higher chance of floating (Ellingham *et al*., 2017). Also, a body will most likely float because of increased buoyancy from putrefactive gas production during bloating (Reisdorf *et al*., 2012).

A body will remain sunken due to factors such as the current, gravitational force of the water on the body, frictional forces, and substrate (Ellingham *et al*., 2017; Mateus *et al*., 2013; Simmons & Heaton, 2013). In cases where a body sinks, frictional forces are increased by a body being snagged within rocks and debris, and there is insufficient water current at greater depths, lesser horizontal displacement will occur because the drag forces of the body will not be able to overcome the gravitational and frictional forces exerted by the water (Mateus *et al*., 2013). When buoyancy increases due to bloat or air trapped in clothing, a body resurfaces, and substantial horizontal displacement can occur because of the increased surface current and reduced frictional and gravitational force on the body (Anderson, 2009; Ellingham *et al*., 2017; Mateus *et al*., 2013).

Bodies submerged in water may be displaced for a distance, creating body marks, disarticulation of body parts and bones, and injuries, while a body that is immobile in water could be preserved due to less impact from objects (Humphreys *et al*., 2013; Palmer, 2020; Stuart & Ueland, 2017). The remains may get caught on transport vessels, rocks, and plants underwater, creating abrasions that may be difficult to distinguish as post-mortem or antemortem trauma on the bones and body (O'Brien, 1997; Stuart & Ueland, 2017). These body displacement processes in water lead to skin sloughing off, soft tissue decomposition, and exposure of underlying muscles, tendons, cartilages, ligaments, and bones, and over time they are disposed to sequential disarticulation; in most cases, this starts with hand and wrist bones, feet, and ankles, followed by the arm and leg bones (Haglund & Sorg, 2002). The mandible

also gets detached from the skull, while the ribs, vertebrae and pelvic bones tend to disarticulate last (Stuart & Ueland, 2017).

Figure 2.24 is a schematic of the forces acting on a body during horizontal and vertical displacement. In part A of the picture, the process of vertical displacement is illustrated; a body with a lower specific gravity or density floats to the top of the box while a body with higher specific gravity or density sinks to the bottom. In part B, the forces responsible for both horizontal and vertical displacement are illustrated. Friction forces (such as a body being snagged within rocks and debris) and drag forces (such as current) act in opposite directions; if the current is higher than the frictional force, the body would be displaced horizontally, and if the drag forces are higher than the current, the body would be immobile. Part C illustrates the phases of movements a body undergoes when deposited in water; a body sinks when deposited in water and resurfaces at the top during the bloating stage of decomposition. During this phase, a substantial amount of horizontal displacement can occur; after the bloating stage, the body sinks again due to a reduced level of buoyancy (Ellingham *et al*., 2017; Mateus *et al*., 2013).

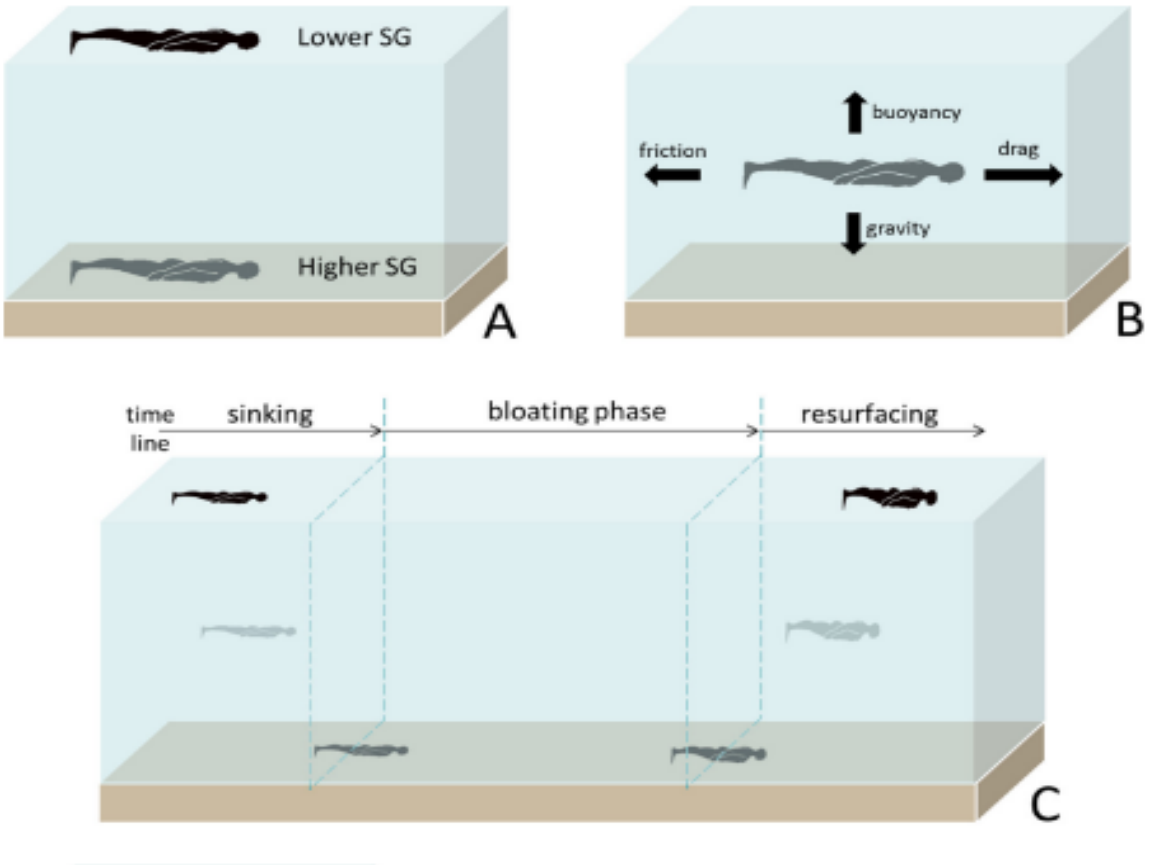


Figure 2.24: Schematics of the forces during horizontal and vertical displacement. a- specific gravity; b- forces acting on the body; c-phases of movement (Mateus et al., 2013).

The movement and behaviour of cadavers in bodies of water, most especially disarticulation, can have implications for their search and recovery from aquatic environments (Haglund, 1993).

2.3.4 Detection, Recovery, and Identification of Drowned Bodies

The entire procedure and process of detecting, recovering, and identifying drowned human remains is complex and requires thorough planning, training, and experience (Ellingham *et al*., 2017). These processes can be hindered due to poor visibility, the difficulty of preserving body parts and the lack of prior knowledge of that specific underwater environment (Becker *et al*., 2013; Winskog, 2012). Traditional methods for detecting drowned bodies include search dogs, divers and draining the water body (Parker *et al*., 2010; Schultz *et al*., 2013).

Water search dogs detect human remains submerged in water by picking up the scent of the body or other materials that float to the surface when released from the body (Erskine & Armstrong, 2021; Osterkamp, 2011). Smell or scent is detected more on water surfaces; therefore, when using search dogs, it is recommended to place the dogs on electric boats (they do not release fumes or gases) when patrolling the water (Rebmann *et al*., 2000). Divers also make use of hand-held solar units when searching for bodies under water (Healy *et al*., 2015); the solar units produce acoustic energy that is fed back off a target, which can be the submerged body, as a sound signal, thereby creating a change in the audio signals based on the distance to the body (Erskine & Armstrong, 2021).

In cases where the location of the body is unknown, sonar exploration of the water beds and waterproof coloured video cameras can be used to detect the body (Dumser & Türkay, 2008; Ellingham *et al*., 2017). Different devices used for terrestrial searches have also been applied to the search for submerged bodies (Ruffell *et al*., 2017). These devices map out the seabed and underwater environs; they include Compressed High-Intensity Radar Pulse [CHIRP] (Plets *et al*., 2009; Vos *et al*., 2012), Ground Penetrating Radar [GPR] (Ruffell, 2006; Schultz *et al*., 2013), Side-Scan Sonar (Healy *et al*., 2015), magnetometry (Parker *et al*., 2010), and seismic reflection and refraction (Ruffell *et al*., 2017).

Remotely Operated Vehicles (ROV) are commonly equipped with cameras to aid in the detection of a body, while the ROVs could have robotic arms to recover remains (Dumser & Türkay, 2008). Figure 2.25 is an example of the ROVs used in the recovery of a submerged body, while Figure 2.26 is a picture of a ROV used in combination with a robotic arm; the robotic arm is used to hold when being lifted from underwater. For optimal recovery, remains are placed in distinctive bags, preferably made of vinyl-coated polyester; this will help with the identification process and draining of water after recovery (Winskog, 2012). During recovery, disarticulated body parts and intact bodies are placed in metal cages or baskets, with the entire body gripped at the lower limbs, shoulders, and waist regions by robotic arms (Schuliar & Perich, 2012; Winskog, 2012). Figure 2.27 shows a metal cage where intact submerged bodies are placed for recovery.



Figure 2.25: A Remotely Operated Vehicle in use (Ellingham et al., 2017).

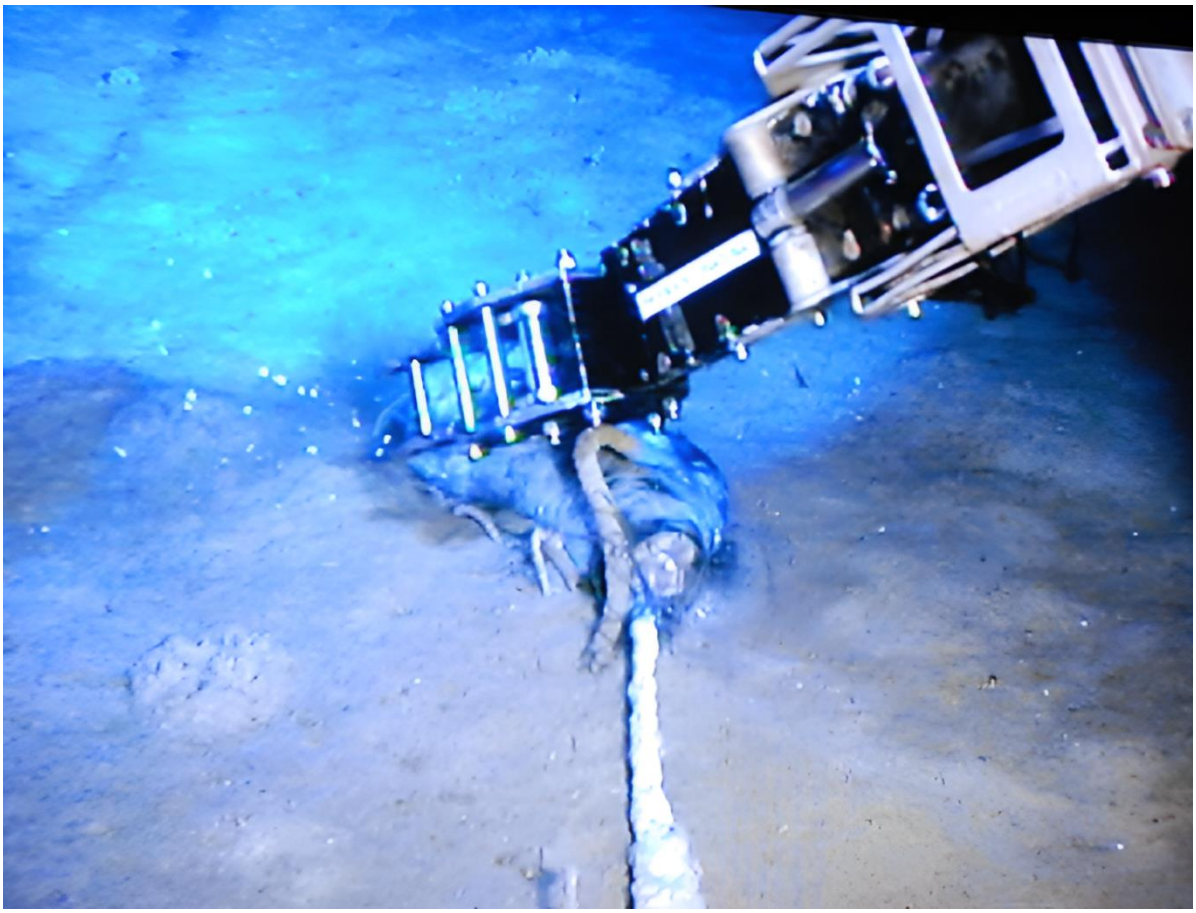


Figure 2.26: ROV equipped with a robotic arm (Ellingham et al., 2017).

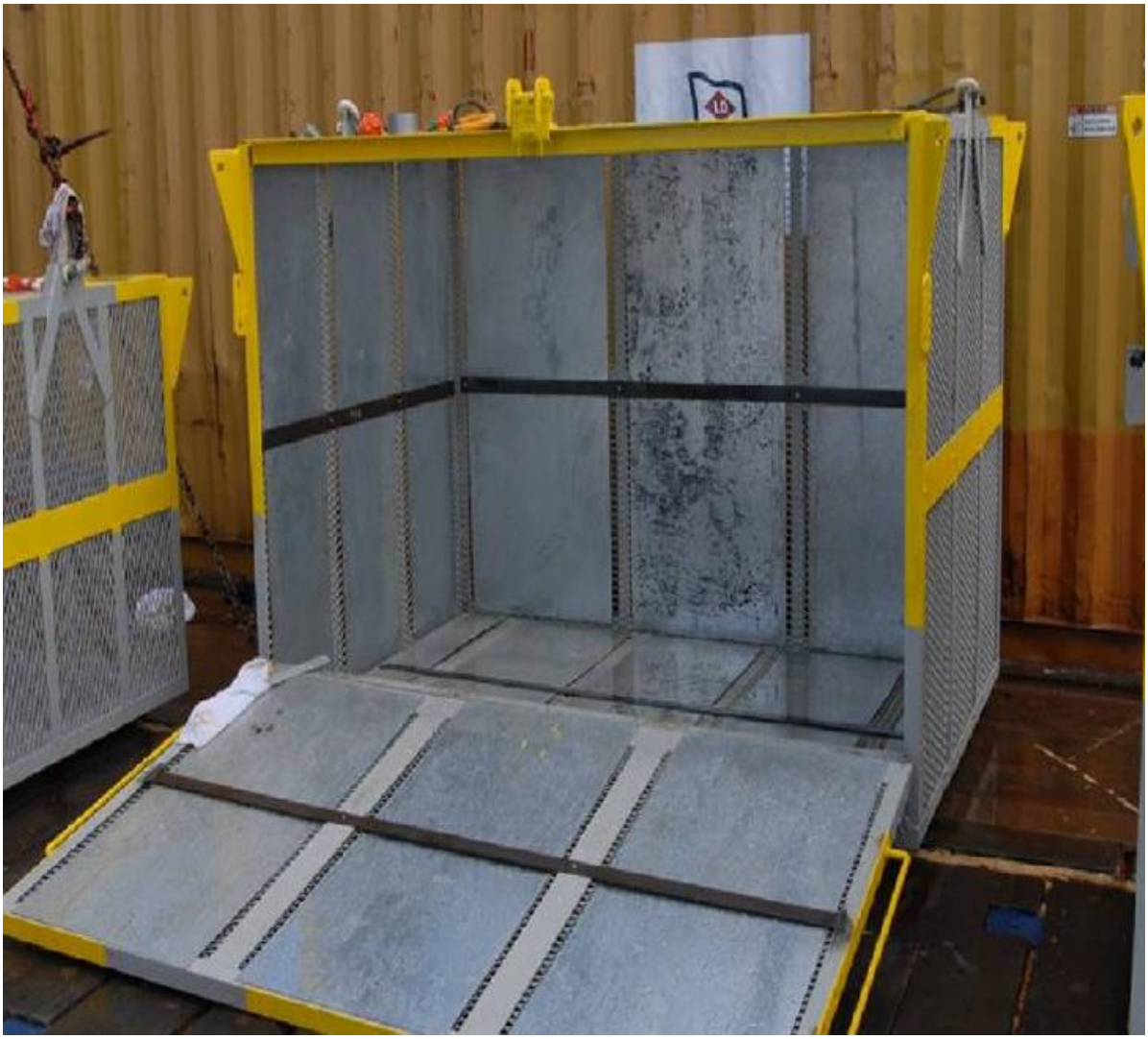


Figure 2.27: A metal cage used to recover submerged remains (Ellingham et al., 2017).

Depending on the available antemortem information, dental records and DNA analysis are the most common and standard procedures for identifying drowned remains (Butler, 2005; Holmlund *et al*., 2008; Fredericks *et al*., 2013; Winskog, 2012). DNA has been successfully extracted and used to identify human remains submerged for up to 35 years (Goodwin *et al*., 2003). Tattoos, piercings, scars, and other visual identifiers have also been used for identifying submerged remains, but DNA samples, dental records and fingerprints are mainly used as the primary identifiers (Ellingham *et al*., 2017).

2.4 Aquatic Decomposition

2.4.1 Process and Chemistry of Aquatic Decomposition

The decomposition chemistry in aquatic environments is similar to terrestrial environments, with a few distinguishable differences (Saukko & Knight, 2015). The differences between the two sets of decomposition are mainly physical markers that are evident at different intervals in only aquatic environs (Heaton *et al*., 2010); they include degloving, "*washerwoman's hand*" (Figure 2.28), and adipocere (Benbow *et al*., 2015; Ubelaker & Zarenko, 2011). The decomposition process continues as soon as a dead body is immersed in water, but the decomposition rate is much slower than terrestrial decomposition (Haglund & Sorg, 2002).



Figure 2.28: Pictorial examples of washerwoman's hand and degloving (van Daalen et al., 2017).

Depending on differing factors, a body immersed in water either sinks or floats (Hobischak & Anderson, 2002). Air in the lungs, clothing, body fat, decomposition gases and density of the water are the factors that affect a body's buoyancy, determining whether a body floats or sinks (Stuart & Ueland, 2017). Every 10 m of water exerts an extra 1 atm of pressure on a body; hence, the deeper a body is placed in water, the lower its chances of floating (Higgs & Pokines, 2013). A higher density of water is experienced in seawater than freshwater due to the high mineral and salt content in the sea; thus, a body will most likely stay afloat in seawater (Stuart & Ueland, 2017).

Within minutes of immersion, there is a shortage of blood flow to the body extremities because of the vasoconstriction of the blood vessels (Stuart & Ueland, 2017). This response in a prolonged state of immersion causes the wrinkling and shrinking of the skin of the limbs due to the loss of blood volume; this phenomenon is known as the *washerwoman's hands* (Saukko & Knight, 2015). Another skin change observed within minutes of immersion is "Cutis anserina or goose pimples"; it is caused by the erector pilli muscles (Di Maio & Di Maio, 2001). As the period of immersion increases, the skin

covering the hands and legs slips off (degloving), and skin slippage may occur in other body parts (Hobischak & Anderson, 2002).

Although rigor mortis and livor mortis are frequently found in bodies retrieved from water, the onset and duration of these post-mortem features are affected by water temperature, current, body position, and amount of muscle and organ activity before death (Karhunen *et al*., 2008). Discolouration of the skin occurs in both terrestrial and aquatic decompositions, but the discolouration of the teeth and gums is unique to aquatic decomposition; once considered to be an indication of drowning, the pink discolouration of the teeth and gums is likely caused by lividity in these tissues happening when the body is in a prone position (Sakuma *et al*., 2015).

Vascular marbling, discolouration of the skin, autolysis and putrefaction also occur during aquatic decomposition at a slower rate due to the low/reduced insect and maggot activity, lower temperature and restriction of other factors that speed up decomposition (Higgs & Pokines, 2013; Karhunen *et al*., 2008). Due to the reduction in oxygen levels in the body after death, the process of putrefaction is mainly stimulated by anaerobic bacteria that result in the breakdown and liquefaction of soft tissues (Janaway *et al*., 2009). The degradative action of anaerobic bacteria in the respiratory and digestive tracts leads to the build-up of gases; this causes bloating, which is observed mainly in the abdomen (Zhou & Byard, 2011).

The products of the metabolic reactions that occur during autolysis and putrefaction are responsible for the discolouration of the skin (Hamilton & Green, 2017; Mayer, 2012; Zhou & Byard, 2011). The green discolouration of the abdomen caused by the release of hydrogen sulphide from sulphur-containing amino acids, which then combine with haemoglobin to create sulfhaemoglobin, is one of the first physical signs of putrefaction (Goff, 2009; Rosier *et al*., 2014). Also, as decomposition progresses, there is evidence of tissue decomposition and reduced moisture; hence the skin becomes dark (Gennard, 2012; Vass *et al*., 2004; Verheggen *et al*., 2017). Due to a reduced possibility of insect colonisation on a body in water, there is little to no maggot mass during putrefaction; therefore, the decomposition rate of soft tissue is significantly reduced in aquatic decomposition compared to terrestrial decomposition (Higgs & Pokines, 2013).

The skin sloughs off due to the release of hydrolytic enzymes (during autolysis) between the dermis and epidermis, resulting in loosening and slippage of the epidermis; vesicles or blisters are initially observed, but over time the nails, hairs and large areas of the skin slough off (Simmons & Cross, 2013). As skin sloughs off and soft tissues decompose, underlying muscles, tendons, cartilages, ligaments, and bones become exposed, and over time they are disposed to sequential disarticulation; in most cases, starting with hand and wrist bones, feet, and ankles, followed by the arm and leg bones (Haglund & Sorg, 2002). The mandible also gets detached from the skull, while the ribs, vertebrae and pelvic bones tend to disarticulate last (Stuart & Ueland, 2017).

Figure 2.29 is an example of the accumulation of gases in the abdominal region due to putrefaction, while vascular marbling and discolouration of the skin are observed in Figures 2.29–2.30.



Figure 2.29: Example of vascular marbling on a body (Gelderman et al., 2018).



Figure 2.30: Pictorial illustration of discolouration of the limbs of a body (Gelderman et al., 2018).

In certain wet, anaerobic circumstances, a fatty acid complex known as “adipocere” can form on remains recovered from aquatic environments. Adipocere is a grey-white, greasy substance often found on bodies recovered from aquatic or damp environments (Lucas *et al*., 2002; Pfeiffer *et al*., 1998). The presence of adipocere on human remains has been said to slow down the decomposition process and preserve remains (Stuart & Ueland, 2017).

Adipocere is a complex of fatty acids; it is produced through the hydrolysis and hydrogenation of triglycerides by the actions of lipases and anaerobic bacteria to form saturated and unsaturated fatty acids (Fiedler *et al*., 2009; O'Brien & Kuehner, 2007). The primary saturated fatty acids include stearic, palmitic, and myristic acids, while the unsaturated fatty acids include palmitoleic, oleic and linoleic acids; these fatty acids can be further broken down by anaerobes like *Clostridium perfringens*, through metabolic reactions such as hydrolysis or hydrogenation (Stuart *et al*., 2016).

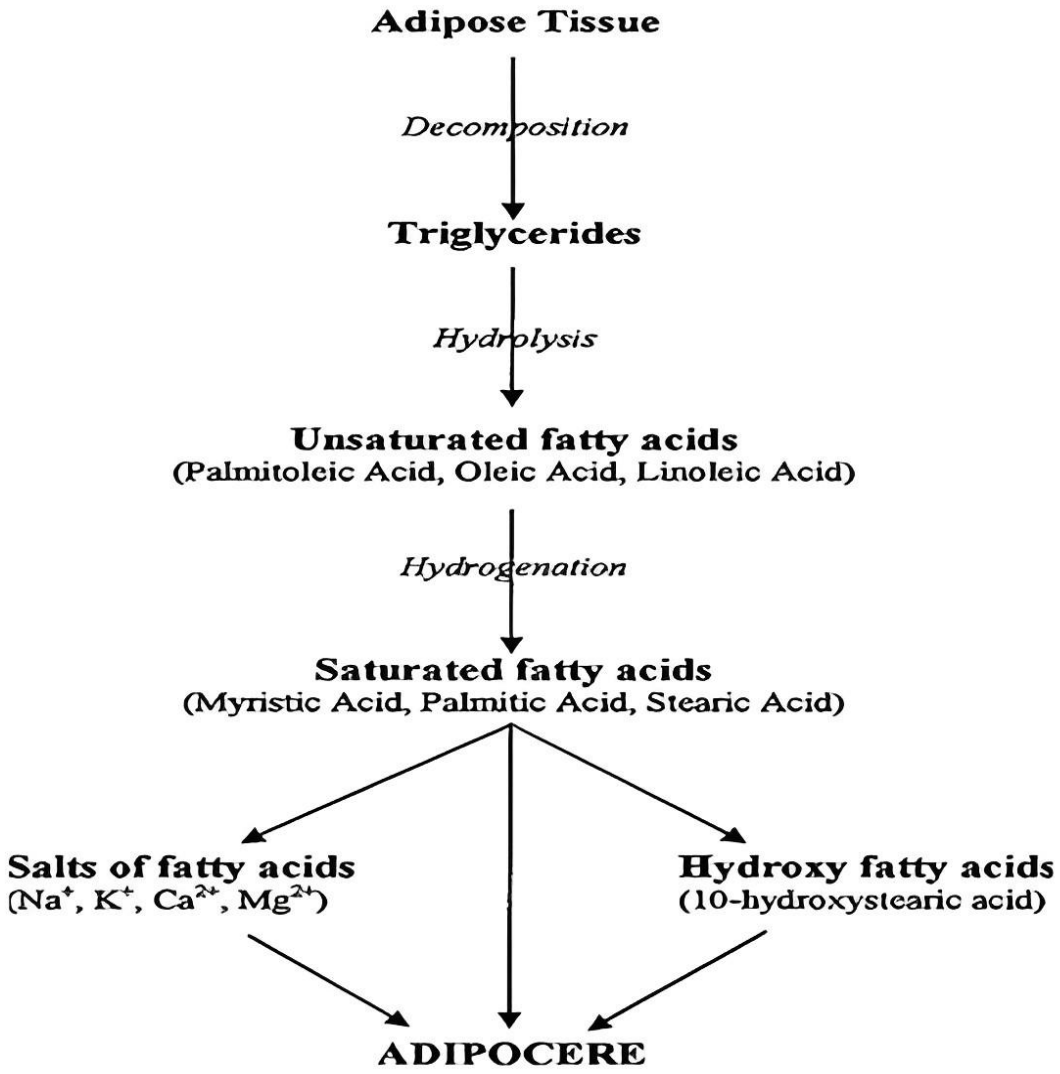


Figure 2.31: The process of adipocere formation (Forbes et al., 2005).

Figure 2.31 above illustrates the process of adipocere formation; as adipose tissues in the body decompose, they release triglycerides. The triglycerides are broken down into unsaturated fatty acids through the process of hydrolysis. Hydrogen ions are added to the unsaturated fatty acids through the process of hydrogenation to form saturated fatty acids; a combination of these fatty acids forms the complex known as adipocere (Forbes *et al*., 2005). Figure 2.32 illustrates triglycerides' hydrolysis process when broken down into glycerol and three fatty acids.

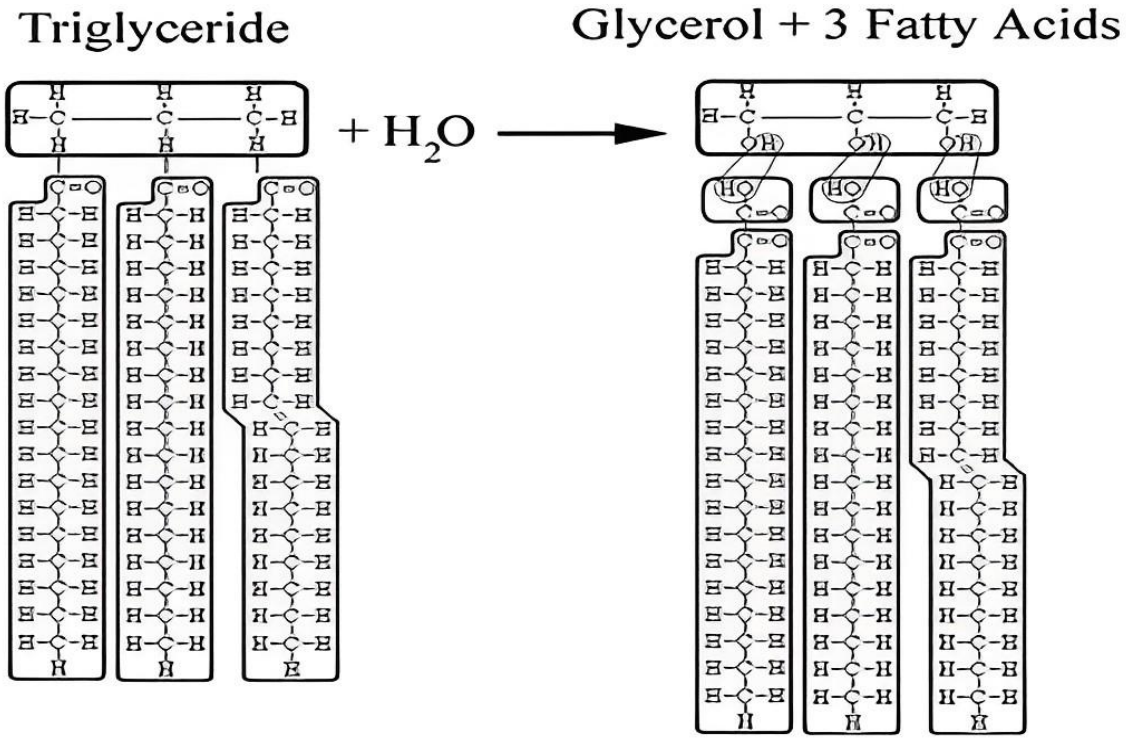


Figure 2.32: An illustration of a hydrolysis reaction during adipocere formation (Moses, 2012).

Adipocere has been observed on bodies recovered from different environmental conditions (Nushida *et al*., 2008; Stuart *et al*., 2016; Widya *et al*., 2012); therefore, various conditions affect the formation of adipocere, but a combination of optimal conditions is necessary for adipocere to form (O'Brien, 1997). The conditions that affect the production of adipocere include moisture content, temperature, humidity, and pH (Widya *et al*., 2012). A Stuart *et al*. (2016) study investigated adipocere formation in various model aquatic environments such as seawater, river, and chlorinated water for 18 months. The study suggested that adipocere formation is significantly inhibited in a saline environment because of reduced microbiological activity, while it was enhanced in the river and chlorinated environment due to bacteria. The enhanced adipocere formation observed in the chlorinated environment was unexpected because hypochlorous acid, which is typically found in chlorinated water, is known for reducing bacterial activities, but the formation of chlorohydrin through the hydrolysis reaction between hypochlorous acid and unsaturated fatty acids could have been responsible for the formation of adipocere observed in the chlorinated environment (Spickett, 2007; Stuart *et al*., 2016)

Adipocere can last centuries on a body, depending on the conditions of the environment (Fiedler *et al*., 2009; Fründ & Schoenen, 2009). The presence of adipocere in a body slows down the process of decomposition; in most cases, it can keep the body preserved by restricting the air to the surface of the body, minimising bacterial activity, and keeping the body moist (Boyle *et al*., 1997; Stuart, 2013; Ueland *et al*., 2013). This could eventually lead to an inaccurate estimation of PMSI (Stuart *et al*., 2016). To improve PMSI estimation for a body with adipocere, Widya *et al*. (2012) conducted a study on wild rabbits submerged for two months to investigate the early formation of adipocere in relation to ADD; results indicated that adipocere formation was most likely to have occurred at an ADD of 630. In the absence of insects, the decomposition rate of bodies is not affected by body size or fat (Simmons *et al*., 2010). Therefore, Widya *et al*. (2012) suggested that the results of their study can be applied to larger-sized remains; remains with adipocere should have a minimum PMSI of 630 ADD when estimating the PMSI. Figure 2.33 is an example of adipocere formation on a cadaver; some facial features are preserved due to the presence of adipocere.



Figure 2.33: Presence of adipocere on recovered remains (Karhunen et al., 2008).

2.4.2 Stages of Aquatic Decomposition

Classifying aquatic decomposition into stages is synonymous with terrestrial decomposition; it relies on grouping the stages based on visual and physical modifications of the decomposing body at different intervals during decomposition (Merritt & Wallace, 2010). The stages of aquatic decomposition were initially divided into six categories: Submerged fresh, Early floating, Floating decay, Bloated deterioration, Floating remains, and Sunken remains (Stuart & Ueland, 2017). After further studies (Haefner *et al*., 2004; Hobischak & Anderson, 2002), it was then reduced to 5 stages of decomposition; *Fresh Submerged*, *Early Floating*, *Early Floating Decay*, *Advanced Floating Decay*, and *Sunken Remains*. Details on each stage of aquatic decomposition are summarised in Table 2.4.

Table 2.4: Stages of aquatic decomposition (Stuart & Ueland, 2017).

Stage	Summary of details of each stage
Fresh submerged	The body remains sunken and has a fresh look with barely any signs of decomposition except the washerwoman's hands, goose pimples and pink discolouration. Rigor, algor and livor mortis could also occur in this stage for a shorter duration than terrestrial decomposition.
Early floating	The body resurfaces as bloating occurs due to the release of gases during putrefaction, skin discolouration (green/purple/pink/brown), marbling, and slippage occur. Due to the body resurfacing, insect and maggot activity is primarily observed in this stage, along with animal predation features or marks.
Early floating decay	Bloating still occurs, skin slippage and sloughing, loss of muscle mass, decomposition and disarticulation of soft tissues become apparent, discolouration darkens towards a greenish-blue or grey colour, and adipocere may occur on the body.
Advanced floating decay	Decay becomes more apparent; the skeleton becomes exposed due to extensive loss of soft tissues, and the disarticulation of the bones begins. Continuous liquefaction causes the bloating to reduce, the skin becomes dry and darker (discolouration can be brown or black), and the body begins to sink.
Sunken remains	At this stage, disarticulation is complete and only bones, nails and hairs remain; the remains also sink.

Figures 2.34–2.36 show some physical changes that occur during the aquatic decomposition stages.



Figure 2.34: Bloated face of a body recovered from water (Kahana et al., 1999).



Figure 2.35: Exposure of the skeleton during the Advanced floating decay stage (Van Daalen et al., 2017).

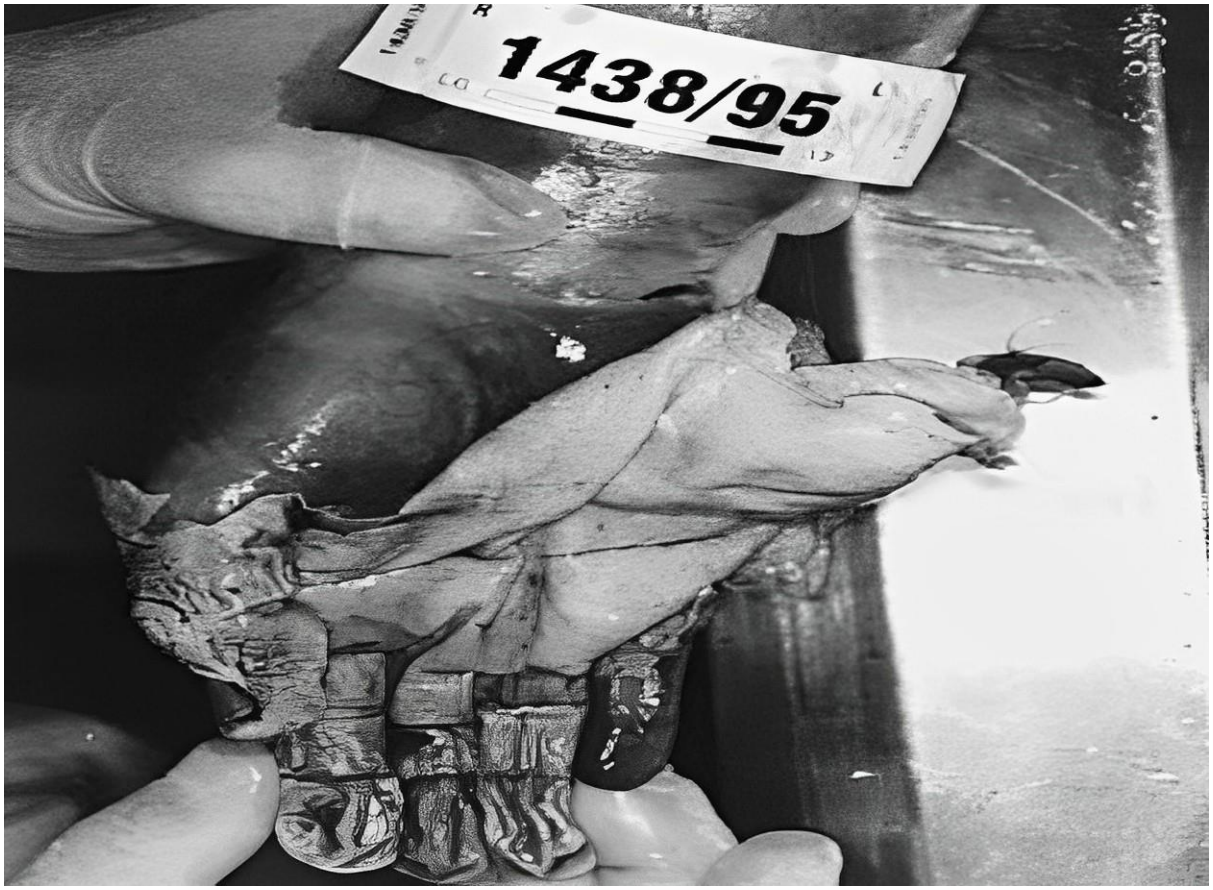


Figure 2.36: Example of the disintegration of soft tissue during the Early floating decay stage (Kahana et al., 1999).

2.5 Water Parameters Affecting Aquatic Decomposition

2.5.1 Temperature

Temperature is the most crucial factor affecting the rate of both aquatic and terrestrial decomposition (De Donno *et al*., 2014; Zhou & Byard, 2011). Water temperature's influence on the decomposition rate has been noted as far back as 56 years ago (Reh, 1967). van 't Hoff's law states that the rate of chemical reactions increases by two or three folds with every 10 ℃ rise in temperature; this law links temperature and the decomposition process because enzymatic, biological, and chemical reactions occur during the decomposition process (Vass, 2011).

Like on land, the higher the water temperature, the faster the decomposition rate (Champaneri, 2006). This is because bacteria and other microorganism activities are increased at elevated temperatures (Vij, 2014); the increase in temperature provides an optimum environment for decomposition (Carter *et al*., 2015; Iancu *et al*., 2015). The autolysis rate increases at a higher temperature (Tsokos, 2005). The cold temperature levels experienced during winter months immensely retard the decomposition rate of a body because of the inhibition of the biological activities of enzymes, bacteria, and insects (Forbes *et al*., 2014). At low temperatures, the eggs laid by insects have little to no appropriate conditions needed to survive; therefore, insect colonisation and activity in decomposing bodies are immensely reduced

(Carter & Tibbett, 2008). The Roberts & Dabbs (2015) study that investigated the differences in the decomposition rates of frozen and never frozen pigs indicated that decomposing occurs at a slower rate in frozen pigs.

ADD represents the accumulation of heat energy needed for biological and chemical reactions in soft tissues for decomposition (Palazzo *et al*., 2020). Like terrestrial decomposition, the ADD in aquatic decomposition is calculated by adding the average daily temperatures exposed to the decomposing body (Humphreys *et al*., 2013). When calculating Accumulated Degree Days (ADD) in the winter months, temperatures below zero degrees are counted as 0 ℃ (Alfsdotter & Petaros, 2021). Temperature also affects other factors that directly affect the rate of decomposition. For example, as the depth of water increases, the temperature reduces, thereby reducing the decomposition rate (Stuart & Ueland, 2017). Also, the level of adipocere formation depends on the temperature and moisture content of the environment in which the body is discovered (Humphreys *et al*., 2013; Widya *et al*., 2012). The process of gas formation and bloating, which will, in turn, make the body float, is affected by temperature; gas formation is reduced at cold temperatures (Stuart & Ueland, 2017).

2.5.2 Salinity

The salt content of a body of water influences the aquatic decomposition rate (Christensen & Myers, 2011; Reijnen *et al*., 2018). The salt concentrations of freshwater, brackish water and seawater are approximately 0.5 ppt, 20ppt and 35 ppt, respectively (Duncan Seraphin *et al*., 2014). Different types of bacteria are essential in the decomposition process, but in the presence of high salt concentration, bacterial activities become immensely reduced (De Donno *et al*., 2014; Rodriguez, 1997). This is supported by the fact that brine (salt solution) is commonly used to preserve and prevent organic substance decay (Barat *et al*., 2005). Hence the reason why decomposition is slower in seawater (high salt content) compared to freshwater (low salt content) like lakes and rivers (Boyle *et al*., 1997).

In freshwater environments, water moves into the lungs by the process of osmosis, resulting in increased blood volume and dilution; this causes haemolysis, and the body organs are damaged, thus, speeding up the process of decomposition (Stuart & Ueland, 2017). It is believed that when a body is submerged in high-saline water, the process of osmosis occurs; there is a loss of water from cells and bacterial cells in the body (Stuart & Ueland, 2017). This causes osmotic shock, leading to death or decelerating bacterial cell growth (Davidson *et al*., 2012). Also, the presence of salt can limit the solubility of oxygen and enzyme activities in microbial cells, thus reducing the growth rate of the cells (Shelef & Seiter, 2005). The loss of water can also cause blood thickening (fermentation), which reduces the oxygen conditions necessary for the growth of bacteria (Stuart & Ueland, 2017). All these processes tend to preserve the submerged body and reduce the decomposition rate.

2.5.3 Depth

The depth of the water in which a body is placed influences other factors that directly affect the decomposition process (Reijnen *et al*., 2018). It has been suggested that sunlight does not penetrate beyond 2 metres deep in water (Heaton *et al*., 2010); therefore, as the depth increases, the temperature reduces, thus slowing down the process of decomposition. At depths greater than 200 metres, the body becomes predominantly scavenged rather than decomposed due to the presence of aquatic invertebrates and animals at greater depths (Anderson & Bell, 2017; Anderson & Hobischak, 2004). In deep water (approximately > 61 m), submerged bodies do not bloat due to the increased pressure of the water, which reduces the gas volume in the soft tissues of the body; therefore, there is a lower chance of the body floating to the surface (Teather, 2013).

During drowning, as a body goes deeper into the water, there is an increased pressure gradient between the air in the lungs and the surrounding tissues, most especially the pulmonary blood vessels (Weenink & Wingelaar, 2021). This increased pressure gradient leads to the leakage of body fluids from the pulmonary vessels into the lungs; this process is said to be one of the possible mechanisms in immersion pulmonary oedema (Wilmshurst, 2019). This pulmonary oedema causes damage and injuries to the lungs and the surrounding vessels, thus, speeding up the process of decomposition (Stuart & Ueland, 2017).

2.5.4 Water Flow

Factors like water current, turbulence and tide that affect the degree of water flow in aquatic ecosystems significantly affect the decomposition rate (Haglund & Sorg, 2002). Water current is the movement of water in a specific direction caused by several forces acting upon the water; these factors include wind, the volume of water flowing, cabbeling stream gradient, riverbed topography, temperature, coriolis effect, and salinity difference (National Geographic Society, 2022; National Oceanic and Atmospheric Administration, 2021; Rahmstorf, 2003). Water current does not directly affect the decomposition rate, but it has a mechanical effect on submerged bodies; the water current creates other occurrences that directly affect the decomposition process (Caruso, 2016). A body deposited in water may be dragged for a distance, creating disarticulation and marks or discolouration that can be mistaken for decomposition changes or injuries, while a body that is immobile in water may be more preserved due to less impact from other objects (Humphreys *et al*., 2013; Palmer, 2020; Stuart & Ueland, 2017).

The remains or clothing may get caught on transport vessels, rocks, sea plant branches and other objects in the water, creating abrasions and lacerations that may be difficult to distinguish as post-mortem or antemortem trauma on the bones and body (O'Brien, 1997; Stuart & Ueland, 2017). These occurrences lead to the exposure and damage of soft tissues, causing an accelerated decomposition rate (Heaton *et al*., 2010; Teather, 2013). Also, the fast flow of water could lead to the removal of the products of

decomposition from the surrounding environment of the body, thus affecting microbial activity (Haglund & Sorg, 2002).

Fast-moving water bodies and areas with concentrated flows frequently experience turbulence, which may impact decomposition (Haglund & Sorg, 2002). There is limited literature on studies that have noticed the significance of turbulence and fast currents on decomposition changes in some forensic drowning cases (Haefner *et al*., 2004; Heaton *et al*., 2010; Reijnen *et al*., 2018; van Daalen *et al*., 2017). The Haefner *et al*. (2004) study suggested that decomposition was faster in "riffle" environments with a faster flow than in water environments with a slower flow. However, decomposition changes in these studies, such as abrasion and disarticulation, likely relate to differences in turbulence, not the differences in the flow rate of the water body (Palmer, 2020). Water flow without turbulence, also known as laminar flow, will not have such decomposition changes on the body. Laminar flow is believed to primarily impact decomposition by washing away decomposition fluids and tissue as it breaks loose (Stuart & Ueland, 2017). The Palmer (2020) study demonstrated that the decomposition rate of rabbits placed in nonflowing water (canal) occurred at a significantly slower rate than rabbits placed in flowing water (river); this is probably because an effect of water flow is skin sloughing and the disarticulation of bones and muscles.

2.5.5 Aquatic Organisms

Like the terrestrial ecosystem, the presence of organisms in the aquatic environment will affect the decomposition rate (Martlin *et al*., 2022). These organisms speed up the decomposition rate by feeding on the exposed soft tissues of the decomposing bodies, exposing the scavenged parts to other agents of decomposition (Stuart & Ueland, 2017). Bacteria, protozoa, fungi, and algae are microorganisms involved in the microbial activities of the process of aquatic decomposition (Lang *et al*., 2016).

Insects and crustaceans are invertebrates that contribute to aquatic decomposition, while vertebrates mainly include fish in the respective water environment (Anderson & Bell, 2017). The presence of these organisms or their scavenging activities on decomposing bodies can also be a valuable tool in PMSI estimation (Benbow *et al*., 2015; Ribéreau-Gayon *et al*., 2018). As stated earlier, insects play crucial roles in the decomposition process, but due to a body of water preventing the access of insects to a submerged body, the influence of insects on the decomposition process is reduced (Wang *et al*., 2022). Insects mainly gain access to bodies undergoing aquatic decomposition during the bloat stage of decomposition, in which the body resurfaces to the top of the water (Merritt & Wallace, 2010). In cases where bodies are submerged in shallow water, ditches and inland water, insects can colonise the bodies, but only the exposed areas.

In maritime environments worldwide, shark trauma is common and results in several fatalities annually (Stock *et al*., 2017). Examples of sharks that have been involved in different human cases of drowning

include the grey reef shark (*Carcharhinus amblyrhynchos*), tiger shark (*Galeocerdo cuvier*) and white shark (*Carcharodon carcharias*) discovered in Hawaiian waters (Ribéreau-Gayon *et al*., 2018). A smaller species of shark involved in shark trauma, though less documented, is the cookiecutter shark (*Isistius* spp.), also found in Hawaiian waters (Ribéreau-Gayon *et al*., 2016). The feeding patterns of these sharks and the shark bites can lead to the loss of body parts and hasten the decomposition process, while seawater can erode or even conceal the borders of the bitemarks (Ribéreau-Gayon *et al*., 2016; Stock *et al*., 2017).

The Anderson & Bell (2014; 2017) studies placed pigs under surveillance in the Saanich Inlet and Strait of Georgia in British Columbia to investigate the nature of the marine decomposition process of these pigs. A wide range of aquatic organisms was scavenging on the pigs at different study intervals. The organism spotted on the pigs include Three Spot Shrimp (*Pandalus platyceros*), Squat Lobster (*Munida quadrispina*), Dungeness crab (*Metacarcinus magister*), amphipods (*Orchomenella obtusa*), herring, dogfish, slender sole, Tanner crab (*Chionectes tanneri*), Arrow worms (*Sagitta elegans*), Sunflower Sea star (*Pycnopodia helianthoides*) and Ruby octopus (Octopus rubescens). Figure 2.37 below is an example of squat lobsters feeding on one of the pigs in the Anderson & Bell (2014) study.



Figure 2.37: Picture of a Squat lobster feeding on a pig underwater (Anderson & Bell, 2014).

The Ribéreau-Gayon *et al*. (2016) study showed that cookiecutter shark bites on victims changed injury patterns and made it difficult to reconstruct what happened at the point of submersion. Figure 2.38 is an example of the different shapes, colours, and sizes of the bite mark the cookiecutter sharks left on submerged bodies. Bite marks made it challenging to differentiate between perimortem and post-mortem injuries than on fresher tissues, thereby complicating the reconstruction of the events leading to death and PMSI estimation (Best & Photopoulou, 2016; Ribéreau-Gayon *et al*., 2016). The *Selachimorpha* species of sharks, galatheae and crabs are other aquatic organisms that have been documented to feed on human remains recovered from water (Ellingham *et al*., 2017).

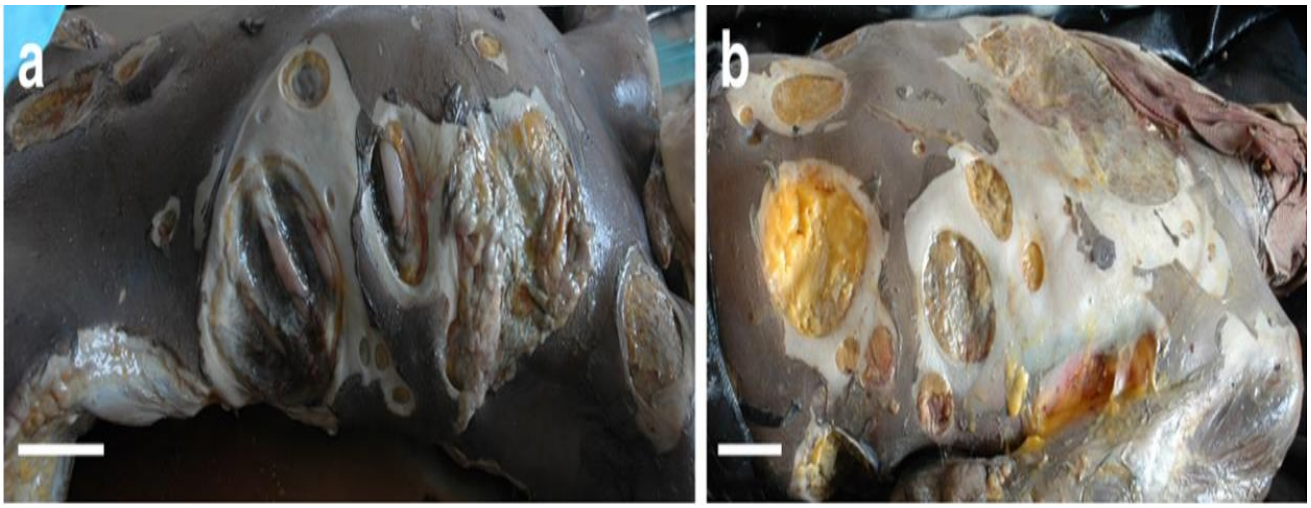


Figure 2.38: Various shapes and sizes of extensive lesions [a-female victim b-male victim] (Ribéreau-Gayon et al., 2016).



(a)-Cranium ***(b)-Femur***

Figure 2.39: Examples of the Galatheae scavenging marks left on bones (Ellingham et al., 2017).

Schuliar *et al*. (2014) conducted a study by carrying out a post-mortem examination of 154 victims recovered from the Atlantic Ocean after the Air France disaster; they found signs of scavenging by Galatheae and amphipods. Figure 2.39 above is an example of the pictorial representations of the predation signs of Galatheae feeding on the bones of victims recovered from the air crash. Figures 2.40–2.41 are other examples of scavenging marks on bones by crabs and sharks.



Figure 2.40: Crab feeding on a bone (Ellingham et al., 2017).



Figure 2.41: Evidence of predation by Shark [Selachimorpha] (Ellingham et al., 2017).

2.5.6 Body Coverings

The presence of body coverings affects the rate of aquatic decomposition (De Donno *et al*., 2014; Notter & Stuart, 2011). The rate of putrefaction is delayed in a well-covered body because decomposition agents such as insects and microorganisms have limited access to the body, and the reduced level of oxygen affects microbial activities (Pakosh & Rogers, 2009; Stuart & Ueland, 2017). Card *et al*. (2015) reported that dipteran species colonization was delayed in pigs because of the presence of clothing. Figure 2.42 shows examples of body parts that are preserved due to the presence of clothing on the bodies prior to when the pictures were taken; the head and neck regions of the body that were exposed exhibit extensive decomposition features such as bone exposure while the trunk regions of the body that were covered with clothing materials look preserved.



Figure 2.42: Bodies exhibiting extensive decomposition in the cervico-cephalic and mandible regions, but the trunk looks preserved due to the prior presence of clothing (Ellingham et al., 2017).

In contrast, Matuszewski *et al*. (2014) argue that the presence of clothing on a decomposing body has a negligible effect on the decomposition rate, but the mass of the body has a more significant effect on the decomposition rate; smaller pigs had a faster active decay rate while larger pigs had a longer duration of the active decay stage. The Matuszewski *et al*. (2014) study suggests that putrefaction takes longer to complete in larger carcasses. The Voss *et al*. (2011) study revealed that colonisation by dipteran larval masses lasted for a longer duration on clothed pigs. The Voss *et al*. (2011) study also suggested that clothed pigs have longer wet decay stages; hence insects that thrive in wet conditions will be attracted to these clothed pigs (Kelly *et al*., 2009).

The presence of body coverings slows down the cooling of a body by keeping it warm and humid; this condition is essential for insect diversity, feeding, abundance and protection from predators and

unfavourable environmental conditions (Campobasso *et al*., 2001; Mashaly *et al*., 2019). However, some researchers contend that clothing is only marginally significant for insect succession on carrion (Matuszewski *et al*., 2016). Also, the types of coverings on the body have differing effects on the decomposition process. Thick coverings can insulate a body from the environment and speed up the formation of adipocere on the body (Notter & Stuart, 2011; Vij, 2014). Natural fibre coverings are more water-absorbent than synthetic fibre coverings; therefore, they accelerate the formation of adipocere (Notter & Stuart, 2011).

The effect of clothing on the decomposition rate was accounted for in the nomogram method developed by Henssge (1988) [See Section 2.2.3.6]. Henssge (1988) formulated constants referred to as corrective factors. These corrective factors consider if a body is naked or clothed (thin or thick layers), still or moving, and submerged (Figure 2.18). The corrective factor is multiplied by the body weight to obtain a corrected weight used in estimating the time since death.

Table 2.5 is a summary showing factors that accelerate and retard aquatic decomposition.

Table 2.5: Summary of conditions that affect the decomposition rate.

Speed Up		**Slow Down**	
Factors	**Process**	**Factors**	**Process**
Temperature	The higher the temperature, the faster the chemical, enzymatic and biological reactions during decomposition.	Temperature	Low temperatures reduce or inhibit the reactions needed for decomposition and reduce the survival of insect eggs.
Current	Fast-flowing water can lead to disarticulation and the dragging of a body against submerged objects—these damages to the body speed up decomposition.	Salinity	Higher salinity concentrations reduce bacterial activity, thereby slowing down the decomposition process.
Organisms	Aquatic organisms feed on submerged bodies, thus exposing soft tissues. These speed up the decomposition of the body.	Depth	There is no sunlight penetration at deeper depths, and temperature levels are reduced. This low temperature slows down the decomposition process.
		Clothing	Clothing can prevent insect colonisation of a body; it can also keep the body warm and preserved

2.6 Water Profile of Water Bodies in the U.K.

This chapter of the PhD research has focused on related subjects on aquatic decomposition extensively; the process of drowning, stages and description of aquatic decomposition and the water conditions that affect the decomposition rate. This PhD research was conducted in the U.K., it is reasonable to understand the water profile of water bodies in the U.K. before the results of this research are produced. Understanding the water profile of water bodies in the U.K. helps to provide further context and justification for the study and to see if the results of this research can be comparable with real-life scenarios that occur in the U.K. Table 2.6 below shows the breakdown of the water profile of seas and rivers in the U.K. These data were collated from various studies such as Banks & Boyce (2023), Dye *et al*. (2020), Findlay *et al*. (2022), Jiang *et al*. (2022) and Jonkers & Sharkey (2016).

Table 2.6: Water profile of seas and rivers in the U.K.

Water Conditions	**Water Body**	
	Sea	**Rivers**
Salinity (ppt)	33–35	~1.0
pH	7.5–8.5	7–8.5
Conductivity (mS/cm)	35–40	< 1.0
Mean Annual Temperature (°C)	6–20	10–15

3 PMSI AND DECOMPOSITION SCORING SYSTEMS

3.1 Methods of Estimation of PMSI

As stated earlier, the interval between immersion/death and the recovery of human remains from a body of water is known as Post-mortem Submersion Interval (Humphreys *et al*., 2013). Different methods have been adapted from techniques for estimating Post-mortem intervals in terrestrial environments to aid in estimating the PMSI of bodies recovered from aquatic ecosystems (Reijnen *et al*., 2018). The most frequently used PMSI estimation strategy is to count the days between when the victim was last known to be alive and when the body was recovered (Hobischak & Anderson, 1999). This approach will only be valid in water death cases with a known time of disappearance or the time the drowning occurred and a known time of recovery (Anderson, 2008). Multiple estimation methods have also been developed for cases where the time of death or immersion is unknown, but a problem encountered in these methods is the variability of environmental conditions across water bodies (Humphreys *et al*., 2013).

3.1.1 Weighing Decomposition

In developing PMSI estimation methods, past studies have weighed human analogue carrions before and after submersion to determine the amount of decomposition that has occurred (Anderson & Hobischak, 2004; Humphreys *et al*., 2013; Zimmerman & Wallace, 2008). The Zimmerman & Wallace (2008) study investigated decomposition rate as a function of weight loss in stillborn piglets submerged in a pond for fifteen days by weighing the piglets on the sampling days. Their study indicated a statistically significantly greater weight loss in the piglets during the early floating stage and the advanced floating decay stage of decomposition. This method had limitations because, to avoid contamination, it required the researcher either to start the experiment with many submerged samples and remove one at each sampling interval or by surfacing a subject, weighing it, and then resubmerging it (Celata, 2015; Humphreys *et al*., 2013). Another limitation is that water absorption and bloating contribute to the mass increase, and this increase in mass is contrary to the amount of decomposition that has occurred in terms of weight loss (Humphreys *et al*., 2013).

3.1.2 Microbial Community Assembly (MCA)

Microorganisms have shown the potential for successional colonisation of submerged remains; the microorganisms include bacteria, algae, protozoa, and fungi (Davis & Goff, 2000; Lang *et al*., 2016; Zimmerman & Wallace, 2008). Numerous studies have been carried out to investigate the use of these microorganisms in estimating PMSI, and they have shown promising forensic value (Benbow *et al*., 2015; Casamatta & Verb, 2000; Haefner *et al*., 2004; He *et al*., 2019).

Dickson *et al*. (2011) conducted a study to investigate marine bacterial succession; they found that bacterial families such as *Firmicutes*, *γ-Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Bacteroidetes* colonised the submerged remains in succession. Dickson *et al*. (2011) also suggested that

if microbes are to be exploited for forensic reasons, various microbial community successional patterns in different seasons should be considered. Benbow *et al*. (2015) conducted a study in a freshwater stream during the summer and found that Proteobacteria predominated early in the decomposition process, but Firmicutes became the dominant communities after 21 days.

MCA profiles and variability in these studies may have crucial forensic context for better understanding the PMSI estimation of drowned bodies and provide additional compelling biological evidence that microbial profiles are distinct among weeks and community changes are consistent among replicate carcasses (Kaszubinski *et al*., 2020).

3.1.3 Entomological Methods

Entomological approaches have been proposed as potential determinants of PMSI estimation; this involves the successional colonisation of submerged remains by insects or invertebrates (Keiper *et al*., 1997; Merritt & Wallace, 2010; Wallace *et al*., 2008). Keiper *et al*. (1997) suggested that the colonising Midge larvae (Diptera: Chironomidae) can be a forensic tool for estimating the PMSI of carcasses in woodland streams. Hobischak & Anderson (2002) also proposed that in pond and stream habitats, the successional colonisation by species like caddisfly, blowflies, carrion beetles (*Silphidae*), nymphal mayflies and stoneflies can be used as PMSI determinants. *Barnacles* are another example of invertebrates used as entomological evidence in PMSI estimation. In a case report, Magni *et al*. (2015) estimated the PMSI of a discovered floating body to be 65 days by evaluating the presence of barnacles *Lepas anatifera* L. (*Crustacea: Cirripedia: Pedunculata*) attached to the garments on the body (Figure 3.1).



Figure 3.1: Photograph of Barnacles attached to the shoe and garment on a recovered body (Magni et al., 2015).

3.1.4 Decomposition Scoring Systems

Lastly, another standard PMSI estimation method involves the use of Decomposition Scoring systems (DSS) that visually measure the extent of decomposition in a body; these DSS scores are then used in combination with the ADD of the recovered bodies to estimate the PMSI of the body (Reijnen *et al*., 2018). Further research is being conducted to validate and improve these methods of PMSI estimation. Some of the standard aquatic DSS are comprised of the Total Aquatic Decomposition Score (TADS) system; observations in the head and neck (Facial Aquatic Decompositional Score [FADS]), trunks (Body Aquatic Decompositional Score [BADS]) and limbs (Limb Aquatic Decompositional Score [LADS]) of each body are noted, scored, and added to give a final TADS value.

The TADS value represents the extent of decomposition of the whole body (van Daalen *et al*., 2017). The most common DSS used for measuring the extent of decomposition in bodies recovered from water were created by Heaton *et al*. (2010), van Daalen *et al*. (2017) and Reh (1967). The Decomposition Scoring Systems (DSS) used for aquatic decomposition are adaptations of the Megyesi *et al*. (2005) Total Body Score (TBS) system that was developed for bodies undergoing terrestrial decomposition. Like the TBS, these DSS assesses the sequential internal and external changes in the body's soft tissues as decomposition processes progress (Hobischak & Anderson, 2002).

3.2 Megyesi *et al*. (2005) TBS

3.2.1 Description

Accurately estimating the PMI is essential in reconstructing the events around death, excluding suspects, and identifying unknown human remains (Gelderman *et al*., 2018). Various forensic fields commonly apply gross observations of the decomposition of soft tissues to estimate the PMI or PMSI of a body because decomposition is a continuous process that can be quantified and split into stages in relation to time and temperature (Gelderman *et al*., 2018; Myburgh *et al*., 2013; Parks, 2010).

To quantify the decomposition process, Megyesi *et al*. (2005) developed a visual and quantitative scoring system that assessed the physical changes observed in the head/neck, trunk and limbs of a body and related them to ADD in terms of the temperature of the body or environment (Table 3.1). These physical changes observed using the TBS system were modified into different stages that are predicted to be frequently sequential; these stages include fresh, early decomposition, advanced decomposition, and skeletonization.

Photographs of 68 human remains were assessed and scored using the TBS system. This scoring system assessed changes ranging from a fresh appearance to skeletonization, which denotes complete decomposition; it also assessed discolouration changes of the skin, skin sloughing off and disarticulating body parts.

Table 3.1: Descriptive stages of decomposition for the TBS system (Megyesi et al., 2005).

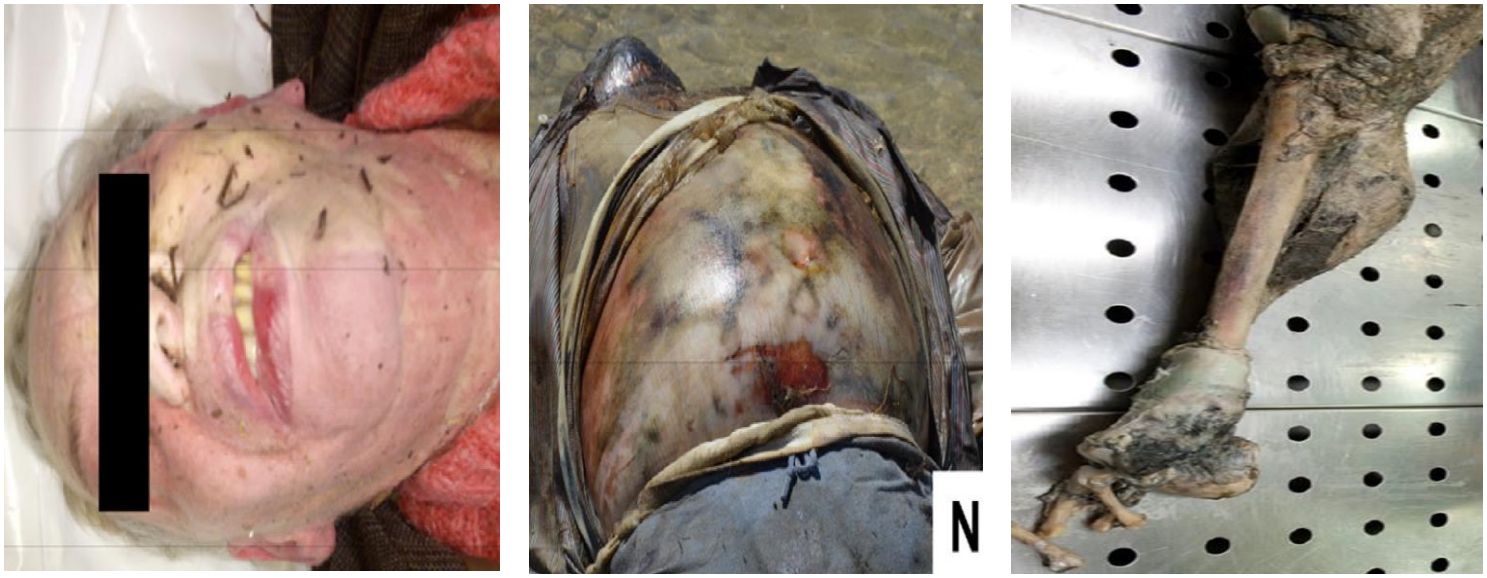
A—Categories and stages of decomposition for the head and neck.	
A. Fresh	
(1pt)	1. Fresh, no discoloration
B. Early decomposition	
(2pts)	1. Pink-white appearance with skin slippage and some hair loss.
(3pts)	2. Gray to green discoloration: some flesh still relatively fresh.
(4pts)	3. Discoloration and/or brownish shades particularly at edges, drying of nose, ears and lips.
(5pts)	4. Purging of decomposition fluids out of eyes, ears, nose, mouth, some bloating of neck and face may be present.
(6pts)	5. Brown to black discoloration of flesh.
C. Advanced decomposition	
(7pts)	1. Caving in of the flesh and tissues of eyes and throat.
(8pts)	2. Moist decomposition with bone exposure less than one half that of the area being scored.
(9pts)	3. Mummification with bone exposure less than one half that of the area being scored.
D. Skeletonization	
(10pts)	1. Bone exposure of more than half of the area being scored with greasy substances and decomposed tissue.
(11pts)	(11pts) 2. Bone exposure of more than half the area being scored with desiccated or mummified tissue.
(12pts)	(12pts) 3. Bones largely dry, but retaining some grease.
(13pts)	(13pts) 4. Dry bone.

B—Categories and stages of decomposition for the trunk.	
A. Fresh	
(1pt)	1. Fresh, no discoloration.
B. Early decomposition	
(2pts)	1. Pink-white appearance with skin slippage and marbling present.
(3pts).	2. Gray to green discoloration: some flesh relatively fresh
(4pts)	3. Bloating with green discoloration and purging of decompositional fluids.
(5pts)	4. Postbloating following release of the abdominal gases, with discoloration changing from green to black.
C. Advanced decomposition	
(6pts)	1. Decomposition of tissue producing sagging of flesh; caving in of the abdominal cavity.
(7pts)	2. Moist decomposition with bone exposure less than one half that of the area being scored.
(8pts)	3. Mummification with bone exposure of less than one half that of the area being scored.
D. Skeletonization	
(9pts)	1. Bones with decomposed tissue, sometimes with body fluids and grease still present.
(10pts)	2. Bones with desiccated or mummified tissue covering less than one half of the area being scored.
(11pts)	3. Bones largely dry, but retaining some grease.

(12pts)	4. Dry bone.

C—Categories and stages of decomposition for the limbs.	
A. Fresh	
(1pt)	1. Fresh, no discoloration
B. Early decomposition	
(2pts)	1. Pink-white appearance with skin slippage of hands and/or feet.
(3pts).	2. Gray to green discoloration; marbling; some flesh still relatively fresh.
(4pts)	3. Discoloration and/or brownish shades particularly at edges, drying of fingers, toes, and other extremities.
(5pts)	4. Brown to black discoloration, skin having a leathery appearance.
C. Advanced decomposition	
(6pts)	1. Moist decomposition with bone exposure less than one half that of the area being scored.
(7pts)	2. Mummification with bone exposure of less than one half that of the area being scored.
D. Skeletonization	
(8pts)	1. Bone exposure over one half the area being scored, some decomposed tissue and body fluids remaining.
(9pts)	2. Bones largely dry, but retaining some grease.
(10pts)	3. Dry bone.

To obtain a TBS for a body, the scores of the head, trunk and limbs were added together; for example, if a body is scored as follows: head and neck = 3 points, trunk = 5 points, and limbs = 8 points, then the TBS would be 3 + 5 + 8 = 16. Pictorial depictions of the TBS of 16 are illustrated in Figure 3.2.



Head=3; discolouration with the skin still fresh **Trunk=5; bloating & black discolouration** **Limbs=8; bone exposure with some tissue left**

Figure 3.2: Pictorial depictions of a TBS of 16 (Pictures from Palazzo et al., 2021; van Daalen et al., 2017).

Megyesi *et al*. (2005) formulated a regression equation for the TBS system that can be used to extrapolate the ADD of the body with a standard error. The TBS is substituted into the regression equation formulated to calculate the ADD. The regression equation formulated by Megyesi *et al*. (2005) is displayed below:

$$Log_{10}ADD = 0.002\ (TBS*TBS) + 1.81 \pm 388.16$$

This can be further expressed as:

$$ADD = 10^{(0.002*TBS*TBS+1.81)} \pm 388.16$$

Using the example of a body with a TBS of 16, the ADD will be calculated as:

$$ADD = 10^{(0.002*16*16+1.81)} \pm 388.16$$

$$ADD = 10^{(0.512+1.81)} \pm 388.16$$

$$ADD = 10^{(2.322)} \pm 388.16$$

$$ADD = 209.89 \pm 388.16$$

The PMI is then inferred by retrospectively adding up the average daily temperatures from when the body was found until the extrapolated ADD is reached.

3.2.2 Limitations

Although the TBS system is one of the fundamental quantitative methods used in PMI estimation, it has flaws and limitations. The TBS system was created using 68 human remains. The study's sample size was small, which could decrease the study's confidence level, reliability, and statistical power and increase the margin of error and bias in the study (Faber & Fonseca, 2014). Vague terms such as “bone exposure of more than half of the area being exposed” and “some flesh relatively fresh”, along with the various skin colour changes listed in descriptions of these scoring systems, add to the subjectivity and inconsistency of these DSS (Gelderman *et al*., 2018; Nawrocka *et al*., 2016).

In addition, photographs of the human remains were used and not the actual bodies; some important decomposition changes might have been missed out in the pictures, which might have affected the descriptions of the DSS. The human remains used in this study were found in various locations that might have affected the decomposition rates significantly. 11 out of the 68 remains were found indoors: houses (3), apartments (3), residential trailer (2), condominium (1), cabin (1), and abandoned building (1). The remaining 57 cases were found outdoors (fields, ditches, and woods) exposed to sunlight in a variety of ways: full sun (13), shaded areas (15), and a mix of sun and shade (29).

Using the example above of a body with a TBS of 16, the calculated ADD was 209.89 ± 388.16. This answer indicates that the lower confidence interval of the ADD is a negative value, 209.89 – 288.16 =

–178.27; this negative ADD makes it impossible to estimate PMSI. Moffatt *et al*. (2016) reviewed the Megyesi *et al*. (2005) TBS study and formula, pinpointed some flaws, and developed an improved equation for TBS and ADD. The first flaw Moffatt *et al*. (2016) highlighted in the TBS system was that the ADD, which is an independent (explanatory) variable, was switched to a dependent (response) variable; the formula predicted ADD from TBS.

The second flaw involved rounding up the regression slope value (0.002) from the formula to one significant number; this overestimates the ADD by 150%. Moffatt *et al*. (2016) excluded indoor cases, cases with an ADD above 3000, and cases with their PMI estimated through entomological methods from the Megyesi *et al*. (2005) TBS study and were left with 15 cases out of 68. These 15 cases were used to develop an Ordinary Least Squares (OLS) linear regression model that had a better line of fit ($r^2 = 0.91$) and narrower confidence interval when compared to the Megyesi *et al*. (2005) model ($r^2 = 0.84$). The model Moffatt *et al*. (2016) created is shown below:

$$TBS_{surf} = (125 \times Log_{10}ADD - 212)^{0.625}$$

Smith *et al*. (2022) reviewed the Moffatt *et al*. (2016) article that highlighted the flaws of the TBS system. Their study found that the Moffatt *et al*. (2016) approach had some flaws. It reduced the Megyesi *et al*. (2005) TBS study sample size from 68 to 15, making the ADD calculation more challenging and the TBS system less useful or applicable in practical applications. Smith *et al*. (2022) suggested an ADD estimation interval model using the formula below:

$$\text{ADD 95\% PI} = 10^{(0.00155 \times TBS \times TBS) + 1.81} \cdot 10^{\pm 2.00 \times 0.201}$$

The Smith *et al*. (2022) model eliminates the estimation of uncertainty error and is a simpler model that can be applied to practical cases when compared to the Moffatt *et al*. (2016) and Megyesi *et al*. (2005) models.

3.3 Heaton *et al*. (2010) DSS

3.3.1 Description

Heaton DSS was derived from a study on 187 human remains recovered from freshwater in the U.K. It assesses the extent of decomposition by scoring decomposition changes observed in the face, head, neck, trunk, and limbs (Table 3.2). For PMSI estimation, the DSS uses a regression equation that extrapolates the ADD from the TADS calculated when assessing the extent of decomposition in the body. The PMSI is inferred by retrospectively adding up the average daily temperatures from when the body was found until the extrapolated ADD is reached (Heaton *et al.*, 2010).

Table 3.2: Descriptive stages of decomposition for the Heaton et al. (2010) DSS.

Descriptive stages for decomposition observed in the face and the assigned facial aquatic decompositional score (FADS).

FADS	Description
1	No visible changes.
2	Slight pink discoloration, darkened lips, goose pimpling.
3	Reddening of face and neck, marbling visible on face. Possible early signs of animal activity ⁄predation—concentrated on the ears, nose, and lips.
4	Bloating of the face, green discoloration, skin beginning to slough off.
5	Head hair beginning to slough off—mostly at the front. Brain softening and becoming liquefied. Tissue becoming exposed on face and neck. Green⁄black discoloration.
6	Bone becoming exposed—concentrated over the orbital, frontal, and parietal regions. Some on the mandible and maxilla. Early adipocere formation.
7	More extensive skeletonization on the cranium. Disarticulation of the mandible.
8	Complete disarticulation of the skull from torso. Extensive adipocere formation.

Descriptive stages for decomposition observed on the torso and the assigned body aquatic decompositional score (BADS).

BADS	Description
1	No visible changes.
2	Slight pink discoloration, goose pimpling.
3	Yellow ⁄ green discoloration of abdomen and upper chest. Marbling. Internal organs beginning to decompose ⁄ autolysis.
4	Dark green discoloration of abdomen, mild bloating of abdomen, initial skin slippage.
5	Green⁄purple discoloration, extensive abdominal bloating—tense to touch, swollen scrotum in males, exposure of underlying fat and tissues.
6	Black discoloration, bloating becoming softer, initial exposure of internal organs and bones.
7	Further loss of tissues and organs, more bone exposed, initial adipocere formation.
8	Complete skeletonization and disarticulation.

Descriptive stages for decomposition observed in the limbs and the assigned limb aquatic decompositional score (LADS).

LADS	Description
1	No visible changes.
2	Mild wrinkling of skin on hands and/or feet. Possible goose pimpling.
3	Skin on palms of hands and/or soles of feet becoming white, wrinkled, and thickened. Slight pink discoloration of arms and legs.
4	Skin on palms of hands and/or soles of feet becoming soggy and loose. Marbling of the limbs—predominantly on upper arms and legs.
5	Skin on hands/feet starting to slough off. Yellow/green to green/black discoloration on arms and/or legs. Initial skin slippage on arms and/or legs.
6	Degloving of hands and/or feet—exposing large areas of underlying muscles and tendons. Patchy sloughing of skin on arms and/or legs.
7	Exposure of bones of hands and/or feet. Muscles, tendons, and small areas of bone exposed in lower arms and/or legs.
8	Bones of hands and/or feet beginning to disarticulate. Bones of upper arms and/or legs becoming exposed.
9	Complete skeletonization and disarticulation of limbs.

Using the Table 3.2, a victim with darkened lips and reddening of the face and neck will get a FAD score of 3, a BAD score of 5 when a purple discolouration and extensive abdominal bloating is observed in the trunk and a LAD score of 5 when the skin on the hands show green/black discolouration, and they start to slough off. Pictorial depictions of these FAD, BAD, and LAD scores are illustrated in Figure 3.3. These scores total a TADS of 13. This TADS is then substituted into the regression equation formulated by Heaton *et al*. (2010) to calculate the ADD:

$$TADS = -3.706 + 7.778 \log_{10}ADD$$

$$13 = -3.706 + 7.778 \log_{10}ADD$$

$$\text{Therefore, } ADD = 10^{\left(\frac{13+3\cdot706}{7\cdot778}\right)}$$

$$ADD = 140.6$$

The PMSI is inferred by retrospectively adding up the average daily temperatures from when the body was found until ADD of 140.6 is reached.



(a) FADS = 3; reddening of the face and neck



(b) LADS = 5; discoloured skin on hand sloughs off



(c) BADS = 5; purple discolouration and abdominal bloating in the trunk

Figure 3.3: Pictorial depiction of a TAD score of 13 (Pictures from Caruso, 2016; Palazzo et al., 2021).

3.3.2 Limitations

Photographs of the human remains were used and not the actual bodies; some vital decomposition changes might have been missed in the pictures, which might have affected the descriptions of the DSS. The bodies used in developing this DSS were recovered from the rivers Mersey and Clyde; there could be a gap in knowledge on whether this scoring system will be suitable for bodies recovered from seawater sources. Heaton *et al*. (2010) suggested that this DSS is unsuitable for estimating PMSI for cases where the presence of adipocere is found, and cases where the ADD value is less than 10. Vague terms such as "mild wrinkling of hands or feet", "more bone exposed", and "small area of bone exposed", along with the various skin colour changes listed in descriptions of this scoring system, add to the subjectivity of this DSS (Gelderman *et al*., 2018; Nawrocka *et al*., 2016).

3.4 van Daalen *et al*. (2017) DSS

3.4.1 Description

The van Daalen DSS was created from a study on 38 human remains recovered from the North Sea borders in the Netherlands. Like the Heaton DSS, it also assesses the extent of decomposition by scoring decomposition changes observed in the face, head, neck, trunk, and limbs (Table 3.3). A summation of the FADS, BADS and LADS to give the TAD score is also conducted when using the van Daalen DSS, but the PMSI is predicted from the TADS using a prediction model and not the ADD.

Table 3.3: Descriptive stages of decomposition for the van Daalen et al. (2017) DSS.

Facial Aquatic Decomposition Score

FADS	Description
1	1.1 No visible changes
2	2.1 Marbling and/or 2.2 Skin slippage and/or 2.3 Hair sloughs off
3	3.1 Bloating of eyelids and/or 3.2 Bloating of lips
4	4.1 Gray, matte discoloration of the skin with a crumbly surface
5	5.1 Partial skeletonization
6	6.1 Complete skeletonization

Body Aquatic Decomposition Score

BADS	Description
1	1.1 No visible changes
2	2.1 Marbling of upper trunk and/or 2.2 Marbling of lower trunk and/or 2.2 Skin slippage and/or 2.3 Hair sloughs off
3	3.1 Bloating of abdomen and/or 3.2 Bloating of genitals
4	4.1 Gray, matte discoloration of the skin with a crumbly surface
5	5.1 Partial skeletonization
6	6.1 Complete skeletonization

Limbs Aquatic Decomposition Score

LADS	Description
1	1.1 No visible changes
2	2.1 Wrinkling and/or white discoloration of the skin of hands and/or feet

3	3.1 Marbling and/or 3.2 Skin slippage and/or 3.3 Hair sloughs off and/or 3.4 Degloving and/or 3.5 Absence of nails
4	4.1 Gray, matte discoloration of the skin with a crumbly surface 4.2 Partial and/or gross skeletonization of the distal part of the limbs (hands and/or feet) 4.3 Partial skeletonization of the more proximal parts of the limbs (arms and/or legs)
5	5.1 Partial skeletonization
6	6.1 Complete skeletonization

3.4.2 Limitations

The DSS was created using 38 human remains. The study's sample size was small, which could decrease the study's confidence level, reliability, and statistical power and increase the margin of error and bias in the study (Faber & Fonseca, 2014). In addition, photographs of the human remains were used and not the actual bodies; some vital decomposition changes might have been missed in the pictures, which might have affected the descriptions of the DSS. The remains used were recovered from the North Sea; there could be a gap in knowledge on whether this scoring system will be suitable for bodies recovered from freshwater sources.

Some fundamental decomposition changes were missed in the descriptions of this DSS; for example, the DSS only considered grey and white skin discolouration changes with no mention of the colour changes expected in livor mortis, marbling, and putrefaction. There was also no mention of other decomposition changes or features such as adipocere formation, animal activity or predation, the disarticulation of body parts, and the initial exposure of soft tissues, organs, and bones. Vague terms such as “grey, matte discolouration of the skin with a crumbly surface” and “partial skeletonization” listed in descriptions of this scoring system add to the subjectivity and inconsistency of this DSS (Gelderman *et al*., 2018; Nawrocka *et al*., 2016).

3.5 Reh DSS

3.5.1 Description

In the 1960s, a German forensic pathologist created a table (Figure 3.4) for PMSI estimation by considering actual water temperature and the external or internal physical changes in bodies immersed in water (Reh *et al*., 1977). The Reh DSS was derived from a study on 277 human remains recovered from the Rhine and Rhine harbour, running waters, lakes, and pools in Germany (Madea, 2015). There are similarities between the Reh DSS and the other two DSS; the Reh DSS also considers physical signs of decomposition on a body. In contrast, the Reh DSS does not consider decomposition scores or ADD but considers the average water temperatures for each month and the water temperature (taken at approximately 0.5–1 m below the water surface) of the place and time of recovery.

Ø	Month	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	Median water temperature (C) of the month according to measurements of Reh	3.5°	3.9°	5.8°	9.9°	13.0°	17.4°	18.6°	18.6°	17.3°	13.2°	8.8°	4.7°
	Signs of putrefaction												
1.	Marbling	32	25	16 (23)	9–10	4–5	2	1–2	2	3	4–5	10	17
2.	Distension of tissues by gas	35	25	16 (23)	10	4–5	2–3	2	3	3–4	7	10	17
3.	Discolouration of the body	35	25	16 (23)	(14)	4–5	2	2	3	3–4	7	10	17
4.	Peeling of the epidermis	35	25	16 (23)	(16)	4–5	3	2	3	3–4	7	10	17
5.	Hair loss	35	25	16 (23)	10–12	4–5	2–3	2–3	3	3–4	7	10	17
6.	Hands: beginning of wrinkling	(1)	(1) 28–30	(12 h)			(6 h)			2 h		2 h	(1)
7.	Nails become loose	>35	(40) 30–32	23	16	5	2–3	3	3	3–4	11	17	28
8.	Peeling of skin in glove form	35	(45)	23	16	10	3	3	3-4	4	7	20	28
9.	Nail loss	>53	45	30 (40)	21	14	8	3	4	10	>11	20	>35
10.	Feet: beginning of wrinkling	(1)	(1)	(12 h)	(1)		(6 h)	½ h		2 h		2 h	(1)
11.	Nails become loose	>53	40	26 (35)	17	10	5	3	4	8	12	17	28
12.	Peeling of skin	>53	60	35	16	10	5	3	5–6	8–9	>11 (14)	20	28
13.	Nail loss	>53	>60	53	>35	>28	>10	3	>10	>10	>11	>20	>35
14.	Transudate in pleural cavities†	35	25 (40)	18 (35)	10	5	3–4	3	3	11	5	>20	
15.	Heart without blood	>39	32–34 (40)	23	14–15	9	4	3	3	5	11	20	28
16.	Brain liquefied	35	30 (40)	(23)	14–	5	3–4	3	3	6	10	17	28

* Minimum time of immersion in days; maximum time in days shown in parentheses.

† >500 ml in adults.

Figure 3.4: Decomposition changes and estimated monthly PMSI figures for the Reh DSS (Madea, 2015).

Unlike the Heaton and van Daalen DSS, the estimated PMSI of a body using the Reh DSS is not extrapolated from an equation. Still, every sign of decomposition is correlated with a minimum time in which this sign can develop. When estimating PMSI, the more the putrefaction signs are considered, the more reliable the Reh DSS becomes (Madea, 2015). The duration of immersion for the last decomposition change is considered the minimum PMSI, while the maximum PMSI can be estimated by considering the putrefaction changes that have not yet occurred in the body. For estimating the

minimum PMSI, the average temperature closest to the actual water temperature at the point of recovery is considered, not the water temperature of the month (Reh, 1969). Using the Reh table in Figure 3.4 for PMSI estimation, a body found in July exhibiting marbling, degloving and nail loss; the minimum PMSI is estimated to be three days.

3.5.2 Limitations

Due to climate changes in the last 40 years, there has been an increase in the water temperature of the river Rhine; therefore, for cases with water temperatures over 20 ℃, there is a decrease in the reliability of the Reh table (Madea & Doberentz, 2010). Further studies are required to investigate the correlation between these higher temperatures and the decomposition rate (Doberentz & Madea, 2010). In addition, vague phrases such as "discolouration of the body" in the descriptions of this scoring system are not specific for the colour changes expected in a body at different stages of decomposition; hence it adds to the subjectivity of this DSS (Gelderman *et al*., 2018; Nawrocka *et al*., 2016).

3.6 Heaton vs van Daalen DSS

Both scoring systems have similar scoring descriptions; these descriptions include skin discolouration, marbling, bloating, skin slippage, hair slough off and skeletonization. The differences between both systems are listed in Table 3.4.

Table 3.4: Differences between the Heaton et al. (2010) & van Daalen et al. (2017) DSS.

Heaton *et al*. (2010)	**van Daalen *et al*. (2017)**
A minimum TAD score of 3 and a maximum of 25.	A minimum TAD score of 3 and a maximum of 18.
FADS and BADS have a maximum score of 8, while LADS has a maximum of 9.	FADS, BADS and LADS all have a maximum score of 6.
It puts more colours into consideration when referring to discolouration. The colours include pink, yellow, red, black, green, white, and purple.	Only considered grey and white colours.
It includes the presence of adipocere on bodies.	No mention of adipocere formation.
There is mention of the possibility of animal activity or predation on the face.	Animal activity or predation is not noted.
The disarticulation of body parts as skeletonization occurs is mentioned.	No mention of the disarticulation of body parts.
The initial exposure of soft tissues, organs and bones is mentioned.	No mention of the initial exposure of soft tissues, organs, and bones.
No mention of the absence of nails.	The absence of nails is mentioned.

3.7 Limitations of Scoring Systems in General

Although these DSS and TBS systems have been validated over the years and passed as reliable, they have a few limitations (Dabbs *et al*., 2016). In some cases, a cadaver could exhibit features representative of more than one stage, especially when decomposition is advanced; this could lead to lesser accuracy of the scores given when using the scoring systems (Nawrocka *et al*., 2016). Also, when scoring limbs, both pairs of the legs and arms could have different decomposition changes leading to difficulties in assigning a score; it has been suggested that each limb is scored, and an average score of the four limbs is calculated as the final score (Dabbs *et al*., 2016). The scoring systems are visual charts assessing physical changes observed in bodies; therefore, they are subjective and depend on the observer's perception. Vague terms such as "heavy maggot activity", "partial skeletonization", "bone exposure", and "some flesh relatively fresh", along with the various skin colour changes listed in descriptions of these scoring systems, add to the subjectivity and inconsistency of these DSS (Gelderman *et al*., 2018; Nawrocka *et al*., 2016).

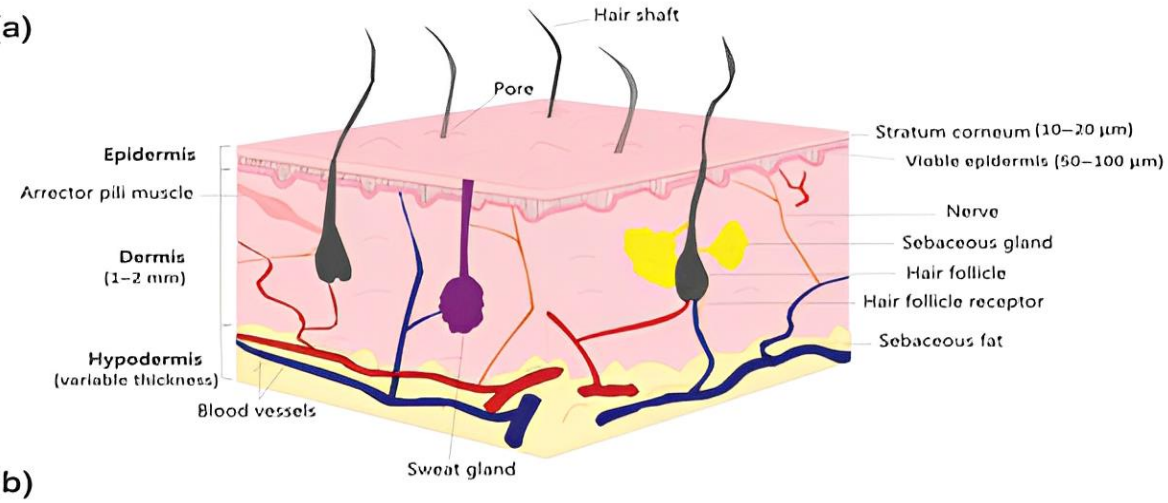
4 SKIN COLOUR AND DECOMPOSITION

Human skin colour is an external feature that is highly significant in medical, anthropological, genetic, and forensic science fields (Walsh *et al*., 2017). In forensic science, skin tone or colour assessment is essential in recreating the events that lead to the death of a victim, age estimation of wounds, scars, and bruises, forensic DNA phenotyping, PMSI estimation and decomposition changes (Chen *et al*., 2021; Hayman & Oxenham, 2016; Heaton *et al*., 2010; Scafide *et al*., 2013; van Daalen *et al*., 2017). The vital roles of skin colour and tone in decomposition and PMSI estimation will be further highlighted in this chapter and other parts of this thesis.

4.1 Physical Structure of the Skin

The skin is the largest human organ, representing approximately 10% of the body mass for adults, with a surface area range of 2–25 m^2; it is the outermost physical interface between the body and its external environment, acting as the first line of defence, controlling what enters and leaves the body (Boxberger *et al*., 2021; Gallo, 2017; Ng & Lau, 2015). The skin is comprised of a complex nexus of cells, microbes and matrix elements providing varying functions such as the protection of the body against infectious agents or UV radiation, sensation and synthesis of vitamin D, thermoregulation, and prevention of dehydration (Lima & Reis, 2018; Prausnitz *et al*., 2012). The skin consists of three primary layers: the hypodermis, dermis, and epidermis (Figure 4.1).

(a)



(b)

Layer	Structure	Cell types	Extracellular matrix components
Hypodermis	Blood vessels, nerves, hair follicles	Adipocytes, fibroblasts, endothelial cells, muscle cells	Elastin, type I collagen
Dermis	Blood vessels, nerves, mechanoreceptors, appendages	Fibroblasts, endothelial cells, Langerhans cells, muscle cells	Elastin, proteoglicans, type I, IV and VII collagen
Epidermis	Stratified keratinized epithelium	Keratinocytes, melanocytes, Merkel cells, Langerhans cells	Keratin, type IVand VII collagen

Figure 4.1: Skin Structure. (a) Schematic representation (b) Characteristics of the skin layers (Moniz et al., 2020).

The hypodermis, the innermost layer, also called the subcutaneous layer, consists of adipose cells, lymphatic and blood vessels placed below the dermis and between the skin, muscles, and bones, but the thickness of this layer differs over various body parts (Joodaki & Panzer, 2018). This layer can be absent in some thin skin like the eyelid (Moniz *et al*., 2020). The hypodermis aids insulation, mechanical integrity and support, and conductance of the vascular and neural signals of the skin and constitutes about 10% of the body mass (Alkilani *et al*., 2015; Joodaki & Panzer, 2018; Ng & Lau, 2015). Other suggestions state that the hypodermis is a connective tissue and is not strictly a layer of the skin (Betts *et al*., 2013).

The dermis is a moderately dense integrated fibrous layer comprised of collagen, elastin, reticulin fibres and a matrix of sweat glands, hair follicles, sebaceous glands, sensory nerve endings, nails, lymphatic vessels, macrophages, dermal dendritic cells, blood capillaries, fibroblasts, and mast cells (Joodaki & Panzer, 2018; Moniz *et al*., 2020). The components of this layer have numerous functions such as being a thermal barrier, protection from physical injury, energy storage, providing flexibility, elasticity, tensile strength, various mechanical behaviours of the skin at small stresses or strains and the recoiling mechanism after deformation (Joodaki & Panzer, 2018; Laurent *et al*., 2007; Lima & Reis, 2018; Moniz *et al*., 2020).

The epidermis is the outermost layer of the skin, approximately 75–150 mm in thickness, and acts as the barrier between the human body and the external environment (Chanda, 2018). It is a stratified

keratinised epithelium of four sub-layers: stratum spinosum, stratum basale, stratum granulosum, and stratum corneum (Moniz *et al*., 2020; Roger *et al*., 2019). The stratum basale of the epidermis comprises various cells: keratinocytes, corneocytes, melanocytes, Merkel, and Langerhans cells (Figure 4.1b); it is one cell thick, and the melanocytes form about 10% of the cells present in the stratum basale (Venus *et al*., 2010). The melanocytes play essential roles in determining skin colour (Jothishankar & Stein, 2019), and these roles will be discussed further in the next section.

In the stratum spinosum, keratinocytes originating from the stratum basale, form a layer attached by desmosomes; these form the "spines or prickles" observed under a microscope (Agarwal & Krishnamurthy, 2022). The stratum granulosum comprises keratinocytes containing dense, ovoid-shaped, lipid-rich keratohyalin granules that release lipids into the intercellular parts of the skin layers, aid barrier function and cohesion within the outer stratum corneum layer (Venus *et al*., 2010). The three inner layers of the epidermis form the "viable epidermis", which is considerably hydrophilic (Forster *et al*., 2009).

There is a fifth sub-layer of the epidermis, known as the stratum lucidum, mainly referred to as a lower part of the stratum corneum; it is found in the sole of the foot or the palms of the hand and accounts for the thickness of these body parts (Moniz *et al*., 2020). The stratum corneum is the outermost layer of the epidermis comprised of about 20 layers of dead anucleate keratinocytes known as corneocytes; it is the protective layer of the skin that regulates water loss and prevents oxidative and mechanical stress (Moniz *et al*., 2020; Ng & Lau, 2015; Venus *et al*., 2010). The stratum corneum contains 10% water and is lipophilic in contrast to the viable epidermis (Forster *et al*., 2009). Figure 4.2 below is a picture of the layers and components of the skin and their respective functions.

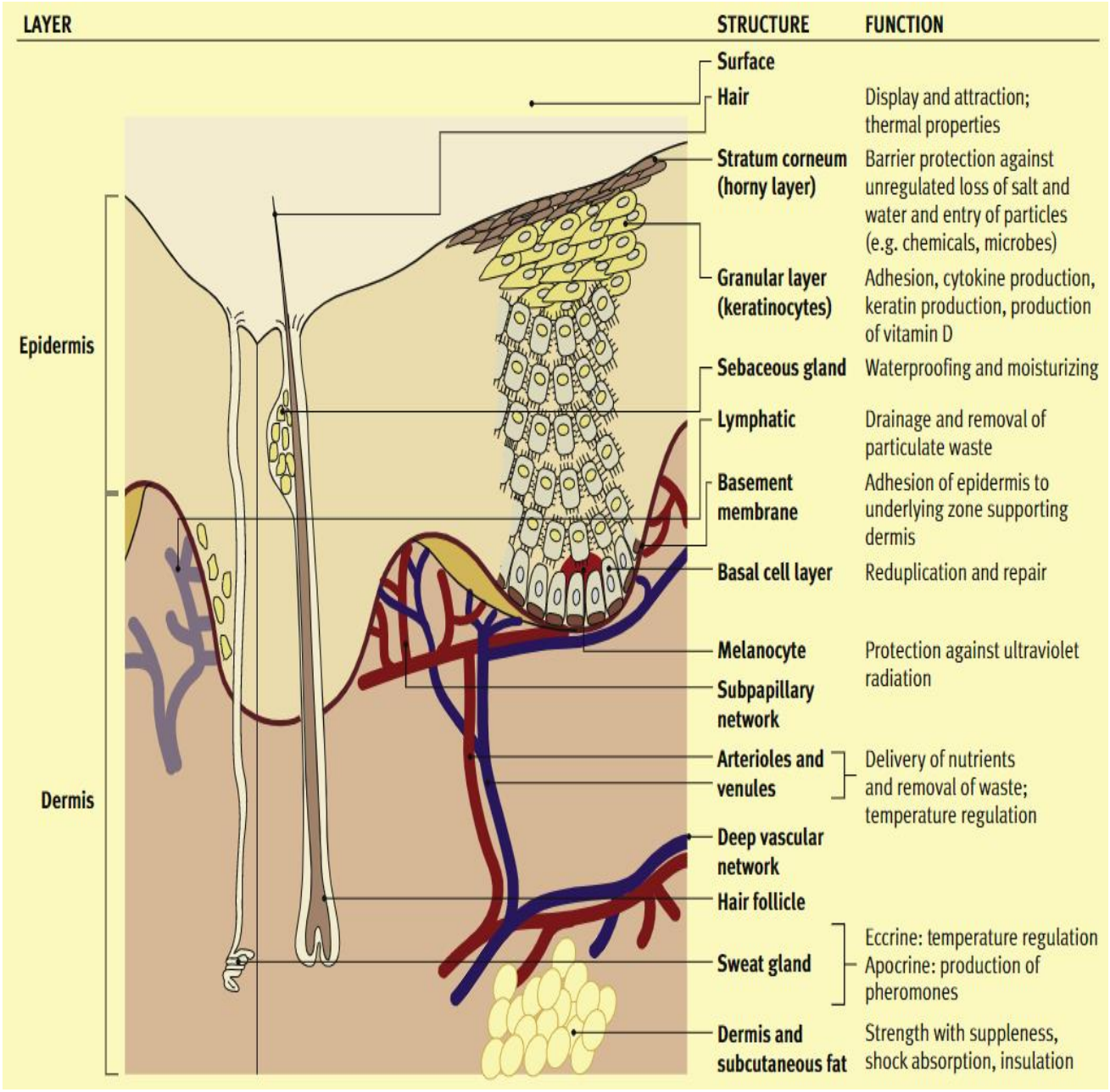


Figure 4.2: Schematic view of the cross-section of the skin layers (Venus et al., 2010).

4.2 Skin Colour and Tone

4.2.1 Determinants of Skin Colour

Different factors influence skin colour in humans: genetics, melanosome, melanin content, UV exposure, and the presence of additional chromophore types in the skin (Taylor, 2002). The differences in the combination of four chromophores: melanin, haemoglobin, carotenoids, and oxyhaemoglobin, impact the perception of human skin colour (Jothishankar & Stein, 2019). The combination of the four

chromophores leads to varying absorption and reflection of light, and they are responsible for the varying shades found in the human skin (Jothishankar & Stein, 2019; Rawlings, 2006). Haemoglobin and oxyhaemoglobin combine to produce a pink tone in pale skin, while melanin and carotenes combine to produce a brown or yellow-orange tone in brown skin (Naik & Farrukh, 2022; Rawlings, 2006).

Melanosomes in melanocytes are essential in synthesising and transferring melanin chromophores in the skin (Jothishankar & Stein, 2019). The variation of skin tones between different ethnicities depends on the size and number of melanosomes in the keratinocytes and melanocytes, not the number of melanocytes that are present in the skin (Naik & Farrukh, 2022). In lighter skin tones, smaller-sized melanosomes are clustered in secondary lysosomes, while in darker skin, melanosomes are larger-sized and dispersed within the lysosomes (Naik & Farrukh, 2022).

The human skin, eyes and hair colours are highly heritable and externally visible traits (Chen *et al*., 2021). Using sets of carefully designated DNA markers known as Single Nucleotide Polymorphisms (SNPs), human hair and eye colours have already been accurately predicted, but the knowledge on predicting skin colour using DNA markers is limited (Walsh *et al*., 2017). This limited knowledge is mainly because of a population-specific or global distribution influence of skin colour; eye and hair colour variations are primarily specific to the European populations, thus, allowing studies to use a homogenous population study, while skin colour variations are not attributed to a specific population; therefore, studies will require a highly heterogeneous global population (Kayser, 2015; Walsh *et al*., 2017). However, various studies have been carried out on SNPs to gain more knowledge on genetics and skin colour determination. The Valenzuela *et al*. (2010) study used 789 individuals of various ethnicities and determined 3 SNPs (SLC45A2 rs16891982, SLC24A5 rs1426654, and ASIP rs2424984) for skin colour determination. The Spichenok *et al*. (2011) study used a set of 7-SNPs to predict "not-white and not-dark skin" colours.

In 2014, the first comprehensive skin colour prediction study was published; the study made use of 285 DNA samples obtained from unrelated individuals from 28 countries [American, African, Eurasian, and Asian roots] (Maroñas *et al*., 2014). 59 SNPs previously associated with skin, eye, and hair colours were investigated in the study and a skin colour predictive test using the ten most strongly associated SNPs in 8 genes linked to skin colouration variations was described; the two most vital DNA markers identified are the SLC45A2 rs16891982 and SLC24A5 rs1426654 SNPs which were identified in the Valenzuela *et al*. (2010) study (Maroñas *et al*., 2014). The Walsh *et al*. (2017) study also identified a set of 36 SNPs from 16 genes that demonstrated that skin colour between and within ethnic groups could be accurately predicted from DNA samples.

Skin tanning after exposure to sunlight or UV is highly variable between ethnic groups and has also been attributed to genetics and melanin pigmentation (Ge *et al*., 2017; Visconti *et al*., 2018). Seven genes already associated with human hair, eye and skin colours, and skin cancer have been identified

from various Genome-wide association studies (GWASs) on European populations to be involved in the tanning ability of the skin after exposure to UV or sunlight (Chahal *et al*., 2016; Eriksson *et al*., 2010; Gerstenblith *et al*., 2010; Han *et al*., 2008; Jacobs *et al*., 2015; Law *et al*., 2015; Liu *et al*., 2015; Zhang *et al*., 2013). These seven genes include ASIP, EXOC2, HERC2, IRF4, MC1R, SLC45A2 and TYR (Nan *et al*., 2009; Sulem *et al*., 2007; Sulem *et al*., 2008; Zhang *et al*., 2013). The Visconti *et al*. (2018) study also identified 20 SNPs significantly associated with tanning ability, with 14 of the SNPs being novel findings and the other 6 SNPs being associated with pigmentation-related phenotypes.

4.2.2 Measuring Skin Colour

A consistent and reproducible skin colour evaluation is crucial and provides essential clues in various scientific fields (Ly *et al*., 2020). The Fitzpatrick Skin Type (FST) scale is widely used for skin tone classification (Table 4.1). It was developed in 1975 to gauge the response of a person's skin tone to sun exposure or ultraviolet radiation in terms of the degree of burning and tanning (Fitzpatrick, 1988). Phototypes I-IV were initially used to categorise skin types in decreasing sensitivity to UV light and increasing tanning ability. Later, V and VI were used for brown and dark-skinned individuals based on their fundamental pigmentation or ethnicity rather than responsiveness to sun exposure (Ly *et al*., 2020).

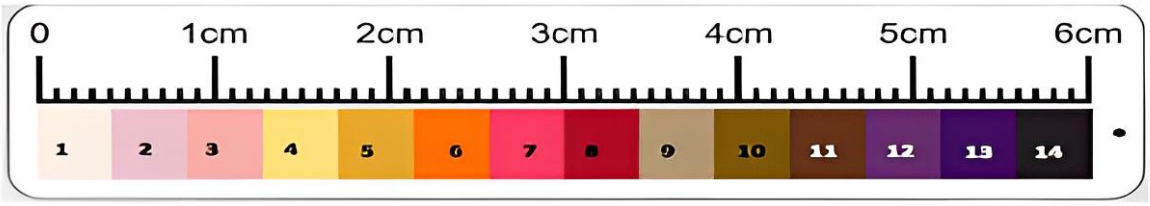
Table 4.1: The Fitzpatrick Skin Type scale (Sachdeva, 2009).

Phototype	**Sunburn and tanning history (defines the phototype)**	**Immediate pigment darkening**	**Delayed tanning**	**Constitutive color (unexposed buttock skin)**	**UV-A MED (mJ/cm^2)**	**UV-B MED (mJ/cm^2)**
I	Burns easily, never tans	None (-)	None (-)	Ivory white	20-35	15-30
II	Burns easily, tans minimally with difficulty	Weak (± to +)	Minimal to weak (± to +)	White	30-45	25-40
III	Burns moderately, tans moderately and uniformly	Definite +	Low +	White	40-55	30-50
IV	Burns minimally, tans moderately and easily	Moderate ++	Moderate ++	Beige-olive, lightly tanned	50-80	40-60
V	Rarely burns, tans profusely	Intense (brown) +++	Strong, Intense (brown) +++	Moderate brown or tanned	70-100	60-90
VI	Never burns, tans profusely	Intense (dark brown) +++	Strong intense (brown) +++	Dark brown or black	100	90-150

The FST is limited, subjective and has observer and recall bias. Darker skin-toned individuals typically have different levels of skin pigmentation, and phrases like "burns minimally to rarely" and "tans deeply" may be unrelatable and culturally insensitive; therefore, objective and relatable techniques were developed (Eilers *et al*., 2013; Pichon *et al*., 2010).

In neonates, a metric graduated colour (MGC) tool has been used along with digital photographs to improve objective assessments of skin wounds (Figure 4.3). The MGC tool placed near the injury would provide a colour reference for the injury bed, help with image clarity, and allow colour correction. The

MGC instrument may also enhance objectivity in infant skin damage's first and subsequent evaluations (August *et al*., 2019).



1- peach
2- blush
3- pink
4- yellow
5- mustard
6- orange
7- fuchsia
8- red
9- tan
10- brown
11- chocolate
12- violet
13- purple
14- black
*- white

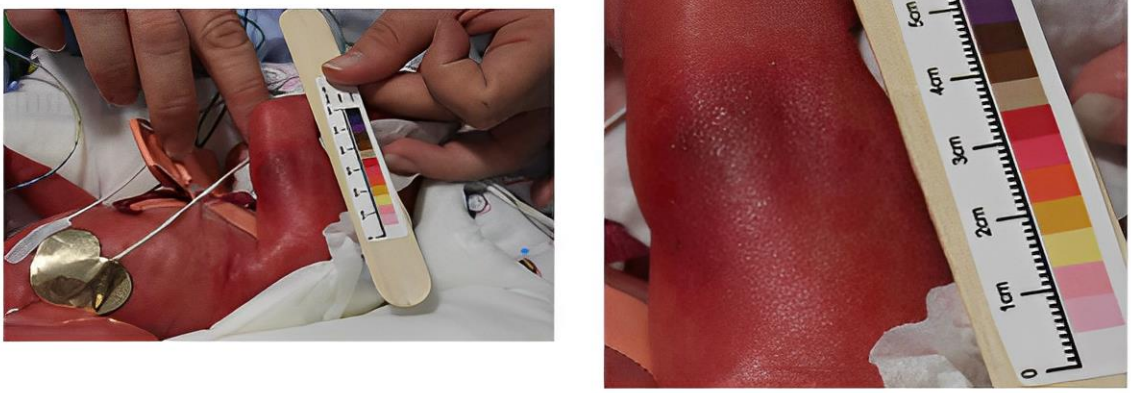


Figure 4.3: MGC tool being used to assess a baby's skin and wound colour (August et al., 2019).

Colourimeters and spectrophotometers are objective, quantitative, and observer-independent devices used in skin colour evaluation; they quantify skin colour, erythema, and tanning (Ly *et al*., 2020). Spectrophotometry provides objective measurements, significantly improves repeatability, and enables precise, quantitative evaluation of skin tone (Sitek *et al*., 2016). These devices were developed under the standardization of the Commission Internationale de l'Eclairage (CIE); the CIELAB measurements are linked with skin tone and related factors, such as erythema (Brainard & Stockman, 2010; Del Bino & Bernerd, 2013; Everett *et al*., 2012). The CIELAB, or CIE L* a* b*, is a 3D colour space. The L* axis is a grey scale with values from 0 to 100. The L* value correlates with the level of pigmentation,

a* is the red/green axis and correlates with erythema, and b* is the yellow/blue axis and correlates with pigmentation and tanning (Figure 4.4).

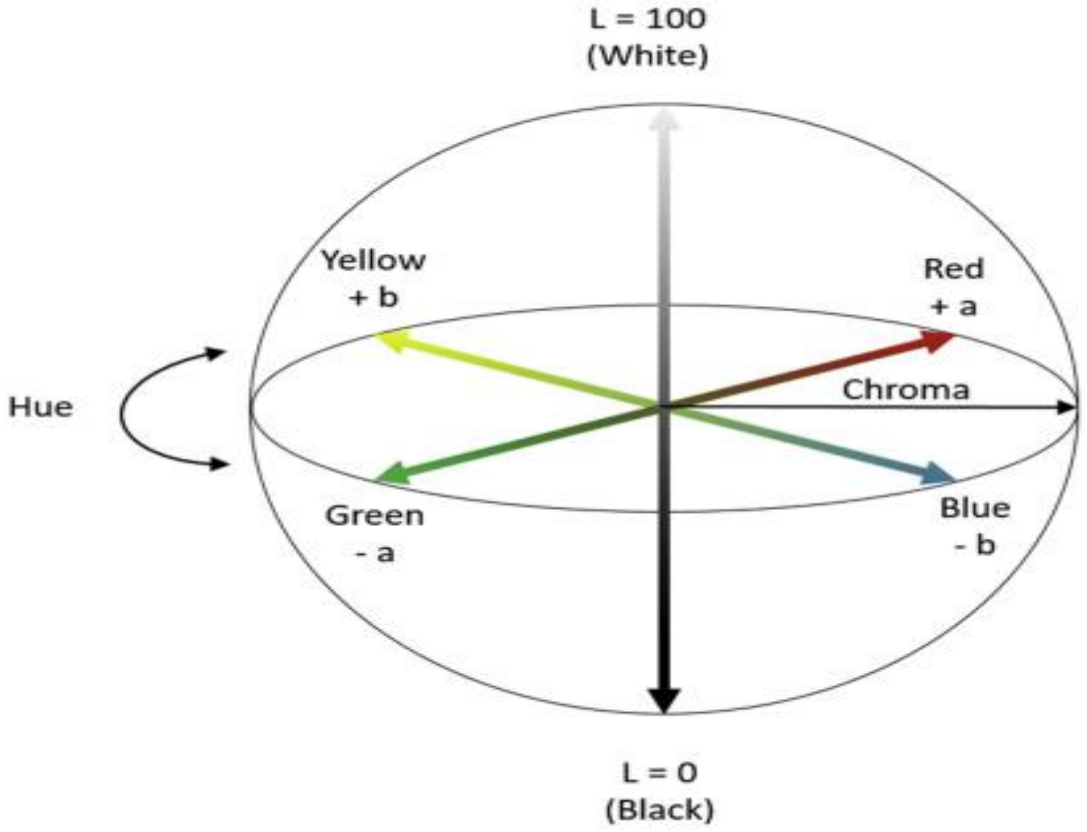
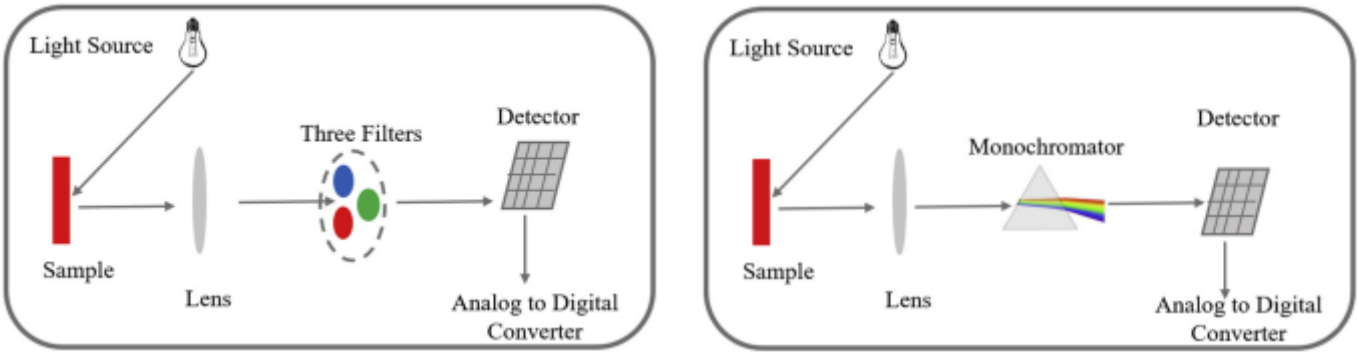


Figure 4.4: The CIELAB colour space diagram (Ly et al., 2020).

Figure 4.5 illustrates a colourimeter and spectrophotometer's essential components and operating principles, while Figures 4.6–4.7 are pictures of both devices in use. In both devices, a light source emits wavelengths onto a sample, and the sample absorbs the wavelengths and reflects the light. The colourimeter filters reflected light through the red, green, and blue trichromatic filters. In contrast, in the spectrophotometer, the reflected light is captured by the device and the multiple spectral sensors process wavelengths between 360–700 nm. A detector analyses the reflected light's spectral characteristics and, therefore, the sample colour.



(a) Colourimeter *(b) Spectrophotometer*

Figure 4.5: Operating principle of a colourimeter and spectrophotometer (Ly et al., 2020).

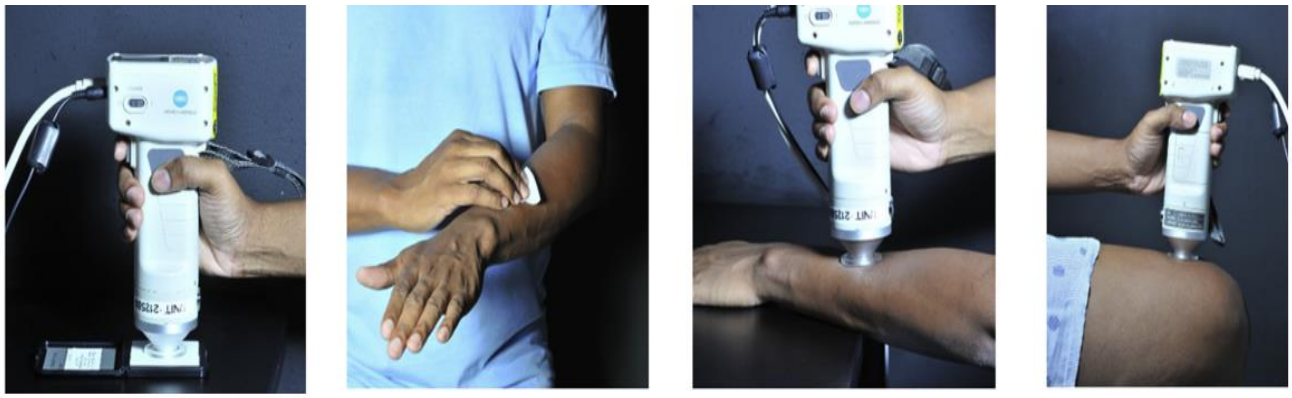


Figure 4.6: A colourimeter in use (Ly et al., 2020).



Figure 4.7: A spectrophotometer in use (Ly et al., 2020).

4.2.3 Importance of Skin Colour in Forensic Science

The ability to predict skin colour is a growing, increasing focus in the forensic genetics field to build DNA-based tools for predicting observable physical features (Chen *et al*., 2021; Kayser & de Knijff, 2011). As an alternative to eyewitness accounts, which frequently lack accuracy or may not be available to investigators, the HIrisPlex-S system and genome-wide association (GWA) studies make it possible to use DNA tests to predict eye, hair, and skin colour from DNA, including low quantity forensic DNA (Chen *et al*., 2021; Maroñas *et al*., 2014). In circumstances where a DNA profile matches with a known suspect is not possible, forensic DNA phenotyping can be used to predict the skin colour and other pigmentation traits of an unknown crime scene sample donor directly from the DNA samples recovered from the scene (Kayser, 2015; Schneider *et al*., 2019).

The criminal court system regularly requests that forensic health professionals examine the skin of victims of assault to estimate the age of their wounds, scars, and other features, particularly bruising (Scafide *et al*., 2013). Clinically, the most frequent factor utilised to establish a rough age estimate for a bruise is its colour (Grossman *et al*., 2011). Therefore, forensic experts and medical professionals have advised using objective measures such as colourimetry and spectrophotometry to evaluate the colour of these bruises (Hughes *et al*., 2004).

Skin colour also plays a crucial role in PMSI estimation, the decomposition process and recreating the events that lead to the death of a victim through a phenomenon known as livor mortis (Figure 4.8). The gravitational pooling of blood develops in the lower dependent areas both externally in the skin capillaries and venules and the internal organs resulting in the red-blue-purple discolouration of the skin after death (Hayman & Oxenham, 2016; Simmons & Cross, 2013). The mechanism of livor mortis is complex and usually occurs within 30 minutes to 3 hours after death (Byard, 2020).

Early livor mortis is pink-red due to oxygenated red blood cells; it becomes blue-purple over time as oxygen levels reduce (Tsokos & Byard, 2016). In cyanide poisoning and hypothermia, the colour is light red-pink, while in carbon monoxide, it is a “cherry pink/red” discolouration due to the formation of carboxyhaemoglobin (Bright *et al*., 2014). Livor mortis may be green with putrefactive alterations because of the accumulation of sulfhaemoglobin or brown in cases of nitrate or nitrite poisoning caused by the production of methaemoglobin (Byard, 2020; Prahlow & Byard, 2012). If the victim was anaemic or had substantial blood loss before death, livor mortis might be reduced or non-existent (Byard, 2020; Tsokos & Byard, 2016). These skin colour diagnoses are more challenging to see in dark-skinned individuals and can lead to misdiagnosis; in people with a dark complexion or those who are heavily sun-tanned, it may be very challenging to distinguish livor mortis (Gelderman *et al*., 2018).

In addition, some physical decomposition changes noted in the Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS are described as the discolouration of the skin of different body parts; these colours include pink, red, green, black, grey, yellow, purple, and white. Depending on whether decomposition

is slower or faster, the skin can become a lighter shade of grey or darker, respectively; this could lead to difficulty in assessing the discolouration changes described in these DSS and the misidentification of bodies when using facial recognition techniques or algorithms (Cornett *et al*., 2019; Morgan *et al*., 2006).



(a) Carbon monoxide poisoning: "cherry-red" livor mortis discolouration.



(b) Nitrate poisoning: brown livor mortis discolouration.



(c) Carcinoma of the rectum: sparse lividity due to fatal anal blood loss.

Figure 4.8: Examples of various livor mortis colours (Tsokos & Byard, 2016).

4.3 Lack of Representation of Skin Tones

The diversity of the skin tone of the human population has been misrepresented in the investigative methods and techniques of many scientific fields (Louie & Wilkes, 2018). In medicine, there is an underrepresentation of the skin tone of "*people of colour*" in lectures or clinical notes, case studies and textbooks (Karnieli-Miller *et al*., 2011). For example, broad ethnic classifications like "Black" fall short of capturing socially significant variations in racial appearances that can significantly impact medical or social outcomes (Hannon & DeFina, 2016). To investigate the depiction of skin tone (coded as light, medium, and dark) in medical books, Louie & Wilkes (2018) conducted a study in which they analysed 4,146 images from four anatomy textbooks. To assess skin tone, they used the Massey & Martin (2003) colour scale comprising ten hands with colour scores ranging from 1 to 10, with each score increasing in skin tone darkness (Figure 4.9).

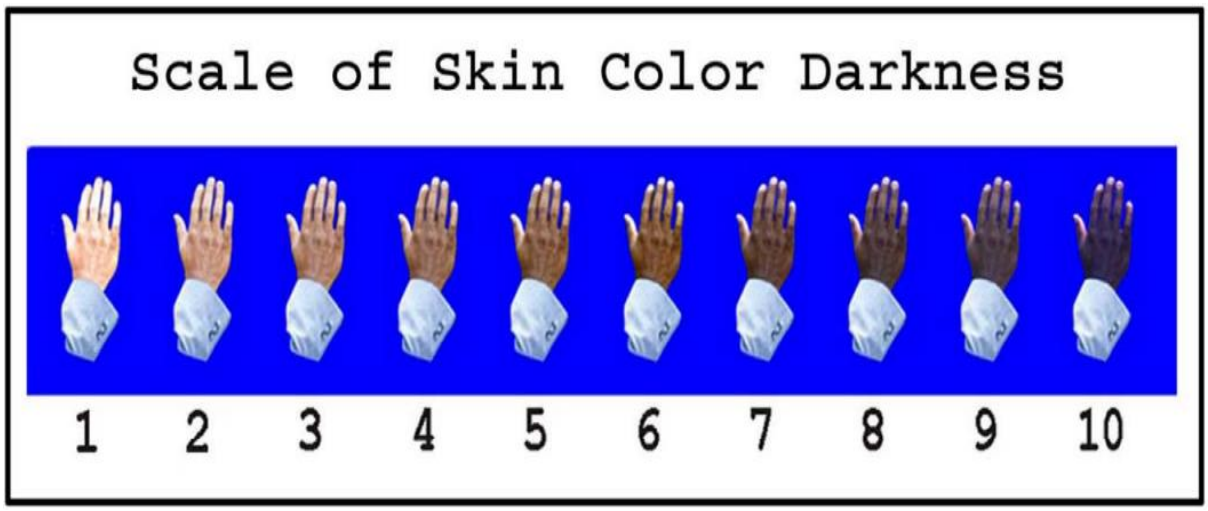


Figure 4.9: The Massey-Martin (2003) skin colour guide (Louie & Wilkes, 2018).

Their research revealed that the skin tones represented in the photos were 4.5% dark, 21% medium and 74.5% light. This underrepresentation of dark skin has led to a lesser diagnosis in cases related to skin symptoms; despite presenting similar symptoms, children with darker skin receive more deficient bacterial infection diagnoses compared to white children (Mangione-Smith *et al*., 2004). Another example of misrepresentation of the diversity of skin tone in diagnostic tools is the subjective FST (Sitek *et al*., 2016). It was created to gauge the response of a person's skin tone to sun exposure or ultraviolet radiation in terms of the degree of burning and tanning and was shown to correspond strongly to "white" skin compared to "brown" skin (Sachdeva, 2009).

Pulse oximeters are essential devices that measure arterial blood oxygen saturation and help in the adjustment of oxygen levels administered to patients (Dyer, 2020; Sjoding *et al*., 2020). These devices depend on the amount of red and infrared light transmitted through the skin, but the measurements should be independent of skin pigmentation (Bickler *et al*., 2005). Recent research suggests that the diagnosis of occult hypoxaemia (arterial oxygen saturation of < 88%) is missed about three times in

black patients compared to white patients (Dyer, 2020). The Sjoding *et al*. (2020) study recorded over 48,000 pulse oximeter readings in 8,675 white and 1,326 black patients. These readings were compared with more accurate arterial oxygen saturation in arterial blood gas that was taken almost ten minutes apart. The results showed that in black patients whose pulse oximeter readings were 92 – 96%, the percentage that had arterial oxygen saturation levels below 88% was 11.4%, while that of white patients was 3.6% (Dyer, 2020; Sjoding *et al*., 2020). These results suggest that black patients are at an increased risk of hypoxaemia (Sjoding *et al*., 2020); therefore, medical assumptions or observations derived from primarily white patient populations may be detrimental to black patients (Noor, 2020).

In forensic science, there is also a lack of consideration of darker skin tones in decomposition scoring systems (DSS) descriptions. The commonly used DSS were created in Europe and North America; in these studies, there is no mention of the ethnic ancestry or skin colour of the human subjects used (Heaton *et al*., 2010; Megyesi *et al*., 2005; van Daalen *et al*., 2017). Due to this, problems may arise when observing discolouration changes in bodies with darker skin tones; in the Gelderman *et al*. (2018) study, it was challenging to identify the presence of livor mortis in darker skin tone bodies. Depending on whether decomposition is slower or faster, the skin can become a lighter shade of grey or darker, respectively; this could lead to difficulty in assessing the discolouration changes described in these DSS and the misidentification of bodies when using facial recognition techniques or algorithms (Cornett *et al*., 2019; Morgan *et al*., 2006).

Also, most decomposition studies have used pigs and other human analogues; therefore, human skin tone's effect on measuring decomposition's extent has not yet been a pressing limitation. Keyes *et al*. (2016) suggested that more decomposition studies should be carried out with human cadavers in regions like Africa. By carrying out these studies in Africa and other areas that predominantly have people with darker skin tones, it will help to improve the suitability of these DSS for bodies with darker skin tones and improve the knowledge of decomposition in such regions.

5 PART ONE: LABORATORY STUDIES ON WATER CONDITIONS

5.1 Introduction

Various factors affect the rate of aquatic decomposition, including temperature, depth of immersion, adipocere, salinity, tidal fluctuations, and water currents (Humphreys *et al*., 2013; Reijnen *et al*., 2018; Saukko & Knight, 2015). Therefore, more studies are being conducted to investigate these factors affecting aquatic decomposition and improve PMSI estimation (Byard, 2018; Mateus & Vieira, 2014; Reijnen *et al*., 2018; van Daalen *et al*., 2017).

The effect of water salinity on aquatic decomposition rate has been discussed in various studies; it is generally believed that decomposition is slower in high-salt content water bodies [sea, lakes and tidepools] compared to low-salt content ones [estuaries, canals, and rivers] (Boyle *et al*., 1997; Reijnen *et al*., 2018). Despite this hypothesis, most decomposition studies on salinity and aquatic decomposition have only been conducted on human analogues deposited in freshwater from a lake (Humphreys *et al*., 2012) or drowning cases from seas (Mateus & Vieira, 2014; van Daalen *et al*., 2017) and rivers (Byard, 2018; Heaton *et al*., 2010; Palazzo *et al*., 2020; Reijnen *et al*., 2018). More studies need to be carried out with different water salinity types, not only freshwater and seawater.

The hardness of water is commonly measured by the high concentration of minerals in the water, primarily magnesium and calcium ions, but iron, strontium, aluminium, barium, zinc, and manganese ions also contribute to water hardness (WHO, 2011). Hardness is expressed as units of mg/L of calcium carbonate; soft water is considered to have below 60 mg/L of $CaCO_3$, moderately hard, 60–120 mg/L, while hard and very hard waters are 120–180 mg/L and more than 180 mg/L respectively (McGowan & Harrison, 2000). In a forensic context, little or no research has been done on water hardness and its effect on decomposition, but there are some promising aspects of the phenomenon of water hardness effects on the human body (Sengupta, 2013).

Cations that cause the hardness of water tend to react with some anions in the water to form stable salts (Sengupta, 2013). There is a probability that these salts slow down the decomposition rate in a body because bacterial activities become immensely reduced in the presence of high salt concentrations (De Donno *et al*., 2014). Calcium is an essential mineral for the body and is known to help keep bones and teeth healthy (Aptel *et al*., 1999; WHO, 2011). Adequate calcium intake and high-calcium water increases bone mass and reduce fracture risk in humans (Chandra *et al*., 2012; Costi *et al*., 1999). Based on these effects of calcium on the bones and teeth, there is a possibility that bones and teeth deposited in hard water might appear more preserved when compared to those deposited in soft water or low calcium-containing water.

When hard water is placed inside a container, it produces a distinguishable accumulation of "precipitate" (WHO, 2011). Consequently, studies have suggested that skin barrier dysfunction and atopic eczema

can occur when the skin is exposed to hard water (Jabbar-Lopez *et al*., 2021). Therefore, the possibility of skin barrier dysfunction and precipitate formation on the body surface could be confused for physical decomposition changes. Thus, difficulties could arise when estimating the PMSI of bodies deposited in hard water, especially in proximity to sedimentary rocks, from which seepage and runoff of calcium ions can occur.

Factors like current, turbulence and tide mainly affect the degree of water flow in aquatic ecosystems, which can significantly affect the decomposition rate (Haglund & Sorg, 2002). A body deposited in water may be dragged for a distance, creating disarticulation and marks or discolouration that can be mistaken for decomposition changes or injuries, while a body that is immobile in water may be more preserved due to less impact from other objects (Humphreys *et al*., 2013; Palmer, 2020; Stuart & Ueland, 2017). These occurrences lead to the exposure and damage of soft tissues, causing an accelerated decomposition rate (Heaton *et al*., 2010; Teather, 2013). Also, a fast water flow could lead to the removal of decomposition by-products from the surrounding environment of the body, thus affecting microbial activity (Haglund & Sorg, 2002). These effects of current on the decomposition rate and process make water current a crucial factor for accurate PMSI estimation.

In forensic science, there is a lack of consideration of the various darker skin tones in the descriptions of decomposition scoring systems (DSS) used for aquatic decomposition. The commonly used DSS were created in Europe and North America; in these studies, there is no mention of the ethnic ancestry or skin colour of the human subjects used (Heaton *et al*., 2010; Megyesi *et al*., 2005; van Daalen *et al*., 2017). This suggests that these existing DSS are primarily limited because they have been developed from studies in countries with comparable climates and waterways and individuals with predominantly pale skin. This leaves a gap in knowledge and uncertainty on whether these scoring methods could be helpful in cases of drowning or body deposition in water environments in regions such as Africa, Asia, the Caribbean, or with darker skin-toned individuals.

These gaps in knowledge on the effect of salinity, hardness, current and skin tone on decomposition rate made it essential to carry out laboratory studies in this research to have a broader understanding of the roles and effects these conditions have on the process of aquatic decomposition. The laboratory studies investigated the effect of salinity, hardness, current and skin tone on the decomposition rate of a human analogue (mice).

5.1.1 Aims

The aims for each water condition studied are listed below; the general aims cut across all the laboratory studies.

5.1.1.1 *General Aims*

1. To investigate the suitability (ease of scoring and observing the descriptive changes noted in the DSS for the various body regions and stages of decomposition) of the adapted Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS for assessing the extent of decomposition in water in a human analogue (mice).

2. To investigate if there is a statistically significant difference in the TADS of the mice when comparing the Heaton and van Daalen DSS.

H_1 = There is a significant difference in the TADS of the mice when comparing the Heaton and van Daalen DSS.

H_0 = There is no significant difference in the TADS of the mice when comparing the Heaton and van Daalen DSS.

5.1.1.2 *Salinity*

To investigate if there is a statistically significant difference between the extent of decomposition (TADS) of mice submerged in various concentrations of water salinity.

H_1 = There is a significant difference in the decomposition rate of mice submerged in fresh, brackish, hypersaline and seawater.

H_0 = There is no significant difference in the decomposition rate of mice submerged in fresh, brackish, hypersaline and seawater.

5.1.1.3 *Hardness*

To investigate if there is a statistically significant difference between the extent of decomposition (TADS) of mice submerged in various concentrations of water hardness.

H_1 = There is a significant difference in the decomposition rate of mice submerged in soft, hard, moderately hard, and very hard water.

H_0 = There is no significant difference in the decomposition rate of mice submerged in soft, hard, moderately hard, and very hard water.

5.1.1.4 *Current*

To investigate if there is a statistically significant difference between the extent of decomposition (TADS) of mice that are completely submerged and immobile in water and mice that are:

(1) Moving under the influence of a turbine

(2) Freely floating.

H_1 = There is a significant difference in the decomposition rate of mice exposed to water current, those left to float freely and those completely immobilised and submerged.
H_0 = There is no significant difference in the decomposition rate of mice exposed to water current, those left to float freely and those completely immobilised and submerged.

5.1.1.5 *Skin/Fur tone*

1. To investigate if there is a statistically significant difference between the decomposition rate of the dark and pale-furred mice.

H_1 = There is a significant difference between the decomposition rate (TADS) of the dark and pale-furred mice.

H_0 = There is no significant difference between the decomposition rate (TADS) of the dark and pale-furred mice.

2. Investigate the effect of water salinity concentration on the decomposition rate of these mice.

H_1 = There is a significant difference between the decomposition rate (TADS) of the mice deposited in the fresh and seawater types.

H_0 = There is no significant difference between the decomposition rate (TADS) of the mice deposited in the fresh and seawater types.

5.1.2 Objectives

1. To carry out laboratory studies using mice to investigate the effect of varying levels of the following water conditions a) water type, b) temperature, c) salinity, d) hardness, e) current on the rate of aquatic decomposition of mice left to decompose for 35 days. By carrying out these experiments and investigating the impact of each water condition, this study aimed to improve and validate the current DSS and have an important implication in understanding the gap in knowledge of the effect of these different water conditions on the decomposition process and PMSI estimation.

2. To carry out a laboratory study, using dark and pale-furred mice as human analogues to investigate the suitability of the adapted Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS for assessing the extent of decomposition in dark and pale furred mice deposited in fresh and seawater for 35 days. By carrying out this experiment, this research aimed to have an important implication in improving the suitability of these adapted DSS for scoring the extent of decomposition in darker skin-toned individuals.

5.2 Methodology

These laboratory studies were broken down into five parts that investigated different water conditions: *Pilot Study, Laboratory Salinity Study, Laboratory Hardness Study, Laboratory Current Study* and *Laboratory Skin Tone Study*. A total of 660 dead, previously frozen but defrosted mice (*Mus musculus*) [22.10 g – 32.40 g, mean = 27.25 g] were used for all the laboratory studies. The mice were purchased (already dead and frozen) from the Northampton Reptile Centre and Kiezebrink U.K. Ltd; they were not killed for the purpose of this study. Mice were chosen as the human analogue for these laboratory studies because of their small size, they are easy to transport, store, and maintain and require less space for conducting research; hence, they can be used for replicate studies on a large scale. Also, over the years mice have been the preferred animal species model for biomedical, scientific, and other clinical research because of their anatomical, physiological, fast reproductive cycles, short lifespan, and their genetic similarity to humans (Bryda, 2013). It is important to note that when words such as "seawater" and "brackish water" are mentioned, they denote simulated seawater and brackish water not actual seawater or brackish water that contain other microorganisms in the water.

5.2.1 Pilot Study

The pilot study[2] comprised two parts. The first part was to test if the Heaton and van Daalen DSS could be adapted (a-TADS [See Table 5.7-5.8]) and be suitable for assessing the extent of decomposition in human analogues such as mice. The second part of the study aimed to investigate the effect of varying salinity concentrations (*fresh, brackish, and hypersaline*) on the decomposition rate of 90 dead, frozen, and then thawed mice. Six mice from each group were sampled and scored with the a-TADS system at 7-day intervals of decomposition for 35 days—[See Appendix 1 for pilot study results].

5.2.2 Laboratory Salinity Study

5.2.2.1 *Group Description*

A total of 210 mice were used for the laboratory salinity studies conducted at the University of Central Lancashire. The laboratory salinity study was divided into two parts, an initial study, and a repeat study. The mice in the Initial study were divided into four groups, while the mice for the Repeat study were divided into three groups; each group was made up of 30 mice (Table 5.1). Each group was divided into three boxes (10 mice per box). 35 L boxes (Really Useful Boxes®, U.K.) were filled with 30 L of appropriately modified water and placed indoors in a laboratory setting (Figure 5.1).

The mice in the boxes were labelled with red, blue, green, yellow, and black cable ties (Gocableties®, U.K.) [Figure 5.2]. For each box, cable ties of one colour were used to label two mice that would be removed on each of the five days for sampling. A fishing weight [85 g] (Fishwithfinn®, U.K.) was attached to each mouse to keep it fully immersed in the water at a depth about three-quarters down the box (Figure 5.1–5.2).

[2] Conducted at the University of Huddersfield before transfer to the University of Central Lancashire in January 2021.

Table 5.1: Breakdown of the groups (30 mice per group) for each salinity study.

Study	Mice Used	Groups	Box Content and Description
Initial	120	Control (fresh)	Mice placed in freshwater
		Brackish	Mice placed in brackish water
		Sea	Mice placed in seawater
		Hypersaline	Mice placed in hypersaline water
Repeat	90	Fresh	Mice placed in freshwater
		Sea	Mice placed in seawater
		Hypersaline	Mice placed in hypersaline water

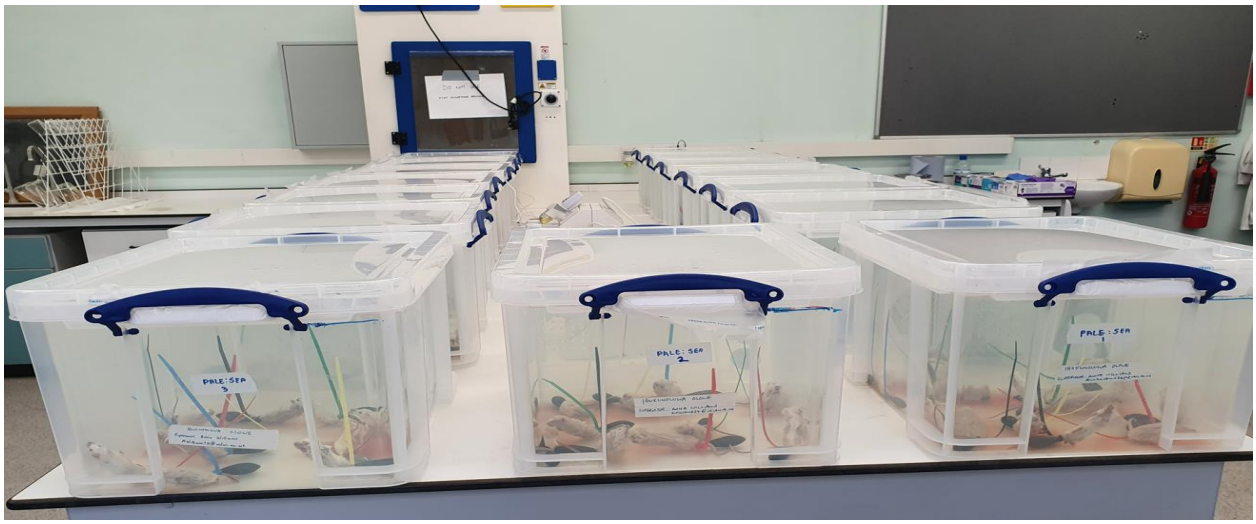


Figure 5.1: Boxes placed indoors in a laboratory setting.

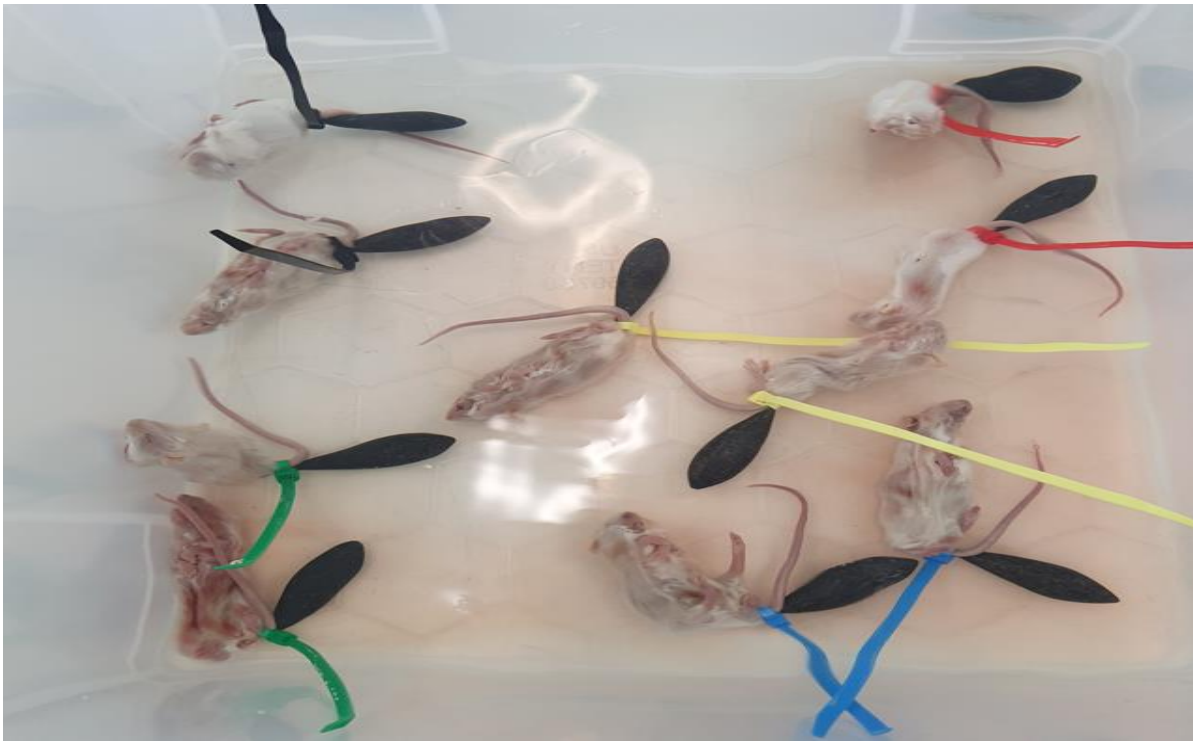


Figure 5.2: Cable ties and fishing weights attached to mice.

5.2.2.2 *Salt-Water Concentration*

Ocean Reef Pro Coral Salt (iQuatics®, U.K.) was used to create the various salinity concentrations in the groups (Table 5.2) based on the recommended standard water concentrations for salinity (Duncan Seraphin *et al*., 2014). Freshwater (Tap) was used for the Control group. Details on the water property and quality of the tap water used in this PhD research are listed in Table 5.3 below. 600 g of salt was dissolved in 30 L of water for each Brackish group, while 1.05 Kg and 1.50 Kg of salt was dissolved in 30 L of water to create the Sea and Hypersaline groups, respectively. The salt was dissolved by stirring the water in the box until there was no trace of salt particles.

Table 5.2: Concentrations of the salts in the various groups.

Groups	Concentration (ppt)
Control (Fresh)	< 1.0
Brackish	20
Sea	35
Hypersaline	50

ppt- parts per thousand.

Table 5.3: Water quality of the tap water used for the studies in this research* (*United Utilities, 2023).

Content	Level	Content	Level
Hardness	Soft	Lead	<0.550 µg Pb/l
Coliform bacteria	0/100 mL	Magnesium	4.61 mg Mg/l
Aluminium	<8.07 µg Al/L	Manganese	<0.765 µg Mn/l
Calcium	28.1 mg Ca/L	Nitrate	<1.90 mg NO3/l
Residual chlorine -Total	0.72 mg/L	Sodium	15.0mg Na/l
Residual chlorine - Free	0.66 mg/L	Turbidity	0.22
Colour	<1.80 Pt/Co scale	E.coli	0 number/100ml
Conductivity	227 uS/cm	Iron	<8.94 µg Fe/l
Copper	<0.0131 mg Cu/l		

5.2.2.3 *Temperature & ADD*

A data logger (RC-5, Elitech®) was placed in a colourless, transparent storage container [3.3 x 3.3 x 1.2"] (SATINIOR®, U.K.) and further stored in a zip-lock bag (Figure 5.3). The bag was then placed underwater in each box to record the water temperature hourly. The ADD for the sampling days was calculated by adding the average daily water temperatures for the days that made up the respective PMSI. For example, if the average temperature of the first seven days of decomposition were 10 ℃, 5 ℃, 10 ℃, 7 ℃, 10 ℃, 8 ℃, and 10 ℃, then the ADD for the PMSI of 7 days was calculated as follows: 10 + 5 + 10 + 7 + 10 + 8 + 10 = 60 ADD.



Figure 5.3: Data Logger placed in a transparent container.

5.2.2.4 *Measuring Water Properties*

Additional water properties were measured in the Repeat salinity study. The pH of the water in the boxes was measured with a digital pH meter (Eletorot®, U.K.), while the salinity and TDS concentrations were measured with a pen-type Salinity/TDS/Temperature meter (GAIN EXPRESS®, U.K.). On the sampling days, the salt content, pH level and Total Dissolved Solids (TDS) concentration of the water in each box were measured and recorded twice; each meter probe was placed in the water and measurements were taken from the left and right edges of the boxes. These water properties were measured to monitor if the decomposition process or by-products would alter their levels.

5.2.3 Laboratory Hardness Study

5.2.3.1 *Group Description*

A total of 120 mice were used for the laboratory hardness studies conducted at the University of Central Lancashire. The mice in the hardness study were divided into four groups (30 mice per group); *Soft* (<60 mg/L), *Moderately hard* (90 mg/L), *Hard* (150 mg/L), *and Very hard* (210 mg/L) groups (Table 5.4). Each group was divided into three boxes (10 mice per box). Twelve 35 L boxes (Really Useful Boxes®, U.K.) were filled with 30 L of appropriately modified water, placed indoors in a laboratory setting and set up like the boxes in the laboratory salinity studies (See Section 5.2.2.1).

Table 5.4: Breakdown of the groups (30 mice per group) for the Hardness study.

Mice Used	Groups	Box Content and Description
120	Soft	Mice placed in soft water
	Moderately hard	Mice placed in moderately hard water
	Hard	Mice placed in hard water
	Very hard	Mice placed in very hard water

5.2.3.2 *Water Hardness Concentration*

$CaCO_3$ salt and baking soda [Bicarbonate of Soda (Sainsbury's, U.K.)] were used to create the varying water hardness concentrations in the hardness groups (Table 5.5), based on the recommended standard water concentrations for salinity and hardness (USGS, n.d.). 60 g of baking soda was dissolved in 30 L of water to create the soft water group. For each Moderately-hard group, 2.7 g of salt was dissolved in 30 L of water, while 4.5 g and 6.3 g of salt were dissolved in 30 L to create the Hard and Very-hard groups, respectively (USGS, n.d.). The $CaCO_3$ and baking soda were dissolved by stirring the water in the box till there was no trace of any particles.

Table 5.5: Hardness concentrations of the various groups.

Groups	$CaCo_3$ Conc. (mg/L)
Soft	-
Moderately hard	90
Hard	150
Very hard	210

5.2.3.3 *Temperature & ADD*

Temperature data was collated the same way as the laboratory salinity study (See Section 5.2.2.3).

5.2.4 Laboratory Current Study

5.2.4.1 *Group Description*

A total of 120 mice were used for the Laboratory Current study conducted at the University of Central Lancashire. The mice were divided into four groups (30 each); *Submerged 1, Floating, Submerged 2* and *Moving* (Table 5.6). Each group was further divided into three boxes (10 mice per box). Twelve 35 L boxes (Really Useful Boxes®, U.K.) were filled with tap water and placed indoors in a laboratory setting. The mice in the boxes were labelled with red, blue, green, yellow, and black cable ties (Gocableties®, U.K.). For each box, cable ties of one colour were used to label two mice that would be removed on each of the five days for sampling. A fishing weight [85g] (Fishwithfinn®, U.K.) was attached to each mouse in the *Submerged* groups to keep it immobile and fully immersed in the water at a depth about three-quarters down the box (Figure 5.4). The mice in the *Floating* group were left to float freely naturally, and no fishing weights were attached to the mice. A turbine (Galapare®, U.K.) rotating at a speed of 200 RPM (21 ms^{-1} or 47 mph) was used to generate movement in the water boxes for the *Moving* group. No fishing weights were attached to the mice in the Moving group, and they were left to float according to the current or flow generated by the turbine. The turbine was attached to the bottom of the boxes (Figure 5.5).

Table 5.6: Breakdown of the groups (30 mice per group) for the Current study.

Mice Used	Groups	Box Content and Description
120	Submerged 1	Mice fully submerged in freshwater with fishing weights
	Floating	Mice placed in freshwater and left to float
	Submerged 2	Mice fully submerged in freshwater with fishing weights
	Moving	Mice placed in freshwater containing turbine device

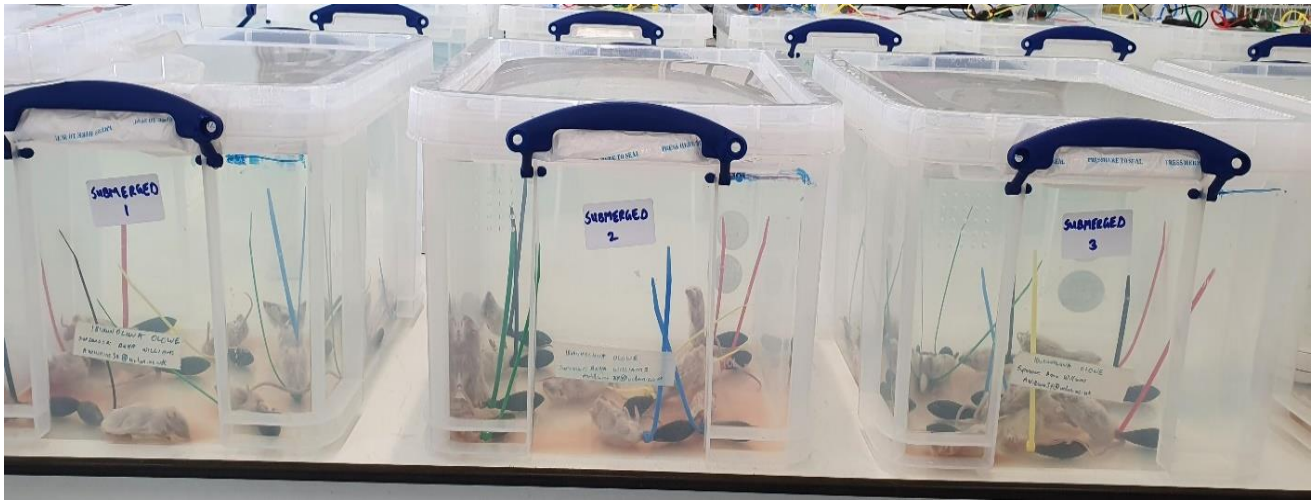


Figure 5.4: Pictures of mice completely submerged with fishing weights.

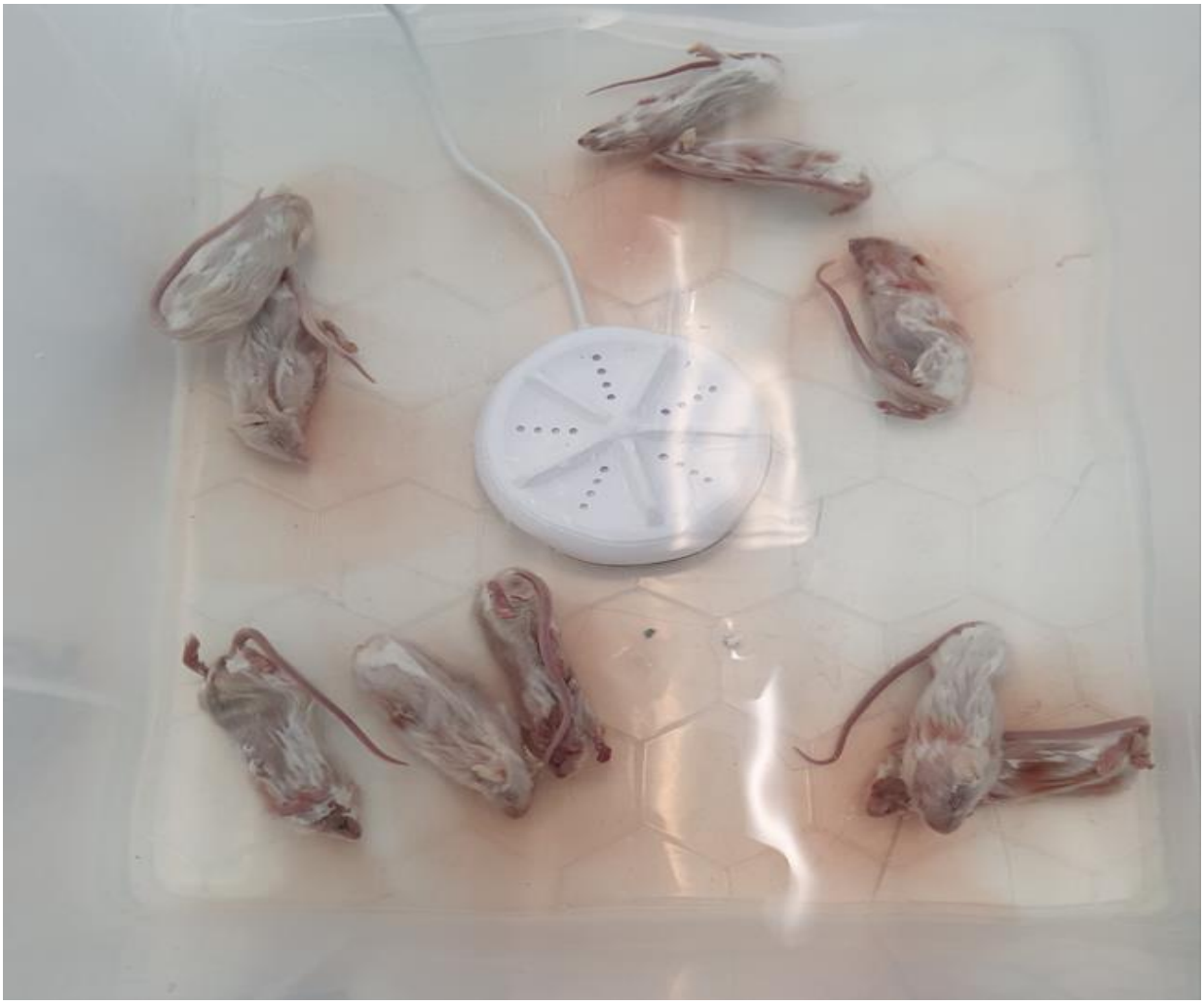


Figure 5.5: Rotating turbine at the bottom of the box.

5.2.4.2 *Temperature & ADD*

Temperature data was collated the same way as the laboratory salinity study (See Section 5.2.2.3).

5.2.4.3 *Measuring Water Properties*

The pH level, salinity, and TDS concentrations of the water in the boxes were measured the same way as the laboratory salinity study (See Section 5.2.2.4).

5.2.5 Laboratory Skin Tone Study

5.2.5.1 *Group Description*

A total of 120 mice were used for the laboratory skin tone study conducted at the University of Central Lancashire. The mice were divided into four groups (30 each) based on the colour of their fur/skin and the water type they were submerged in; *Pale: Fresh, Pale: Sea, Dark: Fresh and Dark: Sea* (Table 5.7). Each group was further divided into three boxes (10 mice per box). Twelve 35 L boxes (Really Useful Boxes®, U.K.) were filled with appropriately modified water and placed indoors in a laboratory setting.

The mice in the boxes were labelled with red, blue, green, yellow, and black cable ties (Gocableties®, U.K.). For each box, cable ties of one colour were used to label two mice that would be sampled on each of the five days of sampling. A fishing weight [85g] (Fishwithfinn®, U.K.) was attached to each mouse to keep it fully immersed in the water at a depth about three-quarters down the box (Figure 5.6). The mice in the Dark: Fresh and Dark: Sea groups were black-furred with a light brown to reddish skin tone, while the mice in the Pale: Fresh and Pale: Sea groups were white-furred with a pale skin tone (Figure 5.7).

Table 5.7: Breakdown of the groups (30 mice per group) for each water condition studied.

Mice Used	Groups	Box Content and Description
120	Pale: Fresh	Pale-furred mice placed in freshwater
	Pale: Sea	Pale-furred mice placed in seawater
	Dark: Fresh	Black-furred mice placed in freshwater
	Dark: Sea	Black-furred mice placed in seawater

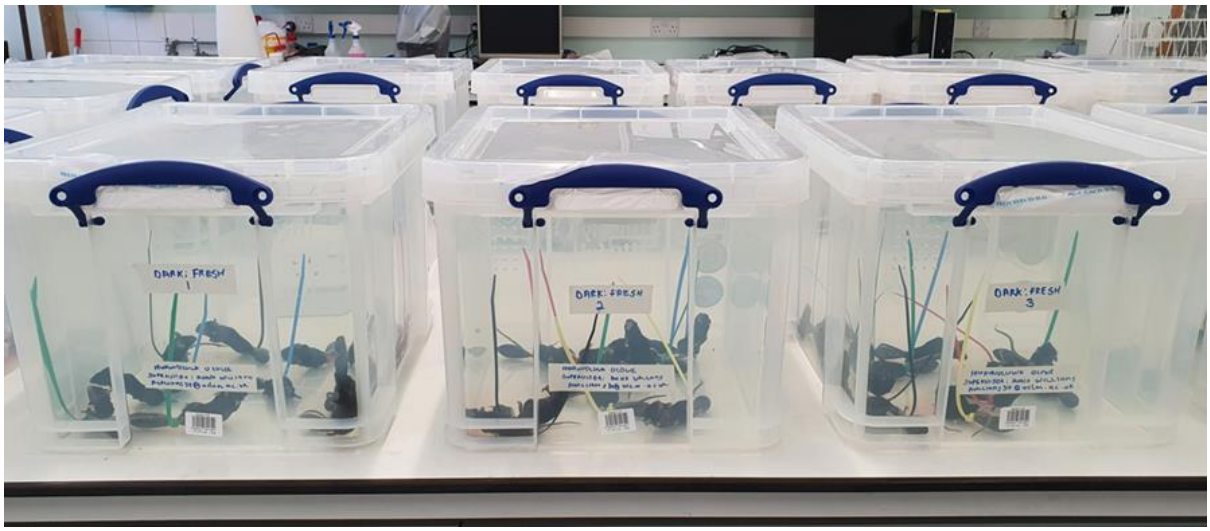


Figure 5.6: Mice immersed in the water at a depth about three-quarters down the box.



Figure 5.7: Dark and Pale mice before they were immersed in water.

5.2.5.2 *Salt-Water Concentration*

Based on the recommended standard water concentrations for salinity (Duncan Seraphin *et al*., 2014), 1.05 Kg of Ocean Reef Pro Coral Salt (iQuatics®, U.K.) was dissolved in 30 L water to create seawater (~35 ppt). Tap water was used for the freshwater (~0.5 ppt).

5.2.5.3 *Temperature & ADD*

Temperature data was collated the same way as the laboratory salinity study (See Section 5.2.2.3).

5.2.5.4 *Measuring Water Properties*

The pH level, salinity, and TDS concentrations of the water in the boxes were measured the same way as the laboratory salinity study (See Section 5.2.2.4).

5.2.6 Measuring Decomposition

The adapted Heaton and van Daalen DSS (a-TADS) for mice were used to score the extent of decomposition in all the mice used in this study. Parts of the DSS concerning changes in human hairs were adapted for animal furs. Features like hair loss, discolouration, skin wrinkling, and slippage were mainly adapted from the scoring systems. The a-TADS systems are detailed in Tables 5.8–5.9; changes made in reference to mice are in red ink. On days 7, 14, 21, 28, and 35 after submersion, two mice were removed from each box (a total of 6 mice per group), not retuned, and scored with their pictures taken.

Table 5.8: Adapted Heaton DSS.

Points	FADS Description
1	Fresh; no visible discolouration or change
2	Marbling, skin discolouration (pink/green), presence of maggots, fur, and whiskers loss
3	Skin slippage, fur/whiskers loss, advanced marbling and discolouration of face and neck, heavy maggot activity or signs of predation/insect activity
4	Bloating of the face especially around offices, advanced skin/fur slough off, drying of the nose and ears
5	Liquefaction, exposure of soft tissue, dark grey/brown/black discolouration, extensive skin slippage around eyes, neck, and head
6	Extensive soft tissue decomposition, bone exposure around eyes, head, mandible, and maxilla
7	Extensive bone exposure, skeletonization, disarticulation of mandible
8	Loss of teeth, complete disarticulation of skull from torso
Points	**BADS Description**
1	Fresh; no visible discolouration or change
2	Skin discolouration (green/pink), marbling, maggots
3	Fur loss, extensive skin discolouration (green/red/brown)
4	Bloating around genitals, skin slippage, discolouration becomes predominantly dark green/grey, maggot activity
5	Bloating – tense around genitals, skin slippage, severe fur loss, liquefaction
6	Discolouration becomes predominantly black, abdomen caves in/collapses due to massive reduction in bloat, exposure of soft tissue and internal organ decomposition
7	Advanced tissue/internal organ decomposition, bone exposure becomes more evident
8	Complete bone exposure, skeletonization and disarticulation
Points	**LADS Description**
1	Fresh; no visible discolouration or change
2	Mild wrinkling, presence of maggots, pink/white discolouration, fur loss
3	Marbling, fur loss, discolouration becomes grey/green, skin on limbs and tail still look fresh
4	Degloving/Skin slippage, bloating, brown discolouration as the skin around fingers and toes dry
5	Black discolouration, advanced skin slippage with leather appearance on skin, extreme fur loss, absence of claws
6	Exposure of soft tissue especially in extremities
7	Bone, muscle, and tendons are exposed
8	Advanced bone exposure and mostly dry
9	Complete skeletonization and disarticulation of tail

Table 5.9: Adapted van Daalen DSS.

Points	FADS Description
1	No visible changes
2	Marbling, Skin slippage and fur and whiskers slough off
3	Bloating of eyelids and/or lips
4	Gray, matte discoloration of the skin with a crumbly surface
5	Partial skeletonization
6	Complete skeletonization
Points	**BADS Description**
1	No visible changes
2	Marbling of upper trunk and/or lower trunk, Skin slippage and fur sloughs off
3	Bloating of abdomen and/or genitals
4	Gray, matte discoloration of the skin with a crumbly surface
5	Partial skeletonization
6	Complete skeletonization
Points	**LADS Description**
1	No visible changes
2	Wrinkling and/or white discoloration of the skin of hands and/or feet
3	Marbling, Skin slippage, fur sloughs off, Degloving and Absence of claws
4	Gray, matte discoloration of the skin with a crumbly surface, Partial or gross skeletonization of the distal and/or proximal parts of the limbs and tail
5	Partial skeletonization
6	Complete skeletonization

Examples of how these adapted DSS were applied in scoring the mice are illustrated in Figures 5.8 and 5.9 below.



Heaton DSS	**van Daalen DSS**
FADS- 2; Discolouration	FADS- 2; Discolouration
BADS- 4; Bloating	BADS- 3; Bloating
LADS- 2; Discolouration	LADS- 2; Discolouration
TADS = 8	**TADS = 7**

Figure 5.8: Example of how a mouse in the fresh stage of decomposition was scored.

Figure 5.8 above shows a slight pink discolouration in this mouse's head and limb regions while bloating is observed in the trunk. The Heaton and van Daalen DSS both assign a score of 2 for discolouration changes observed in these two body regions, while Heaton assigns 4 for bloating and van Daalen assigns 3. Hence the TADS of the mouse using the Heaton and van Daalen DSS equate to 8 and 7, respectively.

In Figure 5.9 below, the mouse is in an advanced state of decomposition. Bone exposure is observed in all three body regions scored. The trunk region shows advanced soft tissue and internal organ decomposition, while the head and limbs exhibit bone exposure and partial skeletonization.



Heaton DSS	van Daalen DSS
FADS- 6; Bone exposure around the mandible	FADS- 5; Bone exposure around the mandible
BADS- 7; Advanced tissue/organ decomposition	BADS- 5; Partial Skeletonization
LADS- 7; Bone and tendon exposed	LADS- 5; Partial Skeletonization
TADS = 20	**TADS = 15**

Figure 5.9: Example of how a mouse in the advanced stage of decomposition was scored.

5.2.7 Statistical analysis

All statistical analyses and plots were conducted using Microsoft Excel and SPSS software [version 28, SPSS Inc.]. ANOVA tests and Tukey's Post Hoc tests (parametric) were used when the data were normally distributed, but the Mann-Whitney U, Friedman, and Wilcoxon tests (non-parametric) were used for data that were not normally distributed (Hopkins *et al*., 2018; Derrick *et al*., 2020). Two-way ANOVA and Friedman tests were done to investigate a significant difference in the TADS between the groups of each water condition on the sampling days as a function of time (Hopkins *et al*., 2018). Post Hoc tests: Tukey's and Wilcoxon tests were conducted to figure out the TADS of which specific groups in each water study significantly differed by comparing the mean of each group with every other mean (Pereira *et al*., 2015). In contrast, the Mann-Whitney U test was used to investigate a significant difference in each mouse's TADS (Heaton vs van Daalen DSS).

5.2.8 Risk Assessment

See Appendix 9 for risk assessment form.

5.3 Results and Discussion

5.3.1 Laboratory Salinity Study

The laboratory salinity study was divided into initial and repeat studies. The initial study investigated the effect of four levels of salinity on the decomposition process: fresh, brackish, sea and hypersaline levels of salinity, while the repeat study investigated three levels of salinity: fresh, sea and hypersaline water. The initial study was conducted between May 11 – June 15, 2021, while the repeat study was conducted between June 28 – August 2, 2022.

5.3.1.1 *Initial Study*

TADS

Table 5.10 shows a breakdown of the average TADS of the mice. Across the 35 days of decomposition, the lowest mean TADS recorded was 6.67 (*n=6, S.D.=0.52*) in the Hypersaline group, and the highest recorded mean was 25.00 (*n=6, S.D.=0*) in the Sea group when using the Heaton DSS. The lowest mean TADS recorded was 7.00 (*n=6, S.D.=0*) in the Hypersaline group, and the highest recorded mean was 18.00 (*n=6, S.D.=0*) in the Sea group when using the van Daalen DSS. The Control group had the highest TAD scores, while the Hypersaline group had the lowest—[See Appendix 2.1.1 for TADS of all the mice]. Figures 5.10–5.11 are graphical representations of the TADS of mice in the groups against the PMSI on the sampling days.

Table 5.10: The average TADS of the salinity group mice on the sampling days.

Average TADS (*n* = *6*)									
DSS		**Heaton DSS** ***[max=25]***				**Van Daalen DSS** ***[max=18]***			
Groups		**Control**	**Brackish**	**Sea**	**Hypersaline**	**Control**	**Brackish**	**Sea**	**Hypersaline**
PMSI (Days)	**7**	8.67	8.33	7.67	6.67	8.17	8.00	7.67	7.00
	14	9.00	9.17	7.67	7.00	8.67	8.50	7.83	8.00
	21	13.17	10.50	9.50	9.17	11.00	10.67	9.83	9.17
	28	15.00	12.83	11.33	10.50	11.17	10.67	10.67	10.17
	35	22.67	21.83	25.00	22.83	14.83	16.33	18.00	16.67

Two-way ANOVA tests were conducted to analyse any statistical difference in the TADS of the four groups across the five PMSI intervals—[See Appendix 2.2.1 for statistics]. The ANOVA results showed that there was a statistically significant difference in the TADS across the four groups when using the Heaton DSS ($r^2 = 0.94$, $p < 0.001$), but there was no statistically significant difference in the TADS when using the van Daalen DSS ($r^2 = 0.88$, $p = 0.178$). A Tukey's Post Hoc test was done to investigate further the specific statistical difference (*p-value*) of the TADS of each group compared to the other three groups (Table 5.11).

For the Heaton DSS, there was a statistically significant difference between the TADS of the Control group and the other three groups: Brackish ($p = 0.029$), Sea ($p = 0.003$) and Hypersaline ($p < 0.001$). There was no significant difference between the TADS of the Brackish and Sea groups ($p = 0.887$), but there was a statistically significant difference between that of the Brackish and Hypersaline groups ($p = 0.012$). Finally, there was no significant difference between the TADS of the Sea and Hypersaline groups ($p = 0.08$). For the van Daalen DSS, there was no significant difference between the TADS of any of the four salinity groups.

Table 5.11: Post Hoc test results of both DSS.

Heaton		**van Daalen**	
Fresh *vs*	Brackish ($p = 0.029$)*	Fresh *vs*	Brackish ($p = 0.997$)
	Sea ($p = 0.003$)*		Sea ($p = 1.000$)
	Hypersaline ($p < 0.001$)*		Hypersaline ($p = 0.318$)
Brackish *vs*	Sea ($p = 0.887$)	Brackish *vs*	Sea ($p = 1.000$)
	Hypersaline ($p = 0.012$)*		Hypersaline ($p = 0.225$)
Sea *vs* Hypersaline ($p = 0.080$)		Sea *vs* Hypersaline ($p = 0.269$)	

- a difference significant to the 5% level.

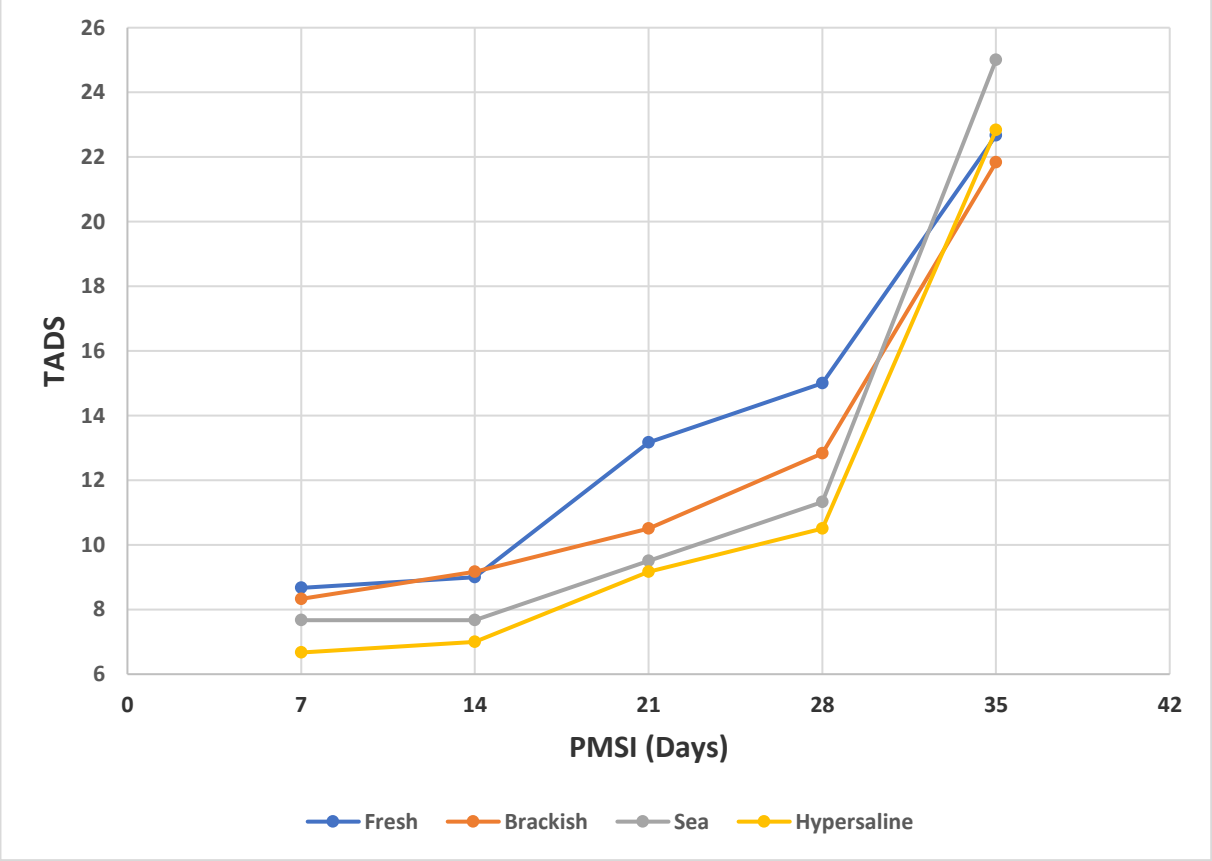


Figure 5.10: Graph of the Average TADS (using Heaton DSS) of the mice against the PMSI of each group.

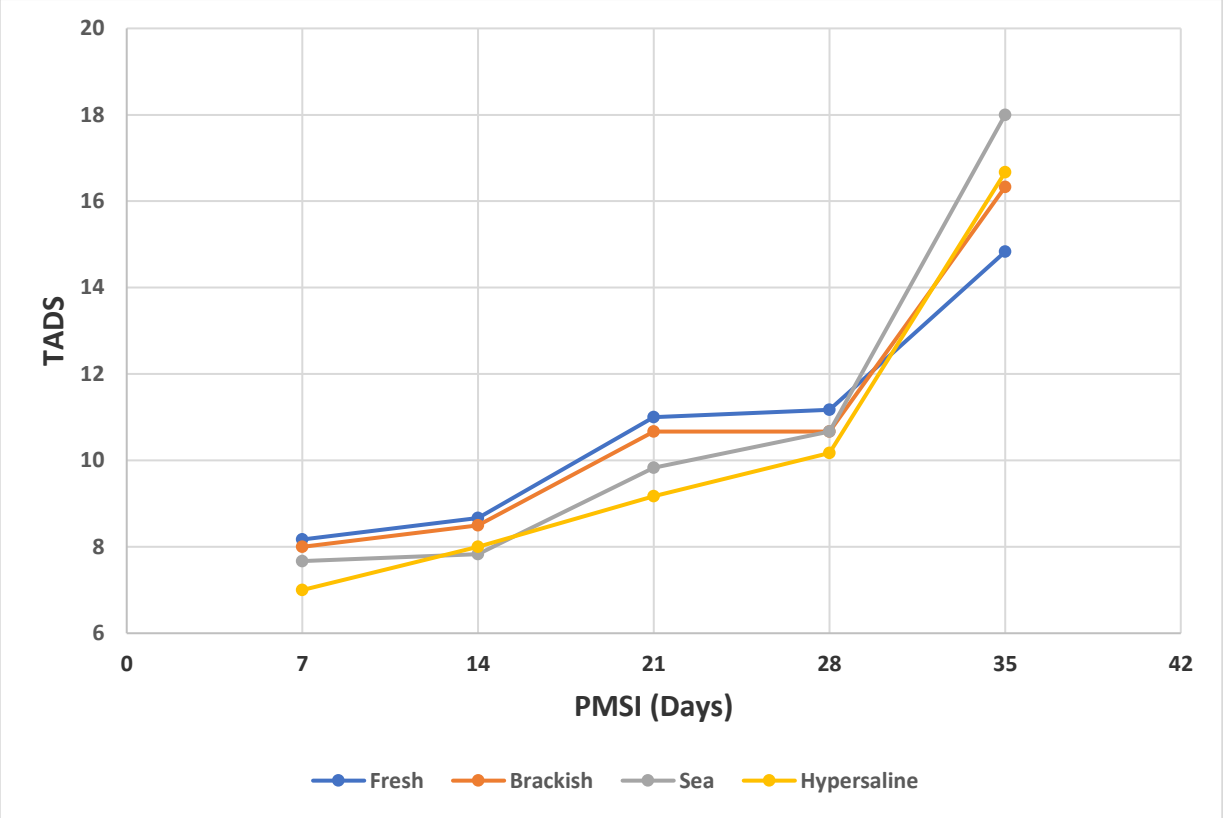


Figure 5.11: Graph of the Average TADS (using van Daalen DSS) of the mice against the PMSI of each group.

DSS Comparison

A Mann-Whitney U test was needed to compare the TADS of the two DSS statistically, but this would not have been possible because they had different maximum scores (Heaton maximum score = 25; van Daalen maximum score = 18). Therefore, Heaton TADS were divided by 25 and the van Daalen by 18 to produce a fraction (e.g., a score of 9/18 = 0.50) to normalise both scores to a ratio of 1. Thus, they both had a maximum score of 1, and the Mann-Whitney U test was used to analyse if there was any significant difference in the TADS obtained when using the Heaton and van Daalen DSS to assess the extent of decomposition in each group. The test results indicate that there was no statistically significant difference between the two DSS for the Fresh group, but there was a statistically significant difference between the two DSS for the other three groups (Table 5.12).

Table 5.12: Mann-Whitney U test results.

Groups	***p-value***
Fresh	0.132
Brackish	0.003*
Sea	< 0.001*
Hypersaline	< 0.001*

****- a difference significant to the 1% level***

Temperature & ADD

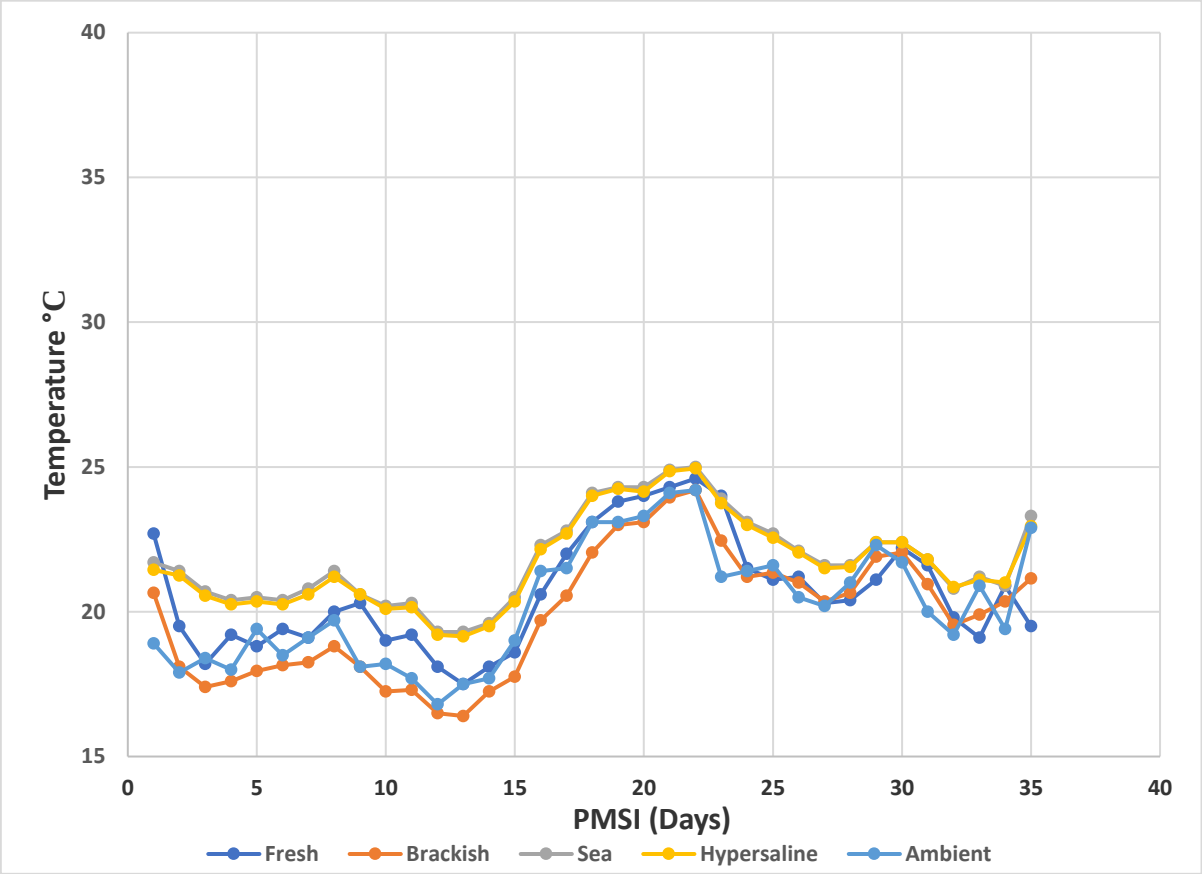


Figure 5.12: Graph showing temperature fluctuation in the water groups across the 35 days.

Figure 5.12 shows the average daily temperatures of the water in each group on the 35 days of decomposition—[See Appendix 2.3.1 for the daily temperature values of each group]. The average daily water temperatures of the boxes in each group were summed up to calculate the ADD values (Table 5.13). The lowest ADD was 128.1 in the Brackish group, and the highest was 762.6 in the Sea group. Figures 5.13–5.14 are graphical representations of the TADS of the mice in the groups against the average Log ADD on the sampling days.

Table 5.13: ADD and Log ADD of the salinity groups across the 35 days.

PMSI (Days)	Control		Brackish		Sea		Hypersaline	
	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD
7	135.8	2.13	128.1	2.11	145.9	2.16	144.7	2.16
14	266.0	2.42	249.7	2.40	286.6	2.46	284.6	2.45
21	424.1	2.63	399.8	2.60	449.8	2.65	447.1	2.65
28	576.1	2.76	551.0	2.74	609.8	2.79	606.4	2.78
35	722.2	2.86	696.9	2.84	762.6	2.88	758.9	2.88

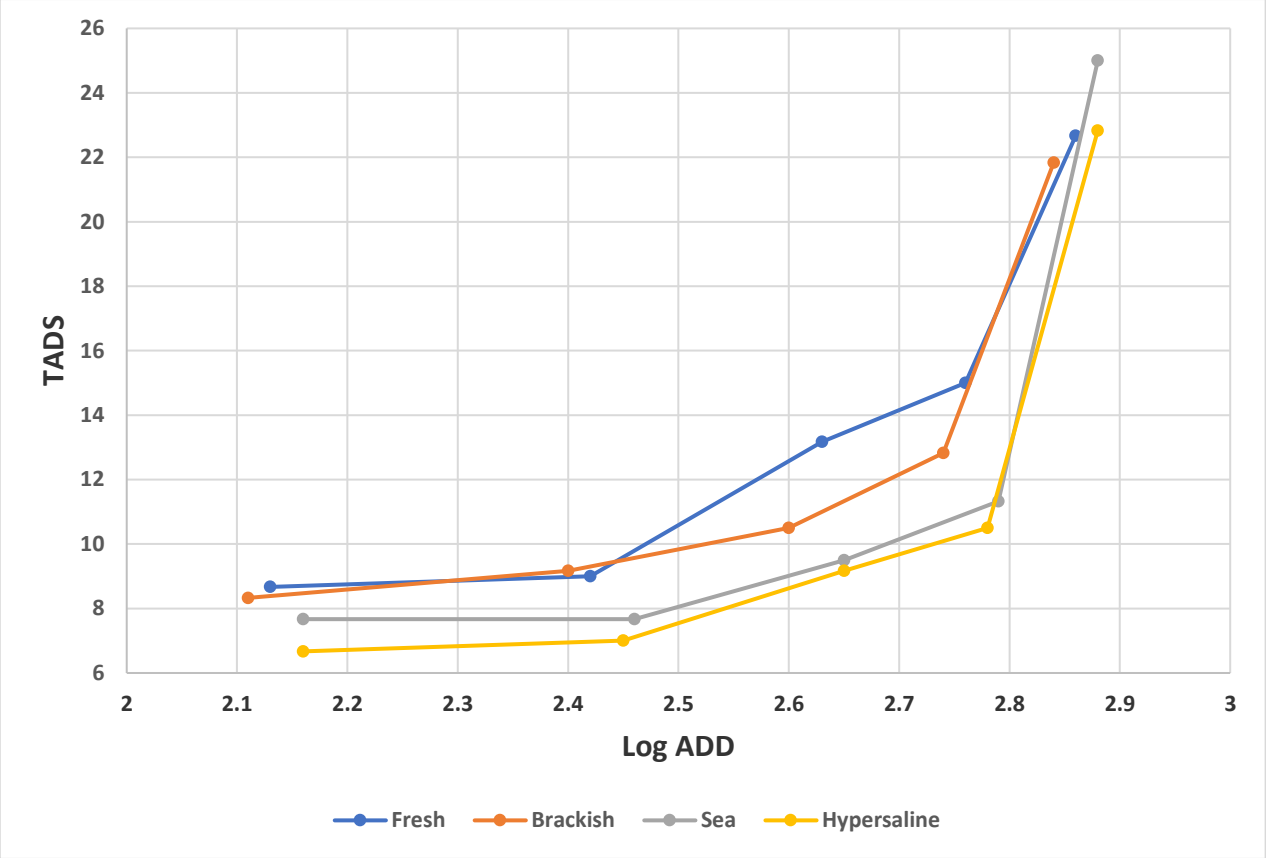


Figure 5.13: Graph of the Average TADS (using Heaton DSS) of the mice against the Log ADD of each group.

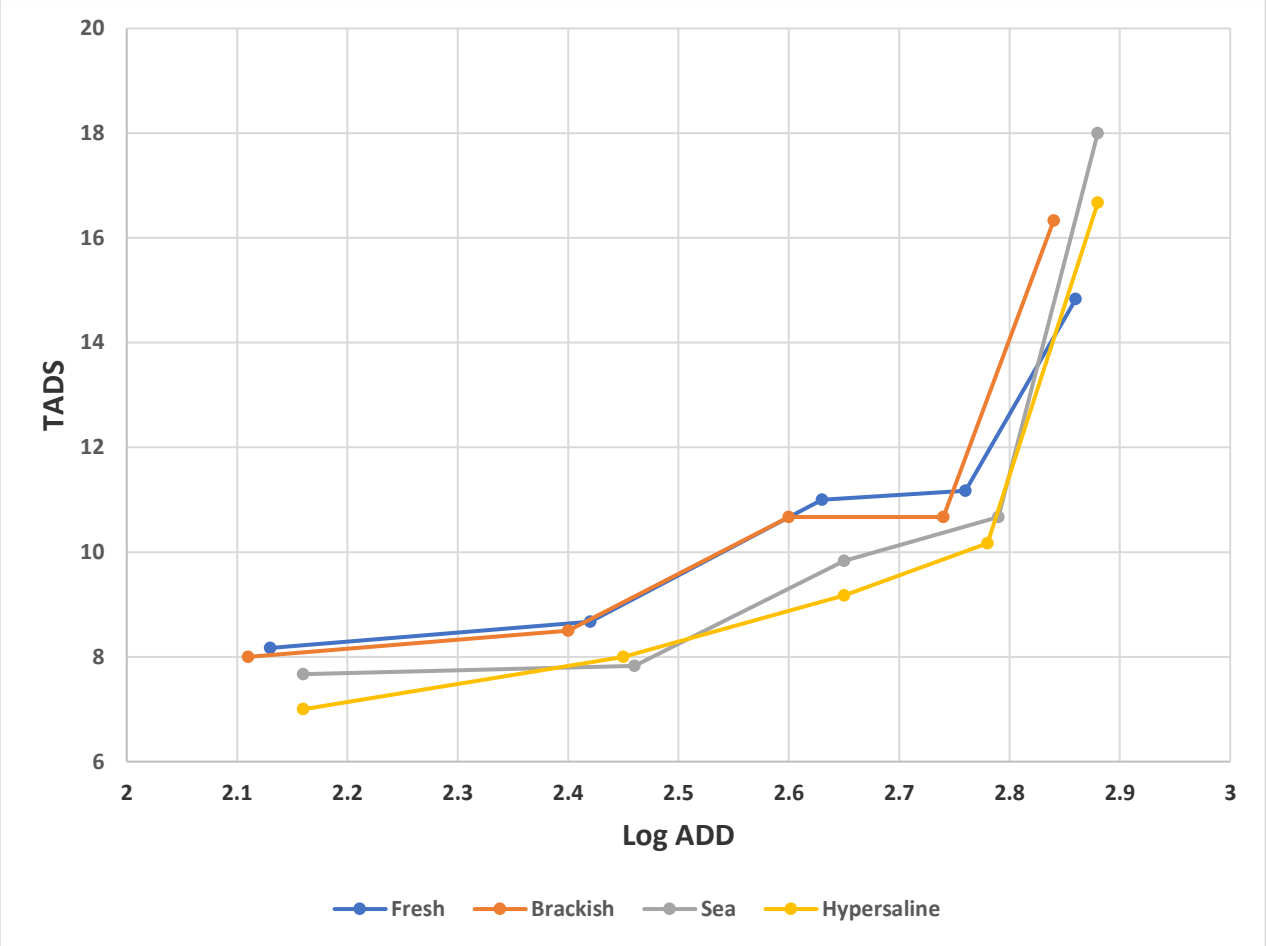


Figure 5.14: Graph of the Average TADS (using van Daalen DSS) of the mice against the Log ADD of each group.

Day	7	14	21	28	35
Fresh					
Brackish					



Figure 5.15: Physical decomposition changes in each salinity group across the 35 days.

5.3.1.2 *Repeat Study*

TADS

Table 5.14 shows a breakdown of the average TADS of the mice. Across the 35 days of decomposition, the lowest mean TADS recorded was 7.00 (*n=6, S.D.=0*) in the Fresh and Hypersaline group, and the highest recorded mean was 24.50 (*n=6, S.D.=0.84*) in the Fresh group, when using the Heaton DSS. The lowest mean TADS recorded was 6.66 (*n=6, S.D.=0.52*) in the Fresh and Hypersaline group, and the highest recorded mean was 17.50 (*n=6, S.D.=0.84*) in the Fresh group when using the van Daalen DSS. The fresh group had the highest TAD scores, while the Hypersaline group had the lowest—[See Appendix 2.1.2 for TADS of all the mice]. Figures 5.16–5.17 are graphical representations of the TADS of mice in the groups against the PMSI on the sampling days.

Table 5.14: Average TADS of the mice in each salinity group on the sampling days.

Average TADS (*n = 6*)							
DSS		**Heaton DSS** ***[max=25]***			**Van Daalen DSS** ***[max=18]***		
Groups		**Fresh**	**Sea**	**Hypersaline**	**Fresh**	**Sea**	**Hypersaline**
PMSI (Days)	**7**	7.00	7.16	7.00	7.00	6.66	7.00
	14	10.50	10.66	9.16	7.80	7.33	6.16
	21	12.30	10.66	11.16	10.00	7.50	7.83
	28	20.00	17.16	12.33	14.60	12.83	8.83
	35	24.50	24.00	18.33	17.50	17.33	13.66

A two-way ANOVA test was conducted to analyse any statistical difference in the Heaton TADS of the three groups across the five PMSI intervals—[See Appendix 2.2.2 for statistics]. The ANOVA result showed that there was a statistically significant difference in the TADS across the three groups ($r^2 = 0.90$, $p < 0.001$). A Tukey's Post Hoc test was done to further investigate the specific statistical difference (*p-value*) of the TADS of each group compared to the other two groups (Table 5.15).

Table 5.15: Post Hoc results of the statistics for the Heaton DSS.

Fresh vs	Sea ($p = 0.200$) Hypersaline ($p < 0.001$)*
Sea vs Hypersaline ($p < 0.001$)*	

****- a difference significant to the 5% level***

There was no statistically significant difference between the TADS of the Fresh group and the Sea group ($p = 0.200$), but there was a statistically significant difference with the Hypersaline group ($p < 0.001$). There was a statistically significant difference between that of the Sea and Hypersaline groups ($p < 0.001$). A Friedman's test was conducted to analyse any statistical difference in the van Daalen

TADS of the three groups across the five PMSI intervals; the result indicated that there was a statistically significant difference between the three salinity groups ($p < 0.001$).

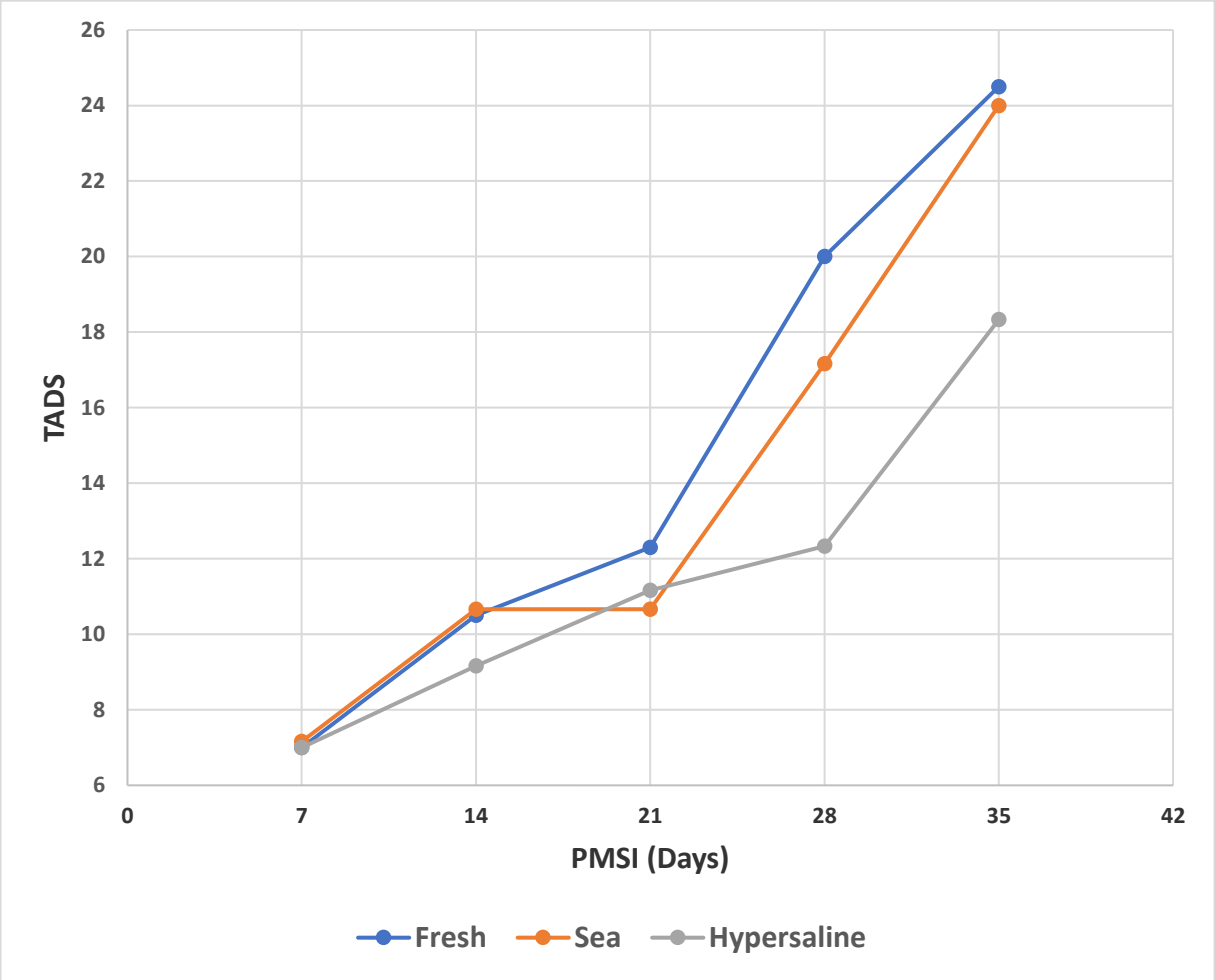


Figure 5.16: Graph of the Average TADS (using Heaton DSS) of the mice against the PMSI of each group.

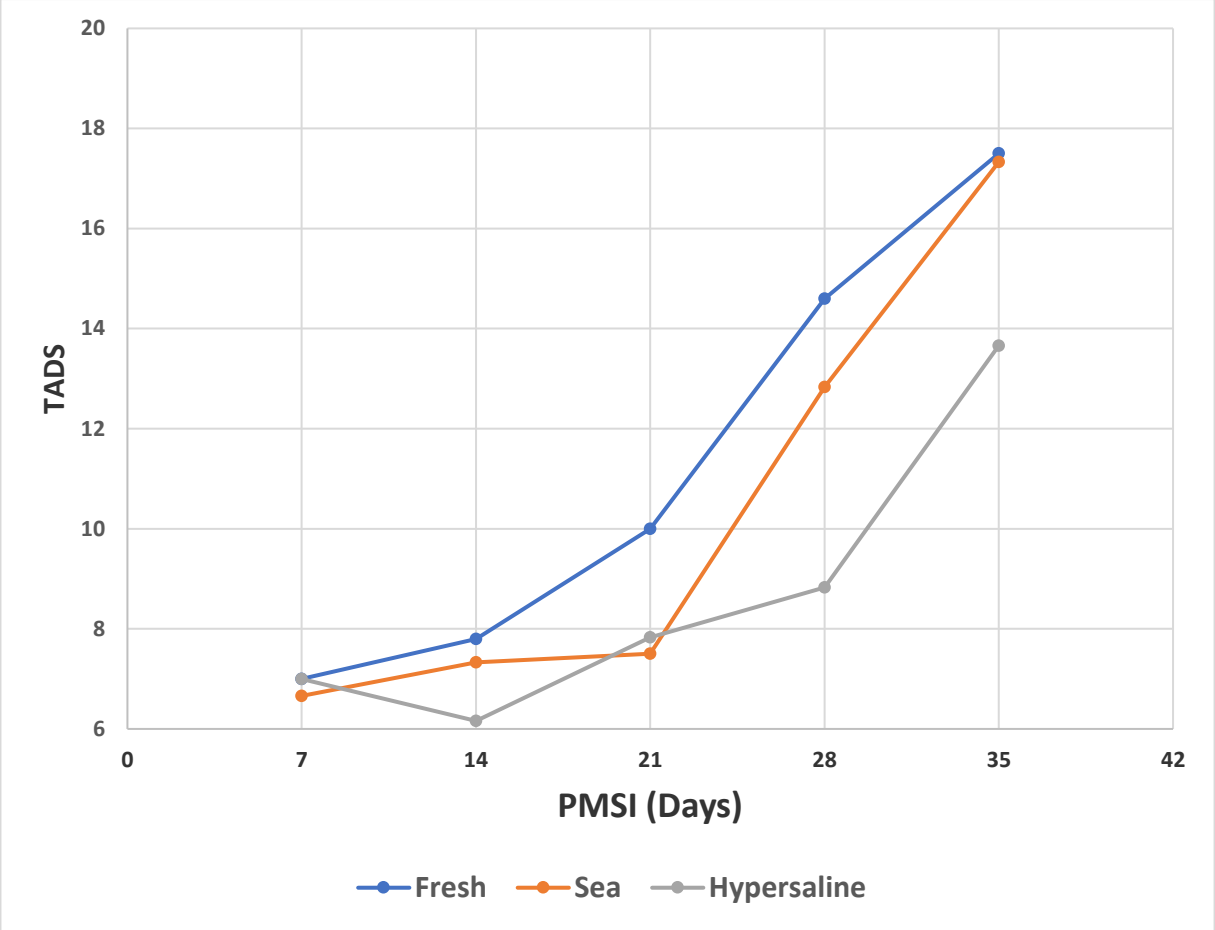


Figure 5.17: Graph of the Average TADS (using van Daalen DSS) of the mice against the PMSI of each group.

DSS Comparison

A Mann-Whitney U test was needed to compare the TADS of the two DSS statistically, but this would not have been possible because they had different maximum scores (Heaton maximum score = 25; van Daalen maximum score = 18). Therefore, Heaton TADS were divided by 25 and the van Daalen by 18 to normalise both scores to a ratio of 1. Thus, they both had a maximum score of 1, and the Mann-Whitney U test was used to analyse if there was any significant difference in the TADS obtained when using the Heaton and van Daalen DSS to assess the extent of decomposition in each group. The results of the test indicate that there was no statistically significant difference between the two DSS for the three groups (Table 5.16).

Table 5.16: Mann-Whitney U test results.

Groups	***p-value***
Fresh	0.583
Sea	0.876
Hypersaline	0.651

****- a difference significant to the 1% level***

Temperature & ADD

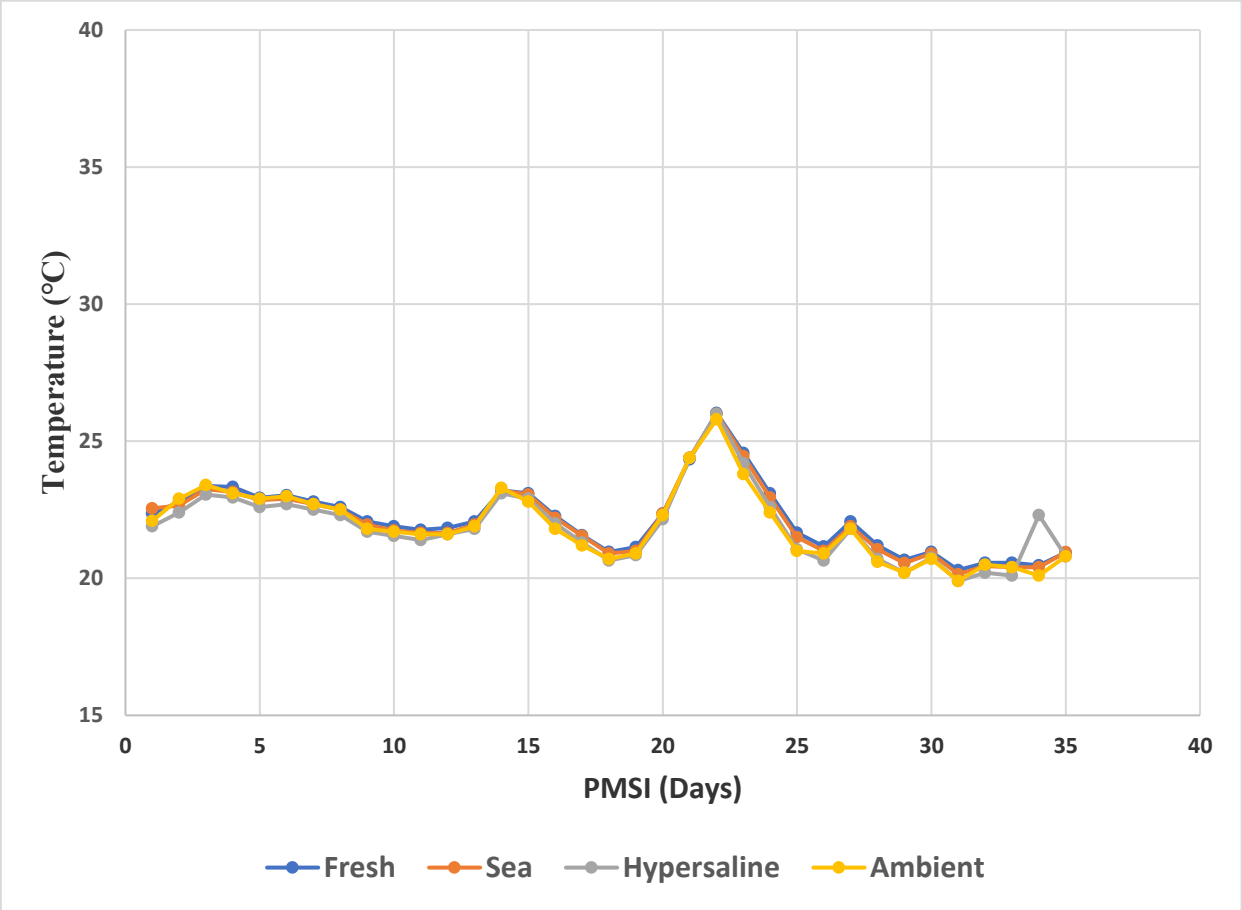


Figure 5.18: Graph showing temperature fluctuation in the water groups across the 35 days.

Figure 5.18 above shows the average daily temperatures of the water in each group on the 35 days of decomposition—[See Appendix 2.3.2 for the daily temperature values of each group]. The average daily water temperatures of the boxes in each group were summed up to calculate the ADD values (Table 5.17). The lowest ADD was 157.9 in the Hypersaline group, and the highest was 776.4 in the Sea group. Figures 5.19–5.20 are graphical representations of the TADS of the mice in the groups against the average Log ADD on the sampling days.

Table 5.17: ADD and Log ADD of the salinity groups across the 35 days.

PMSI (Days)	Fresh		Sea		Hypersaline	
	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD
7	160.8	2.21	160.1	2.20	157.9	2.20
14	316.5	2.50	315.0	2.50	311.1	2.49
21	472.4	2.67	470.5	2.67	466.3	2.67
28	632.0	2.80	629.3	2.80	620.2	2.79
35	776.4	2.89	773.1	2.89	764.5	2.88

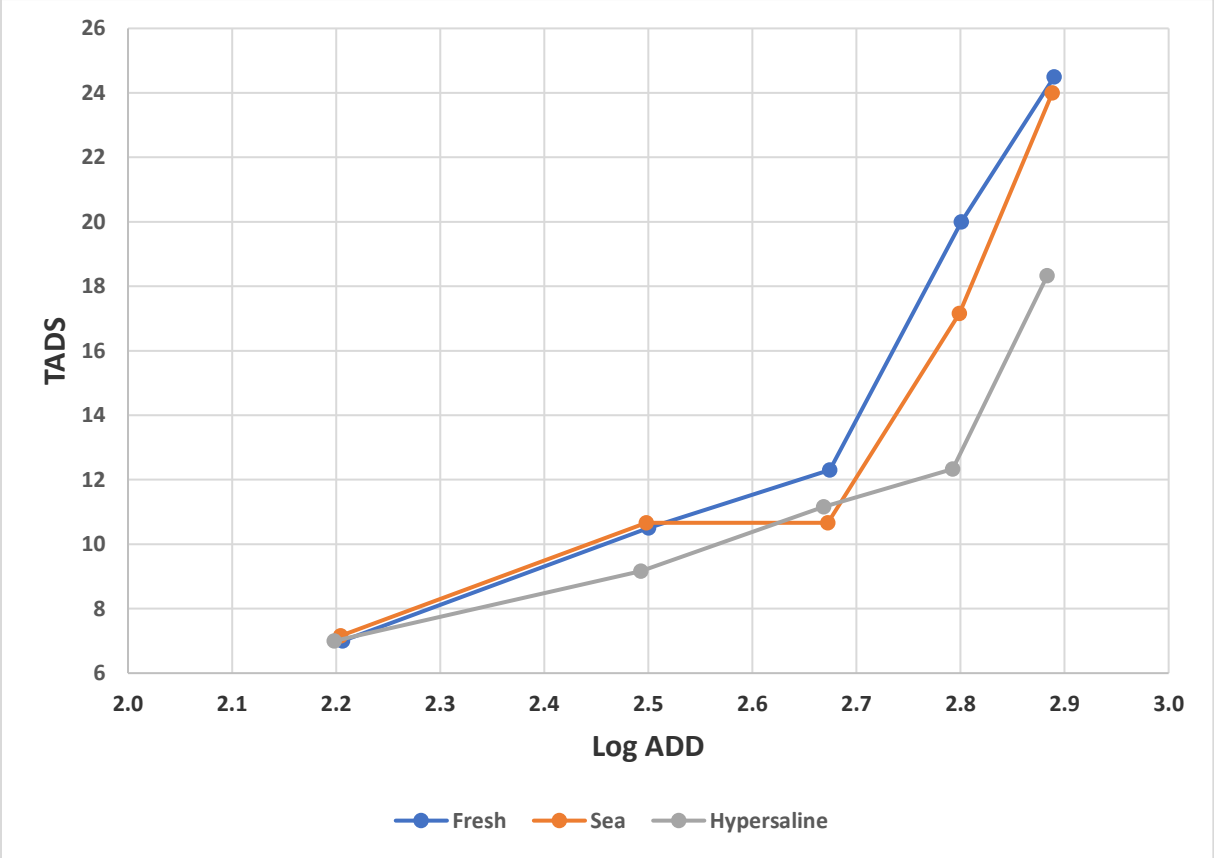


Figure 5.19: Graph of the Average TADS (using Heaton DSS) of the mice against the Log ADD of each group.

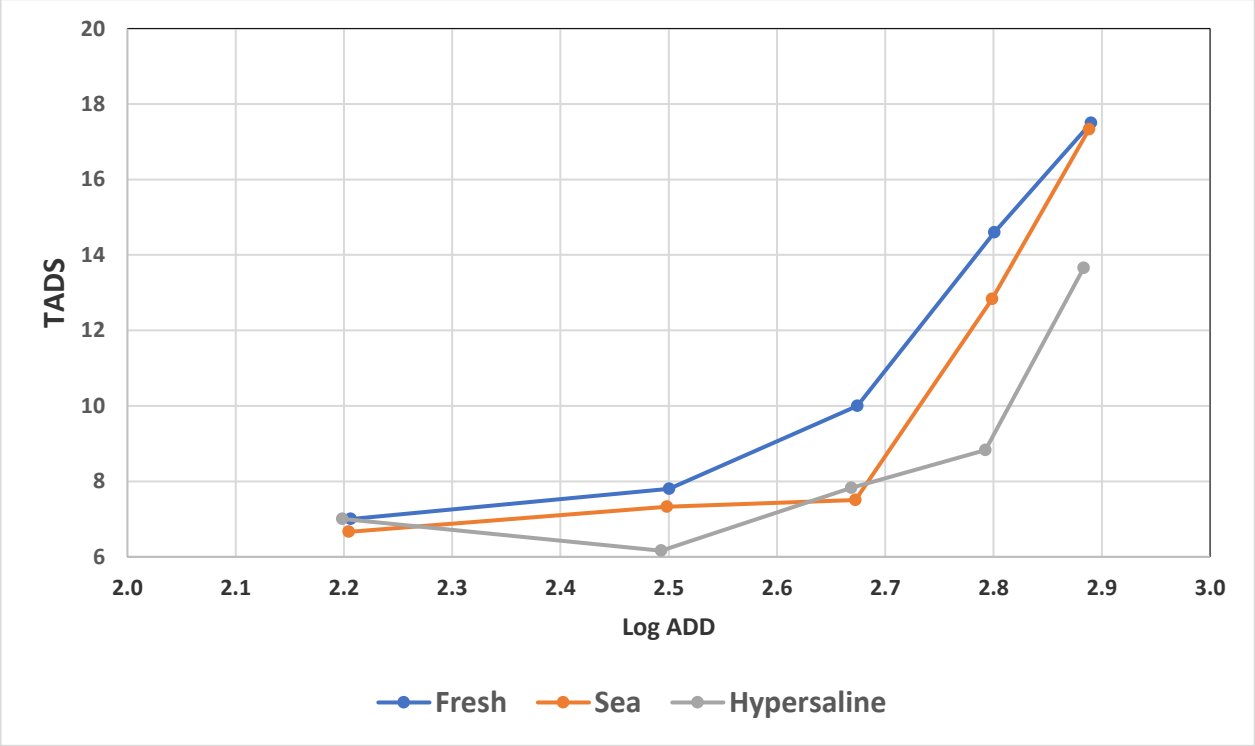


Figure 5.20: Graph of the Average TADS (using van Daalen DSS) of the mice against the Log ADD of each group.

Salinity

The salt concentrations of freshwater, seawater and hypersaline water are approximately 0.5 ppt, 25–35 ppt, and 35–50 ppt, respectively (Duncan Seraphin *et al*., 2014). In this study, the decomposition process and contents did not affect the salinity of the water groups because the mean salinity of each group across the days of decomposition indicates that the salt concentrations of the water in the boxes were in the estimated range (Table 5.18). The freshwater had a minimum salinity of 0.11 ppt and a maximum of 0.62 ppt, seawater had a minimum concentration of 28.3 ppt and a maximum of 29.2 ppt, and hypersaline water had a minimum concentration of 37.9 ppt and a maximum of 40.0 ppt—[See Appendix 2.4 for the salinity concentrations of each group].

Table 5.18: Average salinity concentrations of each group on the sampling days.

Groups	**Average Salinity (ppt)** ***[n=6]***					
	Days of Decomposition					
	0	**7**	**14**	**21**	**28**	**35**
Fresh	0.11	0.16	0.38	0.52	0.60	0.62
Sea	28.5	29.2	28.4	29.1	28.9	28.3
Hypersaline	38.3	39.4	39.1	40.0	39.7	37.9

pH

The pH of freshwater ranges between 6.5–8.0, while seawater is primarily alkaline, and its pH ranges between 7.0–8.4 (Chester & Jickells, 2012), while that of hypersaline water can range from 6.2–10 (Golan *et al*., 2014; Papadimitriou *et al*., 2016). Table 5.19 shows the pH level of each group across the decomposition days. Like the salinity concentrations, the pH level of the fresh and seawater used in this study remained in the estimated pH range, and the decomposition process and contents did not affect the pH of the fresh and seawater. The freshwater had a minimum pH of 6.74 and a maximum of 7.81, seawater had a minimum pH of 6.59 and a maximum of 8.14, and hypersaline water had a minimum pH of 6.64 and a maximum of 8.18—[See Appendix 2.4 for the pH levels of each group].

Table 5.19: Average pH levels of each group on the sampling days.

Groups	**Average pH** ***[n=6]***					
	Days of Decomposition					
	0	**7**	**14**	**21**	**28**	**35**
Fresh	7.67	6.74	6.92	6.89	7.39	7.81
Sea	8.14	6.67	6.76	6.59	7.08	7.42
Hypersaline	8.18	6.80	6.77	6.64	7.09	7.40

TDS

TDS concentration of freshwater sources is approximately 0.5–1.0 ppt, seawater ranges at about 10–35 ppt, and hypersaline water is above 30 ppt (Moran, 2018; USGS, 2018). Table 5.20 shows the TDS concentration of each group across the decomposition days. The TDS concentration of the water groups used in this study remained in the estimated range, and the decomposition process and contents did not affect the TDS concentration. The freshwater had a minimum TDS of 0.14 ppt and a maximum of 0.68 ppt. TDS values of hypersaline and seawater were not recorded at the onset of the study, but from day 7, seawater had a minimum TDS of 21.7 ppt and a maximum of 27.6 ppt, while hypersaline water had a minimum TDS of 28.2 ppt and a maximum of 36.9 ppt—[See Appendix 2.4 for the TDS of each group].

Table 5.20: Average TDS concentrations of each group on the sampling days.

Groups	**Average TDS (ppt)** ***[n=6]***					
	Days of Decomposition					
	0	**7**	**14**	**21**	**28**	**35**
Fresh	0.14	0.18	0.45	0.64	0.66	0.68
Sea	-	21.7	23.4	23.4	27.6	25.0
Hypersaline	-	28.2	29.0	31.3	36.9	33.4

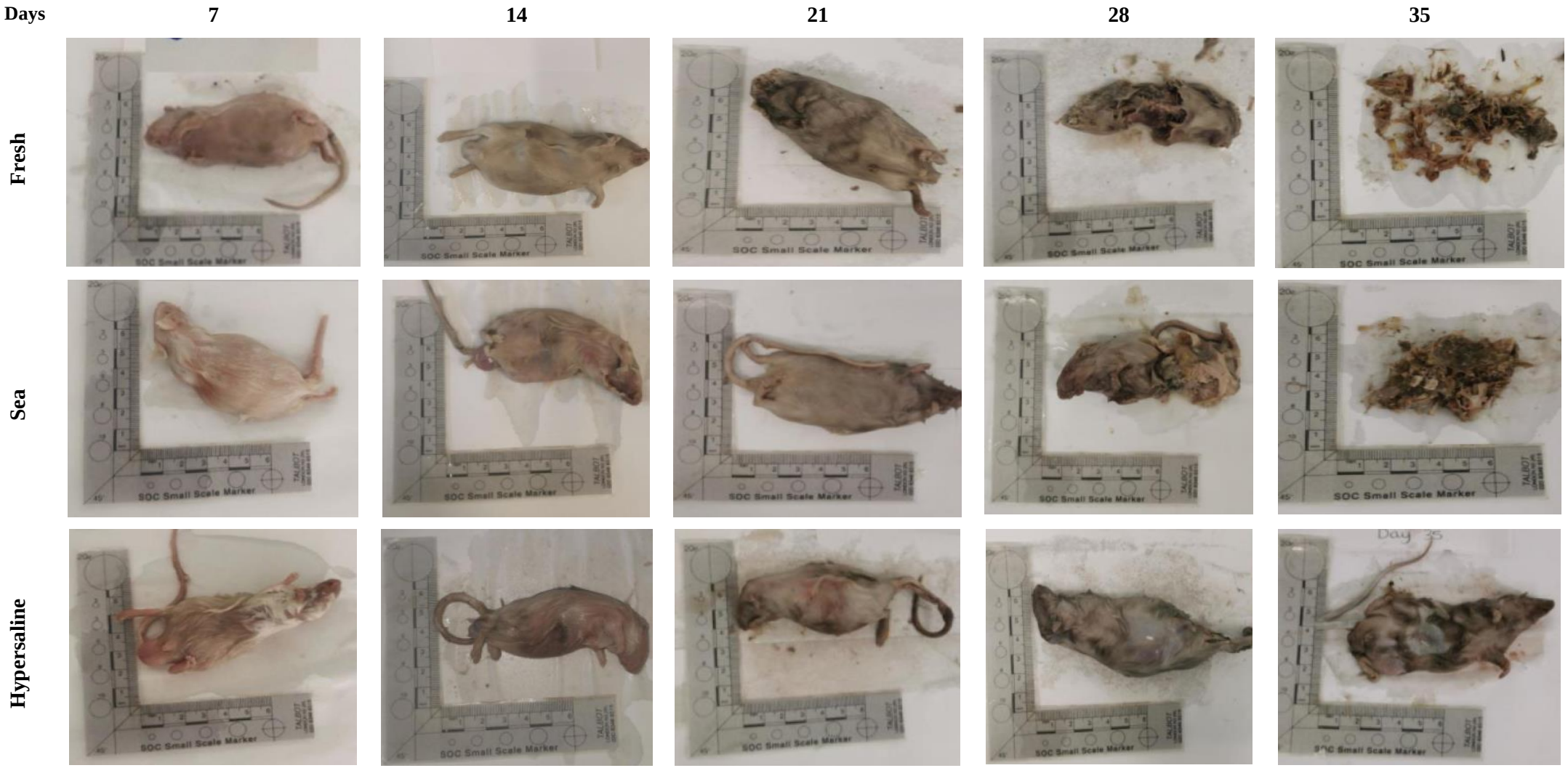


Figure 5.21: Physical decomposition changes in each group across the 35 days.

5.3.1.3 *Discussion*

Initial Study

Decomposition ranged from a "*fresh*" appearance at the onset to "*complete skeletonization*" in some mice at the end, with a minimum observed TADS of 6 and a maximum observed TADS of 25. Physical changes were most observable in the mice's trunk, head, and neck regions, while discolouration changes were the most visible in the tail and limbs. Throughout the study, there was no sign of adipocere formation in any of the mice, which could mainly be because of the low-fat content in mice. See Figure 5.15 for the representation of the decomposition changes recorded. On day 7, the only physical changes observed across all groups were discolouration (pink, green and grey) and a slight fur loss in the mice's head, neck, limbs, and tail regions. There were no signs of bloating in the trunks of the hypersaline group mice, but fur loss and extensive skin discolouration (green, pink, brown and grey) were observed. There were signs of bloating in the trunk and genitals of the brackish, sea and control groups, with the control group showing the most significant level of bloating. Skin slippage and soft tissue exposure were noted in some mice from the fresh and brackish groups.

The extent of decomposition in all groups on day 14 was like that of day 7. There was no sign of bloating in the hypersaline group; the discolouration features observed on day 7 were still consistent on day 14. The only significant physical difference in the sea group was the presence of white patches on the trunks of the mice; these patches resembled spots like rashes or pimples (Figure 5.22).



Figure 5.22: Unknown white patches observed on a mouse in the Sea group.

The cause of the white patches is unclear, but they could have been formed due to osmosis and subsequent salt depositions on the pores of the mice. The control group had more fur loss in the trunk region on day 14 than on day 7.

On day 21, bloating of the face, neck, and orifices was observable in the control group, but the abdominal regions of the mice had caved in due to bursting/compromise of the bloat. There were finally signs of bloating in the trunk of the mice in the hypersaline groups. However, the mice's heads, necks, and limbs in the hypersaline group still exhibited only discolouration. Bloating was still visible in the abdomen of the mice in the sea and brackish groups. Skin slippage around the head and the massive collapse of the trunk of some mice were also observed.

By day 28, the abdominal regions of all the mice in the four groups showed similar decomposition changes. The abdomens had caved in due to the resolution of bloat, and there was an exposure of soft tissue and internal organ decomposition. The mice in the hypersaline and sea groups looked drier and emptier when compared to those in the brackish and control groups. Identifying the organs in the abdominal region of mice in the hypersaline and sea groups was more challenging. This could be a result of the loss of body water from the mice due to the salt content of the water; the osmotic change led to "drying/fermentation" of the body contents (Davidson *et al*., 2012; Stuart & Ueland, 2017). There was evidence of soft tissue decomposition and bone exposure in the head regions of the mice in the control group. In contrast, the brackish and sea groups mainly exhibited signs of bloating in the head region. The hypersaline group only showed signs of discolouration and fur loss in the head region. Only the control group showed signs of bloating for the limbs and tails; the other three groups had only discolouration changes as signs of decomposition.

On day 35, nearly every mouse in the sea and the hypersaline group had reached complete bone exposure and disarticulation. Only 1 out of the 12 mice in both groups did not attain complete skeletonization. This increase in decomposition rate between days 28 and 35 in the sea and hypersaline groups could be due to the drying that occurred in both groups on day 28. Most exposed organs had decomposed extensively beyond recognition on day 28; hence, extensive bone exposure was observed on day 35 as the remaining soft tissue would have washed off or decomposed completely. In contrast, the control and brackish groups did not show complete disarticulation, but there were signs of advanced tissue decomposition and bone exposure. This could be because the “drying/fermentation” was not observed in the control and brackish group. This “drying/fermentation” observation occurs when there is a loss of water (through osmosis) from cells and bacterial cells in a body submerged in high-saline water (Stuart & Ueland, 2017).

Effect of Salinity

Figures 5.13 and 5.14 display the TADS and Log_{10} ADD data points of the four salinity groups on the five sampling days. The TADS increased as the Log_{10} ADD of each group increased because the decomposition rate depended on the temperature (Champaneri, 2006; Megyesi *et al*., 2005). Across days 7–28, the low-salinity groups (Control and Brackish) are seen to have higher TAD scores when compared with the high-salinity groups (Sea and Hypersaline). This shows that the high salt content in the water of the Sea and Hypersaline groups could cause the slowed-down decomposition rate in the mice because the bacterial action in the water would have been immensely reduced (De Donno *et al*., 2014; Rodriguez, 1997).

Decomposition is slower in the presence of higher salinity compared to low salinity (Boyle *et al*., 1997; Christensen & Myers, 2011; Reijnen *et al*., 2018). On day 35, the difference between the TADS of the four groups was not much, with the Sea and Hypersaline groups having slightly higher scores than the Brackish and Control groups. The Control group had the highest TADS, followed by the Brackish and Sea groups. The Hypersaline group had the lowest TADS. Figures 5.10 and 5.11 show the data points of the TADS against the PMSI of the four groups on the five sampling days; like Figures 5.13 and 5.14, they illustrate that the low-saline groups have higher TADS when compared to the high-saline groups.

Comparison of the Repeat and Initial Study

The initial study was conducted to investigate the effect of four salinity levels (fresh, brackish, sea and hypersaline) on the decomposition rate of mice. The results of the initial study suggested that decomposition was statistically significantly faster in freshwater ($p < 0.05$) compared to the salt-containing water groups (brackish, seawater and hypersaline).

A repeat study was conducted to:

(i) Obtain more data on the effect of varying salinity levels on the decomposition rate in mice.
(ii) Investigate the effect of three levels of salinity (fresh, sea and hypersaline) on the decomposition rate of mice.
(iii) The mice in the seawater and hypersaline groups of the initial study exhibited similar features and trends in their extent of decomposition (Figures 5.13 and 5.14). Therefore, the repeat study investigated if there was a statistically significant difference in the effect of seawater and hypersaline water on the decomposition rate of mice.

The results of the repeat study were like that of the initial study; there was a statistically significant difference ($p < 0.001$) in the effect of the salinity concentrations of the three water groups (fresh, sea and hypersaline) on the decomposition rate of mice, with the mice in the freshwater group decomposing faster than the sea and hypersaline groups (Table 5.14-5.15). This result is in accordance with the general phenomenon that bodies submerged in freshwater decompose earlier and faster than bodies

submerged in saline water (Palazzo *et al*., 2021). Decomposition is slowed down in salt water because the bacterial activity is hindered in the presence of salt (Byard, 2018).

In the initial study, there was no statistically significant difference in the effect of seawater and hypersaline water on the decomposition rate of mice (Table 5.11), but in the repeat study, there was a statistically significant difference between both groups (Table 5.15). Unlike the repeat study in which the salinity concentrations of the water groups were measured at the onset and the five PMSI intervals (Table 5.18), the seawater and hypersaline groups of the initial study were prepared at the start of the study and the salinity levels were not measured at any point of the study. It would have been an excellent option to compare the salinity concentrations of the seawater and hypersaline water groups of both studies on the five PMSI interval days (Seawater Initial vs Seawater Repeat or Hypersaline water Initial vs Hypersaline water Repeat) to explore if the differences in the salinity levels of the hypersaline water or seawater of both studies were the reason for the significant difference in the repeat study. Differences in the temperatures of both studies (Figures 5.12 and 5.18) could also be a reason for the differences in the effect of hypersaline water and seawater on the decomposition rate of the mice.

5.3.2 Laboratory Hardness Study

5.3.2.1 *TADS*

Table 5.21 shows a breakdown of the average TADS of the mice. Throughout the study, the lowest mean TADS recorded was 6.50 (*n=6, S.D.=0.84*) in the Soft Water group, while the highest recorded mean was 25.00 (*n=6, S.D.=0*), also in the Soft Water group. The Soft Water group generally had the highest TAD scores, while the Hard and Very Hard water groups had the lowest TADS—[See Appendix 3.1 for TADS of all the mice]. Figure 5.23 is a graphical representation of the TADS of the mice in the groups against the PMSI on the days of sampling.

Table 5.21: Average TADS of the mice in each hardness group on the sampling days.

PMSI (Days)	Average TADS (*n=6*)			
	Soft	**Moderately hard**	**Hard**	**Very hard**
7	6.50	7.50	7.67	8.00
14	12.67	11.33	10.50	10.83
21	19.00	15.33	13.67	13.83
28	24.50	20.17	19.17	18.50
35	25.00	23.00	22.50	21.00

A two-way ANOVA test was conducted to investigate if there was any statistical difference in the effect of the four concentrations of water hardness on the TAD scores of the mice across the five sampling days—[See Appendix 3.2 for statistics]. The ANOVA results showed that there was a statistically significant difference in the TADS across the four groups ($r^2 = 0.94$, $p < 0.001$). A Tukey's Post Hoc test was done to further investigate the specific statistical difference (*p-value*) of the TADS of each group compared to the other three groups (Table 5.22).

Table 5.22: Post Hoc test results.

Soft water *vs*	Moderately Hard Water ($p < 0.001$)*
	Hard Water ($p < 0.001$)*
	Very Hard Water ($p < 0.001$)*
Moderately Hard Water *vs*	Hard Water ($p = 0.255$)
	Very Hard Water ($p = 0.067$)
Hard Water *vs* Very Hard Water ($p = 0.917$)	

- a difference significant to the 5% level

There was a statistically significant difference between the TADS of the Soft Water group and the other three groups: Moderately Hard Water ($p < 0.001$), Hard Water ($p < 0.001$) and Very Hard Water ($p < 0.001$). There was no significant difference between the TADS of the Moderately Hard Water and Hard

Water groups ($p = 0.255$) and the Very Hard Water group ($p = 0.067$) or between the TADS of the Hard Water and Very Hard Water groups ($p = 0.917$).

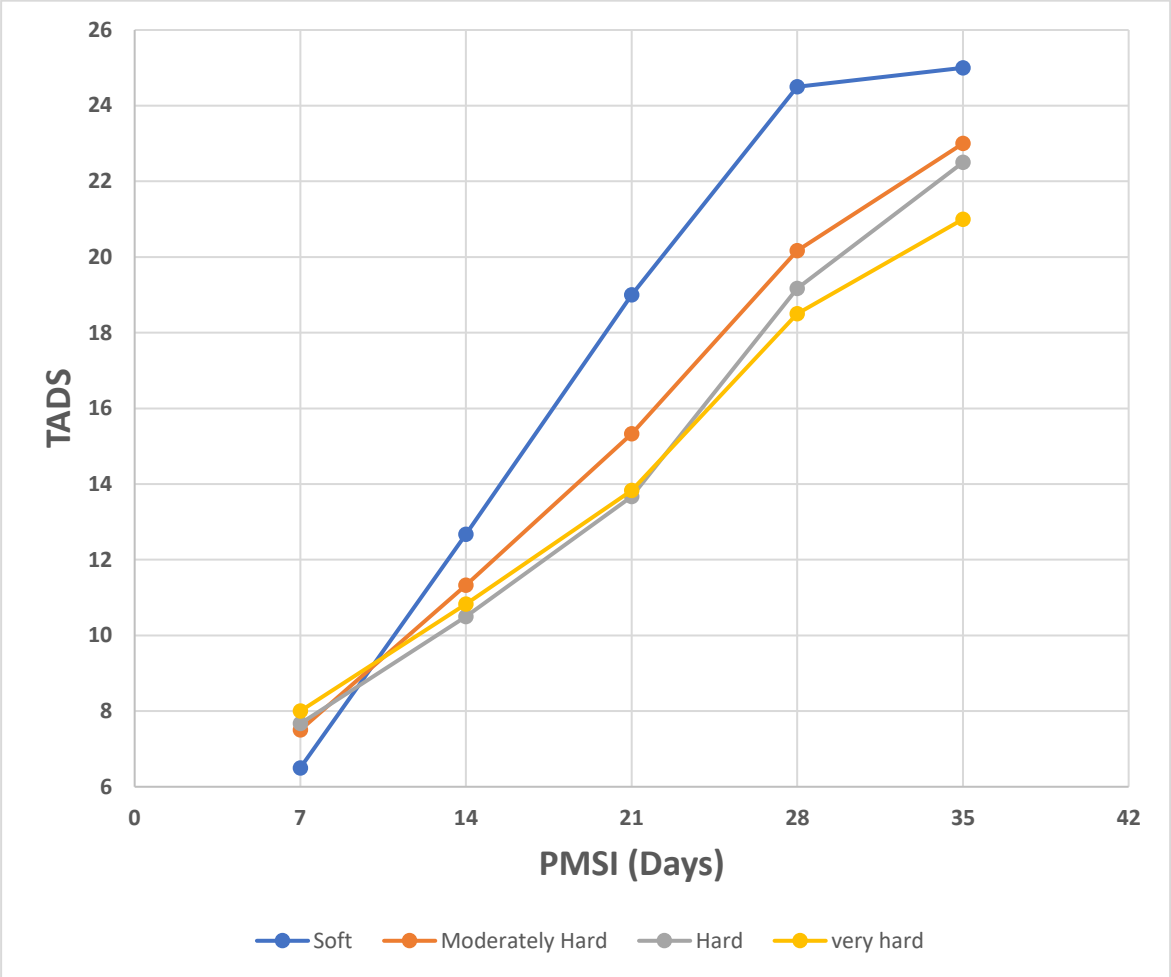


Figure 5.23: Graph of the Average TADS of the mice against the PMSI of each group.

5.3.2.2 *Temperature & ADD*

Figure 5.24 shows the average daily temperatures of the water in each group on the 35 days of decomposition—[See Appendix 3.3 for the daily temperature values of each group].

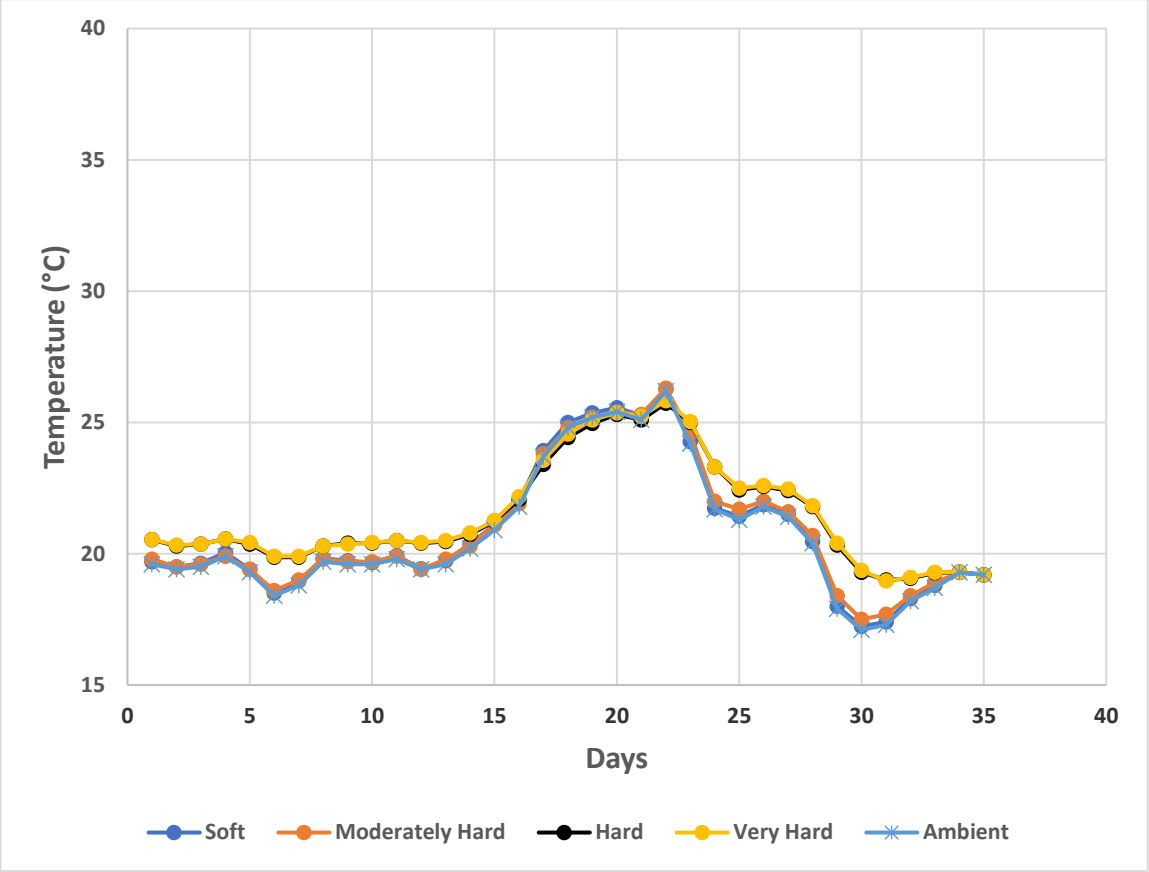


Figure 5.24: Graph showing temperature fluctuation in the water groups across the 35 days.

The lowest ADD was 135.7 in the soft water group, and the highest was 751.8 in the Very hard water group (Table 5.23). The average daily water temperatures of the boxes in each group were summed up to calculate the ADD values. Figure 5.25 is a graphical representation of the TADS of the mice in the groups against the average Log ADD on the sampling days.

Table 5.23: ADD and Log ADD of the salinity groups across the 35 days.

PMSI (Days)	Soft		Moderately hard		Hard		Very hard	
	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD
7	135.7	2.13	135.8	2.13	141.9	2.15	142.0	2.15
14	274.4	2.44	274.4	2.44	285.1	2.45	285.4	2.46
21	442.7	2.65	441.9	2.65	451.6	2.65	452.6	2.66
28	600.2	2.78	600.8	2.78	614.8	2.79	616.2	2.79
35	728.4	2.86	730.2	2.86	750.3	2.88	751.8	2.88

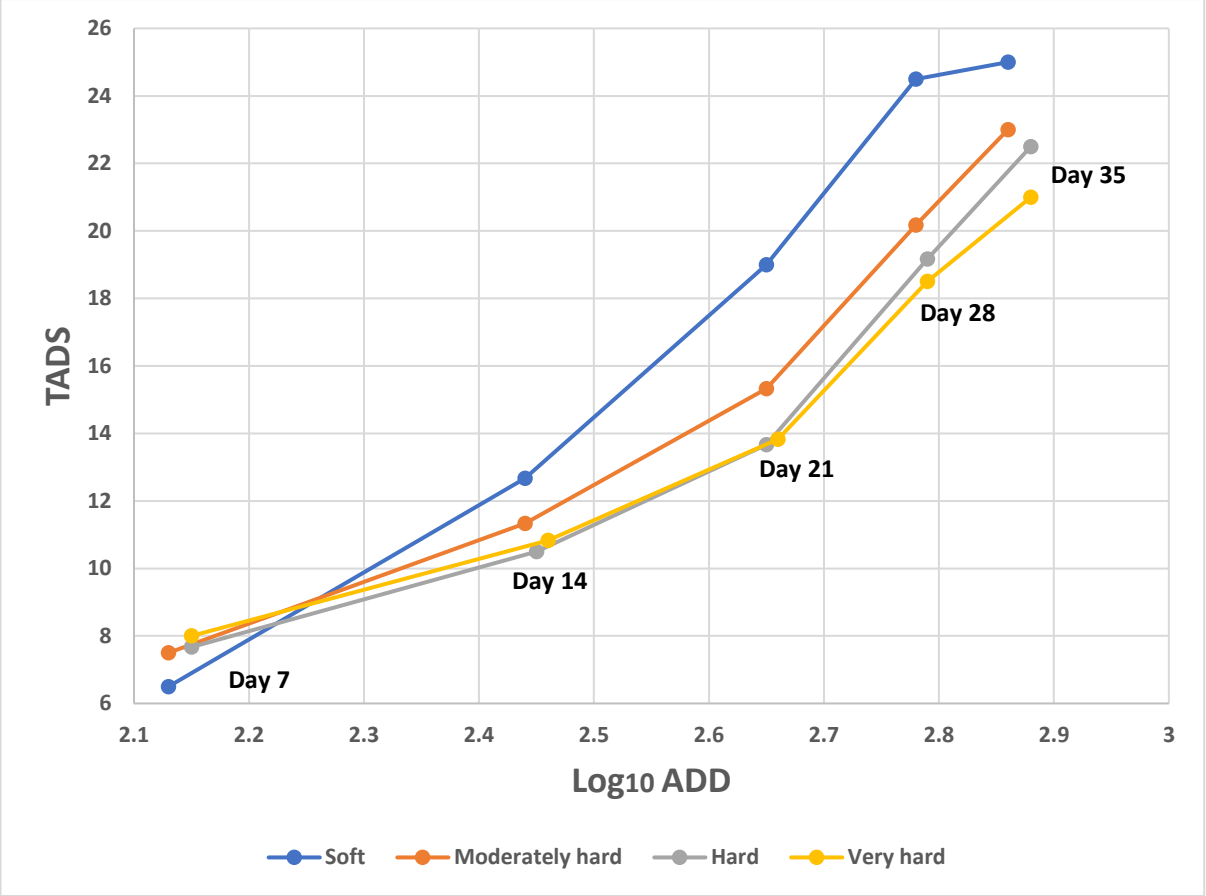


Figure 5.25: Graph of the Average TADS of the mice against the Log ADD of each group.

5.3.2.3 *Pictorial Representations of Hardness Effect*

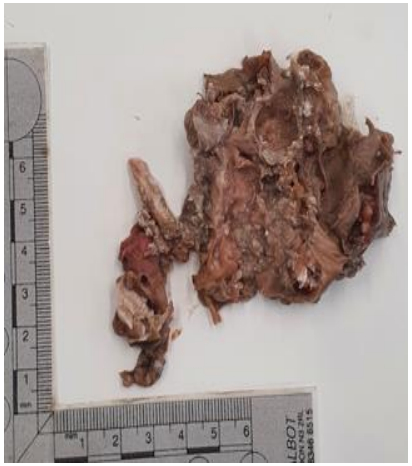
Days	7	14	21	28	35
Soft					
Moderately Hard					



Figure 5.26: Physical decomposition changes in each group across the 35 days.

5.3.2.4 *Discussion*

Decomposition Observations

See Figure 5.26 for the physical representation of the decomposition changes recorded. On day 7, fur loss and pink discolouration were the only observable decomposition changes in the head and limb regions of the mice in all the groups [FADS and LADS=2]. Bloating was observed in the trunk regions of the mice in the Moderately Hard, Hard and Very Hard Water groups [BADS=4]. Only one mouse in the Soft Water group showed bloating in its trunk [BADS=4]; the other mice only presented fur loss and pink discolouration as decomposition changes [BADS=2].

By day 14, bloating was visible in the head region of the mice in all the groups [FADS=4]. Compared to day 7, marbling was the only visible change in the tails and limbs of the mice in all the groups [LADS=3]. Decomposition was advanced in the stomach region of the mice in Soft Water; the abdomens had caved in due to a reduction in bloat, and soft tissues and organs were exposed [BADS=6]. Bloating remained in the Moderately Hard, Hard and Very Hard Water groups [BADS=4].

On day 21, bone exposure and disarticulation of the mandible were prevalent in the head and neck in the Soft Water group [FADS=6-7]. Advanced tissue decomposition and bone exposure were also evident in the abdominal and limb regions [BADS and LADS=7]. In the three other groups, bloating and fur loss were observed in the mice's necks and heads [FADS=4]. Caved abdomens and exposure of soft tissues were also observed in the trunks of the mice in these three groups [BADS=6], while skin slippage and bloating were evident in the limbs [LADS=4]. By day 28, all the mice in the Soft Water group had reached skeletonization and complete disarticulation [FADS=7-8, BADS=8, LADS=9]. In the other three groups, extensive bone exposure, skeletonization and partial disarticulation were evident all over the mice [FADS=6-7, BADS=6-7, LADS=6-8].

On day 35, complete decomposition and disarticulation had occurred in all the mice in the Soft Water group; only a mash-up of lost fur, bones and sloughed-off skin was left in the box [FADS=8, BADS=8, LADS=9]. In the Moderately Hard Water group, four mice had reached complete decomposition [FADS=8, BADS=8, LADS=9], while the remaining two mice showed signs of bone exposure and advanced tissue decomposition [FADS=6, BADS=6-7, LADS=7]. The Hard Water group exhibited similar decomposition changes as the Moderately Hard Water group, ranging from bone exposure, advanced exposure of organs and complete disarticulation [FADS=6-8, BADS=6-8, LADS = 8-9]. The Very Hard Water mice were the least decomposed compared to the other groups. Extensive soft tissue decomposition and bone exposure were observed in the head region [FADS=6]. Skeletonization and disarticulation were evident in the abdomen [BADS=7-8], while skin slippage and exposure of bones, muscles and tendons were observed in the limb [LADS=6-8].

Effect of Hardness Concentrations

Figure 5.25 displays the TADS and Log_{10} ADD data points of the four hardness groups on the five sampling days. On day 7, the four groups had similar TADS, but the Soft Water group had the highest TADS from days 14 – 35, indicating that the decomposition rate was the fastest in the Soft Water group. The Moderately Hard Water group had the second highest TADS and decomposition rate, closely followed by the Hard and Very Hard Water groups.

Cations that cause the hardness of water tend to react with some anions in the water to form stable salts (Sengupta, 2013). It is possible that the Ca^{2+} ions from the $CaCO_3$ added to the Moderately Hard, Hard and Very Hard Water groups have reacted with anions in the water and formed salts. The salts formed could be the reason for the slowed-down decomposition rate in these groups because bacterial activities are immensely reduced in the presence of salt concentrations (De Donno *et al*., 2014).

5.3.3 Laboratory Current Study

The experiment was broken down into two sections. The first section was conducted between April 12 to May 17, 2022, to investigate the differences in the extent of decomposition in mice immobile and completely submerged with fishing weights (Submerged 1) and mice placed in water and left to float freely (Floating). The second was conducted between June 28 to August 2, 2022, to investigate the differences in the extent of decomposition in mice immobile and completely submerged with fishing weights (Submerged 2) and mice placed in water and left to float according to the current or flow generated by a turbine (Moving).

5.3.3.1 *TADS*

Throughout the 35 days of decomposition for each experiment, the extent of decomposition in the *Moving* group was the most advanced; from a PMSI of 7 days till the end of the experiment, the *Moving* group had the highest TADS (Table 5.24). The lowest mean TADS recorded with the Heaton DSS was 6.17 (*n=6, S.D.=0.41*) in the Floating group, while the highest recorded mean was 25.00 (*n=6, S.D.= 0*) in the Moving group. With the van Daalen DSS, the lowest mean TADS recorded was 6.17 (*n=6, S.D.=0.41*) in the Submerged 2 group, while the highest recorded mean was 18.00 (*n=6, S.D.= 0*) in the Moving group—[See Appendix 4.1 for TADS of all the mice]. Figures 5.27–5.28 are graphical representations of the TADS of the mice in the groups against the PMSI on the days of sampling.

Table 5.24: Average TADS of the mice in each group on the sampling days.

PMSI (Days)	Average TADS (*n=6*)							
	Heaton DSS *[max=25]*				Van Daalen DSS *[max=18]*			
	Submerged 1	Floating	Submerged 2	Moving	Submerged 1	Floating	Submerged 2	Moving
7	6.67	6.17	7.33	8.67	7.33	7.00	6.17	6.83
14	9.50	9.67	11.83	17.17	7.17	7.67	9.00	12.83
21	10.50	12.17	12.50	21.83	8.17	8.50	10.00	15.83
28	12.83	16.17	15.33	24.50	8.83	11.17	10.50	17.50
35	17.17	19.67	20.00	25.00	11.50	14.17	14.33	18.00

A two-way ANOVA test was conducted to analyse any statistical difference in the TADS of the four groups across the five sampling days when using the Heaton DSS. The ANOVA results showed that there was a statistically significant difference in the TADS across the four groups ($r^2 = 0.92$, $p < 0.001$). A Tukey's Post Hoc test was done to further investigate the specific statistical difference (*p-value*) of the TADS of each group compared to the other three groups. The results of this test indicate that there was a statistically significant difference between the TADS of all the groups except when the *Floating* and *Submerged 2* groups were compared (Table 5.24).

For the van Daalen DSS, the Friedman test was conducted to analyse any statistical difference in the TADS of the four groups across the five sampling days. Results indicated a statistically significant difference between the TADS of the four groups ($p < 0.001$). A Wilcoxon Post Hoc test was done to further investigate the specific statistical difference (*p-value*) of the TADS of each group compared to the other three groups. The results of this test indicate that there was a statistically significant difference between the TADS of all the groups except when the Floating group was compared to the two Submerged groups (Table 5.25)—[See Appendix 4.2 for statistics].

Table 5.25: Post Hoc test results of both DSS.

Heaton		**van Daalen**	
Moving *vs*	Submerged 1 ($p < 0.001$)*	Moving *vs*	Submerged 1 ($p < 0.001$)*
	Floating ($p < 0.001$)*		Floating ($p < 0.001$)*
	Submerged 2 ($p < 0.001$)*		Submerged 2 ($p < 0.001$)*
Floating *vs*	Submerged 1 ($p = 0.019$)*	Floating *vs*	Submerged 1 ($p = 0.030$)
	Submerged 2 ($p = 0.538$)		Submerged 2 ($p = 0.478$)
Submerged 2 *vs* Submerged 1 ($p < 0.001$)*		Submerged 2 *vs* Submerged 1 ($p < 0.001$)*	

***- a difference significant to the 5% level** ***- a difference significant to the 1% level**

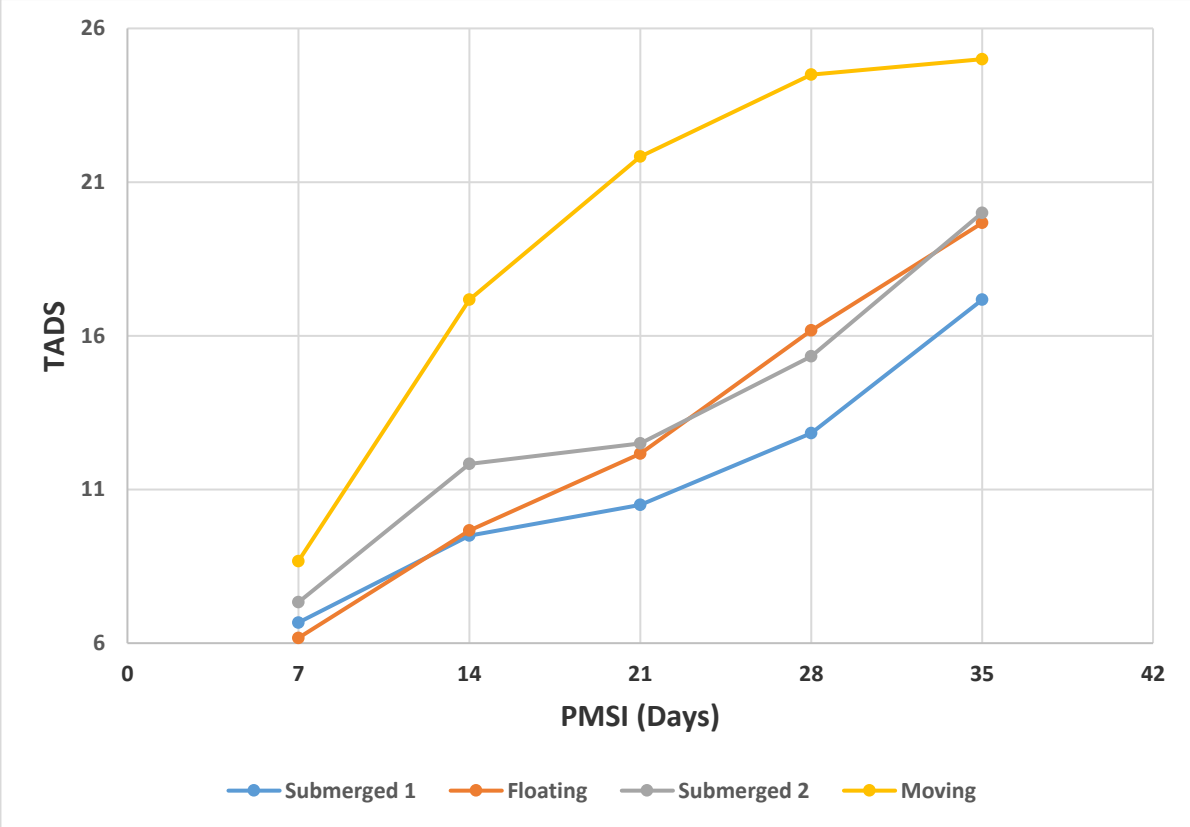


Figure 5.27: Graph of the Average TADS (using Heaton DSS) of the mice against the PMSI of each group.

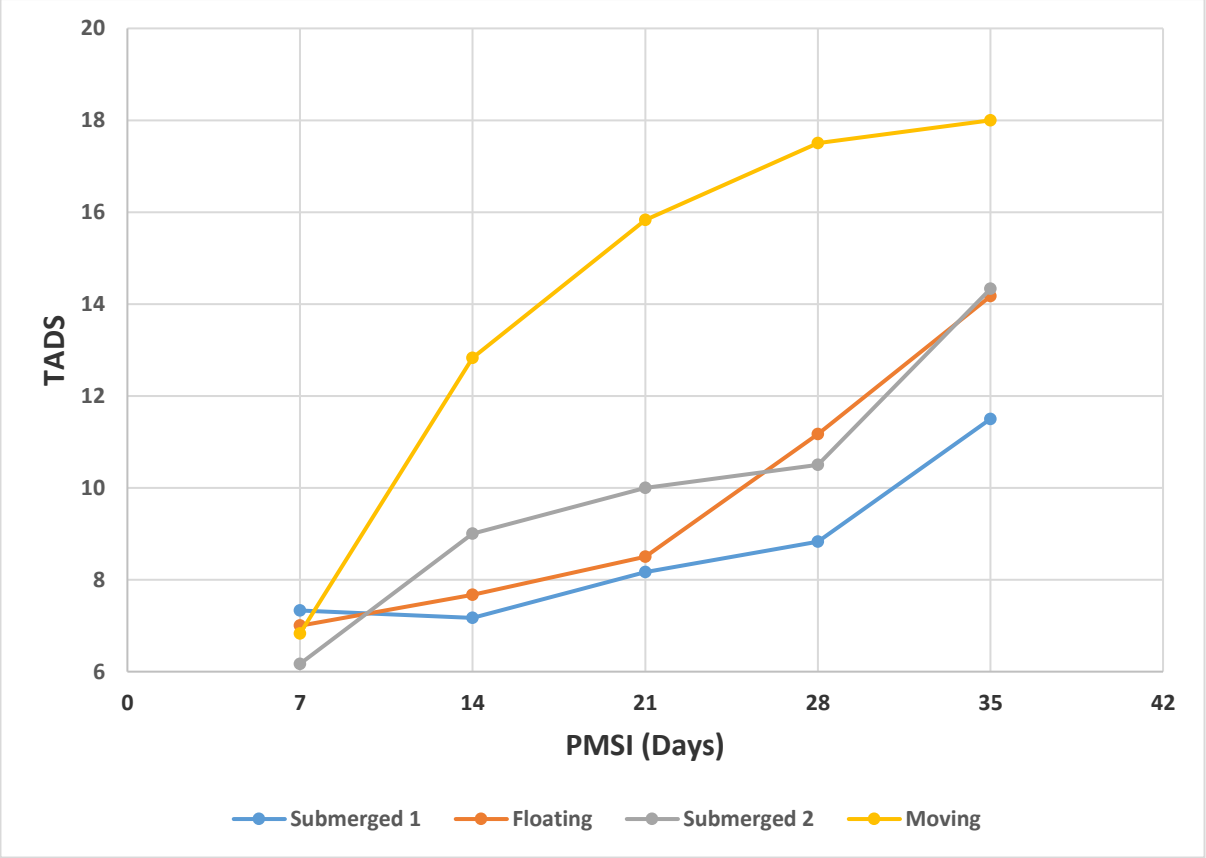


Figure 5.28: Graph of the Average TADS (using van Daalen DSS) of the mice against the PMSI of each group.

5.3.3.2 *DSS Comparison*

A Mann-Whitney U test was needed to compare the TADS of the two DSS statistically, but this would not have been possible because they had different maximum scores (Heaton maximum score = 25; van Daalen maximum score = 18). Therefore, Heaton TADS were divided by 25 and the van Daalen by 18 to normalise both scores to a ratio of 1. Thus, they both had a maximum score of 1, and the Mann-Whitney U test was used to analyse if there was any significant difference in the TADS obtained when using the Heaton and van Daalen DSS to assess the extent of decomposition in each group—[See Appendix 4.3 for statistics]. The results of the tests indicate that there was no statistically significant difference between the Heaton and van Daalen DSS scores for the four groups (Table 5.26).

Table 5.26: Mann-Whitney U test results.

Groups	***p-value***
Submerged 1	0.430
Floating	0.656
Submerged 2	0.593
Moving	0.736

****- a difference significant to the 1% level***

5.3.3.3 *Temperature & ADD*

Figure 5.29 shows the average daily temperatures of the water in each group on the 35 days of decomposition. The temperatures in the second set of the experiment; *Submerged 2* and *Moving* group, were higher compared to the first set; *Submerged 1* and *Floating* group, because the second set was conducted in the hotter summer months—[See Appendix 4.4 for the daily temperature values of each group].

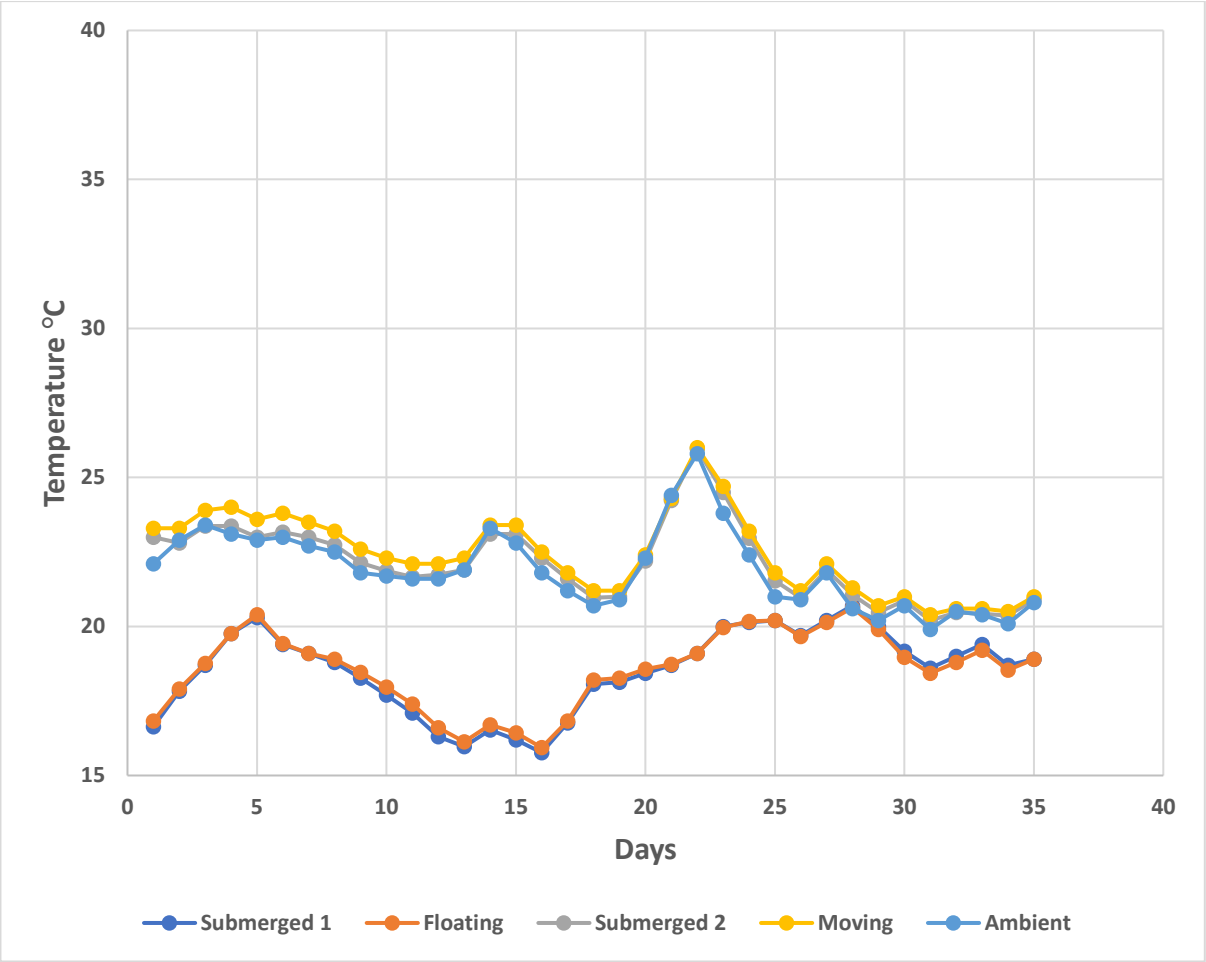


Figure 5.29: Graph showing temperature fluctuation in the water groups across the 35 days.

The average daily water temperatures of the boxes in each group were summed up and averaged to calculate the ADD values. Table 5.27 shows the mean ADD values of each group on the days of sampling. The *Submerged 2* and *Moving* groups had higher ADD compared to the *Submerged 1* and *Floating* groups due to the higher temperatures exhibited in the second set of the experiments (Figure 5.29). The lowest mean ADD was 131.7 (*n=3, S.D.=0.80*) in the Submerged 1 group, and the highest was 785.3 (*n=3, S.D.= 0*) in the Moving group. Figures 5.30–5.31 are graphical representations of the TADS of the mice in the groups against the average Log ADD on the days of sampling.

Table 5.27: ADD and Log ADD of the groups across the 35 days.

PMSI (Days)	Submerged 1		Floating		Submerged 2		Moving	
	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD
7	131.7	2.12	132.2	2.12	161.7	2.21	165.4	2.22
14	252.4	2.40	254.4	2.41	316.8	2.50	323.4	2.51
21	374.5	2.57	377.3	2.58	472.2	2.67	480.2	2.68
28	514.5	2.71	517.2	2.71	631.1	2.80	640.5	2.81
35	648.3	2.81	649.9	2.81	774.8	2.89	785.3	2.90

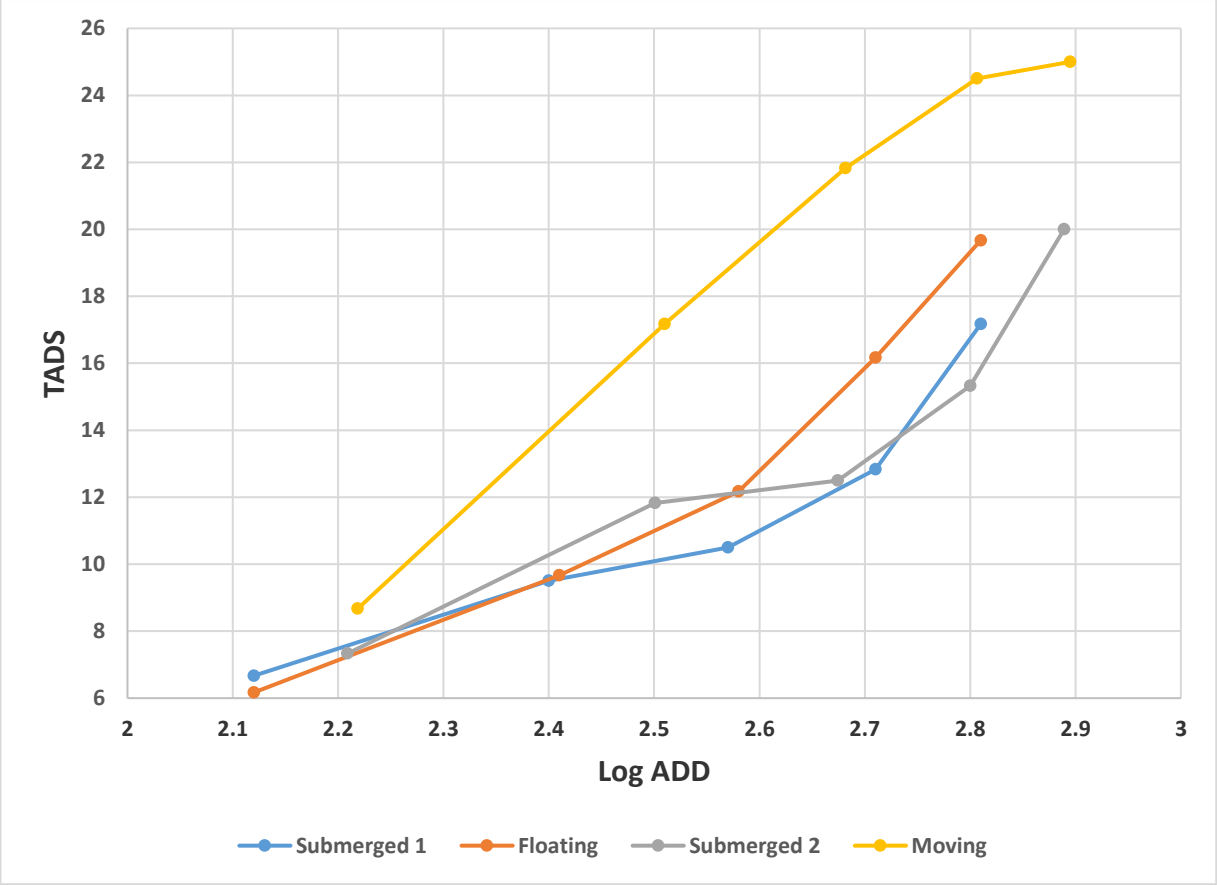


Figure 5.30: Graph of the Average TADS (using Heaton DSS) of the mice against the Log ADD of each group.

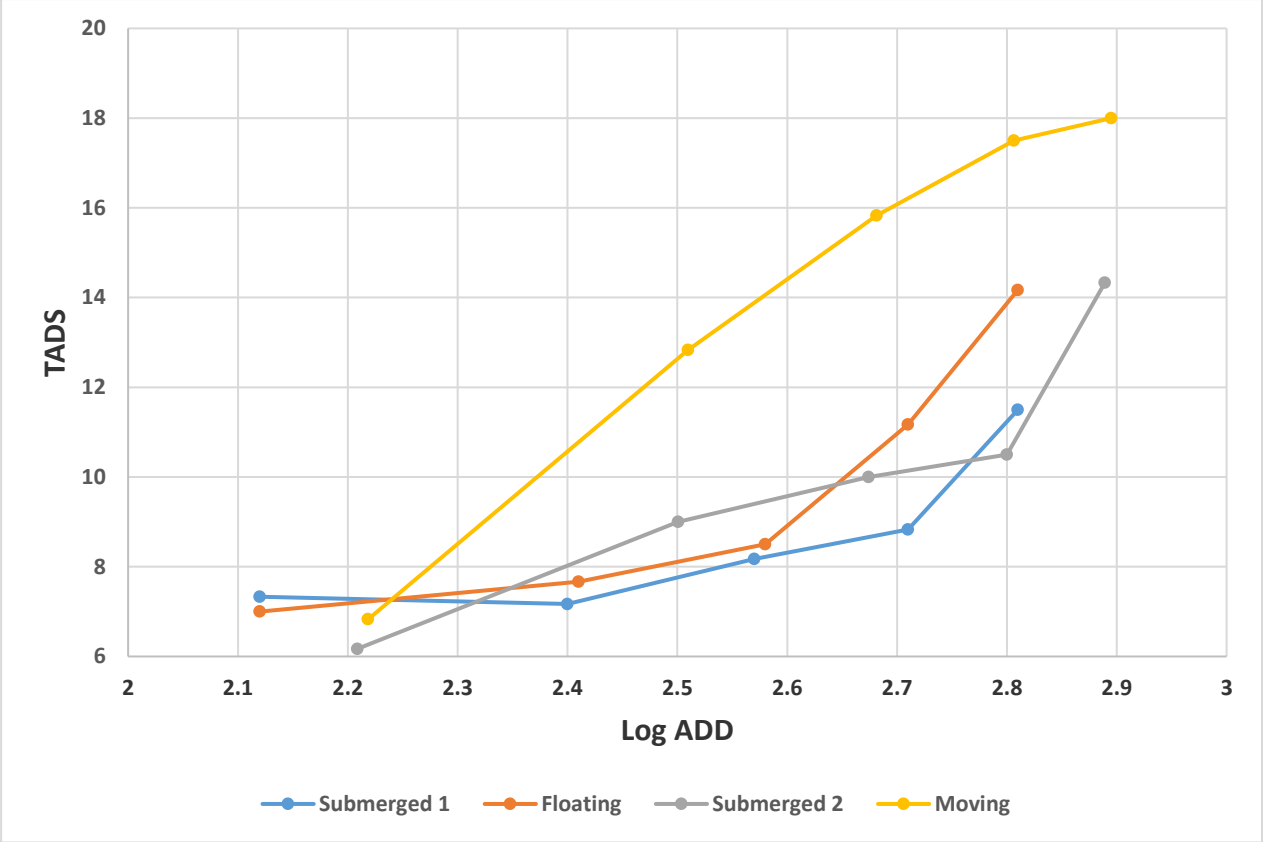


Figure 5.31: Graph of the Average TADS (using van Daalen DSS) of the mice against the Log ADD of each group.

5.3.3.4 *Salinity*

The salt concentration of freshwater is approximately 0.5–1.0 ppt (Duncan Seraphin *et al*., 2014; USGS, 2018). In this study, the decomposition process and contents did not affect the salinity of the freshwater in the boxes because the mean salinity of each group across the days of decomposition indicates that the salt concentrations of the water in the boxes were in the estimated range (Table 5.28). The minimum salinity was 0.11 ppt, and the maximum was 0.82 ppt—[See Appendix 4.5.1 for the salinity concentrations of each group]. These concentrations indicate that the water stayed as freshwater during the experiment; 0.5–1.0 ppt (Duncan Seraphin *et al*., 2014; USGS, 2018).

Table 5.28: Average salinity concentrations of each group on the sampling days.

Groups	**Average Salinity (ppt) *[n=6]***					
	Days of Decomposition					
	0	**7**	**14**	**21**	**28**	**35**
Submerged 1	0.11	0.15	0.20	0.36	0.46	0.51
Floating	0.17	0.24	0.37	0.44	0.49	0.50
Submerged 2	0.11	0.20	0.28	0.42	0.48	0.55
Moving	0.11	0.72	0.74	0.80	0.81	0.82

5.3.3.5 *pH*

The pH of freshwater ranges between 6.5–8.0 (Chester & Jickells, 2012). Table 5.29 shows the pH level of each group across the decomposition days. Like the salinity concentrations, the pH level of water used in this study remained in the standard pH range, and the decomposition process and contents did not affect the pH level. The minimum pH after the onset of the study was 6.92, and the maximum was 7.95—[See Appendix 4.5.2 for the pH levels of each group].

Table 5.29: Average pH levels of each group on the sampling days.

Groups	**Average pH *[n=6]***					
	Days of Decomposition					
	0	**7**	**14**	**21**	**28**	**35**
Submerged 1	8.10	7.03	7.00	6.92	7.07	7.35
Floating	7.95	7.10	6.95	7.21	7.50	7.86
Submerged 2	8.10	6.91	6.98	6.73	7.50	7.54
Moving	7.94	7.04	7.65	7.44	8.12	7.95

5.3.3.6 *TDS*

TDS concentration of freshwater sources is approximately 0.5 ppt (Moran, 2018). Table 5.30 shows the TDS concentration of each group across the decomposition days. The TDS concentration in this study remained in the standard range, and the decomposition process and contents did not affect the TDS concentration. The minimum TDS was 0.13 ppt, and the maximum was 0.92 ppt—[See Appendix 4.5.3 for the TDS concentrations of each group].

Table 5.30: Average TDS concentrations of each group on the sampling days.

Groups	**Average TDS (ppt) *[n=6]***					
	Days of Decomposition					
	0	**7**	**14**	**21**	**28**	**35**
Submerged 1	0.14	0.13	0.21	0.37	0.48	0.52
Floating	0.14	0.25	0.38	0.45	0.50	0.52
Submerged 2	0.14	0.20	0.31	0.47	0.54	0.63
Moving	0.14	0.73	0.83	0.88	0.88	0.92

5.3.3.7 *Pictorial Representations of Current effect*

Days	7	14	21	28	35
Submerged 1					
Floating					

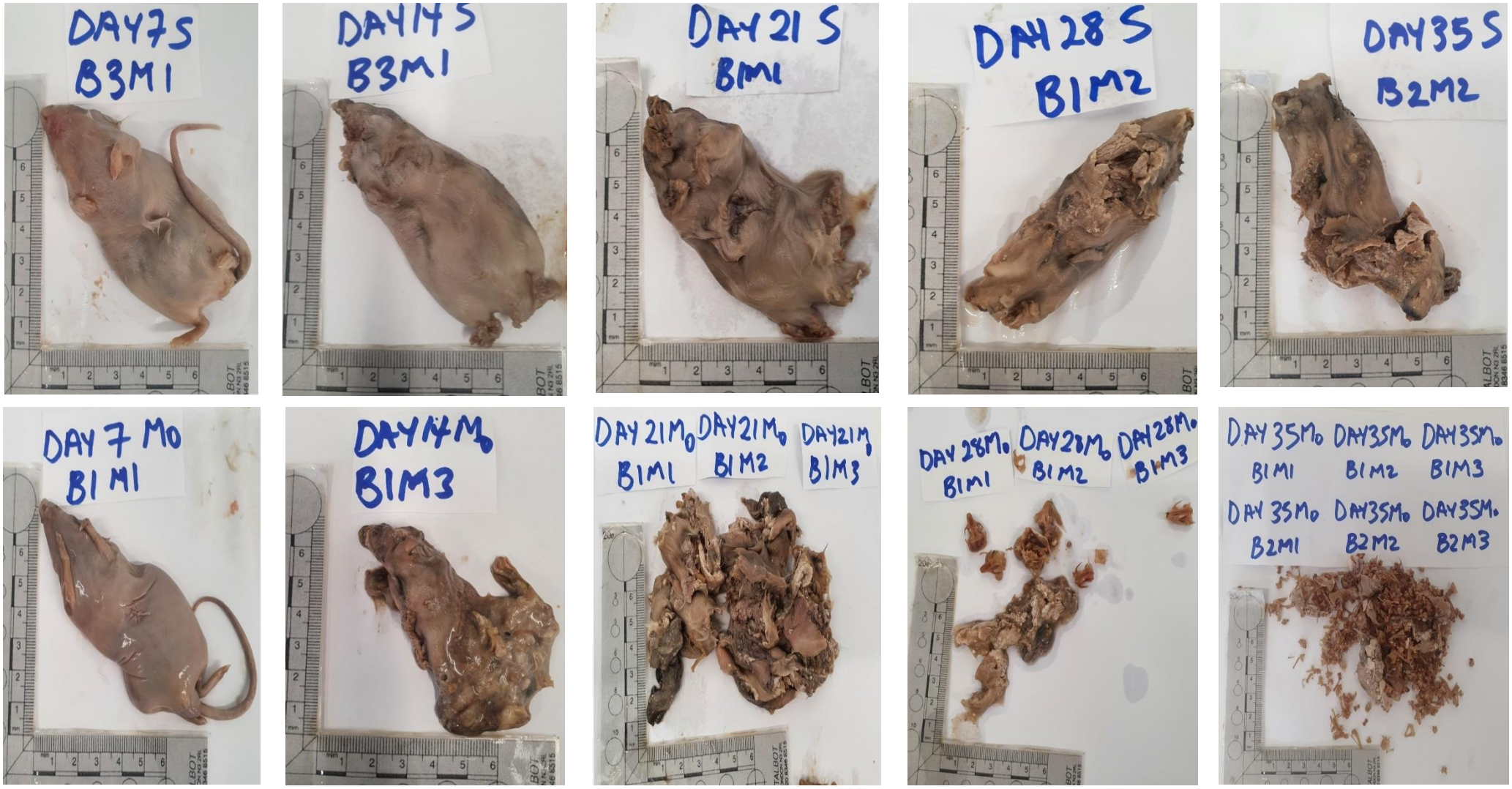


Figure 5.32: Physical decomposition changes in each group across the 35 days.

5.3.3.8 *Discussion*

Decomposition Changes

See Figure 5.32 for the physical representation of the decomposition changes recorded. At the onset of the study, most of the mice had a fresh appearance, while a few exhibited reddening of the face and neck as the only observable decomposition change. Throughout the study, physical changes were most observable in the mice's trunk, head, limb, and neck regions, while discolouration changes were the most visible in the tail. There was no sign of adipocere formation on any mice during the experiment. Decomposition was faster in the *Floating* and *Moving* groups when compared to the *Submerged* groups.

On day 7, the two *Submerged* groups and the *Floating* group showed similar decomposition rates and changes; pink discolouration and fur loss in the head, neck, and limb regions, while bloating was only observed in the trunk of 3 mice in the *Submerged* groups. There was complete fur loss in most of the mice in the *Moving* group on day 7 (Figure 5.32). On day 14, bloating and dark grey discolouration was observed in the trunks of the two *Submerged* groups and the *Floating* group, while pink discolouration, fur loss and skin slippage occurred in the head, neck, and limb regions. Exposure of soft tissue and internal organ decomposition was observed in every region of the mice in the *Moving* group on day 14.

The decomposition rate and changes in the *Submerged* and *Floating* groups started to vary across the groups on day 21, with the *Floating* group being more decomposed than the *Submerged 1* group. The *Floating* group exhibited skin slippage and bloating in the head and limbs, while exposure of soft tissues and internal organ decomposition was observed in the abdomen of the mice. There was no change in the *Submerged 1* group when comparing days 21 and 14; fur loss and skin slippage were observed in the head, neck, and limb while bloating was in the abdominal region. The *Submerged 2* group exhibited exposure of soft tissues and internal organ decomposition, mainly in the abdomen of the mice, while skin slippage and bloating were observed in the head and limbs. Complete disarticulation of the skull from the torso was observed in five out of six mice in the *Moving* group, while extensive bone exposure and advanced organ decomposition were observed in the abdominal and limb regions of all the mice in this group.

By day 28, the decomposition changes in the *Floating* and *Submerged 2* groups were already in the advanced state in all the body regions; soft tissue and bone exposure and advanced internal organ decomposition were observed in every body part. Bloating, liquefaction, skin slippage, degloving and soft tissue exposure were observed in the Submerged 1 group, but the extent of decomposition was not as advanced as in the other three groups. In the *Moving* group, complete disarticulation occurred in the mice's head and limb regions. Only three mice out of 6 in the *Moving* group had not fully disarticulated in the abdominal region.

The *Floating* and *Submerged 2* groups had similar changes on day 35 compared to day 28; bone exposure and advanced internal decomposition were prominent in all the body regions of the mice. In

contrast, decomposition on day 35 in the *Submerged 1* group was more advanced compared to day 28; soft tissue and bone exposure, liquefaction and internal organ decomposition were observed in most body regions of the mice. All the mice in the *Moving* group had attained complete disarticulation and skeletonization on day 35.

Current Effect

The decomposition changes observed suggest that the movement of the mice in the *Moving* group influenced the decomposition rate because the extent of decomposition in the Moving group was statistically more advanced when compared to the *Floating* and *Submerged* groups ($p < 0.001$). Also, when comparing the physical changes observed in the "*Moving* vs *Submerged 2*" and "*Floating* vs *Submerged 1*" groups, it is evident that the decomposition rate was slower in the submerged groups, and the statistical tests also indicated that there was a statistically significant difference in the TADS of the *Submerged* groups compared to the *Moving* and *Floating* groups ($p < 0.001$).

There was a higher level of ADD and temperature in the "*Moving* vs *Submerged 2*" experiment when compared to the "*Floating* vs *Submerged 1*" groups (Figure 5.26, Table 5.27); this higher heat energy could be the reason why there was no significant difference between the *Submerged 2* and *Floating* group ($p = 0.538$). It is reasonable to assume that the *Moving* and *Floating* groups exhibited more advanced decomposition rates compared to their respective Submerged groups because the mice in the box got exposed to the air-containing layer in the boxes when they were floating; this exposure to air could have increased the decomposition rate (Heaton *et al*., 2010) because it facilitates the drying of the exposed regions of the mice in the air.

5.3.4 Laboratory Skin Tone Study

5.3.4.1 *TADS*

Table 5.31 shows the TADS of the mice in the four groups when using the two DSS. Across the 35 days of decomposition, the lowest mean TADS recorded with the Heaton DSS was 6.67 (*n=6, S.D.=1.03*) in all the groups except the Dark: Fresh group, while the highest recorded mean was 21.50 (*n=6, S.D.=4.18*) in the Dark: Fresh group. With the van Daalen DSS, the lowest mean TADS recorded was 7.33 (*n=6, S.D.=0.52*) in all the groups except the Dark: Fresh group, while the highest recorded mean was 15.17 (*n=6, S.D.=3.37*) in the Dark: Fresh group—[See Appendix 5.1 for TADS of all the mice].

Table 5.31: Average TADS of the mice in each salinity group on the sampling days.

PMSI (Days)	Average TADS (*n=6*)							
	Heaton DSS *[max=25]*				van Daalen DSS *[max=18]*			
	Pale: Fresh	Pale: Sea	Dark: Fresh	Dark: Sea	Pale: Fresh	Pale: Sea	Dark: Fresh	Dark: Sea
7	6.67	6.67	7.00	6.67	7.33	7.33	7.50	7.33
14	9.50	9.33	8.67	8.33	7.17	7.33	7.33	7.17
21	10.50	10.17	13.00	8.50	8.17	8.00	8.83	7.67
28	12.83	11.17	20.17	12.17	8.83	9.00	15.00	9.00
35	17.17	14.83	21.50	15.00	11.50	10.17	15.17	11.00

Two-way ANOVA tests were conducted to analyse any statistical difference in the TADS of the four groups across the five PMSI intervals—[See Appendix 5.2 for statistics]. The ANOVA results showed that there was a statistically significant difference in the TADS across the four groups when using the Heaton DSS ($r^2 = 0.87$, $p < 0.001$) and van Daalen DSS ($r^2 = 0.75$, $p < 0.001$). A Tukey's Post Hoc test was done to further investigate the specific statistical difference (*p-value*) of the TADS of each group compared to the other three groups (Table 5.32).

Table 5.32: Post Hoc test results of both DSS.

Heaton		van Daalen	
Dark: Fresh *vs*	Dark: Sea ($p < 0.001$)* Pale: Fresh ($p < 0.001$)* Pale: Sea ($p < 0.001$)*	Dark: Fresh *vs*	Dark: Sea ($p < 0.001$)* Pale: Fresh ($p < 0.001$)* Pale: Sea ($p < 0.001$)*
Dark: Sea *vs*	Pale: Fresh ($p = 0.051$) Pale: Sea ($p = 0.921$)	Dark: Sea *vs*	Pale: Fresh ($p = 0.973$) Pale: Sea ($p = 0.998$)
Pale: Fresh *vs* Pale: Sea ($p = 0.207$)		Pale: Fresh *vs* Pale: Sea ($p = 0.930$)	

- a difference significant to the 5% level

The results for the two DSS indicate a statistically significant difference in the TADS of only the Dark: Fresh group compared to the other three groups. Although the TADS of the Pale: Fresh group are higher

than the Pale: Sea groups (Table 5.31), the post hoc test indicates no significant difference between the TADS of the 2 Pale groups (Table 5.32). Figures 5.33–5.34 are graphical representations of the TADS of the mice in the groups against the PMSI on the days of sampling.

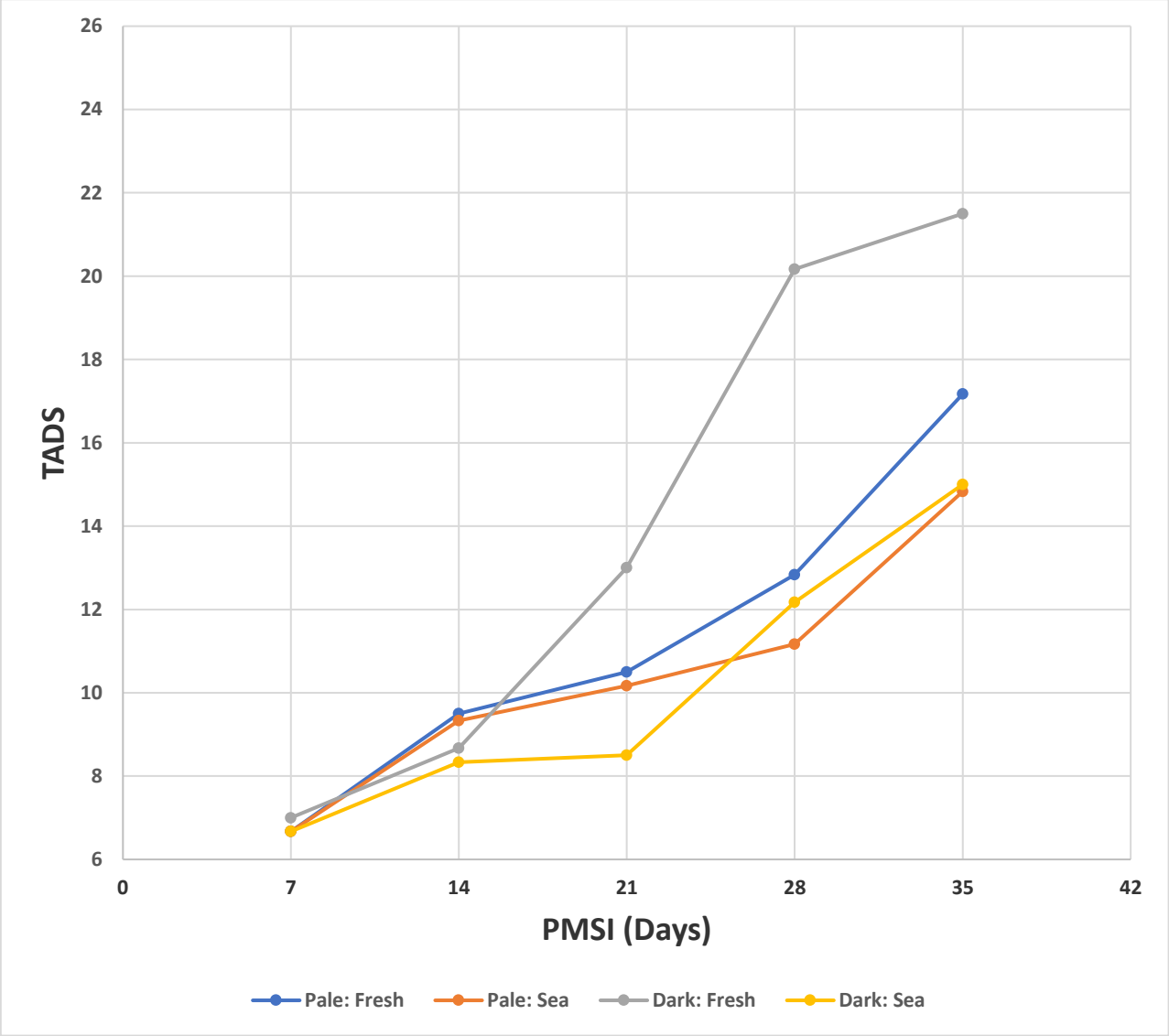


Figure 5.33: Graph of the Average TADS (using Heaton DSS) of the mice against the PMSI of each group.

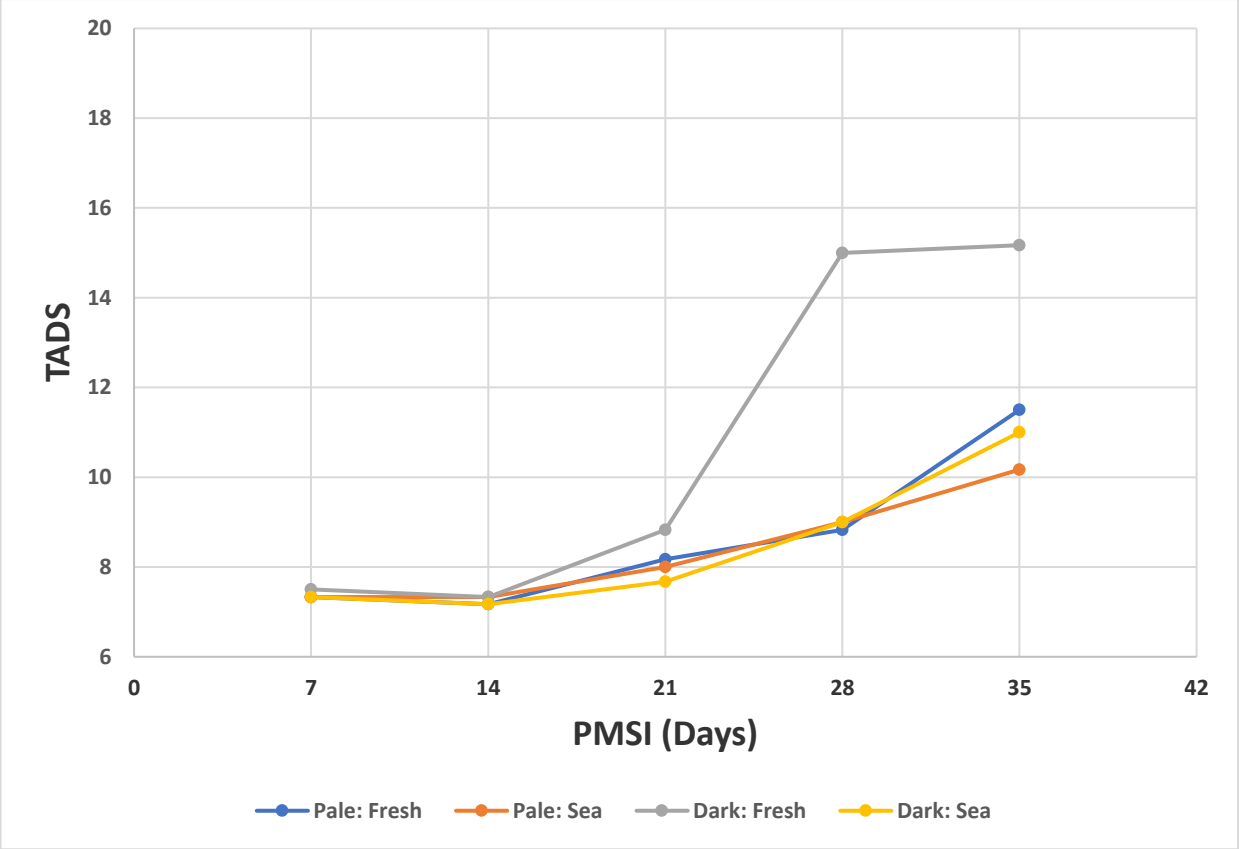


Figure 5.34: Graph of the Average TADS (using van Daalen DSS) of the mice against the PMSI of each group.

5.3.4.2 *DSS Comparison*

A Mann-Whitney U test was needed to compare the TADS of the two DSS statistically, but this would not have been possible because they had different maximum scores (Heaton maximum score = 25; van Daalen maximum score = 18). Therefore, Heaton TADS were divided by 25 and the van Daalen by 18 to normalise both scores to a ratio of 1. Thus, they both had a maximum score of 1, and the Mann-Whitney U test was used to analyse if there was any significant difference in the TADS obtained when using the Heaton and van Daalen DSS to assess the extent of decomposition in each group—[See Appendix 5.3 for statistics]. The results of the test indicate that there was no statistically significant difference between the two DSS across the *Pale: Fresh*, *Pale: Sea* and *Dark: Fresh* groups, but there was a significant difference in the TADS of the *Dark: Sea* group when using the two DSS (Table 5.33).

Table 5.33: Mann-Whitney U test results.

Groups	***p-value***
Pale: Fresh	0.430
Pale: Sea	0.109
Dark: Fresh	0.244
Dark: Sea	0.005*

****- a difference significant to the 1% level***

5.3.4.3 *Temperature & ADD*

Figure 5.35 shows each group's average daily water temperatures on the 35 days of decomposition—[See Appendix 5.4 for the daily temperature values of each group].

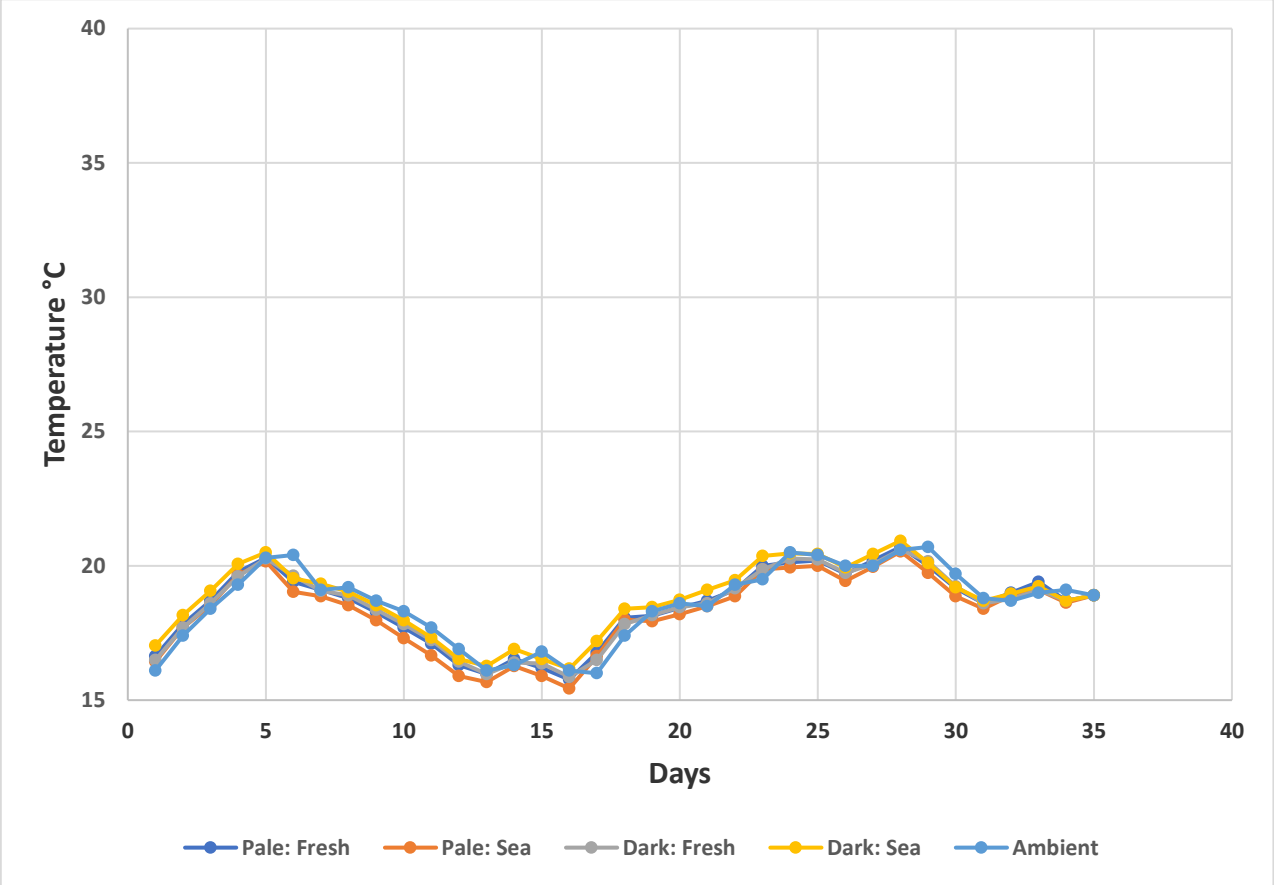


Figure 5.35: Graph showing temperature fluctuation in the water groups across the 35 days.

Table 5.34 shows the ADD of each group on the days of sampling. The lowest mean ADD was 130.4 (*n=3, S.D.=2.91*) in the Pale: Sea group and the highest mean was 656.7 (*n=3, S.D.=3.64*) in the Dark: Sea group. The average daily water temperatures of the boxes in each group were summed up to calculate the ADD values.

Table 5.34: Average ADD and Log ADD of the salinity groups across the 35 days.

PMSI	Pale: Fresh		Pale: Sea		Dark: Fresh		Dark: Sea	
	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD
7	131.7	2.12	130.4	2.12	131.4	2.12	133.7	2.13
14	252.4	2.40	248.7	2.40	252.5	2.40	256.3	2.41
21	374.5	2.57	369.3	2.57	374.3	2.57	380.9	2.58
28	514.5	2.71	507.9	2.71	514.2	2.71	522.9	2.72
35	648.3	2.81	640.4	2.81	647.7	2.81	656.7	2.82

The dark-furred groups had a higher ADD than the pale-furred groups; this could have occurred because darker objects absorb all wavelengths of light, which then get converted into heat, while lighter objects reflect all wavelengths of light, so heat is not absorbed (Massoud, 2005). A Two-way ANOVA test was conducted to investigate if the differences in the ADD of the four groups were statistically significant, but the results showed no statistically significant difference in the daily temperatures of the four groups ($p = 1.000$). Figures 5.36–5.37 are graphical representations of the TADS of the mice in the groups against the average Log ADD on the days of sampling.

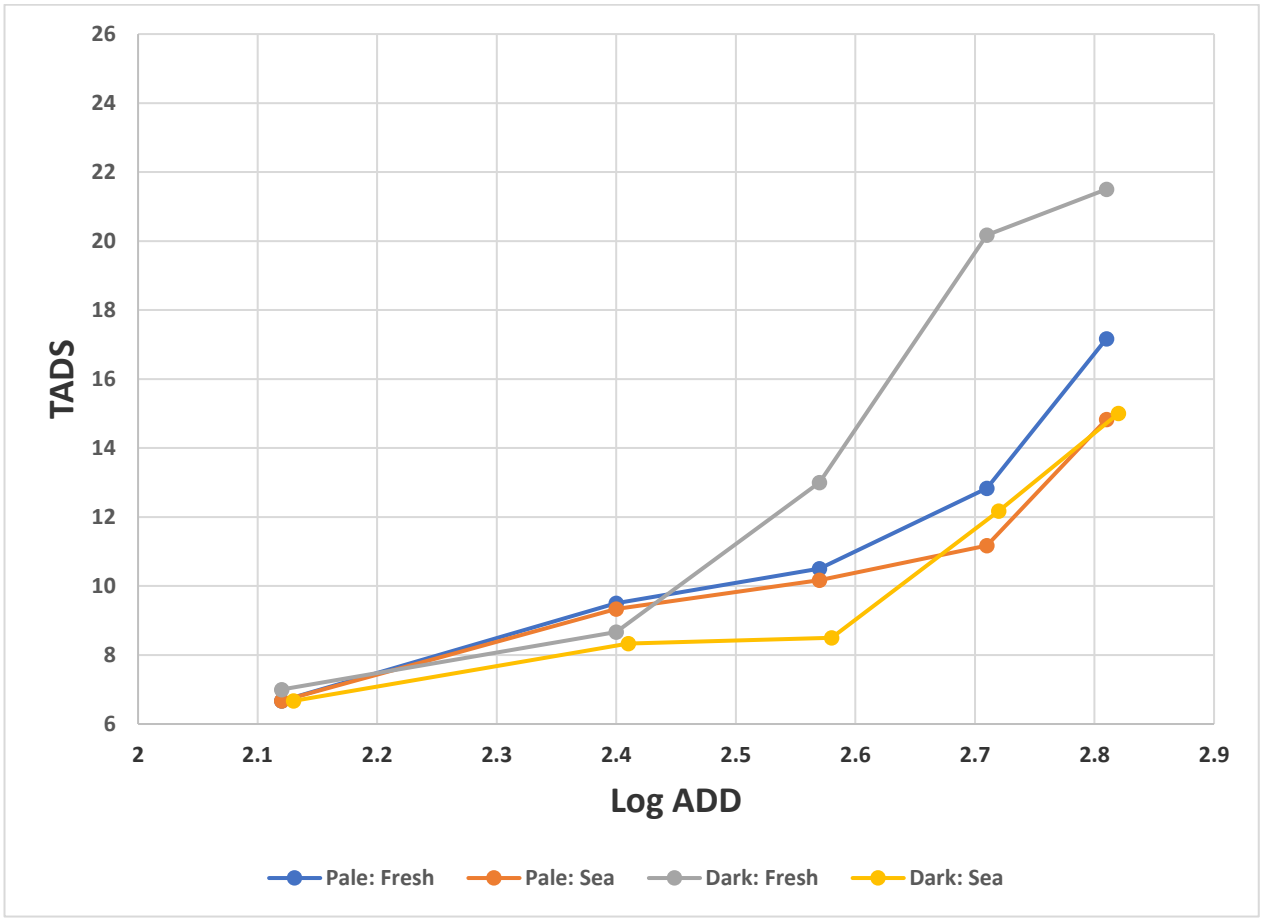


Figure 5.36: Graph of the Average TADS (using Heaton DSS) of the mice against the Log ADD of each group.

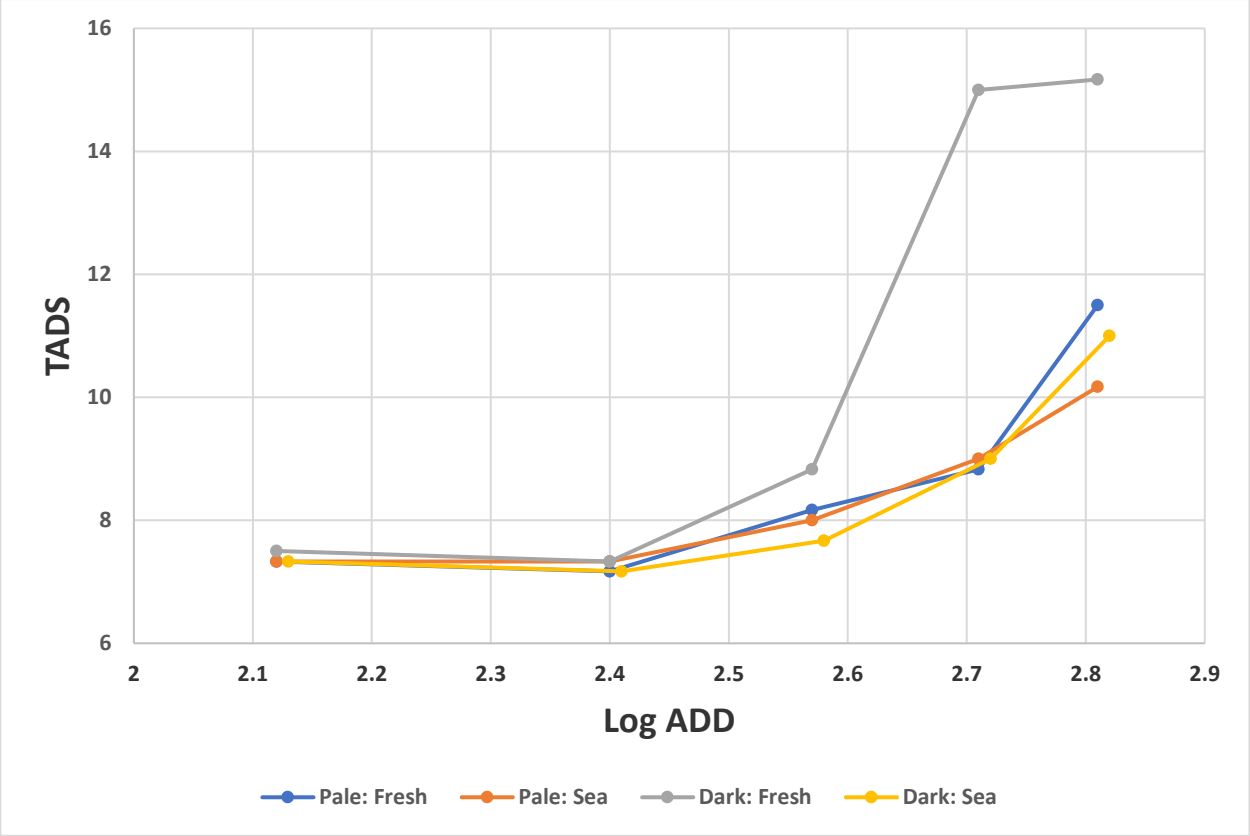


Figure 5.37: Graph of the Average TADS (using van Daalen DSS) of the mice against the Log ADD of each group.

5.3.4.4 *Salinity*

The salt concentration of fresh and seawater is approximately 0.5 ppt and 25–35 ppt, respectively (Duncan Seraphin *et al*., 2014). In this study, the decomposition process and contents did not affect the salinity of the fresh and seawater because the mean salinity of each group across the days of decomposition indicates that the salt concentrations of the water in the boxes were in the estimated range (Table 5.35). The freshwater had a minimum salinity of 0.11 ppt and a maximum of 0.51 ppt. In comparison, the seawater had a minimum concentration of 30.6 ppt and a maximum of 31.6 ppt—[See Appendix 5.5.1 for the salinity concentrations of each group].

Table 5.35: Average salinity concentrations of each group on the sampling days.

Groups	**Average Salinity (ppt) *[n=6]***					
	Days of Decomposition					
	0	**7**	**14**	**21**	**28**	**35**
Pale: Fresh	0.11	0.15	0.20	0.36	0.46	0.51
Pale: Sea	31.4	30.8	31.0	30.6	31.2	30.9
Dark: Fresh	0.11	0.16	0.22	0.36	0.48	0.49
Dark: Sea	31.6	31.2	31.4	31.1	31.4	31.4

5.3.4.5 *pH*

The pH of freshwater ranges between 6.5–8.0, while seawater is primarily alkaline, and the pH ranges between 7.0–8.4 (Chester & Jickells, 2012). Table 5.36 shows the pH level of each group across the decomposition days. Like the salinity concentrations, the pH level of the fresh and seawater used in this study remained in the estimated pH range, and the decomposition process and contents did not affect the pH of the fresh and seawater. The freshwater had a minimum pH of 6.92 and a maximum of 7.52, while the seawater had a minimum pH of 6.91 and a maximum of 7.52—[See Appendix 5.5.2 for the pH levels of each group].

Table 5.36: Average pH levels of each group on the sampling days.

Groups	**Average pH *[n=6]***				
	Days of Decomposition				
	10	**14**	**21**	**28**	**35**
Pale: Fresh	7.03	7.00	6.92	7.07	7.35
Pale: Sea	7.52	7.22	7.01	6.91	7.07
Dark: Fresh	7.00	7.23	7.04	7.17	7.52
Dark: Sea	7.19	7.13	7.00	7.01	7.31

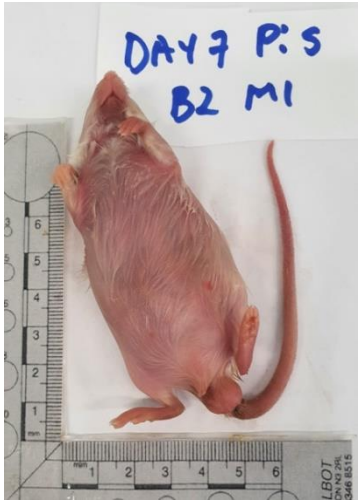
5.3.4.6 *TDS*

TDS concentration of freshwater sources is approximately 0.5 ppt, while seawater ranges from about 15–30 ppt (Moran, 2018). Table 5.37 shows the TDS concentration of each group across the decomposition days. The TDS concentration of the fresh and seawater used in this study remained in the estimated range, and the decomposition process and contents did not affect the TDS concentration. The freshwater had a minimum TDS of 0.13 ppt and a maximum of 0.52 ppt, while the seawater had a minimum of 16.9 ppt and a maximum of 26.5 ppt—[See Appendix 5.5.3 for the TDS concentrations of each group].

Table 5.37: Average TDS concentrations of each group on the sampling days.

Groups	**Average TDS (ppt) *[n=6]***				
	Days of Decomposition				
	10	**14**	**21**	**28**	**35**
Pale: Fresh	0.13	0.21	0.37	0.48	0.52
Pale: Sea	20.5	26.1	25.9	25.9	26.2
Dark: Fresh	0.37	0.22	0.37	0.50	0.51
Dark: Sea	16.9	26.5	26.2	26.4	26.4

5.3.4.7 *Pictorial Representations of Skin Tone and Salinity effect*

Day	7	14	21	28	35
Pale: Fresh					
Pale: Sea					

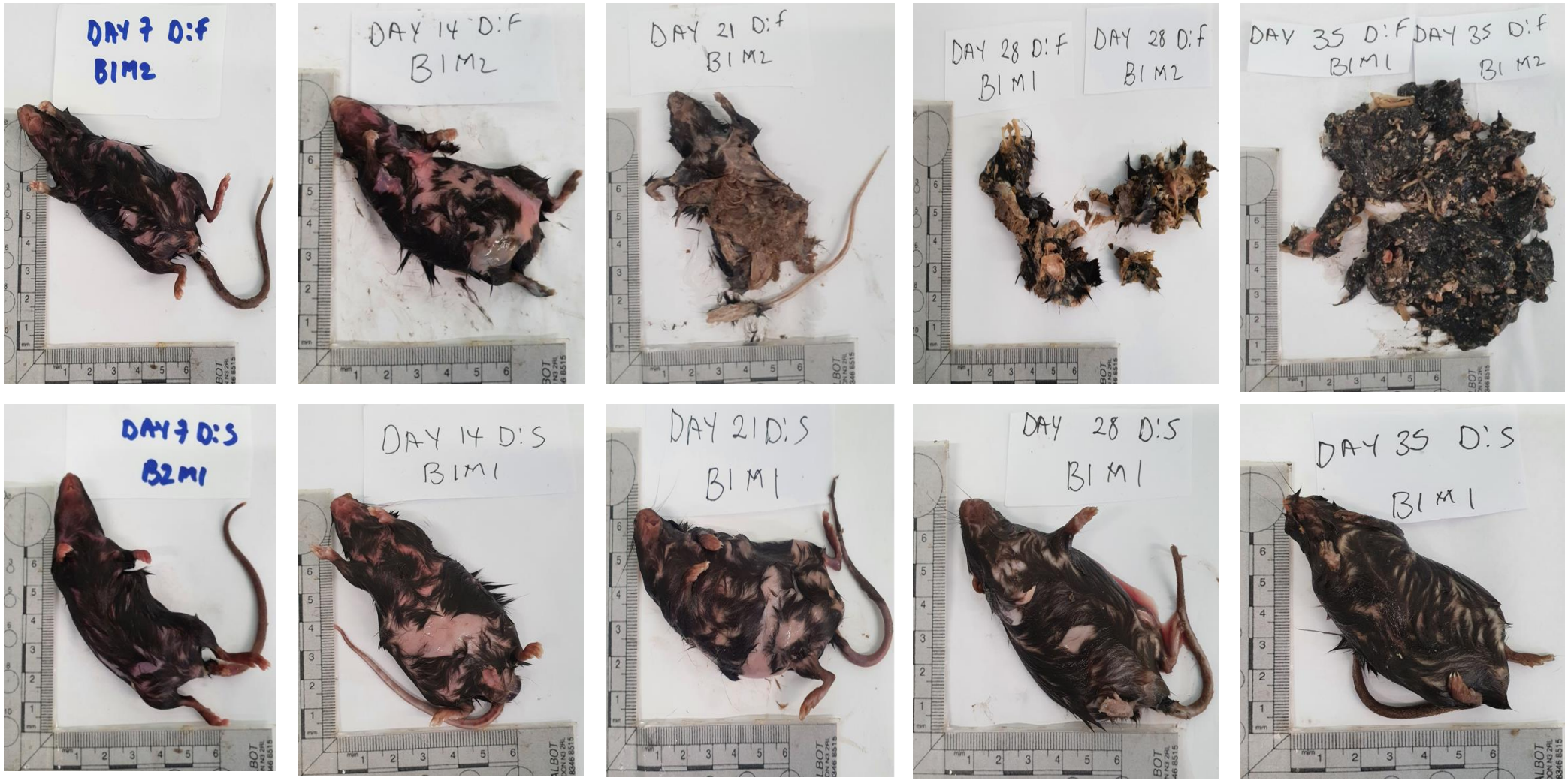


Figure 5.38: Physical decomposition changes in each group across the 35 days.

5.3.4.8 *Discussion*

Decomposition Changes and Rate

See Figure 5.38 for the physical representation of the decomposition changes recorded. At the onset of the study, most of the mice had a fresh appearance, while a few exhibited reddening of the face and neck as the only observable decomposition change. Throughout the study, physical changes were most observable in the mice's trunk, head, limb, and neck regions, while discolouration changes were the most visible in the tail. There was no sign of adipocere formation on any mice during the experiment. Decomposition was faster in the freshwater groups than in the seawater groups, while the dark-furred mice had a higher decomposition rate than the pale-furred mice.

On days 7 and 14, the four groups showed similar decomposition rates and changes; pink discolouration, fur loss and skin slippage in the head, neck, and limb regions, while bloating and dark grey discolouration were observed in the trunk. The decomposition rate and changes started to vary across the groups on day 21, with the freshwater groups being more decomposed than the seawater groups. The *Dark: Fresh* group exhibited the most significant decomposition; liquefaction, extensive skin slippage and soft tissue exposure were observed in the head and limbs, while exposure of soft tissue and advanced internal organ decomposition was observed in the abdomen of the mice. The *Pale: Fresh* and *Pale: Sea* groups showed similar decomposition changes, but the *Pale: Fresh* group had slightly more advanced decomposition changes, such as liquefaction and skin slippage in the abdominal region. The *Dark: Sea* group was the least decomposed group; there were no signs of liquefaction, advanced internal organ decomposition or skin slippage as observed in the other groups.

The same trend in decomposition rate continued in the four groups on days 28 and 35; the *Dark: Fresh* was the most decomposed group, followed by the *Pale: Fresh* and *Pale: Sea* groups, respectively, while the *Dark: Sea* group was the least decomposed group. Extensive soft tissue and bone exposure, severe fur loss and complete disarticulation were the dominant decomposition changes observed in all the body regions of the *Dark: Fresh* group on days 28 and 35. All the mice in the *Pale: Fresh* group exhibited bone exposure in all the body regions. In contrast, most of the *Pale: Sea* and *Dark: Sea* group mice had discolouration, liquefaction and skin slippage as the decomposition changes observed, with only about six mice in these two groups showing bone exposure as the most advanced decomposition change on day 28 and 35.

The mice in the freshwater groups, regardless of their fur colour, were more decomposed than the seawater groups because the high salt content in the seawater groups could have caused the slowed-down decomposition rate in the mice. Bacterial action in the water would have been immensely reduced (Christensen & Myers, 2011; De Donno *et al*., 2014; Reijnen *et al*., 2018). Despite the *Pale: Fresh* group having higher TADS and exhibiting a more advanced extent of decomposition than the *Dark:*

Sea group, the statistics test indicated no significant difference in the TADS of the two groups (Tables 5.31 and 5.32).

It would have been expected that the *Pale: Fresh* group should have a statistically significantly higher TADS than the *Dark: Sea* group because of the high salt content of the *Dark: Sea* group, but this was not the case, probably because of the skin and fur colour of the *Dark: Sea* mice. The *Dark: Sea* group had a higher ADD (656.7) than the *Pale: Fresh* group (648.3); this could have occurred because darker objects absorb all wavelengths of light, which then get converted into heat, while lighter objects reflect all wavelengths of light (Massoud, 2005). This higher ADD in the *Dark: Sea* group could be the reason for the TADS of this group not being significantly lower than that of the *Pale: Fresh* mice because the decomposition rate depends on the temperature (Champaneri, 2006; Megyesi *et al*., 2005). Hence, the decomposition rate in the dark-furred group might have been accelerated because of the absorbed temperature.

DSS Suitability

The adapted Heaton and van Daalen DSS were suitable for scoring the extent of decomposition in the mice. Most of the decomposition changes described in each DSS were observable in the mice, but the Heaton DSS was more suitable for scoring the decomposition changes observed across the various stages of decomposition compared to the van Daalen DSS. After the bloating stage, the van Daalen DSS was not entirely reliable. In the van Daalen DSS, observations such as soft tissue, bone, tendon and muscle exposure, advanced internal organ decomposition and liquefaction were not mentioned. Therefore, in cases where these changes were observed, it was difficult to assign decomposition scores.

Table 5.33 shows the results of the Mann-Whitney U test conducted to investigate any significant difference in the TADS obtained when using the Heaton and van Daalen DSS to assess the extent of decomposition in each group. Only the *Dark: Sea* group differed significantly in the TADS when using the two DSS ($p = 0.005$). This significant difference could have been because of some of the decomposition changes missing in the description of the van Daalen DSS. For example, some mice exhibited changes such as soft tissue exposure and advanced internal organ decomposition, but these changes were not reported in the van Daalen DSS; hence the score with the closest description to these changes was issued.

5.4 Limitations of the Laboratory Studies

The water in the boxes was not changed throughout the study; therefore, the water became murky as decomposition progressed. This obscured the content of the boxes, making it difficult to observe any change occurring in them. The Heaton and van Daalen DSS were designed for human beings; adapting the two systems to be used on the mice is a limitation of the study because the size and composition of the human body and mice are not alike. It was difficult to observe some changes, such as marbling, wrinkling and discolouration in the limbs of the mice because of the size of the mice, their limb morphology, and the presence of fur.

When using the van Daalen DSS, some limitations were encountered because the DSS did not include some decomposition changes observed in the mice. The van Daalen system only considered grey and white colours as discolouration. Despite including skeletonization in the DSS, changes like the disarticulation of body parts and exposure of soft tissues, organs and bones were not noted. Observer bias was also a limitation because only one person assessed the extent of decomposition when using the two DSS; similar results would be expected.

In the laboratory salinity study, there was the presence of a mushroom-like organism found in the Control box (Figure 5.39), the presence of which could have influenced the decomposition rate. It would have helped if DNA or microbial analysis could have been done to identify the species of the organism.

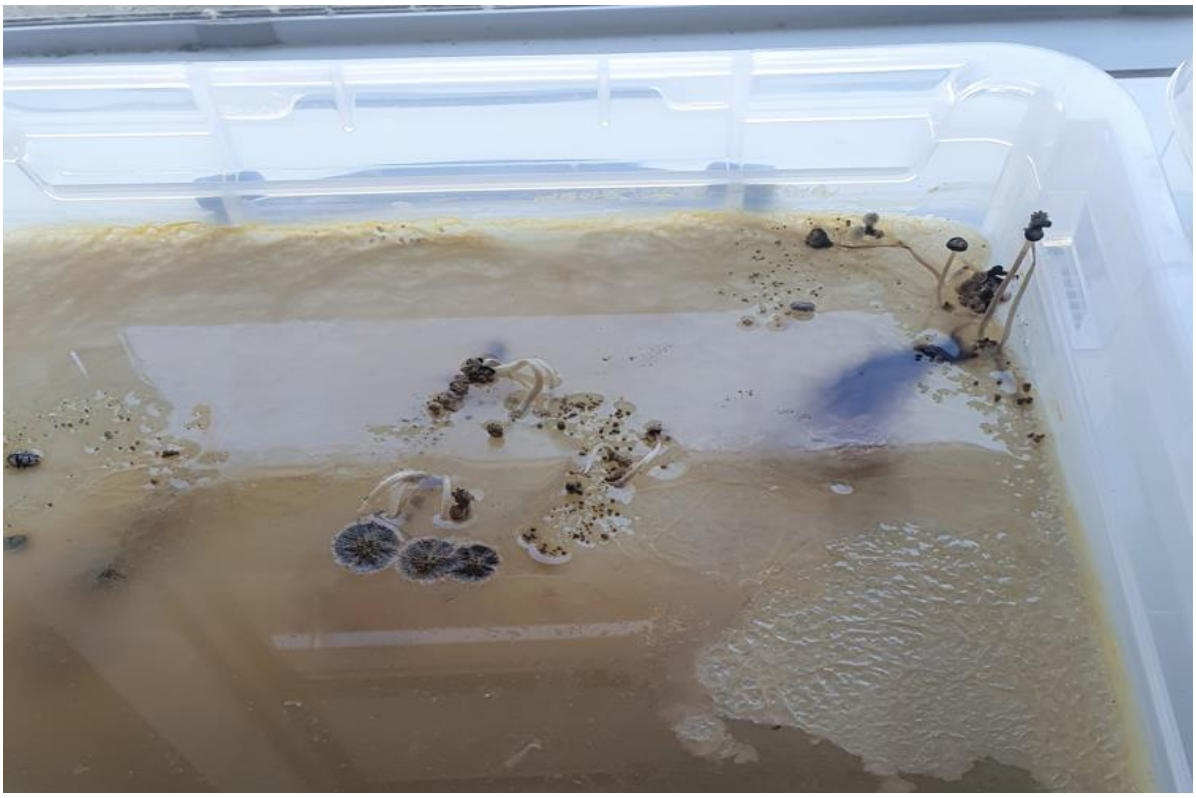


Figure 5.39: Mushroom-like organism found in a freshwater box.

Also, in the skin tone study, the skin tone of the dark mice was not as dark as brown or black, which would have been ideal for this study (Figure 5.40). It was mainly the fur of the dark mice that was black, and the skin of the mice was not black; instead, it was reddish than the pale mice. This reddish colour of the dark-furred mice does not represent the skin colour of dark-skinned individuals. For future studies, mice with dark skin tones, such as brown or black, will be more suitable.



(a) *(b)*

Figure 5.40: Pictures of the skin tone of the two types of mice used. a- pale mice; b- dark mice.

5.5 Conclusions of the Laboratory Studies

This study demonstrated that water salinity, hardness and current significantly affect the decomposition rate of mice submerged in water. It also indicated that decomposition was faster in dark-furred mice compared to pale-furred mice. Key results include:

- **Laboratory salinity experiment**: Control *vs* Brackish ($p = 0.029$); Sea ($p = 0.003$); Hypersaline ($p < 0.001$).
- **Laboratory hardness study:** Soft Water *vs* Moderately Hard Water ($p < 0.001$); Hard Water ($p < 0.001$); Very Hard Water ($p < 0.001$).
- **Laboratory current study:** Moving *vs* Submerged groups ($p < 0.001$); Floating ($p < 0.001$).
- **Laboratory skin tone study:** Dark: Fresh *vs* Dark: Sea ($p < 0.001$); Pale: Fresh ($p < 0.001$); Pale: Sea ($p < 0.001$).

The salinity studies demonstrated that the decomposition rate of mice is significantly different in varying water salinity concentrations (*fresh, brackish, sea and hypersaline water*). It indicates that the decomposition rate is slower in saline water (*hypersaline, sea and brackish*) compared to freshwater; the highest TADS occurred in the low saline groups. These results support the general conclusion that decomposition is slower in high-salt water than in low-salt water (Boyle *et al*., 1997). Water salinity should always be considered when estimating the PMSI of bodies recovered from seawater and marine environments.

In the hardness study, decomposition was fastest in the soft water group and slowed down in the other hard water groups. Stable salts are formed in hard water; it is possible that the salts formed could be the reason for the slowed-down decomposition rate in these hard water groups because bacterial activities become immensely reduced in the presence of salt concentrations (De Donno *et al*., 2014; Sengupta, 2013). Therefore, extra care must be taken when estimating the PMSI of victims recovered from water bodies containing rocks made up of chalk and limestone or other water bodies that get their source from underground aquifers. This is because rocks and aquifers are made up of soluble minerals (such as calcium) that contribute to water hardness.

The current study demonstrated that a moving or floating body decomposes faster than a body left wholly submerged and immobile. Under the same weather conditions, mice left to float decomposed at a significantly faster rate than mice that were completely submerged ($p < 0.001$), while mice moving under the influence of a turbine decomposed at a significantly faster rate than mice completely submerged ($p < 0.001$). The exposure of the Floating and Moving groups to air when the mice floated to the surface of the water could be why decomposition was faster in this group because decomposition is faster in the air than in water (Heaton *et al*., 2010). However, more research must be carried out on this subject to investigate the effect of the current on the decomposition rate. Future research would benefit from using a turbine system to generate higher speed and power to initiate the current flow in

the boxes instead of leaving the mice to float. This automated speed could be chosen to simulate natural currents.

The skin tone study demonstrated that the adapted Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS are suitable for assessing the extent of decomposition in mice with varying fur and skin tones, but the Heaton *et al*. (2010) DSS is more suitable than the van Daalen *et al*. (2017) DSS once the bloating stage of decomposition has been reached. After bloating, decomposition changes such as a collapsed abdomen, soft tissue exposure, liquefaction and advanced internal organ decomposition are expected to be observed in a decomposing body, but the van Daalen DSS does not mention these changes.

This study also demonstrated that a mouse's darker skin or fur tone increases the ADD of the mouse because darker objects tend to absorb all wavelengths of light, which then get converted into heat; this could, in turn, increase the decomposition rate. Despite being placed in the same environment, the dark-furred mice (Dark: Sea group) had a higher ADD [656.7] than the pale-furred mice (Pale: Fresh group) [648.3], but this difference was not statistically different ($p = 1.000$). Extra care should be taken when using these DSS in estimating the PMSI of victims' bodies that have reached or passed the bloating stage or in individuals with darker skin tones because their bodies might absorb more heat. This research proves that there is an essential need to improve the suitability of these DSS for scoring the extent of decomposition in darker-skin-toned individuals.

6 PART TWO: FIELD STUDIES ON SALINITY CONCENTRATION

6.1 Introduction

The salinity of the water a body is submerged in plays a significant role in altering the decomposition rate of that body (Christensen & Myers, 2011; Reijnen *et al*., 2018). The effect of salinity on the decomposition rate has already been investigated in this research using mice as human analogues in controlled laboratory conditions. The laboratory salinity studies demonstrated that the decomposition rate of mice is statistically significantly different in various water salinity concentrations (fresh, brackish, sea and hypersaline water). It indicated that the decomposition rate was slower in saline water (hypersaline, sea and brackish; [$p < 0.05$]) than in freshwater. These results support the general conclusion that decomposition is slower in high-salt water than in low-salt water and that the salinity of water should always be considered when estimating the PMSI of bodies recovered from seawater, estuaries, and marine environments (Boyle *et al*., 1997).

To compare the results of the laboratory salinity studies for mice with larger human analogues, pigs were used for this research section. It must be noted that the mice in the laboratory study were placed in a box with the same water content for the entire study. In a real-life scenario, a body deposited in water is moved around the water or exposed to flowing water even when completely submerged or immobile (Byard *et al*., 2008). This means that the body deposited is exposed to a larger volume of water with changing or varying salinity concentrations compared to the mice in the laboratory study exposed to constant salinity concentrations and a smaller volume of water. Also, the flowing water in the real-life scenario means the body deposited may be dragged for a distance, creating disarticulation and marks or discolouration that can be mistaken for decomposition changes (Humphreys *et al*., 2013; Palmer, 2020; Stuart & Ueland, 2017).

This potential variation in salinity concentrations in real-life cases leaves a gap in knowledge on whether changing the salinity concentration of water affects or alters the decomposition rate of a body deposited in the water. Therefore, empirical studies need to be conducted to investigate this phenomenon. These field studies were conducted in an attempt to address this gap in knowledge by carrying out two sets of studies with pigs placed in water that had:

1. Fixed water salinity concentration for the entire study.
2. Fixed salinity concentrations refreshed weekly.

6.1.1 Aims

This study aimed to:

1. Investigate the effect of water salinity concentration on the decomposition rate of these pigs.

H_1 = There is a significant difference in the pigs' decomposition rate (TADS) deposited in the fresh, brackish and seawater types.

H_0 = There is no significant difference in the pigs' decomposition rate (TADS) deposited in the fresh, brackish and seawater types.

2. Investigate if there is a statistically significant difference between the extent of decomposition (TADS) of human analogues (pigs) deposited in constant water for the entirety of the study and the ones deposited in water changed weekly.

H_1 = There is a significant difference between the decomposition rate (TADS) of the pigs deposited in constant water and those in changing water.

H_0 = There is no significant difference between the decomposition rate (TADS) of the pigs deposited in constant water and those in changing water.

3. Investigate if there is a statistically significant difference in the TADS of the pigs when comparing the Heaton and van Daalen DSS.

H_1 = There is a significant difference in the TADS of the mice when comparing the Heaton and van Daalen DSS.

H_0 = There is no significant difference in the TADS of the mice when comparing the Heaton and van Daalen DSS.

6.1.2 Objectives

To carry out two field studies using pigs as human analogues to investigate the effect of fixed water salinity levels and the effect of refreshing the salt/fresh water weekly on the decomposition rate of pigs deposited in water for 70 days. This research would have important implications for understanding the effect of changing water salinity concentrations on decomposition and PMSI estimation.

6.2 Methodology

6.2.1 Breakdown of Field Studies

Two field studies were conducted at the UCLan TRACES Taphonomy Facility in Lancashire, United Kingdom. These studies used a total of 35 dead domestic pigs (*Sus scrofa*). Ethical approval [Ref 21/04] was granted by the UCLan Animal Welfare Ethical Review Board (AWERB) committee. Pigs were provided dead (the cause of death was a bolt to the frontal bone) from a local supplier, and the PMI was less than 2 hours. Pigs were chosen as the human analogue for these field studies because pigs and humans have various similarities such as size, physiology, anatomy, and metabolic profile (Gutierrez *et al*., 2015). In terms of skin thickness and hair spacing, pig and human skin are similar, while the renal and digestive system of pigs are similar to that of humans making them a viable option for medical studies (Dalgaard, 2014; Sangild *et al*., 2014). The first experiment was the "Initial Salinity" study which investigated the effect of water salinity concentration on the decomposition rate of pigs; it was conducted between July 14 and September 22, 2021. The second experiment was the "Changing Salinity Concentration" study which investigated if there was a statistically significant difference between the extent of decomposition (TADS) of human analogues (pigs) deposited in constant, unchanging water for the entirety of the study and one deposited in water which was refreshed weekly; it was carried out between June 8 and August 17, 2022. It is important to note that when words such as "seawater" and "brackish water" are mentioned, they denote simulated seawater and brackish water not actual seawater or brackish water that contain other microorganisms in the water.

6.2.2 Initial Salinity Study

6.2.2.1 *Group Descriptions*

Fifteen pigs (8.8 Kg – 14.8 Kg, mean = 11.8 Kg, S.D. = 1.7) were used for this study. The pigs were divided into three groups (5 pigs per group); *Control, Brackish and Seawater* (Table 6.1). Fifteen 84 L plastic lidded boxes (Argos Home®, U.K.) were filled with 60 L of appropriately modified water and placed outdoors in a field setting (Figure 6.1). Each pig was placed on a plastic butterfly netting mesh that lay underwater and extended out of each box (Figure 6.2). The box lid was then clipped into place on top of the box, and heavy stones were used to weigh it down to prevent water leakage or water ingress from rain. These boxes were not fully sealed despite placing stones on the lids; therefore, insects gained access to the pigs in the boxes.

Table 6.1: Breakdown of the groups (5 pigs per group) for the Initial salinity study.

S/N	Groups	Box Content and Description
1	Fresh (< 1.0 ppt)	Pigs placed in freshwater
2	Brackish (~20 ppt)	Pigs placed in brackish water
3	Sea (~35 ppt)	Pigs placed in seawater

6.2.2.2 *Salt Concentration*

Based on the recommended standard water concentrations for salinity (Duncan Seraphin *et al*., 2014), 1.2 Kg of Ocean Reef Pro Coral Salt (iQuatics®, U.K.) was dissolved in 60 L of water to create brackish water (~20 ppt) while 2.1 Kg was dissolved in 60 L water to create seawater (~35 ppt). Tap water was used for the freshwater group and the details of the water properties of the tap water can be found in Table 5.3. The salt was dissolved by stirring the water in the box until there was no trace of salt particles or any settling of salt.



Figure 6.1: Argos boxes placed outdoors in a field setting for the Initial salinity study.



Figure 6.2: Pictures of the mesh, data logger and pig in the box for the Initial salinity.

6.2.2.3 *Temperature & ADD*

A data logger (EL-USB-3, EasyLog®) was placed in a 50 mL conical-bottom centrifuge tube (Cole-Parmer®, U.K.) and further stored in a zip-lock bag. The bag was then placed underwater in each box to record the water temperature hourly. The ADD for the sampling days was calculated by summing the average daily water temperatures for the days that made up the respective PMSIs. For example, if the average temperature of the first seven days of decomposition were 10 ℃, 5 ℃, 10 ℃, 7 ℃, 10 ℃, 8 ℃, and 20 ℃, then the ADD for the PMSI of 7 days was:

10 + 5 + 10 + 7 + 10 + 8 + 20 = 70 ADD.

6.2.2.4 *Measuring Decomposition*

The adapted Heaton and van Daalen DSS were used to score the extent of decomposition in the pigs on days 7, 14, 21, 28, 35, 42, 48, 56, 62 and 70 of decomposition in the initial-salinity study. Examples of how these adapted DSS were applied in scoring the pigs are illustrated in Figures 6.3 and 6.4.



Heaton DSS	**van Daalen DSS**
FADS- 2; Marbling and discolouration	FADS- 2; Marbling and discolouration
BADS- 2; Discolouration	BADS- 2; Discolouration
LADS- 2; Discolouration	LADS- 2; Discolouration
TADS = 6	**TADS = 6**

Figure 6.3: Illustration of scoring a pig with a low extent of decomposition using the two adapted DSS.



Heaton DSS	van Daalen DSS
FADS- 8; Complete Skeletonization	FADS- 6; Complete Skeletonization
BADS- 7; Partial Skeletonization	BADS- 5; Partial Skeletonization
LADS- 9; Complete Skeletonization	LADS- 6; Complete Skeletonization
TADS = 24	**TADS = 17**

Figure 6.4: Illustration of scoring a pig with an advanced extent of decomposition using the two adapted DSS.

The lids of the boxes were taken off to assess the extent of decomposition in the pigs, and in some cases, when the water in the boxes was too murky to see through, the pigs were brought out of the boxes to assess the extent of decomposition. It is possible that flies and insects may have gained access to the pigs when measuring decomposition.

6.2.3 Changing Salinity Study

6.2.3.1 *Group Descriptions*

Twenty pigs (9.6 Kg – 17.0 Kg, mean = 13.0 Kg, S.D. = 2.2) were used for this study. The pigs were divided into four groups (5 pigs per group); *Sea-Constant*, *Fresh-Constant*, *Sea-Changing* and *Fresh-Changing* (Table 6.2). Thirty 85 L boxes (Really Useful Boxes®, U.K.) placed on wooden pallets outdoors at TRACES were used for the study (Figures 6.5–6.6). At the onset of the study, twenty of these boxes were filled with 60 L of appropriately modified water, while ten boxes were left empty. At seven-day intervals, the pigs in the *Sea-Changing* and *Fresh-Changing* groups were transferred into the empty boxes, which, by this time, had been filled with 60 L of the appropriately modified water for each group. Each pig was placed on a mesh that lay underwater and extended out of each box (see Figure 6.6). The box lid was clipped into place on top of the box to prevent insects, water leakage and water ingress from rain. These boxes were not fully sealed despite the clipped lids; therefore, insects gained access to the pigs in the boxes.

Table 6.2: Breakdown of the groups (5 pigs per group) for the Changing salinity study.

S/N	Groups	Box Content and Description
1	Sea-Constant (~35 ppt)	Pigs placed in the same seawater for the entire study
2	Fresh-Constant (< 1.0 ppt)	Pigs placed in the same freshwater for the entire study
3	Sea-Changing (~35 ppt)	Pigs placed in seawater that was renewed weekly
4	Fresh-Changing (< 1.0 ppt)	Pigs placed in freshwater that was renewed weekly



Figure 6.5: 85 L Really Useful boxes placed outside on the pallets for the Changing salinity study.



(a)- Pigs before being submerged



(b)- Boxes being filled with water.



(c)- Data loggers with Pigs submerged and held in place with mesh.



(d)- Overview of the field

Figure 6.6: Set-up procedures for the Changing salinity study.

6.2.3.2 *Salt-Water Concentration*

Based on the recommended standard water concentrations for salinity (Duncan Seraphin *et al*., 2014), 2.1 Kg was dissolved in 60 L water to create seawater (~35 ppt), and tap water was used as freshwater (~0.5 ppt). The salt was dissolved by stirring the water in the box till there was no trace of salt particles.

6.2.3.3 *Temperature & ADD*

A data logger (EL-USB-3, EasyLog®) was placed in a 50 mL conical-bottom centrifuge tube (Cole-Parmer®, U.K.) and further stored in a zip-lock bag. The bag was then placed underwater in each box to record the water temperature hourly. The ADD for the sampling days was calculated by summing the average daily water temperatures for the days that made up the respective PMSIs.

6.2.3.4 *Measuring Water Properties*

Additional water properties were measured in the changing-salinity study. The pH of the water in the boxes was measured with a digital pH meter (Eletorot®, U.K.), while the salinity and TDS concentrations were measured with a pen-type Salinity/TDS/Temperature meter (GAIN EXPRESS®, U.K.). On the sampling days, the salt content, pH level and Total dissolved solids (TDS) concentration of the water in each box were measured and recorded twice; each meter probe was placed in the water and measurements were taken from the left and right edges of the boxes. These water properties were measured to monitor if they were affected by the decomposition process or accumulation of by-products in the water. In the *Sea-Changing* and *Fresh-Changing* groups, the three water properties were measured before taking out the pigs from the boxes with the previous week's water content and measured before placing the pigs in the boxes with the replenished modified water.

6.2.3.5 *Measuring Decomposition*

In the changing-salinity study, the adapted Heaton and van Daalen DSS were used to score the extent of decomposition in the pigs on days 7, 14, 20, 27, 35, 41, 49, 56, 63 and 70 after submersion.

6.2.4 Statistical Analysis

All statistical analyses and plots were conducted using Microsoft Excel and SPSS software [version 28, SPSS Inc.]. ANOVA tests and Tukey's Post Hoc tests (parametric) were used when the data were normally distributed, but the Mann-Whitney U, Friedman, and Wilcoxon tests (non-parametric) were used for data that were not normally distributed (Hopkins *et al*., 2018; Derrick *et al*., 2020). Two-way ANOVA and Friedman tests were done to investigate a significant difference in the TADS between the salinity groups on the sampling days as a function of time (Hopkins *et al*., 2018). Post Hoc tests: Tukey's and Wilcoxon tests were conducted to figure out the specific TADS that significantly differed by comparing the mean of each group with the mean of the other groups (Pereira *et al*., 2015). In contrast, the Mann-Whitney U test was used to investigate a significant difference in each pig's TADS (Heaton vs van Daalen DSS).

6.3 Results

6.3.1 Initial-Salinity Study

6.3.1.1 *TADS*

Table 6.3 shows a breakdown of the average TADS of the pigs; they were scored with the Heaton and van Daalen DSS. After a PMSI of 1 day, the lowest mean TADS recorded was 13.4 (*n=5, S.D.=0.9*) and the highest recorded mean was 24.8 (*n=5, S.D.=0.5*) when using the Heaton DSS. The lowest mean TADS recorded was 10.4 (*n=5, S.D.=1.1*) and the highest recorded mean was 17.8 (*n=5, S.D.=0.4*) when using the van Daalen DSS. The Control group had the highest set of TAD scores, while the Sea group had the lowest—[See Appendix 6.1.1 for TADS of all the pigs].

Table 6.3: Average TADS of the pigs [Possible Maximum Score: Heaton=25, van Daalen=18].

DSS		**Average TADS (*n*=5)**					
Groups		**Heaton**			**van Daalen**		
		Fresh	**Brackish**	**Sea**	**Fresh**	**Brackish**	**Sea**
PMSI (Days)	**0**	6.8	5.8	6.4	7.2	6.4	7.2
	7	14.6	13.6	13.4	11.6	10.4	10.4
	14	21.4	18.0	19.0	15.0	13.6	13.4
	21	22.0	20.0	19.8	15.4	14.6	14.6
	28	22.6	20.4	20.8	15.8	14.8	14.8
	35	23.2	21.6	21.4	16.2	15.4	15
	42	24.0	22.2	22.4	16.8	15.8	15.6
	48	24.2	22.6	22.8	17.0	16.2	16.2
	56	24.6	23.4	23.2	17.6	16.6	16.6
	62	24.8	23.8	23.2	17.8	16.6	16.6
	70	24.8	23.8	23.6	17.8	16.8	16.8

Two separate two-way ANOVA tests were conducted to investigate any statistical difference in the effect of the three varying water salinity groups on the TAD scores of the pigs across the ten sampling days—[See Appendix 6.2.1 for statistics]. The first test was carried out on the TADS obtained with the Heaton DSS, while the second was for the van Daalen DSS. The ANOVA results showed a statistically significant difference in the TADS across the three groups (Heaton: $r^2 = 0.74, p < 0.001$; van Daalen: $r^2 = 0.62, p < 0.001$). Since $p < 0.05$, the null hypothesis was rejected. Hence, it was concluded that there is a significant difference in the decomposition rate of human analogues (pigs) between the fresh, brackish and seawater types.

A Tukey's Post Hoc test was done to further investigate the specific statistical difference (*p-value*) of the TADS of each group compared to the other two groups. For the Heaton DSS, there was a statistically significant difference between the TADS of the Control group and the other two groups: Brackish ($p = < 0.001$) and Sea ($p < 0.001$), but there was no significant difference between the TADS of the Brackish and Sea groups ($p = 0.999$). For the van Daalen DSS, there was a statistically significant difference between the TADS of the Control group and the other two groups: Brackish ($p = 0.006$) and Sea ($p = 0.003$), but there was no significant difference between the TADS of the Brackish and Sea groups ($p = 0.968$). Figures 6.7–6.8 are graphical representations of the TADS of the pigs in the groups against the PMSI on each sampling day in the study.

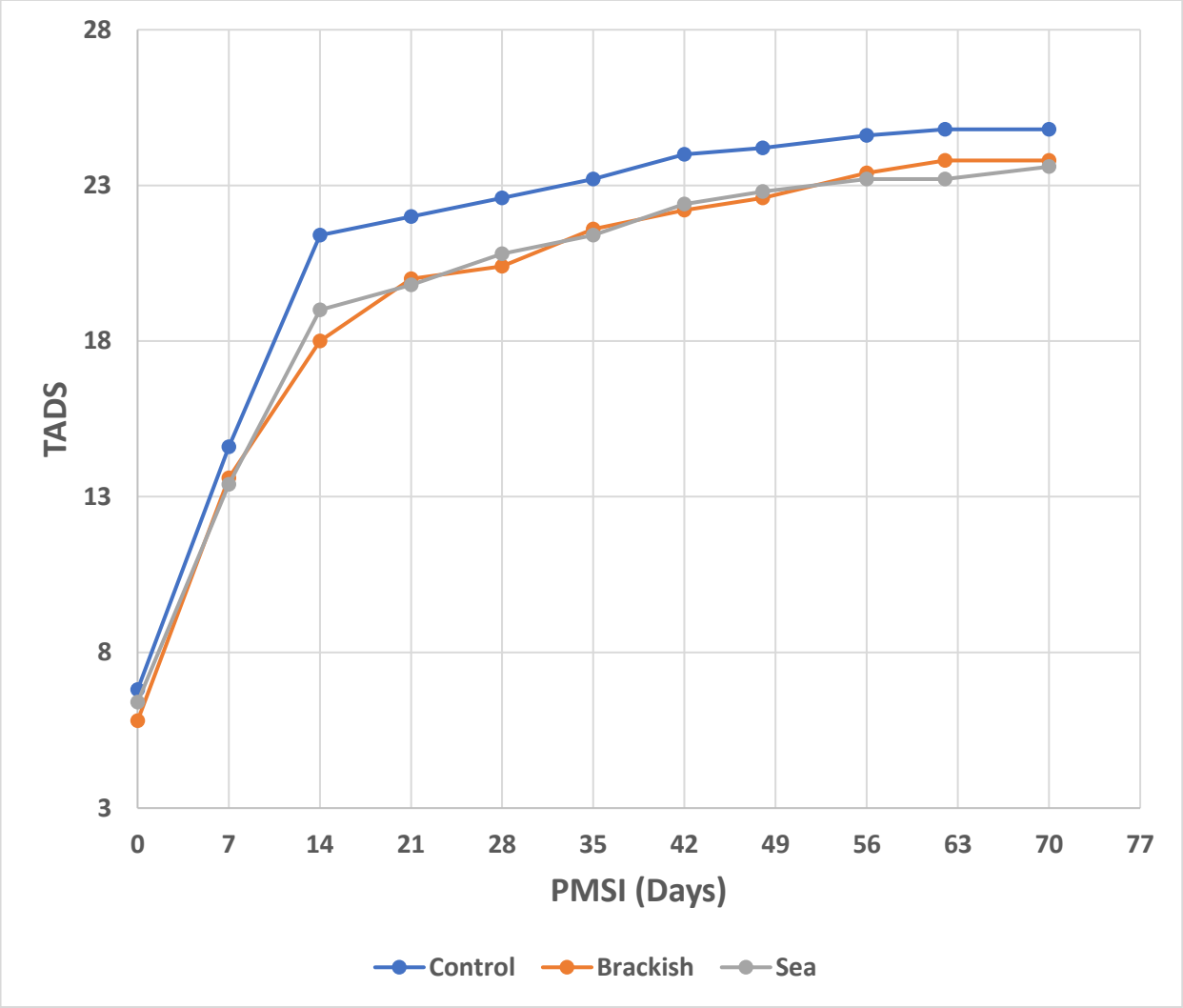


Figure 6.7: Graph of the TADS (using Heaton DSS) of the pigs against the PMSI of each salinity group.

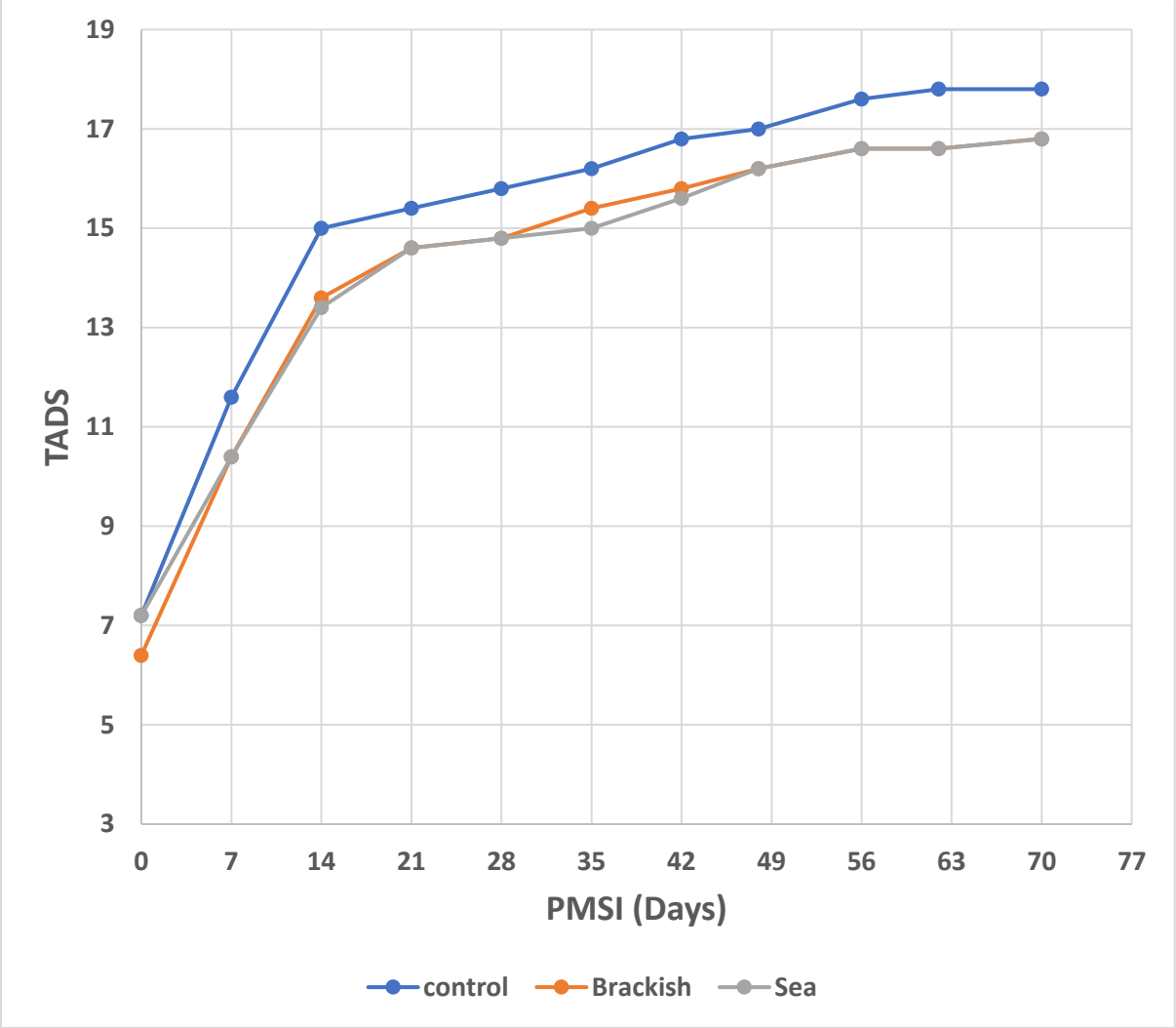


Figure 6.8: Graph of the pigs' TADS (using van Daalen DSS) against the PMSI of each salinity group.

6.3.1.2 *DSS Comparisons*

The ANOVA and Tukey's tests indicated that the Heaton and van Daalen DSS had identical results since both TADS obtained had statistically significant differences across the three salinity groups. An Independent Sample t-test was needed to compare the two DSS statistically, but this would not have been possible because they had different maximum scores (Heaton = 25; van Daalen = 18). Therefore, Heaton TADS were divided by 25 and the van Daalen by 18 to normalise both scores to a ratio of 1. Thus, they both had a maximum score of 1. The t-test was carried out between the TADS of the two DSS for each group—[See Appendix 6.3.1 for statistics]. There was no statistically significant difference between the two DSS across the three groups: Control ($p = 0.853$), Brackish ($p = 0.617$) and Sea ($p = 0.746$). Hence, the Heaton and van Daalen DSS had very similar results and were both suitable for the study.

6.3.1.3 *Temperature & ADD*

Figure 6.9 shows the average daily temperatures of the water in each group across the 70 days of decomposition—[See Appendix 6.4.1 for the daily temperature values of each group]. The average daily water temperatures of the boxes in each group were summed up to calculate the ADD values (Table 6.4). The lowest ADD was 186.2 (*n=3, S.D.=3.4*), and the highest was 1407.6 (*n=3, S.D.=13.7*). Figures 6.10–6.11 are graphical representations of the TADS of the pigs in the groups against the average Log ADD across the 70 days of decomposition.

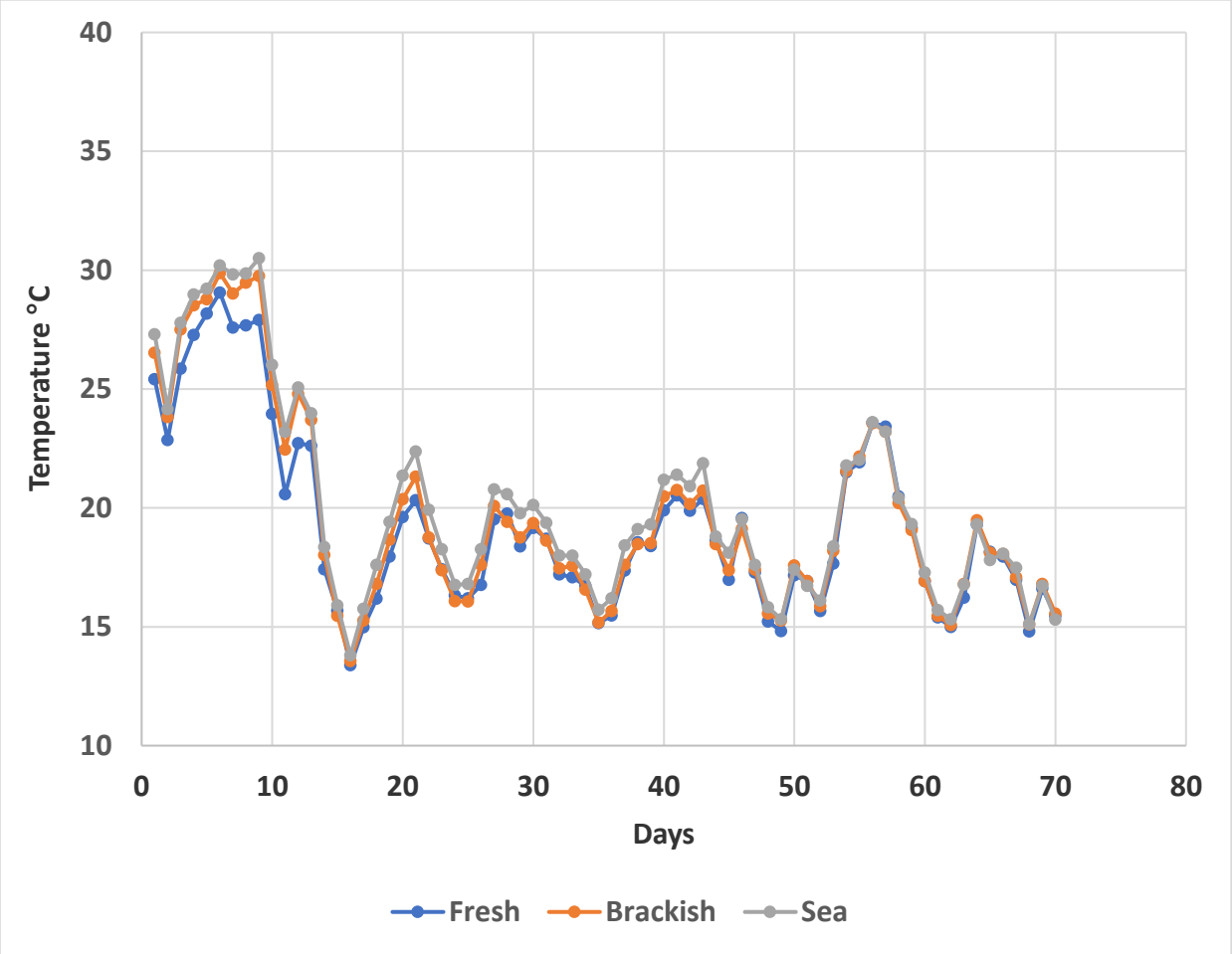


Figure 6.9: Graph showing temperature fluctuation in the water groups across the 70 days.

The temperature values recorded in the first ten days of this study occurred during a heat wave in the U.K. Despite the increase in average sea and river temperatures because of climate change, the temperature values observed in this study exceed the current average water temperatures observed in ideal real-life scenarios; the average sea and river temperatures in the U.K. range between 6–20°C (Tinker *et al*., 2020). The high water temperatures in this study are akin to rare occurrences of hot water drownings in baths or showers (Nishiyama *et al*., 2011; Suzuki *et al*., 2017; Suzuki *et al*., 2019).

Table 6.4: ADD and Log ADD of the three salinity groups across the 70 days of decomposition.

PMSI (Days)	Control		Brackish		Sea	
	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD
7	186.2	2.27	194.0	2.29	197.5	2.30
14	349.1	2.54	367.4	2.57	374.4	2.57
21	467.2	2.67	488.9	2.69	500.7	2.70
28	591.9	2.77	614.2	2.79	632.0	2.80
35	714.3	2.85	737.7	2.87	760.2	2.88
42	844.4	2.93	869.4	2.94	896.7	2.95
48	952.5	2.98	978.1	2.99	1008.5	3.00
56	1101.7	3.04	1129.2	3.05	1159.8	3.06
62	1212.1	3.08	1239.1	3.09	1271.0	3.10
70	1347.6	3.13	1376.1	3.14	1407.6	3.15

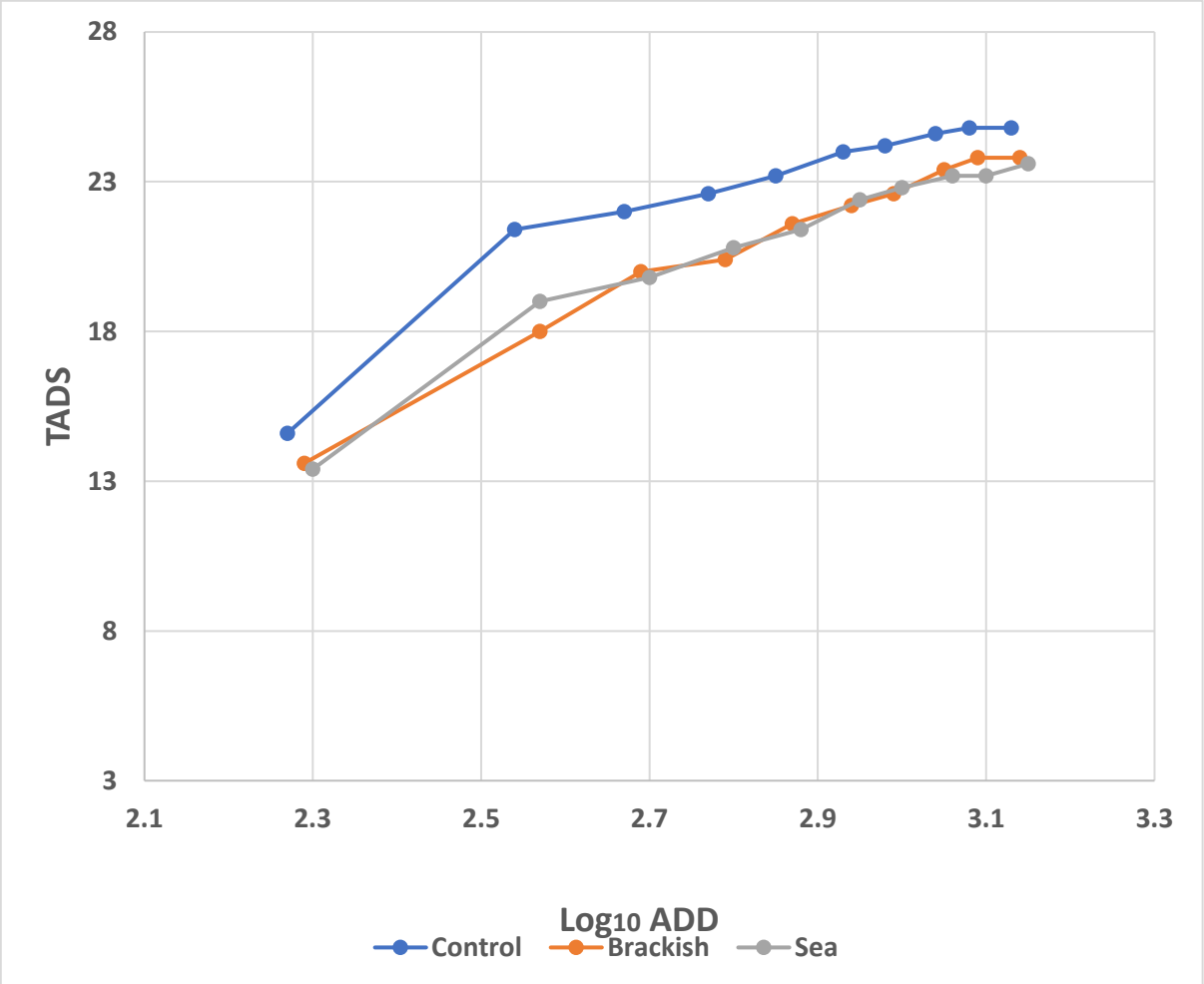


Figure 6.10: Graph of the TADS (using Heaton DSS) of the pigs against the Log ADD of each salinity group.

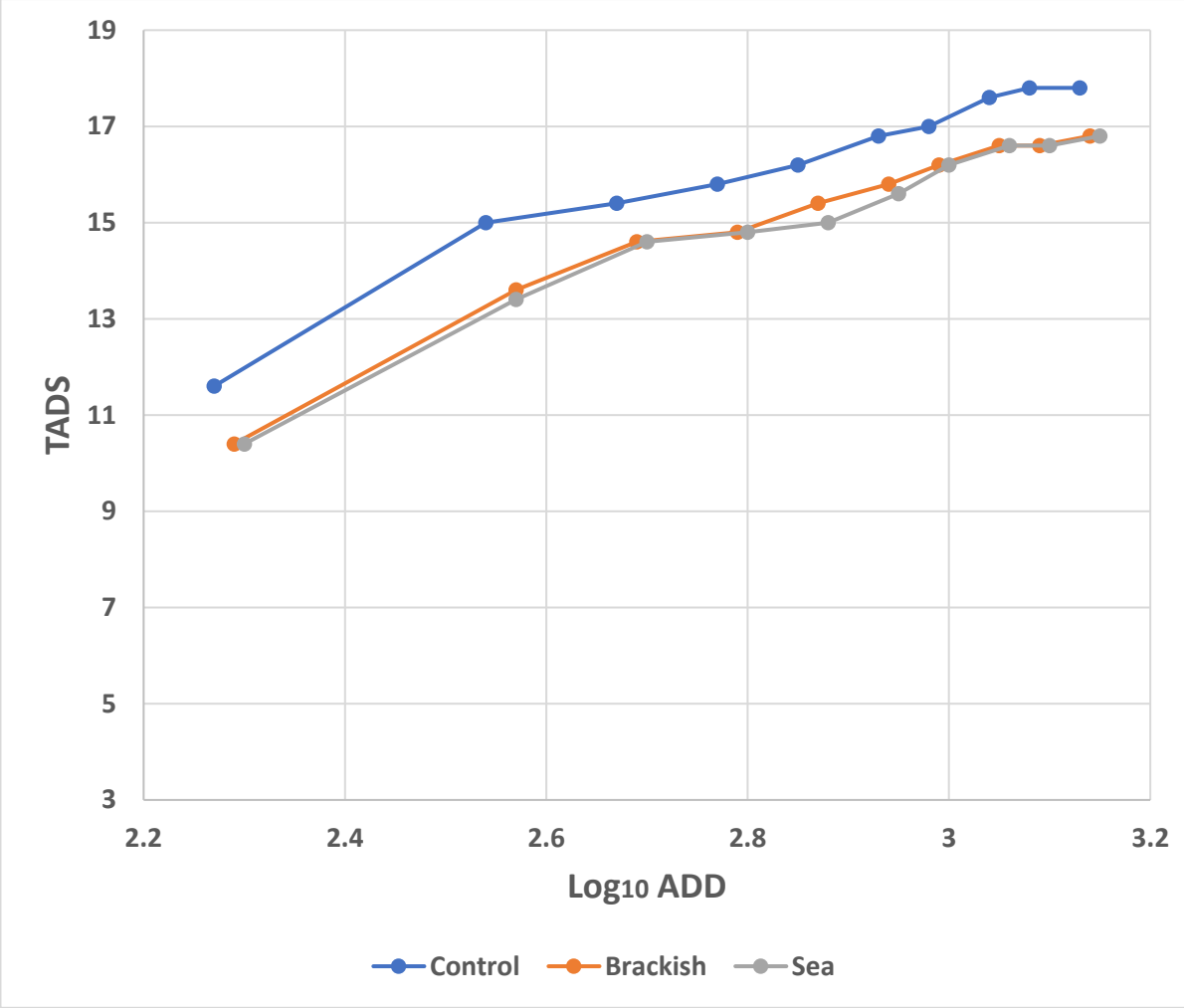


Figure 6.11: Graph of the pigs' TADS (using van Daalen DSS) against the Log ADD of each salinity group.

6.3.1.4 *Pictorial Representations of salinity effect*

Day	Control	Brackish	Sea
7			
14			

21







28







35







42







48







56







62







70







Figure 6.12: Physical decomposition changes in the pigs of each group across the 70 days.

6.3.2 Changing-Salinity Study

6.3.2.1 *TADS*

Table 6.5 shows a breakdown of the average TADS of the pigs; they were scored with the Heaton and van Daalen DSS. After the first week of the study, the lowest mean TADS recorded was 10.6 (*n=5, S.D.=0.5*) in the sea-constant group, and the highest recorded mean was 24.4 (*n=5, S.D.=0.5*) in the sea-changing group when using the Heaton DSS. The lowest mean TADS recorded was 8.6 (*n=5, S.D.=0.5*) in the sea-constant group, and the highest recorded mean was 17.4 (*n=5, S.D.=0.5*) in the sea-changing group when using the van Daalen DSS—[See Appendix 6.1.2 for TADS of all the pigs].

Table 6.5: Average TADS of the pigs.

PMSI (Days)	Average TADS (*n=5*)							
	Heaton DSS *[max=25]*				van Daalen DSS *[max=18]*			
	Sea-Constant	Fresh-Constant	Sea-Changing	Fresh-Changing	Sea-Constant	Fresh-Constant	Sea-Changing	Fresh-Changing
0	6.2	5.0	4.6	6.0	5.2	4.4	4.0	4.2
7	10.6	11.4	10.8	11.2	8.6	9.0	8.6	8.8
14	13.6	14.6	14.6	15.2	9.8	9.8	10.4	10.4
20	16.4	17.6	19.4	18.6	11.6	12.6	14.0	13.4
27	18.2	19.8	21.4	21.2	13.4	13.8	15.4	15.2
35	20.6	21.8	22.0	23.0	15.0	15.8	15.8	16.0
41	21.8	23.0	22.4	23.0	15.4	16.0	16.0	16.0
49	21.8	23.6	23.0	23.0	15.4	16.6	16.0	16.0
56	22.8	23.8	23.4	23.0	16.4	16.8	16.4	16.0
63	23.0	24.0	24.0	23.4	16.6	17.0	17.0	16.4
70	23.4	24.2	24.4	23.8	16.8	17.2	17.4	16.8

Two separate Friedman's tests were conducted to investigate any statistical difference in the effect of the four water salinity groups on the TAD scores of the pigs across the ten sampling days—[See Appendix 6.2.2 for statistics]. The first test was carried out on the TADS obtained with the Heaton DSS, while the second was for the van Daalen DSS. The results showed a statistically significant difference in the TADS across the four groups (Heaton: $p < 0.001$; van Daalen*:* $p = 0.006$). A Wilcoxon Post Hoc test was done to further investigate the specific statistical difference (*p-value*) of the TADS of each group compared to the other three groups (Table 6.5). For the Heaton DSS, there was a statistically significant difference between the TADS of the Sea-Constant group and the other three groups: Fresh-Constant ($p = < 0.001$), Sea-Changing ($p = 0.001$) and Fresh-Changing ($p < 0.001$), but there was no statistically significant difference when comparing the other three groups with each other.

In contrast, there was no statistically significant difference between the TADS of any of the four groups when using the van Daalen DSS (Table 6.6). Figures 6.13–6.14 are graphical representations of the TADS of the pigs in the groups against the PMSI on the sampling days of the study.

Table 6.6: Post Hoc test results of both DSS.

Heaton		**van Daalen**	
Sea-Constant *vs*	Fresh-Constant ($p < 0.001$)*	Sea-Constant *vs*	Fresh-Constant ($p = 0.016$)
	Sea-Changing ($p = 0.001$)*		Sea-Changing ($p = 0.013$)
	Fresh-Changing ($p < 0.001$)*		Fresh-Changing ($p < 0.078$)
Fresh-Constant *vs*	Sea-Changing ($p = 0.842$)	Fresh-Constant *vs*	Sea-Changing ($p = 0.411$)
	Fresh-Changing ($p = 0.520$)		Fresh-Changing ($p = 0.868$)
Sea-Changing *vs* Fresh-Changing ($p = 0.374$)		Sea-Changing *vs* Fresh-Changing ($p = 0.109$)	

****- a difference significant to the 1% level***

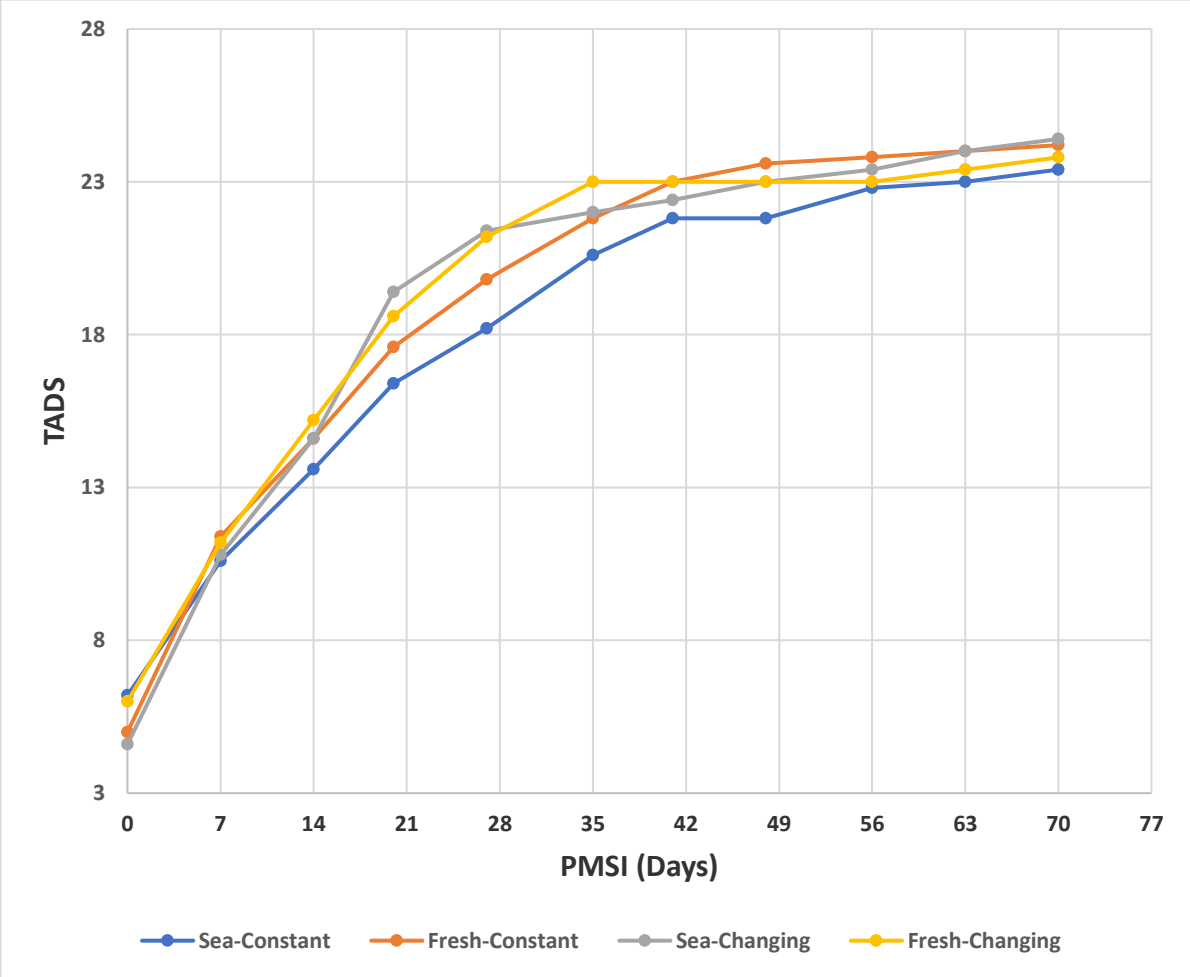


Figure 6.13: Graph of the TADS (using Heaton DSS) of the pigs against the PMSI of each salinity group.

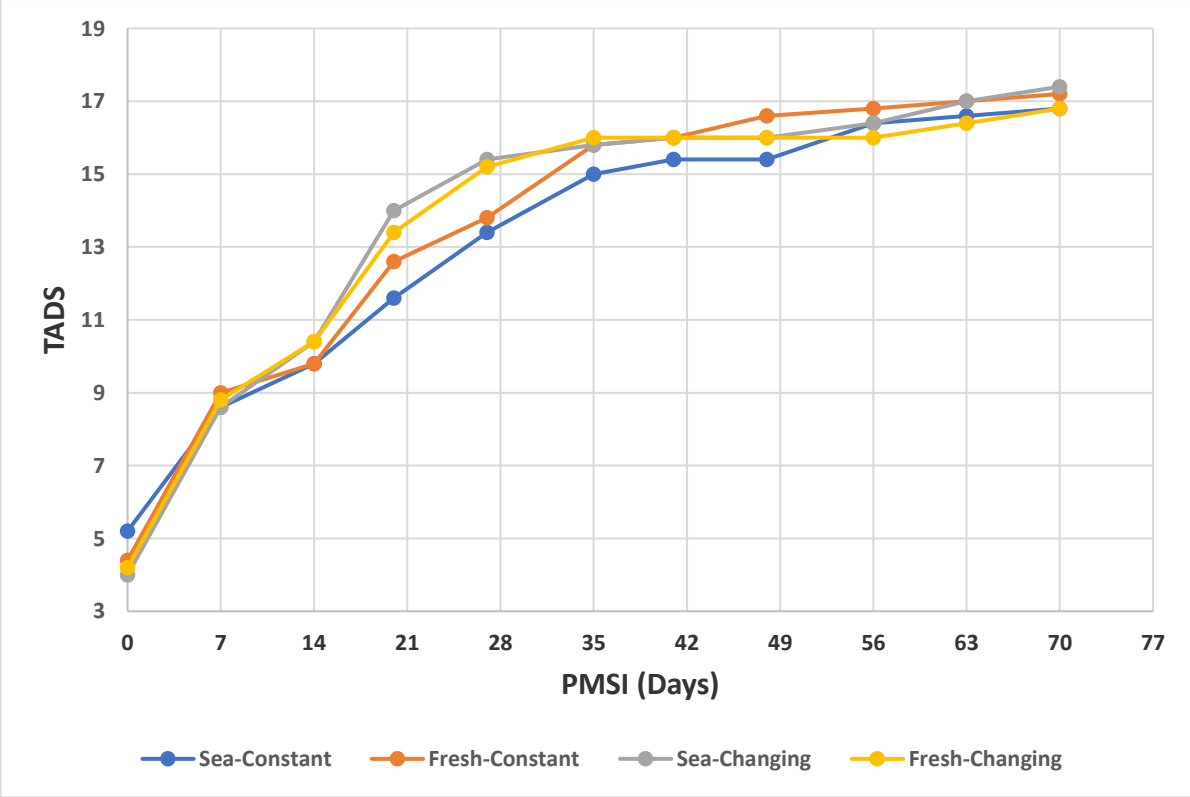


Figure 6.14: Graph of the pigs' TADS (using van Daalen DSS) against the PMSI of each salinity group.

6.3.2.2 *DSS Comparisons*

A Mann-Whitney U test was needed to compare the TADS of the two DSS statistically, but this would not have been possible because they had different maximum scores (Heaton = 25; van Daalen = 18). Therefore, Heaton TADS were divided by 25 and the van Daalen by 18 to produce a fraction (e.g., a score of 4/18 = 0.22) to normalise both scores to a ratio of 1. Thus, they both had a maximum score of 1, and the Mann-Whitney U test was used to analyse if there was any significant difference in the TADS obtained when using the Heaton and van Daalen DSS to assess the extent of decomposition in each group—[See Appendix 6.3.2 for statistics]. The results of the test indicated that there was no statistically significant difference between the two DSS for all the groups (Table 6.7).

Table 6.7: Mann-Whitney U test results comparing the normalised Heaton DSS scores with the normalised van Daalen DSS scores.

Groups	***p-value***
Sea-Constant	0.967
Fresh-Constant	0.568
Sea-Changing	0.632
Fresh-Changing	0.024

****- a difference significant to the 1% level***

6.3.2.3 *Temperature & ADD*

Figure 6.15 shows the average daily temperatures of the water in each group across the 70 days of decomposition—[See Appendix 6.4.2 for the daily temperature values of each group]. The average daily water temperatures of the boxes in each group were summed up to calculate the ADD values (Table 6.8). The lowest ADD was 135.3 (*n=4, S.D.=8.7*), and the highest was 1653.9 (*n=4, S.D.=41.4*). Figures 6.16–6.17 are graphical representations of the TADS of the pigs in the groups against the average Log ADD across the 70 days of decomposition.

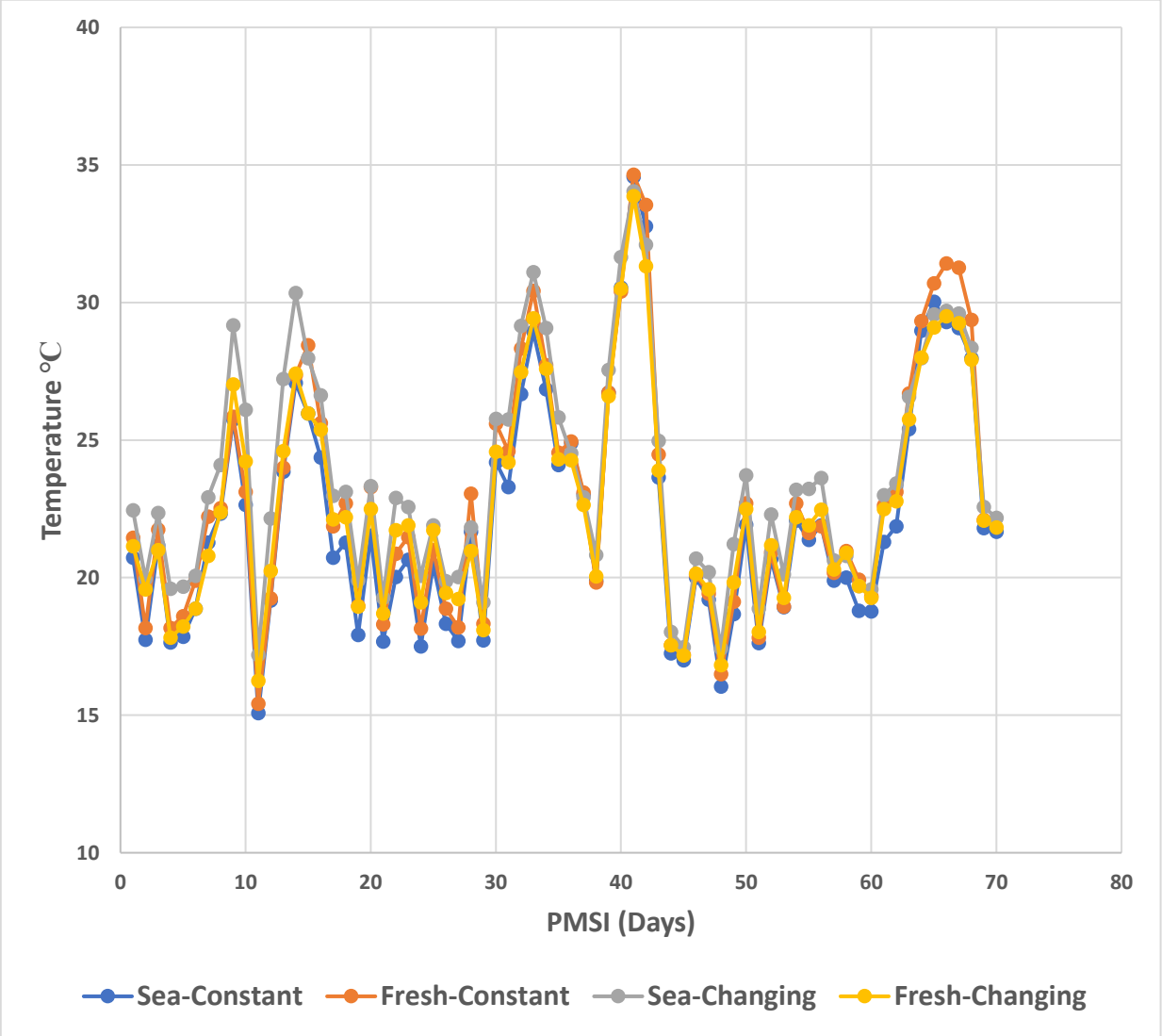


Figure 6.15: Graph showing temperature fluctuation in the water groups across the 70 days.

Table 6.8: ADD and Log ADD of the three salinity groups across the 70 days of decomposition.

PMSI (Days)	Sea-Constant		Fresh-Constant		Sea-Changing		Fresh-Changing	
	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD	ADD	Log_{10} ADD
7	135.3	2.13	140.3	2.15	147.0	2.17	137.5	2.14
14	291.2	2.46	297.8	2.47	323.3	2.51	299.6	2.48
20	423.0	2.63	438.7	2.64	467.3	2.67	436.7	2.64
27	555.3	2.74	575.6	2.76	613.9	2.79	578.6	2.76
35	748.8	2.87	778.2	2.89	821.5	2.91	775.2	2.89
41	908.5	2.96	937.9	2.97	983.0	2.99	933.2	2.97
48	1054.4	3.02	1086.7	3.04	1133.9	3.05	1079.7	3.03
56	1217.6	3.09	1252.4	3.10	1310.2	3.12	1247.0	3.10
63	1363.6	3.13	1405.2	3.15	1463.9	3.17	1398.2	3.15
70	1552.5	3.19	1602.0	3.20	1653.9	3.22	1585.9	3.20

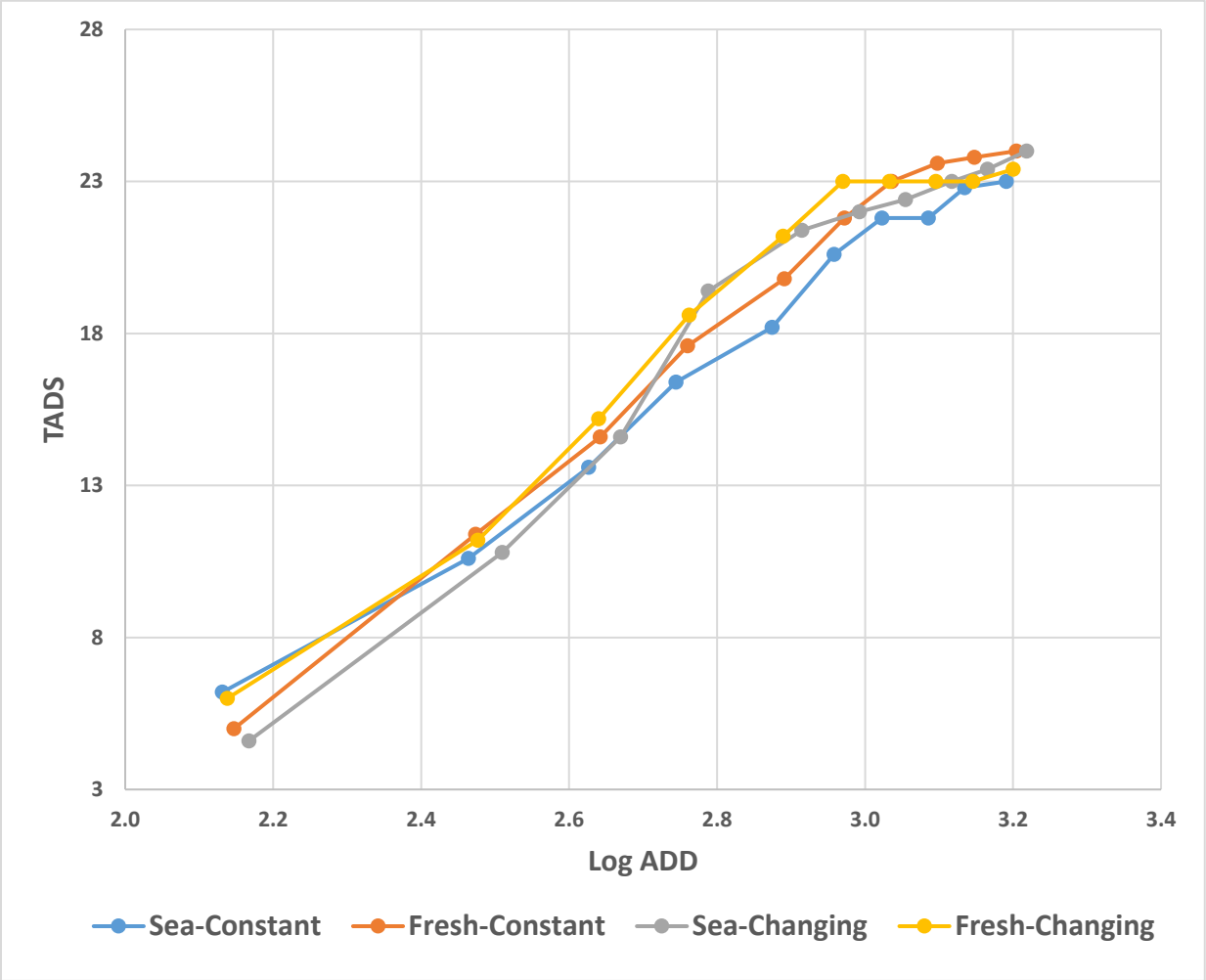


Figure 6.16: Graph of the TADS (using Heaton DSS) of the pigs against the Log ADD of each salinity group.

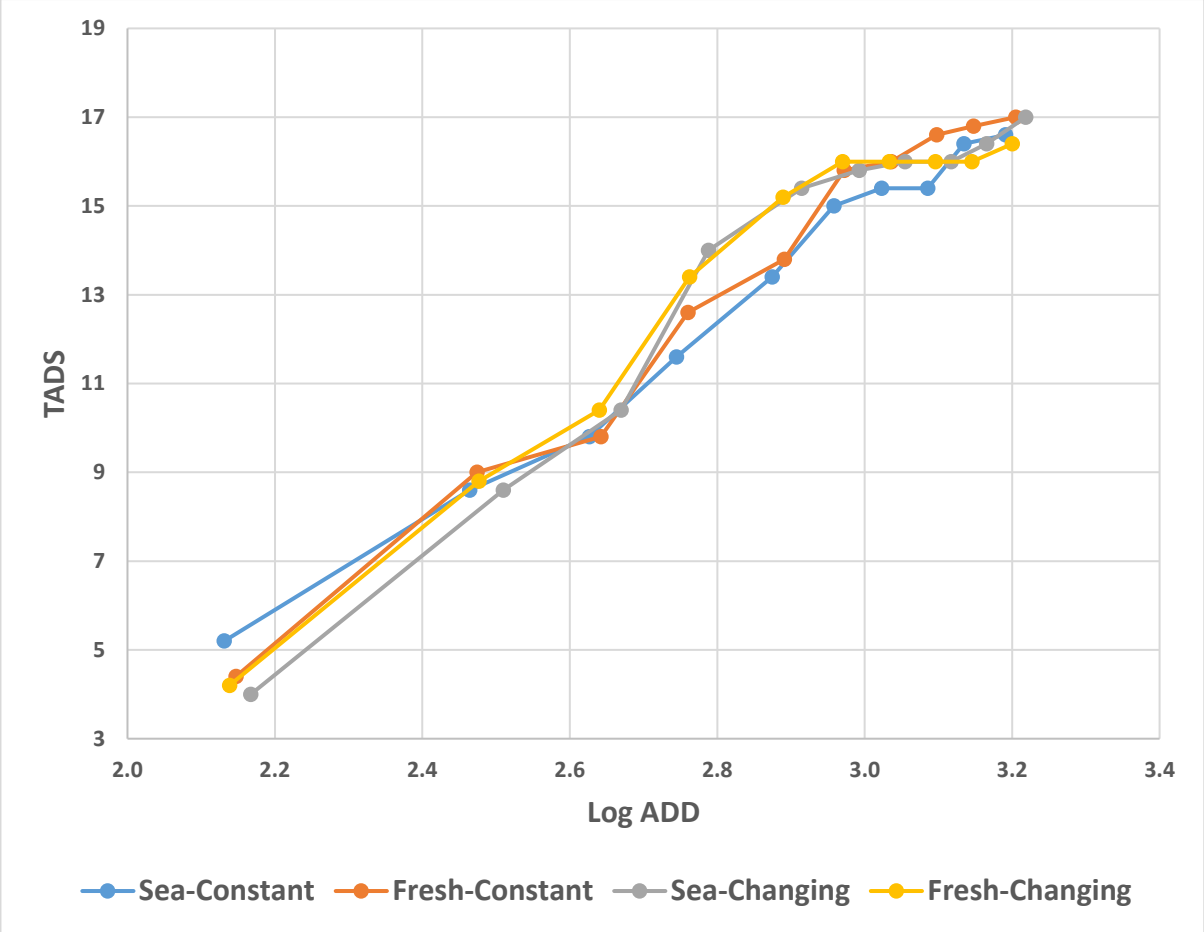


Figure 6.17: Graph of the pigs' TADS (using van Daalen DSS) against the Log ADD of each salinity group.

6.3.2.4 *Salinity*

In the *Sea-Changing* and *Fresh-Changing* groups, salinity concentrations were measured before taking out the pigs from the boxes with the previous week's water content ("Out" heading in Table 6.9) and measured before placing the pigs in the boxes with the replenished modified water ("In" heading in Table 6.9) —[See Appendix 6.5.1 for the salinity concentrations of each group]. The salt concentration of fresh and seawater is approximately 0.5–1.0 ppt and 25–35 ppt, respectively (Duncan Seraphin *et al*., 2014). In this study, the decomposition process and contents did not affect the salinity of the two seawater groups because the mean salinity of each group across the days of decomposition indicates that the salt concentrations of the water in the boxes were in the estimated range (Table 6.9). The decomposition process and contents affected the salinity of the two freshwater groups; the salinity of the Fresh-Constant group was 4.77 ppt on day 20 and peaked at 12.97 ppt on day 70, while the Fresh-Changing group salinity peaked at 4.03 ppt. A salinity of 12.97 ppt indicates that the water in the box had attained concentrations similar to that of brackish water that could range between 1.0–30 ppt (Duncan Seraphin *et al*., 2014).

Table 6.9: Average salinity concentrations of each group on the sampling days.

Groups	**Average Salinity (ppt) *[n=5]***																		
	Days of Decomposition																		
	0	**7**		**14**		**20**		**27**		**35**		**41**		**48**		**56**		**63**		**70**
Sea-Constant	31.3	32.2		31.2		32.6		26.8		33.2		33.6		32.8		34.1		33.4		33.5
Fresh-Constant	0.04	0.12		1.82		4.77		6.67		10.48		12.54		12.92		12.52		12.34		12.97
	Days of Decomposition																		
	0	**7**		**14**		**20**		**27**		**35**		**41**		**48**		**56**		**63**		**70**
	In	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**
Sea-Changning	32.4	32.3	30.3	30.2	29.9	32.8	29.3	21.3	19.6	27.8	29.8	29.9	26.2	26.3	27.7	28.9	30.1	30.1	30.1	29.6
Fresh-Changing	0.04	0.16	0.04	2.66	0.04	4.03	0.04	2.08	0.05	2.02	0.04	0.89	0.04	0.41	0.04	0.15	0.04	0.08*		0.11

- pigs were left in the boxes for two weeks due to shortage of water and faulty pumps.

6.3.2.5 *pH*

Like the salinity concentrations in the *Sea-Changing* and *Fresh-Changing* groups, pH levels were measured before taking out the pigs from the boxes with the previous week's water content ("Out" heading in Table 6.10) and measured before placing the pigs in the boxes with the replenished modified water ("In" heading in Table 6.10). The pH of freshwater ranges between 6.5–8.0, and seawater is primarily alkaline, and its pH ranges between 7.0–8.4 (Chester & Jickells, 2012). It was difficult to tell if the pH level of the freshwater and seawater groups was affected by the decomposition process and contents because the pH levels fluctuated weekly across all the groups in no pattern—[See Appendix 6.5.2 for the pH levels of each group].

Table 6.10: Average pH levels of each group on the sampling days.

Groups	**Average pH *[n=5]***																		
	Days of Decomposition																		
	0	**7**		**14**		**20**		**27**		**35**		**41**		**48**		**56**		**63**		**70**
Sea-Constant	8.24	6.33		6.09		6.30		6.49		6.64		6.92		7.01		7.25		7.12		7.32
Fresh-Constant	7.34	6.20		6.29		6.56		6.81		6.92		6.90		6.98		6.82		6.81		6.82
	Days of Decomposition																		
	0	**7**		**14**		**20**		**27**		**35**		**41**		**48**		**56**		**63**		**70**
	In	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**
Sea-Changning	8.24	6.07	8.24	6.15	8.34	6.59	8.00	6.84	8.71	6.90	7.88	6.77	7.64	6.79	8.26	6.83	8.32	6.94	7.80	5.89
Fresh-Changing	7.34	6.04	7.34	6.35	6.97	6.82	7.57	7.13	7.59	6.93	6.71	7.01	7.07	7.22	7.13	7.33	7.62	6.95*		7.30

- pigs were left in the boxes for two weeks due to a shortage of water and faulty pumps.

6.3.2.6 *TDS*

TDS concentrations of the *Sea-Changing* and *Fresh-Changing* groups were measured before taking out the pigs from the boxes with the previous week's water content ("Out" heading in Table 6.11) and measured before placing the pigs in the boxes with the replenished modified water ("In" heading in Table 6.11). TDS concentration of freshwater sources is approximately 0.5–1.0 ppt, while seawater ranges at about 10–35 ppt (Moran, 2018; USGS, 2018). The TDS concentration of the seawater groups used in this study remained in the accepted range, and the decomposition process and contents did not affect the TDS concentration; seawater groups had a minimum TDS of 18.7 ppt and a maximum of 29.8 ppt—[See Appendix 6.5.3 for the TDS concentrations of each group]. The decomposition process and products affected the TDS concentrations of the two freshwater groups; the TDS concentration of the Sea-Constant group was 4.65 ppt on day 20, and it peaked at 12.22 ppt on day 70, while the Sea-Changing group salinity peaked at 3.97 ppt on day 20. A TDS concentration of 12.22 indicates that the water in the box had attained concentrations synonymous to that of seawater that could range between 10–35 ppt (Moran, 2018; USGS, 2018).

Table 6.11: Average TDS concentrations of each group on the sampling days.

Groups	**Average TDS (ppt) *[n=5]***																		
	Days of Decomposition																		
	0	**7**		**14**		**20**		**27**		**35**		**41**		**48**		**56**		**63**		**70**
Sea-Constant	26.5	27.2		26.4		27.7		22.9		27.7		28.2		27.8		29.8		28.7		28.1
Fresh-Constant	0.04	0.13		1.84		4.65		6.42		9.55		11.31		11.68		12.10		11.72		12.22
	Days of Decomposition																		
	0	**7**		**14**		**20**		**27**		**35**		**41**		**48**		**56**		**63**		**70**
	In	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**
Sea-Changning	27.4	27.1	25.5	25.3	25.1	27.4	25.7	18.7	17.4	23.6	25.4	25.3	22.4	20.8	23.5	24.9	25.5	25.3	25.2	24.9
Fresh-Changing	0.04	0.17	0.04	2.67	0.04	3.97	0.04	2.09	0.05	2.05	0.04	0.92	0.04	0.43	0.04	0.19	0.04	0.08*		0.11

***- pigs were left in the boxes for two weeks due to a shortage of water and faulty pumps.**

6.3.2.7 *Pictorial Representations of salinity effect*

Day	Sea-Constant	Fresh-Constant	Sea-Changing	Fresh-Changing
7				
14				

20






27






35






41






48






56






63

70

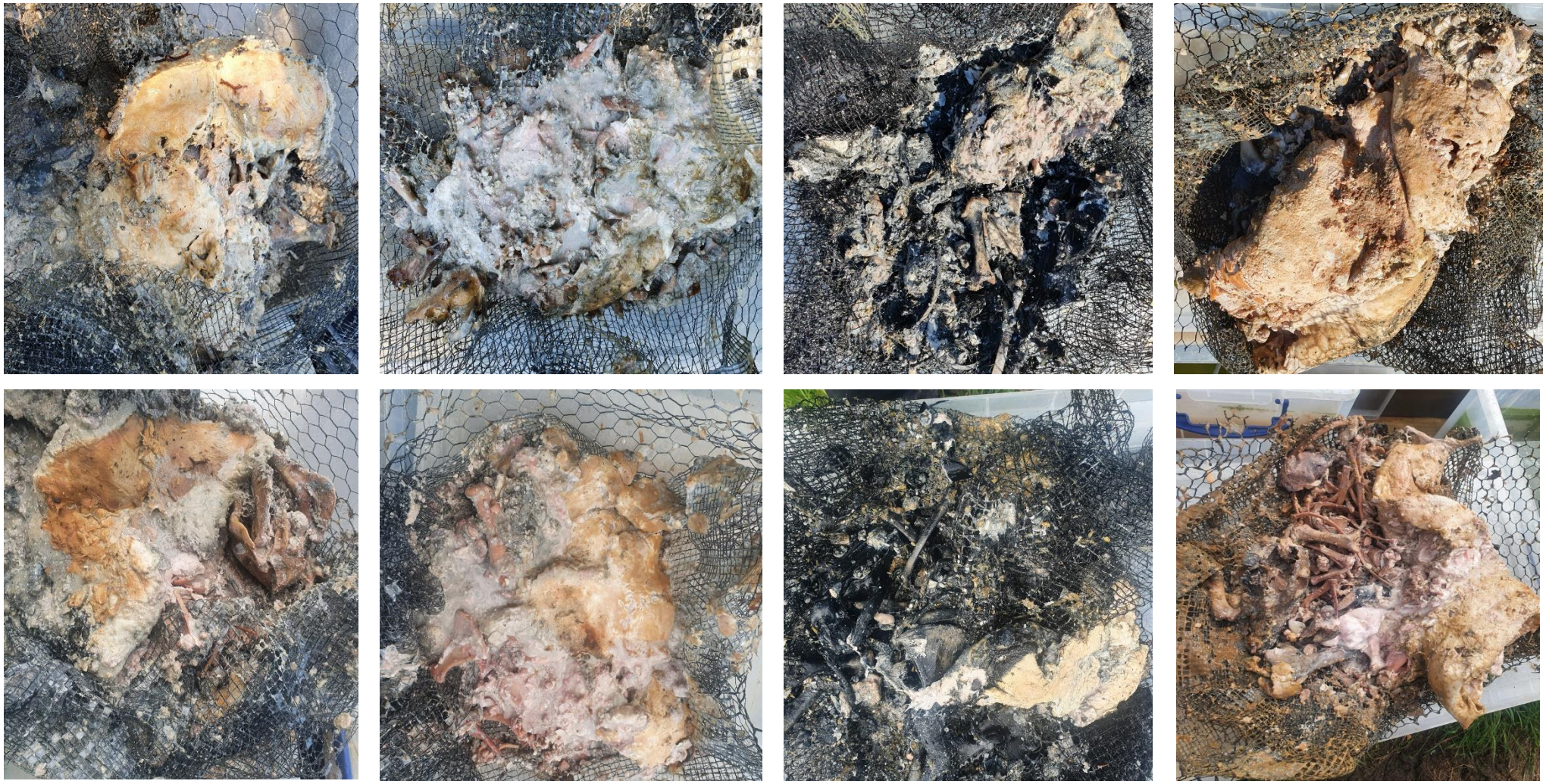


Figure 6.18: Physical decomposition changes in the pigs of each group across the 70 days.

6.4 Discussion

6.4.1 Initial-Salinity Study Discussion

See Figure 6.12 for the physical representation of the decomposition changes recorded. The decomposition process in the pigs was advanced due to the heatwave experienced in the U.K. in July 2021. As early as day 7, bone exposure and heavy maggot activity were observed in some body parts of all the 15 pigs across the three groups. By day 21, some pigs had already reached the skeletonization stage and adipocere was observed on the pigs (Figure 6.19).

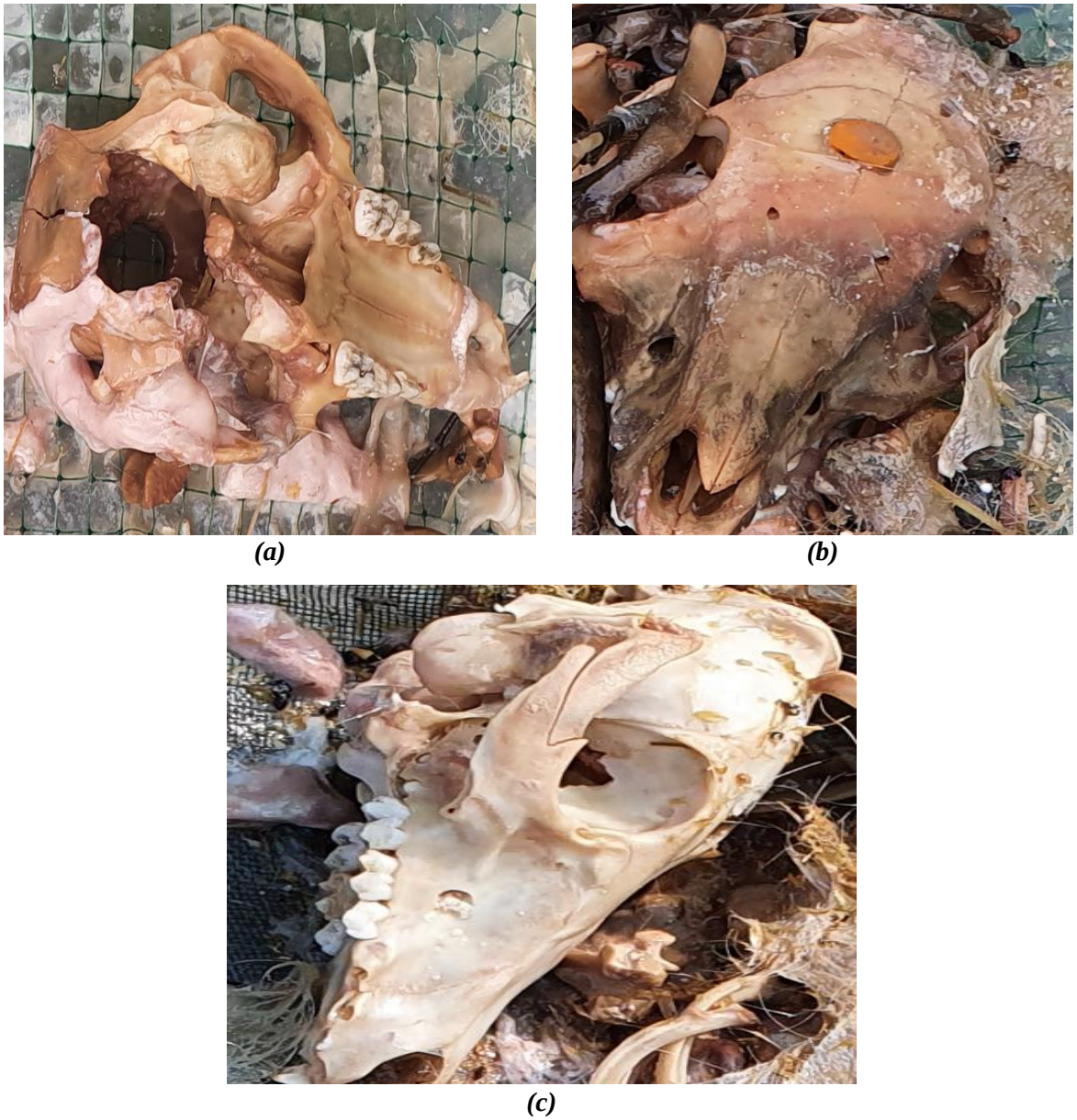


Figure 6.19: Presence of adipocere on the back and trunk of a pig as at day 21.

On each sampling day of the study, decomposition was most advanced in the Control group. By day 42, complete skeletonization disarticulation was evident in most parts of the five pigs in the Control group. On day 70, only one pig in this group had not attained complete disarticulation; parts of the spine were still articulated. Partial skeletonization and disarticulation were observed in all the pigs in the Brackish group on day 35. On day 56, 2 pigs in this group had reached complete skeletonization and

disarticulation. By day 70, the pigs were fully skeletonised, but only partial disarticulation was observed in the spine, with a few ligaments still attached to the vertebrae.

The Sea group showed a similar decomposition trend to that of the Brackish group. The difference between the Sea group and the other two groups was the bleached appearance of the bones of the pigs in the Sea group, which could be due to the high salt content in the Sea group (Figure 6.20). The orange bung seen in the pig's skull in Figure 6.20b is the rubber bolt shot to the frontal lobe of the pigs when they were killed at the local supplier. Also, the limbs of one of the pigs in the Sea group looked fresh for the first 56 days of the study (Figure 6.21).



(a) ***(b)*** ***(c)***

Figure 6.20: Pictures of the skulls of the pigs from the three groups. Control (a); Brackish (b); Sea (c).



Figure 6.21: Fresh lower limbs of the pig in the Sea group as of Day 56.

6.4.2 Changing-Salinity Discussion

The pigs had a PMI of 2 hours before they were submerged at the onset of the study. These pigs were placed on the floor for weighing and labelling before being placed in water; it is possible that flies could have gained access to the pigs during these procedures or soon after death. Three of the pigs were already exhibiting bloating features in their trunk regions, while all the pigs had pink discolouration and marbling in their head, neck, trunk, and limbs. On day 7, all the pigs were bloated in their head, neck, trunk, and abdomen, while pink and green discolouration, marbling and skin slippage were the decomposition features observed in their limbs. Insect activity became prominent on day 14 (Figure 6.22). Bloating was reduced in most pigs, and liquefaction had started to occur. Green, grey, and black discolouration was also observed in the trunk of the pigs.



Figure 6.22: Presence of insect activity on the pigs by day 14.

There was an increased presence of maggots by day 20 (Figure 6.23), especially in 9 out of 10 pigs in the changing groups. This increased maggot presence in the changing groups could be because flies could have gained access to the pigs when they were being moved into new boxes weekly. All the abdominal regions of the pigs had passed the liquefaction stage, with some showing signs of advanced internal and organ decomposition. Bone exposure was also observed in the head and limb regions of some of the pigs.



Figure 6.23: Maggots infestation by day 20.

By day 27, decomposition was more advanced in the Fresh and Sea Changing groups when compared to the Fresh and Sea Constant groups; all the pigs in the changing groups exhibited advanced organ decomposition and bone exposure, with all pigs having disarticulated mandibles, while some had complete disarticulation of their skull from the torso. The pigs in the Changing groups were exposed to air and insects when being moved between boxes, this exposure, though for a short duration, could have contributed to the drying of the exposed parts and led to a faster decomposition rate in the pigs (Heaton *et al*., 2010). The Fresh-Constant group decomposed faster than the Sea-Constant group; complete disarticulation of the skull from the torso was observed in 3 pigs in the Fresh-Constant group. The Sea-Constant group decomposed at the slowest rate when compared to the other three groups. The presence of salt in the Sea-Constant group would have slowed down bacterial activity hence the reason why decomposition was faster in the freshwater group (Byard, 2018; Palazzo *et al*., 2021). Adipocere was also observed in some pigs by day 27 (Figure 6.24).

Only 2 out of 15 pigs in the Fresh-Constant, Sea-Changing, and Fresh-Changing groups had not attained complete disarticulation and decomposition in the head region by day 35 of decomposition. In contrast, only 1 out of 5 in the Sea-Constant group had attained complete disarticulation in the head. All the pigs in the four groups had extensive bone and soft tissue exposure in all body regions. Although all the pigs were already exhibiting extensive bone exposure in all the body regions by day 35, their decomposition rate was immensely reduced from day 35 to day 70. None of the pigs attained complete decomposition in all body regions throughout the study; there were still signs of articulation in all the trunks of the pigs

and the limbs of some pigs. This slowed-down decomposition rate observed in the pigs could have been because of the presence of adipocere observed on the pigs, especially in the trunk regions. The presence of adipocere on a body slows down the process of decomposition; in most cases, it can keep the body preserved by restricting the air to the surface of the body, minimising bacterial activity, and keeping the body moist (Boyle *et al*., 1997; Stuart, 2013; Ueland *et al*., 2013). The presence of green algae was noticed in the Fresh-Changing groups on day 63 of decomposition (Figure 6.25).



Figure 6.24: Adipocere observed on pigs on days 27 (Sea-Constant) and 35 (Fresh-Changing).



Figure 6.25: Green algae build up in the boxes of the Fresh-Changing group.

6.4.3 Study Comparisons

The Initial-Salinity study was conducted to investigate the effect of three levels of salinity [fresh (< 1.0 ppt), brackish (~20 ppt), and sea (~35 ppt)] on the decomposition rate of pigs, while the Changing-Salinity study focused on the effect of refreshing the water by investigating the difference between the extent of decomposition of pigs deposited in constant water for the entirety of the study and the ones deposited in water that was refreshed weekly. The results of both studies suggested that decomposition was statistically significantly faster in freshwater groups ($p < 0.001$) when compared to the salt-containing water groups (Table 6.3, 6.5–6.6). Insect and maggot activities were already pronounced in both studies between days 14-21. Adipocere was also observed on the pigs in all water types between days 21-28, and most of the pigs had already attained partial skeletonization by days 42-49.

In the Changing-Salinity study, the Sea-Changing and Fresh-Changing groups decomposed at a faster rate when compared to the Sea-Constant and Fresh-Constant groups, respectively (Table 6.5–6.6). Reasons for this could have been exposing the pigs to air when scoring them; transferring them to a new box which in turn exposes the pigs to external bacteria species and allows insect access, as well as causing a disturbance that can lead to the disarticulation of body parts. When collecting data in decomposition studies, repeated disturbance of the cadaver(s) can affect insect activity and other factors that are crucial in the decomposition process (Adlam & Simmons, 2007).

One major difference between the Initial-Salinity study and the Changing-Salinity study, and the Fresh-Constant group and Fresh-Changing group of the Changing-Salinity study was the presence of green algae in the Fresh-Changing group only. Algae are aquatic organisms capable of growing into colonies and performing photosynthesis in the presence of suitable conditions such as warm temperature, moisture, appropriate sunlight exposure and necessary nutrients such as phosphorus and nitrogen (Gubelit & Berezina, 2010; Wang *et al*., 2017). The salt in the seawater groups and the Fresh-constant group (which had its salinity concentrations rise above freshwater salinities from a PMSI of 14 days [Table 6.9]) could have been the reason why algae were not found in these groups as it could have hindered any bacteria activity, which could have broken down body tissues and provided the optimal nutrients (nitrogen, phosphorus, and carbon dioxide) and condition for the formation of algae.

6.5 Limitations of the Field Studies

For the Initial-Salinity study, Really Useful Boxes® were not available for purchase. Therefore, Argos Home® boxes were used, but they were not solid and big enough to successfully hold the pigs. The walls of the boxes bulged out under the outward pressure of the water when the water and pigs were placed inside, making it difficult for the boxes to be closed to prevent the entry of insects. Stones had to be placed on the boxes to keep them closed once the lids had been clipped into place. Also, metal grills were placed above the pigs to immerse them in the water entirely, but the grill could not keep

them submerged because the side walls of the boxes gaped. The water in the boxes was not changed throughout the study; therefore, the water became murky as decomposition progressed. This obscured the content of the boxes, making it difficult to observe any decomposition change occurring in the boxes.

For the Changing-Salinity study, the mesh placed below the pigs, which was used to aid their removal from the water, unexpectedly released black ink into the boxes, thereby obscuring the content of the boxes, making it difficult to observe any change occurring in the boxes and the decomposition changes on the bones and tissues of some pigs (Figure 6.26). It also had the effect of dying some of the tissues and bones black under the water (Figure 6.26). In addition, it was difficult to observe any change occurring in the boxes of the Fresh-Constant and Sea-Constant boxes because the water became murky as decomposition progressed, and it was not changed throughout the study.



Figure 6.26: Bones of pigs turned black because of the mesh bleaching.

In both Salinity studies, when using the van Daalen DSS, some limitations were encountered because the DSS did not include some decomposition changes observed in the pigs. The van Daalen system only considered grey and white colours as discolouration, and there was no mention of adipocere formation. Despite including skeletonization in the DSS, changes such as “the disarticulation of body parts” and “exposure of soft tissues, organs and bones” were not offered as options in the DSS descriptions.

6.6 Conclusions of the Field Studies

These field studies demonstrated that the salinity of water significantly affects the decomposition rate of pigs submerged in water. Key results include:

- **Initial-Salinity study:** When using the Heaton and van Daalen DSS, the extent of decomposition in the Control [fresh] group was statistically significantly faster when compared to the Brackish and Sea groups ($p < 0.001$).
- **Changing-Salinity study:** When using the Heaton DSS, the extent of decomposition in the Sea-Changing group was statistically significantly faster when compared to the Sea-Constant group ($p = 0.001$), but they did not differ significantly when using the van Daalen DSS ($p = 0.013$). There was no significant difference between the extent of decomposition in the Fresh-Changing and Fresh-Constant groups when using the Heaton DSS ($p = 0.520$) and van Daalen DSS ($p = 0.868$).

The Initial-salinity study demonstrated that the decomposition rate of pigs is significantly different in varying water salinity concentrations (*fresh, brackish, and seawater*). It indicated that the decomposition rate was slower in salt water (*sea and brackish*) compared to freshwater; the highest TADS occurred in the freshwater group. The Changing-Salinity study demonstrated that pigs submerged in water with renewed water salinity decomposed faster than pigs submerged in water with constant salinity; the Sea-Changing and Fresh-Changing groups decomposed at a faster rate when compared to the Sea-Constant and Fresh-Constant groups, respectively.

Exposing the pigs to air when scoring them and transferring them to a new box which exposes the pigs to external/exogenous bacteria and insect species and causes a disturbance that can lead to the disarticulation of body parts, could have been the reasons for the faster decomposition rate observed in the pigs submerged in water with changing salinity (Adlam & Simmons, 2007). The pigs in the Changing groups were exposed to conditions akin to real-life scenarios (such as open seas and rivers with different water sources) because bodies deposited in water are exposed to a larger volume of water with changing or varying salinity concentrations and the flowing water in real-life scenarios means the body deposited may be dragged for a distance, creating disarticulation and marks or discolouration that can be mistaken for decomposition changes (Humphreys *et al*., 2013; Palmer, 2020; Stuart & Ueland, 2017).

The pigs in the Constant group were exposed to conditions analogous to real-life scenarios, such as bathtubs, pools, and enclosed water bodies without different sources; in these conditions, the body is exposed to the same water, thus the same salinity. The results of these studies support a general conclusion that extra caution should be taken, and the water salinity level should always be considered when estimating the PMSI of bodies recovered from seawater and marine environments.

7 PART THREE: COMPARATIVE HUMAN IMAGE ANALYSIS

7.1 Introduction

Skin colour is a physical feature that plays a crucial role in the diagnosis and interpretation of many phenomena in various scientific fields (Ha *et al*., 2009; Lindholm *et al*., 2008), but the diversity of the skin tone of the human population has been misrepresented or underrepresented in many investigative methods and techniques (Louie & Wilkes, 2018). Healthcare workers diagnose infections or conditions such as jaundice and cyanosis based on skin colour changes, but there is an underrepresentation of the skin tone of "*people of colour*" in clinical or lecture notes, case studies and textbooks (Everett *et al*., 2012; Karnieli-Miller *et al*., 2011). This underrepresentation of dark skin has led to a lesser diagnosis in cases related to skin symptoms; despite presenting similar symptoms, children with darker skin receive more deficient bacterial infection diagnoses than children with paler skin (Mangione-Smith *et al*., 2004).

In forensic science, there is also a lack of consideration of darker skin tones in the descriptions of decomposition scoring systems (DSS). The commonly used DSS were created in Europe and North America; in these studies, there is no mention of the ethnic ancestry or skin colour of the human subjects used (Heaton *et al*., 2010; Megyesi *et al*., 2005; van Daalen *et al*., 2017). Also, some physical decomposition changes noted in the Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS are described as the discolouration of the skin of different body parts; these colours include pink, red, green, black, grey, yellow, purple, and white. Observing these colour changes in darker skin tones is difficult because they are not relatively visible to the naked eye, so problems may arise when observing discolouration changes in bodies with darker skin tones (Yapo Etté *et al*., 2006). In the Gelderman *et al*. (2018) study, it was challenging to identify the presence of livor mortis in darker skin tone bodies. During the decomposition process, the skin can become a lighter shade of grey or darker; this could lead to difficulty in assessing the discolouration changes described in these DSS and the misidentification of bodies when using facial recognition techniques or algorithms (Cornett *et al*., 2019; Morgan *et al*., 2006).

These challenges suggest that these existing DSS are potentially limited because they have been developed from studies in countries with comparable temperate climates and waterways and individuals with predominantly pale skin. This leaves a gap in knowledge and uncertainty on whether these scoring methods could be helpful in cases of drowning or body deposition in water environments from regions such as Africa, Asia, the Caribbean, or with darker skin-toned individuals. Keyes *et al*. (2016) suggested that more decomposition studies should be carried out with human cadavers in regions like Africa. Carrying out these studies in Africa and other areas that predominantly have people with darker skin tones will help to improve the suitability of these DSS for bodies with darker skin tones and improve the knowledge of human decomposition in such regions.

7.1.1 Aims

This study aimed to:

1. Investigate the suitability (ease of scoring and observing the descriptive changes noted in the DSS for the various body regions and stages of decomposition) and validate whether the Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS are suitable to achieve accurate PMSI estimates for drowning cases from regions or cases involving individuals with darker skin tones.

H_1 = There is a significant difference between the estimated PMSI and case note PMSI values when applying the Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS to drowning cases involving darker skin tone victims.

H_0 = There is no significant difference between the estimated PMSI and case note PMSI values when applying the Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS to drowning cases involving darker skin tone victims.

2. Investigate if there is a statistically significant difference between the estimated PMSI and the PMSI values stated in the case notes.

H_1 = There is a significant difference between the estimated PMSI and the case note PMSI values.

H_0 = There is no significant difference between the estimated PMSI and the case note PMSI values.

3. Investigate if there is a statistically significant difference in the TADS of the human remains when comparing the Heaton and van Daalen DSS scores.

H_1 = There is a significant difference between the TADS of the remains when comparing the Heaton and van Daalen DSS scores.

H_0 = There is no significant difference between the TADS of the remains when comparing the Heaton and van Daalen DSS scores.

4. Investigate if it is possible to score the extent of decomposition and estimate the PMSI of a body in photographs with the non-visible or missing head, trunk or limbs, and test if there is a statistically significant difference between this estimated PMSI and the PMSI values stated in the case notes.

H_1 = There is a significant difference between the estimated PMSI of bodies with non-visible or missing heads, trunks or limbs and the case note PMSI values.

H_0 = There is no significant difference between the estimated PMSI of bodies with non-visible or missing heads, trunks or limbs and the case note PMSI values.

7.1.2 Objective

To assess and compare case files, pictures and autopsy reports of bodies recovered from water regions in the U.K., Netherlands, and South Africa. The comparative study was conducted by analysing the images obtained from these countries; the Heaton and van Daalen DSS were used to assess the degree of decomposition in these pictures. Temperature data of each case or its location and the decomposition scores formulated were used to estimate the PMSI; this estimated PMSI was compared to the stated PMSI in the case files to see if there was a significant difference between both PMSI values. These analyses determined if:

- The use of the DSS results in a misleading PMSI estimate for victims with darker skin tones.
- The use of the DSS must be adapted regionally (regardless of the skin tone of the victim).
- The current DSS may need to be amended, or a new DSS needs to be formulated for darker skin tone victims or cases from locations with predominantly darker skin-toned individuals.

7.2 Methodology

7.2.1 Collation of Images and Case Notes

From 2020 to 2022, numerous prospective emails were sent out, and meetings online and in person were held with a range of professionals across the world to join this collaborative research. These professionals included pathologists, medical doctors, healthcare administrative officers, police officers and other forensic scientists from countries such as South Africa, Nigeria, Zambia, the Democratic Republic of Congo, Japan, Australia, Finland, Netherlands, Germany, Italy, and the United Kingdom. Positive responses were received during these meetings, but an official collaboration was only possible with professionals from South Africa, the Netherlands, and the United Kingdom. Memoranda of Understanding were created between the University of Central Lancashire and Maastricht University and the University of Central Lancashire and the University of Cape Town for the sharing and transfer of information and images from forensic cases.

Hard copies and electronic copies of images, closed case files, autopsy files and case notes of a total of 143 cases concerning bodies recovered over a 9-year period from 2014-2022 were initially obtained from databases in the U.K., Netherlands, and South Africa. The Netherlands database used was the Maastricht University/Netherlands Forensic Institute "Water Decomposition Atlas". The cases from South Africa were obtained through an MoU and collaboration with the University of Cape Town Forensic Pathology Unit; case note data was retrieved from the “Office Autopsies database” (Ethics reference- HREC: R036/2014), while the pictures for each case were retrieved from electronic records stored on “Livelink (Open Text, Canada)”. For the cases from the United Kingdom, the researcher was able to see recent and more historic photographic evidence from cases investigated by the Blackpool and Fylde Coroner’s service.

Keywords relevant to drowning and this research were identified and used to interrogate the databases to initially select some cases (Table 7.1). After searching, 32 cases (from 2015–2021) were selected from the Water Decomposition Atlas, while 89 cases (from 2017-2021) were retrieved from the Office Autopsies database and 22 cases (from 2014–2022) from the Blackpool and Fylde Coroner’s service.

Table 7.1: Identified keywords used to search the databases for cases.

Relevance	Keywords
Essential	Water; Aquatic; Drown; Drowning; Drowned; Submerged; Immersed; Underwater; River; Canal; Pool; Pond; Bath; Sea; Lake; Stream; Marine; Ocean; Boat; Ship; Death by drowning; Shower;
Additional	Swim; Swimming; Dingy; Inflatable; Barge; Houseboat; Speedboat; Sink; Beach; Bay; Bridge; Jetty; Propeller; Outboard motor; Diving; Diver; Surf; Lifeguard; capsize;

7.2.2 Exclusion Criteria

A total of 73 cases met the criteria for this study; details of these cases are listed in Table 7.2 below. Cases were excluded from the study when:

- The victim was a child (below 18 years; 18 is the age of majority in the U.K.).
- The victim was skeletonised with no trace of skin attached to the body.
- Cases where the body was dismembered with missing limbs, head, or trunk.
- Cases with no images of the deceased and a lack of information on the description of the physical conditions or descriptions of decomposition changes of the remains.
- Cases with no images of one or more of the following: head, neck, limbs, and trunk.
- There was no available estimated PMSI or uncertainty regarding the time of death, the date and time of the body's entry and exit from the water.
- The victim received medical intervention: CPR confirmed the victim was still alive after the body was retrieved from the water, or the victim died in the hospital (or ambulance) or at the scene but not in the water.

Table 7.2: Breakdown of cases that met the study criteria.

Country	Database	No. of Cases	Year Period
Netherlands	Water Decomposition Atlas	20	2015-2021
South Africa	Office Autopsies database/Livelink	44	2017-2021
United Kingdom	Blackpool and Fylde Coroner’s Service	9	2017-2022

7.2.3 Collated Information

This study received ethical approval from UCLAN (SCIENCE 0172). All data provided were kept in password-protected files on secure, private hard drives, and only the study investigators had access to this information. The details about the individuals' identities were not required, and all forms of identification were kept anonymous throughout the research. Various details of the characteristics of the drowned victims were collected from the case notes, and these variables were divided into required and optional variables (Table 7.3). The Required variables were needed for PMSI estimations and to build a demographic profile for the victims. In contrast, the Optional variables were needed for additional information on the water conditions and physical profile.

Table 7.3: Collated characteristics of the drowned victims.

Required Variables	
Age: in years (DOB is also sufficient)	
Sex/gender	
The type of body of water the victim was recovered from: river, pool, lake, sea, bath etc	
Location (town/city) of the water body from which the body was recovered.	
Skin tone/Ethnicity/ancestral affiliation (White/Caucasian/European, Black/African/Afro-Caribbean)	
Estimated PMSI: hours or days. If the Estimated PMSI is unavailable, then the "date the individual went missing/Last seen alive" and the "date the body was recovered" will be sufficient for this research.	
Optional Variables	
Height (cm/m) and Weight (kg/st/lb)	Post-mortem examination date
Body, water, and ambient temperature	Water current (rpm/ms^{-1}/mph^{-1})
Type of clothing or objects covering the body	the pH of the water
Position in the water column and if body displacement occurred	Cause of death
Whether the body was found floating or submerged by being weighted down (stones or heavy objects tied around feet) or obstructed under the water (tangled in netting or vegetation underwater)	Health history
Weather conditions (storm/wind)	

A skin colour score was assigned to each case to quantify the skin tone of the victim by assessing the least decomposed part of the body using the NIS Skin Colour Scale (Figure 7.1) created by Massey & Martin (2003).

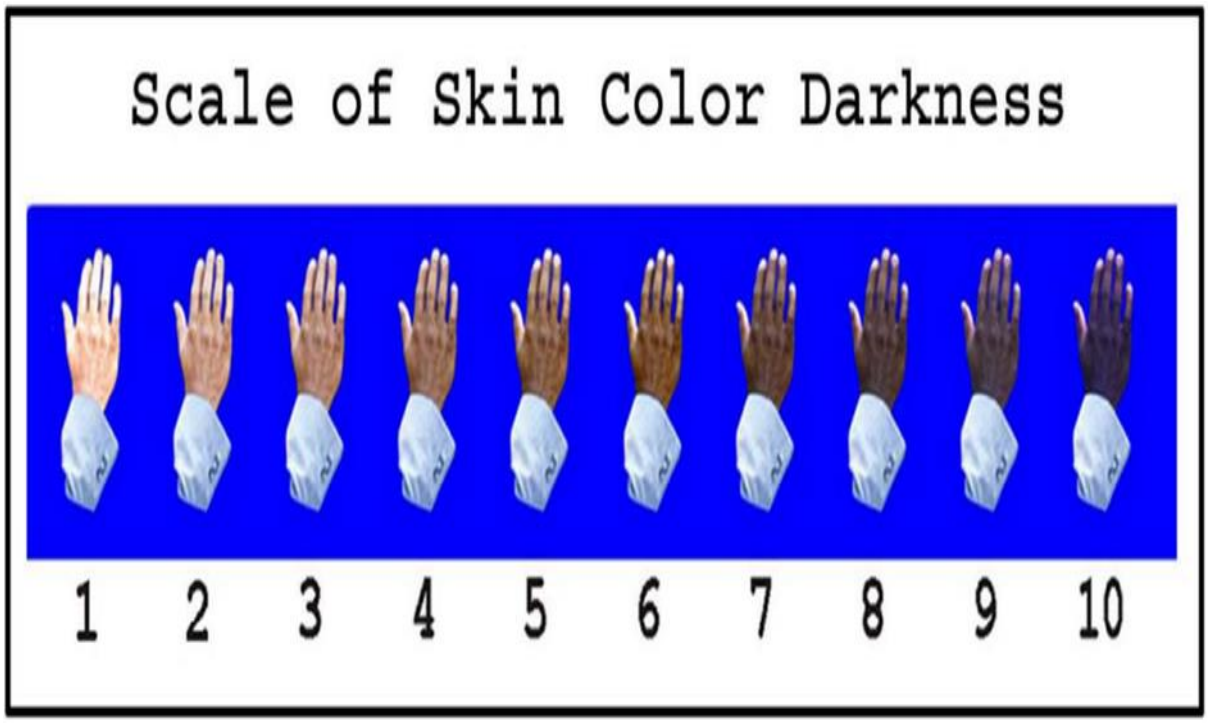
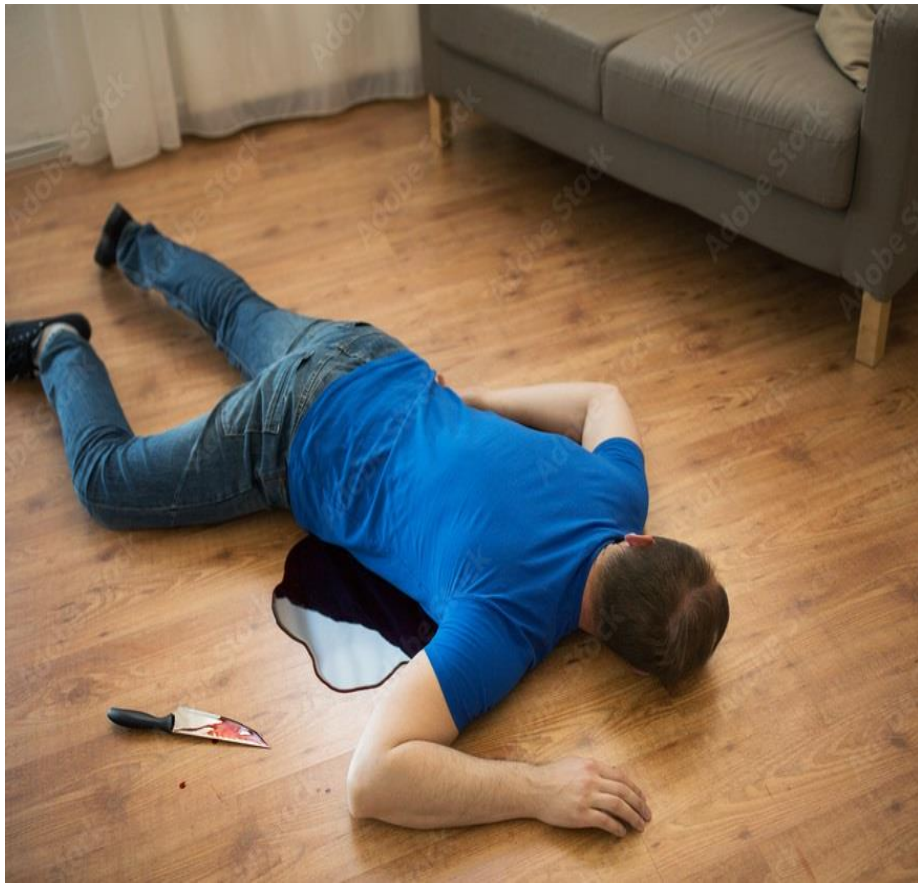


Figure 7.1: The Massey-Martin (2003) skin colour guide (Louie & Wilkes, 2018).

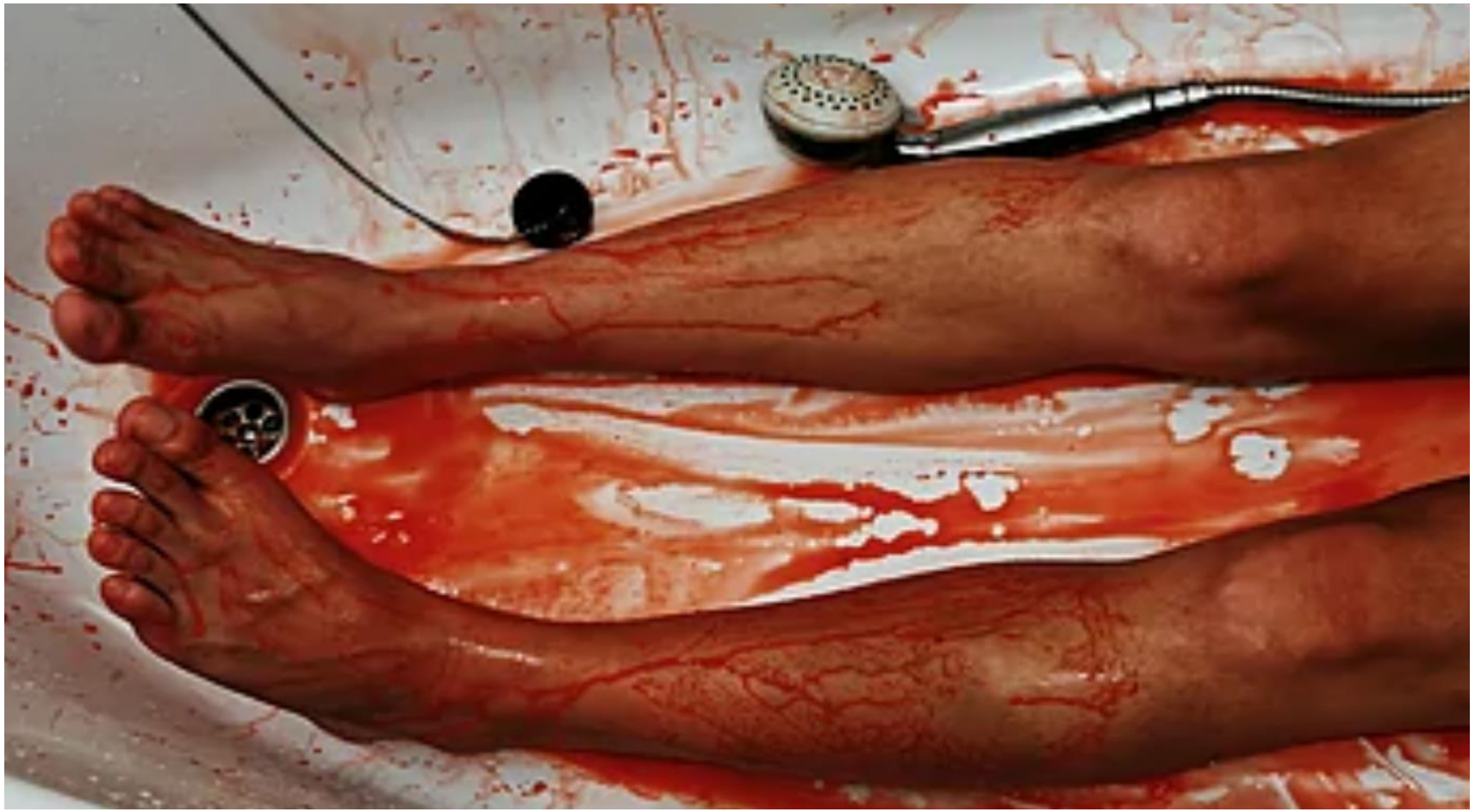
Examples of pictures and the skin colour scores assigned to them are listed in Figure 7.2 below.



(a) 3: This image was given a score of 3 when compared with the images in the Massey-Martin (2003) skin colour guide.



(b) Skin colour guide score of 2.



(c) Skin colour guide score of 5.

Figure 7.2: Pictures and the skin colour score assigned to them.

A schematic of the various variables collated from case notes is detailed in Figure 7.3 below—[See Appendix 7.1 for the full cases information spreadsheet].

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	Country	S/N	Case No.	Date Rec.	Sex	Age	PMSI	Ancestry	Colour Scale	Weight (kg)	Height (cm)	Body Temp.	Water temp	Amb. Temp	Body Coverings	Last Seen	Date Recovered	Water Type	Location
2		1	263082		M	49	3 hrs	-	6	65	175	43.8	37.8	20.0	None	30/07/15	30/07/15	Bath	Deventer
3		2	457606		M	70	2 hrs	-	1	-	-	-	-	-	1-2 thin layers	03/12/17	03/12/17	Canal	Zwolle
4		3	518882		M	69	20 hrs	-	1	-	-	-	-	-	1-2 thin layers	03/07/18	04/07/18	Canal	Steenwijkerwold
5		4	539337		M	23	7 days	-	2	-	-	-	-	-	None	05/10/18	11/10/18	Lake	Hardenberg
6		5	579251	07/10/20	M	57	1 hr	-	2	-	-	-	-	-	None	17/04/19	17/04/19	Canal	Zwolle
7		6	602651		M	36	-	-	2						1-2 thin layers	-	29/07/19	Canal	Schalkhaar
8		7	606018		M	59	1 day	-	1	100	180	22.2	21.5	-	1-2 thin layers	12/08/19	13/08/19	River	Zwolle
9		8	611760		F	50	8 days	-	2	-	-	-	18.7	20.3	1-2 thin layers	30/08/19	07/09/19	Canal	Ommen
10		9	632404		M	39	2 hrs	-	2	-	-	-	-	-	None	03/12/19	03/12/19	Ditch (fresh)	Zwolle
11	Netherlands	10	1		M	56	18 days	-	2	-	178	-	20.5	19.7	1-2 thin layers	15/07/21	03/08/21	Canal	Wijhe
12		11	3		M	74	11 hrs	-	1	70	170	-	-	-	1-2 thin layers	15/08/20	16/08/20	Canal	Staphorst
13		12	4	02/11/21	M	45	27 days	-	2	75	180	-	9.6	-	2-3 thick layers	-	05/04/20	River	Overijssel
14		13	5		M	36	29 days	-	4	-	-	-	-	-	2-3 thick layers	04/12/19	02/01/20	River	's Heerdenbroek
15		14	7		F	74	2 hrs	-	2	-	-	23.1	0.5	-7.0	1-2 thick layers	12/02/21	12/02/21	Canal	Staphorst
16		15	8		M	24	3 days	-	6	95	180	7.5	4.9	5.8	2-3 thick layers	19/01/21	22/01/21	Ditch (fresh)	Giethoorn
17		16	9		M	80	5 hrs	-	2	-	-	24.0	-	-	Swim suit	26/07/16	26/07/16	River	Deventer
18		17	12		M	71	2 days	-	2	85	175	7.8	-	-	1-2 thin layers	17/03/16	19/03/16	River	Kampen
19		18	14	07/12/21	F	62	5 days	-	1	-	-	-	-	-	2-3 thick layers	24/01/16	30/01/16	Canal	Ommen
20		19	15		M	70	7 days	-	2	90	185	-	-	24.0	1-2 thin layers	21/07/16	27/07/16	Canal	Staphorst
21		20	16		F	67	7 days	-	2	78	170	6.7	-	6.7	3-4 thin layers	25/02/17	03/03/17	Canal	Hardenberg
22		21	345800		M	N/R	1 hr	-	1	-	-	-	-	-	-	01/08/21	01/08/21	Sea	Blackpool
23		22	345784		M	75	2 days	-	2	-	-	-	-	-	-	14/08/20	16/08/20	Brook (Fresh)	Blackpool
24		23	345778		M	N/R	1 day	-	1	-	-	-	-	-	-	-	-	Sea	Blackpool
25		24	345778	15/08/22	M	N/R	1 day	-	1	-	-	-	-	-	-	-	-	Sea	Blackpool
26	United Kingdom	25	344778		M	64	8.5 hrs	-	2	-	-	-	-	-	-	11/10/19	12/10/19	Sea	Blackpool
27		26	344648		M	49	0.5 hrs	-	2	-	-	-	-	-	-	31/08/19	31/08/19	Sea	Blackpool
28		27	342214		M	18	2 hrs	-	2	-	-	-	-	-	-	13/12/17	13/12/17	Sea	Blackpool
29		28	13775004	22/08/22	F	59	28 days	-	2	49	173	-	-	-	-	07/01/22	04/02/22	Sea	Blackpool
30		29	14569864		M	54	2 days*	-	2	-	-	-	-	-	-	-	09/06/22	Sea	Blackpool
31		30	21/001		M	51	6 hrs	Coloured	6	104	183	-	-	-	1 thick layer	05/01/21	06/01/21	Sewage	Breede Valley Municipality
32		31	21/002		M	28	6 hrs	Coloured	6	54	160	-	-	-	1 thick layer	05/01/21	06/01/21	Sewage	Breede Valley Municipality
33		32	21/009		M	18	10 min	Black	6	68	174	-	-	-	Shorts	10/02/21	10/02/21	Sea	Hout Bay, Cape Town
34		33	21/016		M	19	1 day	Coloured	5	57	170	-	-	-	1 thin layer	-	06/03/21	Sea	Grotto Bay
35		34	21/020		M	68	6 days	Caucasian	4	-	-	-	-	-	1 thin layer	21/03/21	27/03/21	Dam	Silver Mine, Cape Town
36		35	21/024		M	36	1 hr	Coloured	5	67.5	160	-	-	-	1-2 thin layers	12/05/21	12/05/21	Sea	Cape Peninsula, Cape Town
37		36	21/025		M	48	2 hrs	Caucasian	3	66	180	-	-	-	None	13/05/21	14/05/21	Bath	Mowbray, Cape Town
38		37	21/033		M	50	1 day	Black	5	64	173	-	-	-	1 thick layer	29/06/21	30/06/21	Marsh	Llandudno, Cape Town
39		38	21/036		M	21	3 hrs	Black	7	-	-	-	-	-	1-2 thick layers	24/07/21	24/07/21	Sea	Woodstock, Cape Town
40		39	21/038		M	70	1 day	Coloured	5	-	-	-	-	-	1-2 thin layers	-	30/07/21	Sea	Milnerton Rural, Cape Town
41		40	21/046		M	39	10 mins	Coloured	5	-	-	-	-	-	1-2 thick layers	17/08/21	18/08/21	Sea	Victoria & Alfred Waterfront, Cape Town

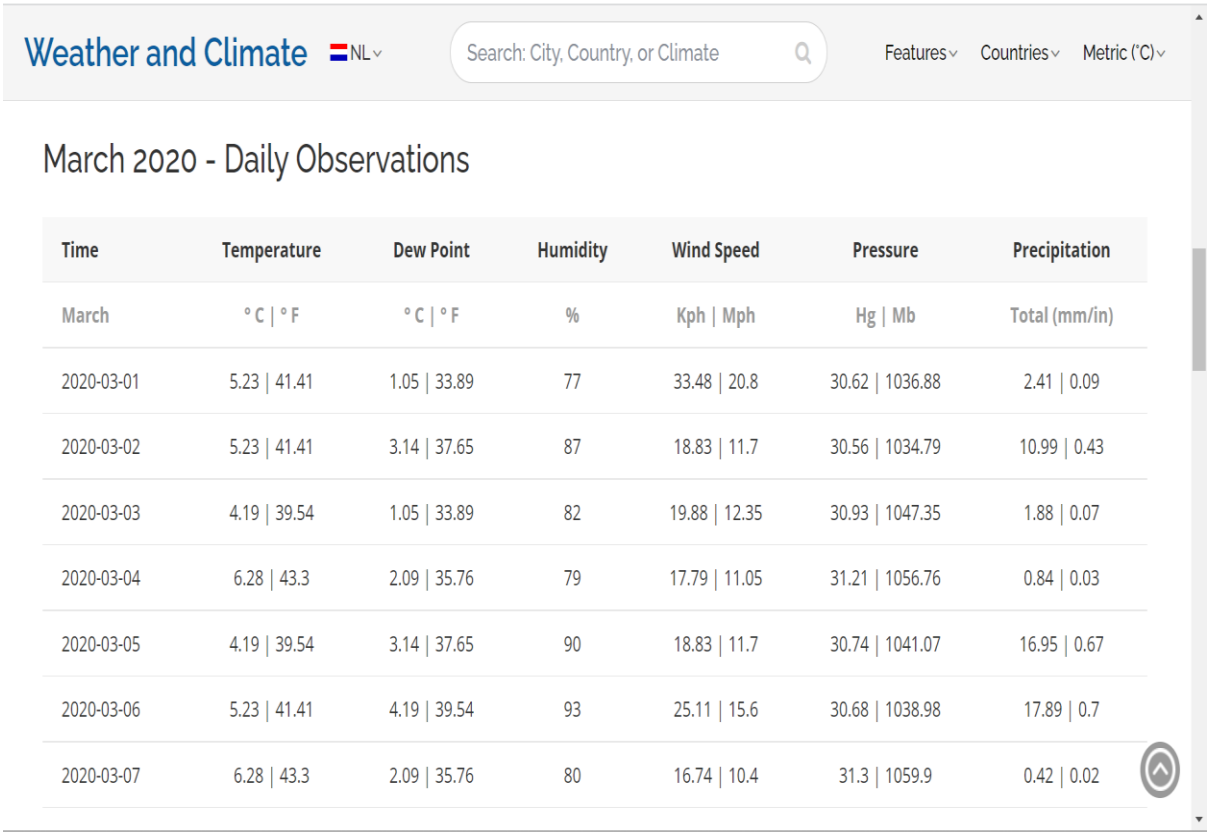
Figure 7.3: Collated information obtained from case notes.

7.2.4 Measuring Decomposition

The extent of decomposition of the bodies in the photographs, along with the description of the physical conditions or decomposition changes of the remains in the case notes, was visually assessed and scored with the Heaton, van Daalen and Reh DSS (Tables 3.2 and 3.3 and Figure 3.4). These DSS were used to assess the post-mortem changes observed in the head, neck, trunk, and limbs of the bodies. Where possible, the images were scored by two observers to reduce any observer bias and account for interobserver error.

7.2.5 Temperature Data

Where possible, a case's body, water and ambient temperatures were recorded. The retrospective daily ambient or water temperatures of the location of each case were also obtained from online sources such as the Global Historical Weather and Climate (The Global Historical Weather and Climate, n.d.) and Weather Underground (Weather Underground, n.d.) websites. The name of the city where a case was located was inputted into the websites, and the date the body was retrieved was used to obtain the average daily temperatures. Figure 7.4 below is an example of a search conducted for the average daily temperatures in Zwolle, Netherlands, for the first week in the month of March 2020.



March 2020 - Daily Observations

Time	Temperature	Dew Point	Humidity	Wind Speed	Pressure	Precipitation
March	°C | °F	°C | °F	%	Kph | Mph	Hg | Mb	Total (mm/in)
2020-03-01	5.23 | 41.41	1.05 | 33.89	77	33.48 | 20.8	30.62 | 1036.88	2.41 | 0.09
2020-03-02	5.23 | 41.41	3.14 | 37.65	87	18.83 | 11.7	30.56 | 1034.79	10.99 | 0.43
2020-03-03	4.19 | 39.54	1.05 | 33.89	82	19.88 | 12.35	30.93 | 1047.35	1.88 | 0.07
2020-03-04	6.28 | 43.3	2.09 | 35.76	79	17.79 | 11.05	31.21 | 1056.76	0.84 | 0.03
2020-03-05	4.19 | 39.54	3.14 | 37.65	90	18.83 | 11.7	30.74 | 1041.07	16.95 | 0.67
2020-03-06	5.23 | 41.41	4.19 | 39.54	93	25.11 | 15.6	30.68 | 1038.98	17.89 | 0.7
2020-03-07	6.28 | 43.3	2.09 | 35.76	80	16.74 | 10.4	31.3 | 1059.9	0.42 | 0.02

Figure 7.4: Average daily temperatures in Zwolle, Netherlands, for the first week of March 2020.

7.2.6 PMSI Estimations

7.2.6.1 *Heaton DSS*

The TAD scores calculated when using the Heaton DSS were substituted into the regression formula developed by Heaton *et al*. (2010) to extrapolate an ADD value.

$$TADS = -3.706 + 7.778 \log_{10} ADD$$

This can be further expressed as:

$$ADD = 10^{((TADS+3{\cdot}706)/(7{\cdot}778))}$$

To estimate the PMSI, the average daily ambient temperature values obtained from the weather forecast websites were added retrospectively from the day the body was discovered until the ADD value was reached. In a situation where the extrapolated ADD was smaller than the first average daily temperature (which is the day the body was found), it indicates that the estimated PMSI was not up to 24 hours because ADD is a summation of average daily temperatures. A modified equation was created to calculate the estimated PMSI in hours:

$$Est.PMSI = \left(\frac{24hrs \times Ext.ADD}{T_{ave.}}\right)$$

Where, Est. PMSI = Estimated PMSI

Ext. ADD = Extrapolated ADD

$T_{ave.}$ = Average Daily Temperature

For example, in a case where the extrapolated ADD was 15 and the average daily temperature on the day the body was recovered was 20 ℃, the estimated PMSI would be:

Est. PMSI = (24hrs × 15)/ 20℃

Est. PMSI = 18 hours.

7.2.6.2 *van Daalen DSS*

The Heaton *et al*. (2010) study extrapolates the ADD from the TADS and uses the extrapolated ADD to estimate the PMSI, but in the van Daalen *et al*. (2017) study, the PMSI was predicted from the TADS using a prediction model and not the ADD. Unfortunately, the study did not provide the prediction formula or equation used to predict the PMSI. Hence, two alternative options were attempted to estimate each case's PMSI. These are explained below.

New Regression Formula

A graph of the TADS of all the cases against the log of their ADD was plotted to develop a regression formula using the equation of the best line of fit (Figure 7.5). This regression formula was then used to extrapolate an ADD for each case.

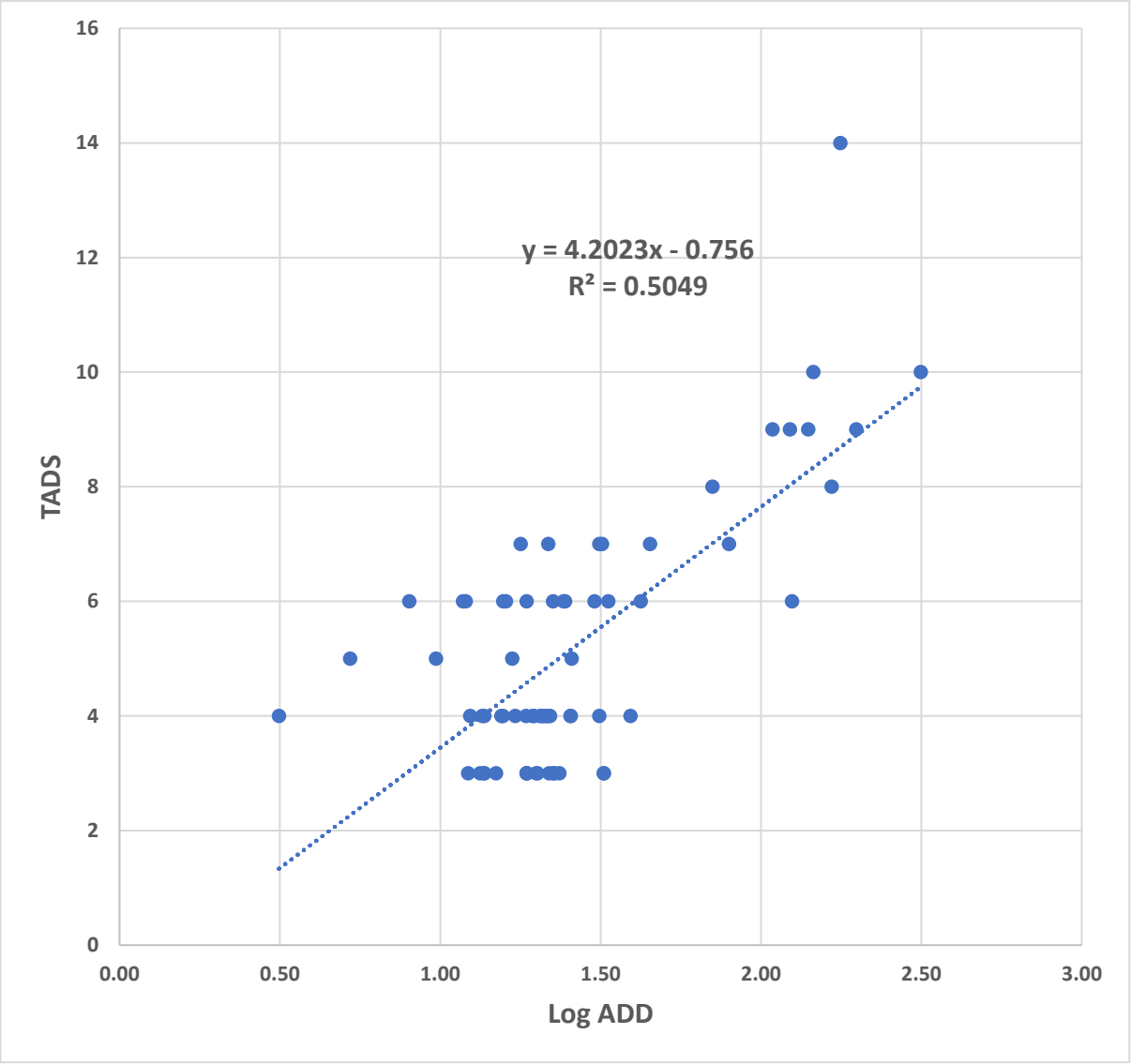


Figure 7.5: Graph used to develop a regression formula for the cases.

The equation of the line was y = -0.756 + 4.2023x

Where y = TADS and x = Log ADD. The equation can be rewritten as:

$$TADS = -0.756 + 4.2023 \log_{10} ADD$$

This can be further expressed as:

$$ADD = 10^{((TADS+0.756)/(4.2023))}$$

The van Daalen TADS values were substituted into this regression formula, but the extrapolated ADD values obtained were extremely high compared to the actual ADD values. For example, a case with an actual ADD of 830.3 had its extrapolated ADD value as 3246.50. In addition, the r^2 value, which is the best line of fit of the graph, was 0.50. This r^2 value is too low for extrapolating and indicates that the model does not fit well with the data. Therefore, this regression formula was not used in PMSI estimations.

Converting van Daalen TADS to 25

A conversion factor was needed to convert the van Daalen scores to the 25-point scale used for the Heaton scores (Heaton maximum score = 25, van Daalen maximum score = 18). To do this conversion, each van Daalen score was divided by 0.72 (which is 18/25). For example, a van Daalen score of 18 was divided by 0.72 to give 25, and a van Daalen score of 10 was divided by 0.72 to give 13.9 [or (10/18) × 25]. These converted values were then substituted into the Heaton regression formula to extrapolate an ADD for each case. The PMSI of each case was estimated by cumulatively adding the average daily ambient temperature values retrospectively from the day the body was discovered until the ADD value was reached, just like it was applied in the Heaton DSS. Like for the Heaton DSS, in a situation where the extrapolated ADD was smaller than the first average daily temperature (which is the day the body was found), it indicates that the estimated PMSI was not up to 24 hours, because ADD is a summation of average daily temperatures. A modified equation was created to calculate the estimated PMSI in hours:

$$Est.PMSI = \left(\frac{24hrs \times Ext.ADD}{T_{ave.}}\right)$$

Where, Est. PMSI = Estimated PMSI

Ext. ADD = Extrapolated ADD

$T_{ave.}$ = Average Daily Temperature

This conversion option was more suitable than the new regression formula. For example, the case with an actual ADD of 830.3 had its extrapolated ADD value as 947.1 when using this conversion option.

7.2.6.3 *Reh DSS*

Unlike the Heaton and van Daalen DSS, the estimated PMSI of a body using the Reh DSS is not extrapolated from an equation. Instead, Reh created a table (Figure 3.4) for PMSI estimation by considering the month the body was discovered, the actual water temperature and the external or internal physical changes in the bodies immersed in water. In the Reh Table, decomposition changes were correlated with a minimum time in which this sign can develop. The immersion time it has taken for the last decomposition feature to be visible is considered the minimum PMSI, while the maximum PMSI can be estimated by considering the putrefaction changes that have not yet occurred in the body.

7.2.7 Evaluating the Sufficiency of FADS, BADS and LADS

When estimating PMSI, the TADS of a body is substituted into the regression equations. Assessing the extent of decomposition or calculating the TADS of a body and estimating the PMSI when a body part is missing or not visible enough to assess will most likely lead to incorrect/inaccurate PMSI estimates because one or more of the FADS, BADS and LADS of the body is missing. In this study, cases in which the body was dismembered with missing limbs, head, or trunk and cases with no images of one or more of the following: head, neck, limbs, and trunk were initially excluded from the study. The available parts of these bodies were scored with the Heaton and van Daalen DSS, and the incomplete TADS (Inc-TADS) was used to estimate the PMSI.

For example, in a body where the head was visible in the picture, and this head exhibited bloating signs (FADS = 4), but the trunk and limbs were covered with clothes (BADS = 0, LADS = 0), the Inc-TADS would be: 4 + 0 + 0 = 4. BADS and LADS are 0 because they were not visible to score. The Inc-TADS of 4 was then substituted in the regression formula and used to estimate the PMSI. This estimated PMSI and the PMSI values stated in the case notes were compared to see if there was any significant difference between both PMSIs. This process was conducted to investigate if scoring one or two of the body parts for assessing the extent of decomposition of a body was sufficient for estimating the PMSI of the body; to determine whether it is possible to use one or two of the FADS, BADS and LADS against the TADS when estimating PMSI.

7.2.8 Statistical and Further Analysis

Depending on if the data were normally distributed, the estimated PMSIs for the cases with complete body parts and the ones with missing body parts were compared to the stated PMSIs in the case files by conducting an independent sample t-test or a Mann-Whitney U test to see if there was a significant difference between the estimated PMSI and the recorded PMSI values. The Heaton and van Daalen TAD scores of each case were normalised to a ratio of 1, and independent sample t-tests or the Mann-Whitney U test were conducted to investigate if there was a significant difference between the two DSS scores. This determined if both DSS could be substituted for each other when assessing the extent of decomposition in a drowned body.

7.3 Results

7.3.1 Demographic Profiling

Of the 73 cases used for this study, 61 (84%) were male, while 12 (16%) were female. Only 69 out of the 73 cases had their ages recorded; 18 (25%) of the cases were in the age range of 18–29, 13 (18%) were 30–39 years old, 5 (7%) in ages 40–49 range, 12 (16%) in the 50–59 age range, 21 (29%) were aged 60 and above, and 4 (5%) of the cases did not have their ages recorded.

The bodies used for this study were recovered from different types of water bodies. 32 (44%) bodies were found in seas, 6 (8%) in rivers, 15 (21%) retrieved from canals, 2 (3%) from lakes, 1 (1%) from a brook, 6 (8%) from dams, 5 (7%) were found in bathtubs, and 6 (8%) bodies were recovered from other sources such as a ditch and sewages.

The ethnicities or ancestral affiliations of most of the cases were not mentioned in the case files. Therefore, this study decided to stratify the cases based on the Massey-Martin (2003) skin colour guide. Cases with a skin colour score between 1-3 were tagged as “Pale”, while those with a score between 4-10 were tagged as “dark”. The results obtained from scoring the skin tone of each body indicated that 38 (52%) of the cases were stratified as Pale, while 35 (48%) cases were tagged as Dark.

7.3.2 TADS

The results of the TAD scores for each case when using the Heaton and van Daalen DSS are listed in Table 7.4 below. The TADS values in the table indicate that the decomposition across the cases ranged from a fresh appearance to an advanced state of decomposition. The lowest TADS value obtained when using the Heaton and van Daalen DSS was a score of 3; this indicates that when assessing the extent of decomposition of the body, there was no visible decomposition change observed in the head, neck, trunk, and limb regions of that body. The highest TADS obtained when using the Heaton DSS was 19, while that of the van Daalen DSS was 14; these scores indicate that bone exposure and advanced internal organ decomposition were the decomposition features observed in the body with those scores.

In this study, most of the cases were in the fresh stage of decomposition. Some of the fresh cases in the study had no visible decomposition change when assessed. At the same time, some fresh cases only showed signs of mild wrinkling of the flesh of the hands and feet, epidermis peeling off, and discolouration changes when assessed. The advanced decomposed cases exhibited decomposition features such as skin slippage, bone exposure, liquefaction, degloving of the hands and feet, and advanced soft tissue and internal organ decomposition. A further breakdown of the TADS of each case can be seen in Figure 7.6.

7.3.3 DSS Comparison

A Mann-Whitney U test was needed to compare the TADS of the two DSS statistically, but this would not have been possible because they had different maximum scores (Heaton maximum score = 25; van Daalen maximum score = 18) [Table 7.4]. Therefore, the Heaton TADS were divided by 25 and the van Daalen TADS were divided by 18 to normalise both scores to a ratio of 1. Thus, they both had a maximum score of 1, and the Mann-Whitney U test was used to analyse if there was any significant difference in the TADS obtained when using the Heaton and van Daalen DSS to assess the extent of decomposition in each case. The Mann-Whitney U test result indicated a statistically significant difference between the TADS of the Heaton DSS and the van Daalen DSS for the cases in the study ($p = 0.002$).

Table 7.4: TADS of all the cases used. S/N stands for sample number.

S/N	TADS		S/N	TADS		S/N	TADS		S/N	TADS	
	Heaton	**van Daalen**		**Heaton**	**van Daalen**		**Heaton**	**van Daalen**		**Heaton**	**van Daalen**
1	4	4	**19**	15	10	**37**	4	4	**55**	3	3
2	4	4	**20**	7	7	**38**	6	5	**56**	5	4
3	5	4	**21**	4	3	**39**	4	4	**57**	4	4
4	14	9	**22**	9	7	**40**	3	3	**58**	3	3
5	6	6	**23**	6	6	**41**	6	5	**59**	4	3
6	15	12	**24**	6	6	**42**	10	7	**60**	3	3
7	5	5	**25**	6	6	**43**	3	3	**61**	3	3
8	16	9	**26**	6	6	**44**	15	9	**62**	7	4
9	5	5	**27**	6	6	**45**	4	4	**63**	4	4
10	17	10	**28**	19	14	**46**	6	6	**64**	5	4
11	6	5	**29**	7	6	**47**	3	3	**65**	5	4
12	16	9	**30**	4	4	**48**	6	7	**66**	6	6
13	11	8	**31**	4	4	**49**	3	3	**67**	3	3
14	6	5	**32**	3	3	**50**	4	4	**68**	14	8
15	5	4	**33**	3	3	**51**	5	4	**69**	4	4
16	6	5	**34**	9	6	**52**	3	3	**70**	7	6
17	6	5	**35**	3	3	**53**	13	7	**71**	3	3
18	5	5	**36**	4	4	**54**	5	4	**72**	4	4
									73	3	3

7.3.4 PMSI Estimates

The PMSI of each case was estimated using the Heaton, van Daalen and Reh DSS, and these three estimates are listed in Figure 7.6 and Table 7.5 below. Figure 7.6 illustrates the complete breakdown of each case; it shows the sex/gender, age, ancestry, skin colour score, location, and date a body was recovered from the water, the breakdown of the TADS and the maximum PMSI estimates obtained using the Heaton, van Daalen and Reh DSS.

Country	S/N	Case No.	Sex	Age	PMSI	Water Type	Location	Ancestry	Colour Scale	Recovered	Heaton						van Daalen							Reh		
											FADS	BADS	LADS	TADS	Est. ADD	Est. PMSI	FADS	BADS	LADS	TADS	H.TADS	Est. ADD	Est. PMSI	Descriptions No.	Month	Est. PMSI
Netherlands	1	263082	M	49	3 hrs	Bath	Deventer	-	6	30/07/15	1	1	2	4	9.8	13 hrs	1	1	2	4	5.6	15.5	22 hrs	6, 10	July	min 0.5 hrs
	2	457606	M	70	2 hrs	Canal	Zwolle	-	1	03/12/17	1	1	2	4	9.8	5 days	1	1	2	4	5.6	15.5	7 days	6	December	1 day max
	3	518882	M	69	20 hrs	Canal	Steenwijkerwold	-	1	04/07/18	2	1	2	5	13.2	14 hrs	1	1	2	4	5.6	15.5	17 hrs	1, 6, 10	July	2 days max
	4	539337	M	23	7 days	Lake	Hardenberg	-	2	11/10/18	4	5	5	14	189.0	14 days	3	3	3	9	12.5	121.2	8 days	1, 3, 4	October	7 days max
	5	579251	M	57	1 hr	Canal	Zwolle	-	2	17/04/19	2	2	2	6	17.7	2 days	2	2	2	6	8.3	35.3	3 days	1	April	9-10 days max
	6	602651	M	36	-	Canal	Schalkhaar	-	2	29/07/19	5	5	5	15	254.1	11 days	4	4	4	12	16.7	416.2	20 days	1, 2, 3, 4, 12, 16	July	3 days max
	7	606018	M	59	1 day	River	Zwolle	-	1	13/08/19	2	1	2	5	13.2	19 hrs	2	1	2	5	6.9	23.4	2 days	1, 2, 6, 10	August	3 days max
	8	611760	F	50	8 days	Canal	Ommen	-	2	07/09/19	5	5	6	16	341.6	17 days	3	3	3	9	12.5	121.2	8 days	1, 2, 3, 4, 5, 12, 14, 16	September	11 days max
	9	632404	M	39	2 hrs	Ditch (fresh)	Zwolle	-	2	03/12/19	1	2	2	5	13.2	3 days	1	2	2	5	6.9	23.4	5 days	1	December	17 days max
	10	1	M	56	18 days	Canal	Wijhe	-	2	03/08/21	5	5	7	17	459.3	24 days	3	3	4	10	13.9	182.9	11 days	1, 2, 3, 4, 9, 12	August	10 days max
	11	3	M	74	11 hrs	Canal	Staphorst	-	1	16/08/20	2	1	3	6	17.7	18 hrs	2	1	2	5	6.9	23.4	23 hrs	1, 6, 10	August	2 days max
	12	4	M	45	27 days	River	Overijssel	-	2	05/04/20	5	5	6	16	341.6	49 days	3	3	3	9	12.5	121.2	19 days	2, 3, 4, 8, 12, 13	April	35 days max
	13	5	M	36	29 days	River	's-Heerdenbroek	-	4	02/01/20	4	4	3	11	77.8	16 days	3	2	3	8	11.1	80.4	15 days	1, 3, 4	January	35 days max
	14	7	F	74	2 hrs	Canal	Staphorst	-	2	12/02/21	2	2	2	6	17.7	17 days	2	1	2	5	6.9	23.4	22 days	6, 10	February	1 day max
	15	8	M	24	3 days	Ditch (fresh)	Giethoorn	-	6	22/01/21	2	1	2	5	13.2	3 days	1	1	2	4	5.6	15.5	3 days	6, 10	January	1 day max
	16	9	M	80	5 hrs	River	Deventer	-	2	26/07/16	2	2	2	6	17.7	19 hrs	2	1	2	5	6.9	23.4	2 days	1, 3, 6, 10	July	2 days max
	17	12	M	71	2 days	River	Kampen	-	2	19/03/16	2	2	2	6	17.7	3 days	2	1	2	5	6.9	23.4	4 days	6	March	12 hrs
	18	14	F	62	5 days	Canal	Ommen	-	1	30/01/16	2	1	2	5	13.2	2 days	2	1	2	5	6.9	23.4	4 days	6, 10	January	1 day max
	19	15	M	70	7 days	Canal	Staphorst	-	2	27/07/16	5	5	5	15	254.1	13 days	4	3	3	10	13.9	182.9	9 days	1,2,3,4,5,8,12,16	July	3 days max
	20	16	F	67	7 days	Canal	Hardenberg	-	2	03/03/17	2	2	3	7	23.8	4 days	2	2	3	7	9.7	53.3	9 days	6, 10	March	12 hrs
United Kingdom	21	345800	M	N/R	1 hr	Sea	Blackpool	-	1	20/08/20	2	1	1	4	9.8	12 hrs	1	1	1	3	4.2	10.3	13 hrs	1	August	2 days max
	22	345784	M	75	2 days	Brook (Fresh)	Blackpool	-	2	16/08/21	3	3	3	9	43.0	3 days	2	2	3	7	9.7	53.3	4 days	1,3,6,10	August	3 days max
	23	345778	M	N/R	1 day*	Sea	Blackpool	-	1	16/08/20	2	2	2	6	17.7	2 days	2	2	2	6	8.3	35.3	3 days	Fresh	August	1 day max
	24	345778	M	N/R	1 day*	Sea	Blackpool	-	1	16/08/20	2	2	2	6	17.7	2 days	2	2	2	6	8.3	35.3	3 days	fresh	August	1 day max
	25	344778	M	64	8.5 hrs	Sea	Blackpool	-	2	12/10/19	2	2	2	6	17.7	2 days	2	2	2	6	8.3	35.3	3 days	1,3,6,10	October	7 days max
	26	344648	M	49	0.5 hrs	Sea	Blackpool	-	2	31/08/19	2	2	2	6	17.7	2 days	2	2	2	6	8.3	35.3	3 days	1,6,10	August	2 days max
	27	342214	M	18	2 hrs	Sea	Blackpool	-	2	13/12/17	2	2	2	6	17.7	6 days	2	2	2	6	8.3	35.3	9 days	6,10	December	1 hour min
	28	13775004	F	59	28 days	Sea	Blackpool	-	2	04/02/22	6	7	6	19	830.3	107 days	5	5	4	14	19.4	947.1	117 days	1,3,4,8	February	45 days max
	29	14569864	M	54	2 days*	Sea	Blackpool	-	2	09/06/22	3	2	2	7	23.8	2 days	2	2	2	6	8.3	35.3	3 days	1,3,6,10	June	2 days max
	30	21/001	M	51	6 hrs	Sewage	Breede Valley Municipality	Coloured	6	06/01/21	1	1	2	4	9.8	11 hrs	1	1	2	4	5.6	15.5	18 hrs	6,10	January	1 day max
	31	21/002	M	28	6 hrs	Sewage	Breede Valley Municipality	Coloured	6	06/01/21	1	1	2	4	9.8	11 hrs	1	1	2	4	5.6	15.5	18 hrs	6,10	January	1 day max
	32	21/009	M	18	10 mins	Sea	Hout Bay, Cape Town	Black	6	10/02/21	1	1	1	3	7.3	8 hrs	1	1	1	3	4.2	10.3	11 hrs	fresh	February	1 day max
	33	21/016	M	19	1 day	Sea	Grotto Bay, Cape Town	Coloured	5	06/03/21	1	1	1	3	7.3	9 hrs	1	1	1	3	4.2	10.3	12 hrs	fresh	March	1 day max
	34	21/020	M	68	6 days	Dam	Silver Mine, Cape Town	Caucasian	4	27/03/21	3	3	3	9	43.0	3 days	2	2	2	6	8.3	35.3	2 days	1,6,10	March	23 days max
	35	21/024	M	36	1 hr	Sea	Cape Peninsula, Cape Town	Coloured	5	12/05/21	1	1	1	3	7.3	11 hrs	1	1	1	3	4.2	10.3	17 hrs	fresh	May	1 day max
	36	21/025	M	48	2 hrs	Bath	Mowbray, Cape Town	Caucasian	3	14/05/21	1	1	2	4	9.8	15 hrs	1	1	2	4	5.6	15.5	1 day	6,10	May	1 day max

39		**37**	21/033	M	50	1 day	Marsh	Llandudno, Cape Town	Black	5	30/06/21	1	1	2	**4**	9.8	17 hrs	1	1	2	**4**	**5.6**	15.5	2 days	6,10	June	6 hrs
40		**38**	21/036	M	21	3 hrs	Sea	Woodstock, Cape Town	Black	7	24/07/21	3	1	2	**6**	17.7	2 days	2	1	2	**5**	**6.9**	23.4	3 days	fresh	July	1 day max
41		**39**	21/038	M	70	1 day	Sea	Milnerton Rural, Cape Town	Coloured	5	30/07/21	1	1	2	**4**	9.8	19 hrs	1	1	2	**4**	**5.6**	15.5	2 days	3,10	July	2 days max
42		**40**	21/046	M	39	10 mins	Sea	Cape Town	Coloured	5	18/08/21	1	1	1	**3**	7.3	13 hrs	1	1	1	**3**	**4.2**	10.3	19 hrs	fresh	August	1 day max
43		**41**	21/049	F	69	2 days	Bath	Lansdowne, Cape Town	Coloured	3	01/09/21	2	1	3	**6**	17.7	2 days	2	1	2	**5**	**6.9**	23.4	2 days	3,6,10	September	3 days max
44		**42**	21/058	M	35	5 days	Sea	Cape Town	Coloured	2	29/09/21	4	3	3	**10**	57.8	4 days	2	2	3	**7**	**9.7**	53.3	4 days	1,2,6,10,12	September	8-9 days max
45		**43**	21/059	M	N/R	1 day	Sea	False Bay, Cape Town	Coloured	5	03/10/21	1	1	1	**3**	7.3	14 hrs	1	1	1	**3**	**4.2**	10.3	20 hrs	fresh	October	1 day max
46		**44**	21/060	M	35	8 days	Sea	Woodstock, Cape Town	Coloured	4	04/10/21	5	5	5	**15**	254.1	17 days	4	2	3	**9**	**12.5**	121.2	8 days	1,3,4,12	October	14 days max
47		**45**	21/065	M	20	1 day	Dam	Table Mountain, Cape Town	Caucasian	2	11/10/21	2	1	1	**4**	9.8	12 hrs	2	1	1	**4**	**5.6**	15.5	19 hrs	1,3	October	7 days max
48		**46**	21/073	F	27	2 days	Sea	Kommetjie, Cape Town	Caucasian	2	01/11/21	2	2	2	**6**	17.7	2 days	2	2	2	**6**	**8.3**	35.3	3 days	3	November	10 days max
49		**47**	21/079	M	26	3 hrs	Dam	Silver Mine, Cape Town	Coloured	3	20/11/21	1	1	1	**3**	7.3	9 hrs	1	1	1	**3**	**4.2**	10.3	12 hrs	fresh	November	1 day max
50		**48**	21/080	F	73	1 day	Sea	Simon's Town, Cape Town	Caucasian	3	25/11/21	2	2	2	**6**	17.7	19.5 hrs	2	2	3	**7**	**9.7**	53.3	3 days	1,3	November	10 days max
51		**49**	21/084	M	57	12 hrs	Bath	Cape Town	Coloured	6	18/12/21	1	1	1	**3**	7.3	8 hrs	1	1	1	**3**	**4.2**	10.3	11 hrs	fresh	December	1 day max
52		**50**	21/085	M	32	1 day	Dam	Darling, Cape Town	Coloured	6	20/12/21	1	1	2	**4**	9.8	9 hrs	1	1	2	**4**	**5.6**	15.5	15 hrs	6,10	December	1 day max
53	**South Africa**	**51**	21/087	F	79	1 day	Bath	Melkbosstrand, Cape Town	Caucasian	2	29/12/21	1	1	3	**5**	13.2	17 hrs	1	1	2	**4**	**5.6**	15.5	20 hrs	6,10	December	1 day max
54		**52**	20/009	M	32	8 hrs	Ditch (Fresh)	Pinelands, Cape Town	Black	6	20/02/20	1	1	1	**3**	7.3	8 hrs	1	1	1	**3**	**4.2**	10.3	12 hrs	fresh	February	1 day max
55		**53**	20/020	M	27	2 days	Sea	Bakoven, Cape Town	Black	7	23/05/20	4	4	5	**13**	140.6	8 days	2	2	3	**7**	**9.7**	53.3	4 days	4	May	4-5 days max
56		**54**	20/023	M	50	15 hrs	Canal	Ottery, Cape Town	Black	8	09/06/20	2	1	2	**5**	13.2	15 hrs	1	1	2	**4**	**5.6**	15.5	2 days	6,10	June	6 hrs
57		**55**	20/028	M	27	3 hrs	Dam	Retreat, Cape Town	Coloured	6	05/07/20	1	1	1	**3**	7.3	13 hrs	1	1	1	**3**	**4.2**	10.3	18 hrs	fresh	July	1 day max
58		**56**	20/041	M	70	2 days*	Canal	Diep River, Cape Town	Coloured	5	07/09/20	2	1	2	**5**	13.2	18 hrs	1	1	2	**4**	**5.6**	15.5	1 day	6,10	September	2 hrs
59		**57**	20/064	M	21	2 days	Sea	Muizenberg, Cape Town	Black	8	30/11/20	1	1	2	**4**	9.8	12 hrs	1	1	2	**4**	**5.6**	15.5	21 hrs	6	November	2 hrs
60		**58**	20/065	M	21	1 day	Canal	Bonteheuwel, Cape Town	Black	7	05/12/20	1	1	1	**3**	7.3	9 hrs	1	1	1	**3**	**4.2**	10.3	11 hrs	fresh	December	1 day max
61		**59**	20/066	M	20	1 day*	Canal	Langa, Cape Town	Black	7	05/12/20	2	1	1	**4**	9.8	13 hrs	1	1	1	**3**	**4.2**	10.3	11 hrs	fresh	December	1 day max
62		**60**	18/006	M	60	1 hr	Sea	Clifton, Cape Town	Black	6	04/01/18	1	1	1	**3**	7.3	5 hrs	1	1	1	**3**	**4.2**	10.3	12 hrs	fresh	January	1 day max
63		**61**	18/007	M	25	10 mins	Sea	Kommetjie, Cape Town	Black	5	04/01/18	1	1	1	**3**	7.3	5 hrs	1	1	1	**3**	**4.2**	10.3	12 hrs	fresh	January	1 day max
64		**62**	18/010	M	74	1 day	Lake	Cape Town	Caucasian	2	06/01/18	2	2	3	**7**	23.8	22 hrs	1	1	2	**4**	**5.6**	15.5	17 hrs	6	January	1 day max
65		**63**	18/018	M	27	3 hrs	Dam	Cape Town	Indian	5	25/01/18	1	1	2	**4**	9.8	11 hrs	1	1	2	**4**	**5.6**	15.5	17 hrs	6,10	January	1 day max
66		**64**	18/028	M	78	1 day	Sea	Grotto Bay, Cape Town	Caucasian	2	04/03/18	2	1	2	**5**	13.2	15 hrs	1	1	2	**4**	**5.6**	15.5	18 hrs	6,10	March	12 hrs
67		**65**	18/032	F	56	1 day	Sea	Kommetjie, Cape Town	Caucasian	2	25/03/18	2	1	2	**5**	13.2	20 hrs	1	1	2	**4**	**5.6**	15.5	22 hrs	6,10	March	12 hrs
68		**66**	18/045	M	33	2 days*	River	Hazendal, Cape Town	Coloured	6	05/07/18	2	2	2	**6**	17.7	2 days	2	2	2	**6**	**8.3**	35.3	4 days	3,6,10	July	2 days max
69		**67**	18/064	M	33	1 hr	Sea	Camps Bay, Cape Town	Black	7	07/10/18	1	1	1	**3**	7.3	8 hrs	1	1	1	**3**	**4.2**	10.3	11 hrs	fresh	October	1 day max
70		**68**	17/002	M	20	3 days	Sea	Strandfontein, Cape Town	Black	6	11/01/17	5	4	5	**14**	189.0	8 days	3	2	3	**8**	**11.1**	80.4	4 days	1,2,3,4,5	January	35 days max
71		**69**	17/003	M	37	4 hrs	Sea	Sea Point Promenade, Cape Town	Coloured	4	15/01/17	1	1	2	**4**	9.8	11 hrs	1	1	2	**4**	**5.6**	15.5	19 hrs	6,10	January	1 day max
72		**70**	17/005	M	62	1 day	Sea	Cape Town	Caucasian	2	05/02/17	2	2	3	**7**	23.8	2 days	2	2	2	**6**	**8.3**	35.3	2 days	3,6,10	February	25 days max
73		**71**	17/016	F	53	2 hrs	Sea	Hout Bay, Cape Town	Coloured	5	20/05/17	1	1	1	**3**	7.3	9 hrs	1	1	1	**3**	**4.2**	10.3	19 hrs	fresh	May	1 day max
74		**72**	17/018	M	34	3 hrs	Sea	Cape Town	Black	6	11/06/17	1	1	2	**4**	9.8	17 hrs	1	1	2	**4**	**5.6**	15.5	2 days	6	June	6 hrs
75		**73**	17/019	F	44	3 hrs	Sea	Hout Bay, Cape Town	Black	6	14/06/17	1	1	1	**3**	7.3	13 hrs	1	1	1	**3**	**4.2**	10.3	19 hrs	fresh	June	1 day max

Figure 7.6: Breakdown of the TADS, PMSI estimates and demographic profile of each case.

Table 7.5: Recorded rounded-up PMSI in the case notes and the Estimated PMSIs using the three DSS. S/N stands for sample number.

S/N	PMSI (Days)					S/N	PMSI (Days)					S/N	PMSI (Days)				
	Skin Tone	Notes	Heaton	Van Daalen	Reh		Skin Tone	Notes	Heaton	Van Daalen	Reh		Skin Tone	Notes	Heaton	Van Daalen	Reh
1	Dark	1	1	1	1	**14**	Pale	1	17	22	1	**27**	Pale	1	6	9	1
2	Pale	1	5	7	1	**15**	Dark	3	3	3	1	**28**	Pale	28	107	117	45
3	Pale	1	1	1	2	**16**	Pale	1	1	2	2	**29**	Pale	2	2	3	2
4	Pale	7	14	8	7	**17**	Pale	2	3	4	1	**30**	Dark	1	1	1	1
5	Pale	1	2	3	9	**18**	Pale	5	2	4	1	**31**	Dark	1	1	1	1
6	Pale	-	11	20	3	**19**	Pale	7	13	9	3	**32**	Dark	1	1	1	1
7	Pale	1	1	2	3	**20**	Pale	7	4	9	1	**33**	Dark	1	1	1	1
8	Pale	8	17	8	11	**21**	Pale	1	1	1	2	**34**	Dark	6	3	2	23
9	Pale	1	3	5	17	**22**	Pale	2	3	4	3	**35**	Dark	1	1	1	1
10	Pale	18	24	11	10	**23**	Pale	1	2	3	1	**36**	Pale	1	1	1	1
11	Pale	1	1	1	2	**24**	Pale	1	2	3	1	**37**	Dark	1	1	2	1
12	Pale	27	49	19	35	**25**	Pale	1	2	3	7	**38**	Dark	1	2	3	1
13	Dark	29	16	15	35	**26**	Pale	1	2	3	2	**39**	Dark	1	1	2	2

40	Dark	1	1	1	1	**53**	Dark	2	8	4	4	**66**	Dark	2	2	4	2
41	Pale	2	2	2	3	**54**	Dark	1	1	2	1	**67**	Dark	1	1	1	1
42	Pale	5	4	4	8	**55**	Dark	1	1	1	1	**68**	Dark	3	8	4	35
43	Dark	1	1	1	1	**56**	Dark	2	1	1	1	**69**	Dark	1	1	1	1
44	Dark	8	17	8	14	**57**	Dark	2	1	1	1	**70**	Pale	1	2	2	25
45	Pale	1	1	1	7	**58**	Dark	1	1	1	1	**71**	Dark	1	1	1	1
46	Pale	2	2	3	10	**59**	Dark	1	1	1	1	**72**	Dark	1	1	2	1
47	Pale	1	1	1	1	**60**	Dark	1	1	1	1	**73**	Dark	1	1	1	1
48	Pale	1	1	3	10	**61**	Dark	1	1	1	1						
49	Dark	1	1	1	1	**62**	Pale	1	1	1	1						
50	Dark	1	1	1	1	**63**	Dark	1	1	1	1						
51	Pale	1	1	1	1	**64**	Pale	1	1	1	1						
52	Dark	1	1	1	1	**65**	Pale	1	1	1	1						

Table 7.5 shows the rounded-up estimated PMSI values of each case; the PMSI values below 24 hours were rounded up to 1 day to have all the PMSI values uniformly expressed in days for statistical analyses. Statistical analyses were conducted to investigate if there was a statistically significant difference between the estimated PMSI (when using the three DSS) and the PMSI values stated in the case notes. Some of the estimated PMSI values were in hours (Figure 7.6). These values had to be rounded up to the nearest day figure to have all the PMSI reported in days (Table 7.5); PMSI values of less than 24 hours were rounded up to 1 day.

A Two-way ANOVA test was used to compare the rounded-up PMSI values—[See Appendix 7.4 for statistics]. The results of the test indicated that there was no statistically significant difference between the four PMSI values ($p = 0.612$). A Tukey's Post Hoc test was done to further investigate the specific *p-value* between each PMSI compared to the other three sets of PMSI (Table 7.6). Results of the post hoc test indicated no statistically significant difference between each PMSI compared to the other PMSI values.

Table 7.6: Post Hoc test results of the four sets of PMSI.

Case notes *vs*	Heaton ($p = 0.644$) van Daalen ($p = 0.784$) Reh ($p = 0.671$)
Heaton *vs*	van Daalen ($p = 0.995$) Reh ($p = 1.000$)
van Daalen *vs* Reh ($p = 0.998$)	

7.3.5 PMSI Estimations with Missing Body Parts

Thirty-seven cases in which the body was dismembered with missing limbs, head, or trunk and cases with no images of one or more of the following: head, neck, limbs, and trunk, were initially excluded from the study. The available parts of these bodies were scored with the DSS, and the Inc-TADS was used to estimate the PMSI (Table 7.7)—[See Appendix 7.3 for the Spreadsheet of TADS & PMSI of Missing Body Parts]. This process was conducted to investigate if scoring one or two of the body parts used in scoring the extent of decomposition of a body (Head, Trunk, and Limbs) will be sufficient for estimating the PMSI of the body. In Table 7.7, the available body parts that were scored are listed as H for Head, T for Trunk, and L for Limbs in brackets beside the sample number of each case.

Table 7.7: Estimated PMSI using Inc-TADS. S/N: sample number, H: Head, T: Trunk, L: Limbs.

S/N & Body Region	PMSI (Days)			S/N & Body Region	PMSI (Days)			S/N & Body Region	PMSI (Days)		
	Notes	Heaton	Van Daalen		Notes	Heaton	Van Daalen		Notes	Heaton	Van Daalen
1 (H)	1	1	1	13 (H, L)	1	1	1	25 (H, L)	1	1	1
2 (H)	1	1	1	14 (H, L)	12	3	2	26 (L)	1	1	1
3 (H)	1	1	1	15 (H, L)	1	1	1	27 (H, L)	1	1	1
4 (H, L)	1	1	1	16 (H)	4	1	1	28 (H, L)	1	1	1
5 (T, L)	7	3	2	17 (H)	1	1	1	29 (H, T)	1	1	1
6 (H)	1	1	1	18 (H)	6	1	1	30 (H)	7	1	1
7 (H, L)	1	1	1	19 (H, T)	5	2	2	31 (H, T)	1	1	1
8 (H)	1	1	1	20 (H)	1	1	1	32 (T)	1	1	1
9 (H)	5	1	1	21 (L)	1	1	1	33 (L)	9	1	1
10 (H)	1	1	1	22 (H)	2	1	1	34 (H, L)	1	3	3
11 (H)	1	1	1	23 (L)	1	1	1	35 (H)	1	1	1
12 (H, L)	1	1	1	24 (L)	1	1	1	36 (H)	1	1	1
								37 (H, L)	1	1	1

The results in Table 7.7 indicate that the PMSI estimates of the Heaton and van Daalen DSS when using one or two of the FADS, BADS and LADS to make up the TADS were not as accurate as the case note PMSIs. A Two-way ANOVA test was conducted to compare and investigate if there was a statistically significant difference between the case note PMSIs and the Heaton and van Daalen PMSIs—[See Appendix 7.5 for statistics]. The results showed that there was a statistically significant difference between the three PMSI values ($p < 0.001$). A Tukey's Post Hoc test was conducted to investigate the statistical difference (*p-value*) between the case note PMSIs and the other two PMSIs (Table 7.8). Results of the Post Hoc test indicated that there was a statistically significant difference between the case note PMSI when compared to the Heaton and van Daalen PMSI values, but there was no statistically significant difference between the Heaton PMSIs and van Daalen PMSIs.

Table 7.8: Post Hoc test results of the three sets of PMSI.

Case notes *vs*	Heaton ($p = 0.010$)* van Daalen ($p = 0.007$)*
Heaton *vs*	van Daalen ($p = 0.989$)

***- a difference significant to the 5% level.**

7.4 Discussions

7.4.1 Demographic Profile

Seventy-three cases met the exclusion criteria and were used in this study, with 61 (84%) of the cases being male and 12 (16%) being female. Despite this small sample size, this sex/gender statistic of the study follows the trend that a higher proportion of drowned victims tend to be male (El Sibai *et al*., 2018; Franklin *et al*., 2020; Roberts *et al*., 2021). It has been suggested that men and boys are known to intentionally partake in more high-risk activities around water bodies compared to women and girls; therefore, this could be the reason more men and boys drowned in the cases used for this study (Ajibade *et al*., 2013; Peden *et al*., 2018).

There is a wide variation in the mortality rate of victims of different age brackets and ranges worldwide; some countries record the highest mortality rates in children, while some record the highest rates in younger adults (Lin *et al*., 2015). The Peden *et al*. (2019) study revealed that from 2005 to 2014, the percentage of drowning deaths in children aged 0–17 in Australia, Canada, and New Zealand was 17.6%, 12.1%, and 17.0%, respectively. The Morgan *et al*. (2008) study on the epidemiology of drownings fatalities in Australia indicated that the 20–29 years age group were the most involved in beach drownings. Also, in victims below the age of 29 years, drowning is among the three major leading causes of unintentional deaths (Moreland *et al*., 2022). There was also a variation in the number of deaths for different age brackets in this study, with the highest mortality rates occurring in the age range of 18–29 [18 cases (25%)] and the 60 and above category [21 cases (29%)].

The bodies of 56 out of the 73 cases were retrieved from natural water sources such as seas, rivers, canals, brooks, and lakes, while 17 bodies were retrieved from artificial sources such as dams, sewage channels, and ditches. These results also follow the trend that drowning frequently occurs in natural water sources compared to artificial sources (Koon *et al*., 2021). In the Lin *et al*. (2015) study, they investigated unintentional drowning cases in 60 countries. They found out that in most countries, drowning occurs more in natural water bodies such as lakes, seas, and ponds, compared to man-made water sources such as swimming pools and bathtubs, with Lithuania (85%), Finland (93%), and Panama (87%) having the highest occurrence of drowning at natural water sources.

In the 110 cases used for this study (PMSI estimates and PMSI estimates for missing body parts), the skin tone of 51 cases (46%) was stratified as pale, while 59 cases (54%) were tagged as dark. A Clemens *et al*. (2021) study reviewed the drowning cases involving people below the age of 29 years in the United States from 1999–2019. Their study found that drowning was more prevalent among victims from Black or African American (1.5 times more) and American Indian or Alaska Native ethnicities (2.0 times more) when compared to non-Hispanic White persons. A 2022 study indicated that in the United States, there was a 23.73% increase in the rate of death by drowning in Black or African American persons from 2019 to 2020 (Moreland *et al*., 2022). Also, Africa accounts for the highest

frequency of drowning incidents and low- and middle-income countries (LMICs) contribute about 90% of the global burden of drowning (Cenderadewi *et al*., 2020; Scarr *et al*., 2022; Tyler *et al*., 2017). This high drowning rate in Africa and LMICs is due to the limited drowning preventive measures, regulations and policies, basic swimming skills and education and awareness campaigns available in these countries and regions (Leavy *et al*., 2016; Mott & Latimer, 2016; Tyler *et al*., 2017). It is important to note that these LMICs, African American/American Indian/Alaska native-containing regions, and Africa are predominantly made up of individuals with dark skin tones. Therefore, this statistic indicates/reiterates the importance of the aims of this chapter because of the correlation between drowning and dark-skinned individuals.

7.4.2 PMSI Estimation

The PMSI estimation results (Figure 7.6 and Table 7.5) indicate that the three DSS were relatively accurate for estimating the PMSI of both dark and pale-skinned bodies in a fresh state of decomposition, while they were not accurate for bodies in advanced decomposition states. The results also showed that the three DSS did not accurately estimate the PMSI of bodies recovered in the cold or winter months. Despite the inaccuracy of these three DSS in estimating the PMSIs of bodies recovered in cold conditions or bodies in advanced decomposition states, there was no statistically significant difference ($p = 0.612$) between the PMSIs of the three DSS and the case note PMSIs (Table 7.6). This statistical result could be because of the study's high number of fresh body cases; the high accuracy of these DSS for these fresh body cases is why the *p values* are close to 1. The PMSI estimation of each DSS is further explained below in the following subsections.

7.4.2.1 *Heaton DSS*

The Heaton DSS and regression formula were relatively accurate for estimating the PMSI of both dark and pale-skinned bodies in a fresh state of decomposition. A body was termed "*fresh*" when it had a maximum TADS of 7–9; such bodies had discolouration features such as pink, red, yellow, green, and white and the wrinkling of the palms and the sole of the feet. For most of the bodies in a fresh state, the case notes PMSIs and estimated PMSIs were the same, while there was a difference of 1 day between the case note PMSIs and the estimated PMSIs for a few cases. Only four fresh cases (Cases 2, 9, 14 and 27; all pale-skinned victims from Netherlands and U.K.) had more than a difference of 1 day between the case note PMSIs and the estimated PMSIs; these cases occurred during the European winter months of February and December. It is possible that the low temperatures experienced during the winter months could be the reason for this major difference between the case note PMSIs and the estimated PMSIs of these cases. There were days when the average daily temperatures recorded were below 0 ℃, leading to an overestimated PMSI value because temperatures below 0 ℃ are added as 0 when calculating the ADD.

For bodies that exhibited advanced decomposition features, none of the estimated PMSIs were accurate for both the dark and pale-skinned bodies recovered in the cold or warm months of both Europe and South Africa. Seven bodies recovered in the warmer months had their estimated PMSI approximately two times longer when compared to the case note PMSI (For example, Case note PMSI = 7 days, Estimated PMSI = 14 days). The case numbers, skin tone, and the month and countries the bodies were recovered from for each of the seven cases include: case 4 (Pale skinned, October- Netherlands), case 8 (Pale skinned, September- Netherlands), case 10 (Pale skinned, August- Netherlands), case 12 (Pale skinned, April- Netherlands), case 19 (Pale skinned, July- Netherlands), case 44 (Dark skinned, October- South Africa), and case 53 (Dark skinned, May- South Africa). These bodies had TADS values in the range of 13–17, and the decomposition features observed in them include bone exposure in the limbs, brain liquefaction and compromise in the bloat experienced in the trunk. The reason the estimated PMSIs were double that of the ones in the case notes could be because these advanced decomposition features led to a high TADS value that was substituted into the Heaton DSS regression formula; the high TADS, in turn, resulted in a high extrapolated ADD from the regression formula. Therefore, a lot of average daily temperatures were needed to add up to the high ADD values, thereby increasing the days of the estimated PMSI.

Three bodies recovered in the European winter months had their estimated PMSIs approximately half as long as the case note PMSIs. The case numbers, skin tone, and the month and countries the bodies were recovered from for each of the four cases include: case 13 (Dark skinned, January- Netherlands), case 18 (Pale skinned, January- Netherlands), and case 20 (Pale skinned, March- Netherlands). These bodies had TADS in the range of 5–11, and their decomposition features include discolouration changes and skin slough-off. It is possible that the cold temperature levels experienced during the winter months immensely retarded the decomposition rate of these bodies (Forbes *et al*., 2014). This slow decomposition rate would have led to the low TADS values obtained when scoring the extent of decomposition in the bodies. The low TADS would have led to a low extrapolated ADD. A low ADD value would have shortened the estimated PMSIs compared to the ones in the case notes because the summation of a lot of average daily temperatures would not be required to add up to these low ADD values. Case 34 (Dark skinned, March- South Africa) also had its estimated PMSI half of that of the case note PMSI, but it was recovered in one of the South African summer months. It is possible that the high temperatures recorded in this month could be the reason why the estimated PMSI was half because the high temperatures would have summed up quickly to the extrapolated ADD.

There were two cases that had exceptions with their estimated PMSIs; cases 28 and 68. Their estimated PMSIs were approximately more than two times longer. For example, case 28 (Pale-skinned, February- U.K.) had its estimated PMSI approximately four times longer than its case note PMSI, while case 68 (Dark-skinned, January- South Africa) had its estimated PMSI approximately three times longer than its case note PMSI. The bodies of these cases were recovered in advanced decomposed states with a

TADS of 14 and 19. These high TADS values led to a high extrapolated ADD value. Due to the low temperatures experienced in winter, a lot of average daily temperatures were added up to equal the high ADD value extrapolated, hence the more extensive duration obtained for the estimated PMSIs when compared to the case note PMSIs.

7.4.2.2 *van Daalen DSS*

The van Daalen DSS was similar to the Heaton DSS in relation to its accuracy. The van Daalen DSS was also relatively accurate for estimating the PMSI of both dark and pale-skinned bodies in a fresh state of decomposition. Like the Heaton DSS, only four fresh cases (Cases 2, 9, 14 and 27; all pale-skinned victims from Netherlands and U.K.) had more than a difference of 1 day between the case note PMSIs and the estimated PMSIs, but when using the van Daalen DSS in these four cases, there was a higher difference between the case note PMSIs and the estimated PMSIs compared to the Heaton DSS.

The van Daalen DSS was slightly more accurate than the Heaton DSS when estimating the PMSI of bodies that exhibited advanced decomposition features. Two (Cases 8 and 44) out of the seven bodies in advanced decomposition states that had their PMSIs overestimated by the Heaton DSS had accurate PMSI estimations with the van Daalen DSS. In contrast, Cases 4, 10, 12, 19 and 53 had overestimated PMSIs. The van Daalen DSS also could not accurately estimate the PMSIs of the three bodies (Cases 13, 18 and 20) recovered in the European winter months that had their estimated PMSIs approximately half as long as the case note PMSIs when using the Heaton DSS. The van Daalen DSS was also not accurate for both cases 28 and 68, which had their PMSIs overestimated four and three times longer, respectively, by the Heaton DSS. The van Daalen DSS overestimated case 28 longer than the van Daalen DSS with extra ten days.

7.4.2.3 *Reh DSS*

Like the Heaton DSS, the Reh table was relatively accurate for estimating the PMSI of both dark and pale-skinned bodies in a fresh state of decomposition, most especially bodies with decomposition features such as the wrinkling of the feet and palms. The Reh table was slightly more accurate than the Heaton and van Daalen DSS for bodies in advanced decomposition states; there was a shorter difference in days between the estimated PMSIs and the case note PMSIs when using the Reh table.

For the bodies that primarily exhibited decomposition features such as discolouration changes and were recovered between November and April in the cold European months and South African summer months, the Reh table overestimated their PMSIs. The following cases are examples of cases with discolouration changes and overestimated PMSIs; case 5 (Pale skinned, April) had an estimated PMSI of 9–10 days, while its case note PMSI was 1 day, case 9 (Pale skinned, December) had an estimated PMSI of 17 days while its case note PMSI was 17 days, case 34 (Dark skinned, March) had an estimated PMSI of 23 days while its case note PMSI was 6 days. Case 68 (Dark skinned, January) had an estimated

PMSI of 35 days while its case note PMSI was 3 days and case 70 (Pale skinned, February) had an estimated PMSI of 25 days while its case note PMSI was 1 day.

7.4.3 Validating the Sufficiency of a Complete TADS

This study has shown that to accurately estimate the PMSI of a body from the TADS, the minimum requirement is for all three body regions (head/neck, trunk, and limbs) to be scored and added up to form a complete TADS. In this study, it was challenging to assess the extent of decomposition in the bodies with dismembered or missing limbs, heads, or trunks and cases with no images of one or more of the following: head, neck, limbs, and trunk. It was also challenging to decide the body regions out of the head, trunk and limbs that were better for estimating PMSI, alone or in combination with a second body region. In addition, statistical results indicated that the estimated PMSI obtained with the Heaton and van Daalen DSS for bodies with a missing FADS, BADS or LADS significantly differed ($p < 0.001$) from the case note PMSIs. This indicates that an accurate PMSI estimation cannot be made without including all three components of the TAD score. Therefore, the study has demonstrated and validated the sufficiency of a complete TADS for PMSI estimation.

7.4.4 Skin Tone, Scoring Observations, and Recommendations on the DSS

While assessing the extent of decomposition in the human remains, some observations were noted on the diversity of the decomposition features observed in the dark-skinned and pale-skinned remains and the discrepancies in the three DSS in relation to the skin tone of a body. These discrepancies and diversities show how the three DSS assess decomposition changes differently in different skin tones. The differences in the decomposition features for the pale and dark-skinned human remains were mostly observed as skin discolouration changes in the fresh stage of decomposition. These observations are highlighted in the following subsections, along with recommendations to help improve these DSS and resolve the discrepancies when assessing decomposition in varying dark skin tones.

7.4.4.1 *Heaton DSS*

For a FAD or BAD score of 2 or 3, the Heaton DSS has a description of “slight pink discolouration”, “reddening of the face and neck”, “yellow/green discolouration of the abdomen and the upper chest”, and “marbling”. In dark skin-toned individuals, the skin did not appear pink/red/yellow/green, but it appeared lighter or paler, with the blood vessels beneath the skin appearing more visible (Figure 7.7). During wrinkling and skin slippage of the limbs, the Heaton DSS also describes discolouration changes such as white/pink/yellow/green, but in a dark-skinned victim, the limbs appeared to be grey or a paler colour of their original skin tone (Figure 7.7). In most of the dark-skinned human remains assessed in this study, pale underlying tissue was observed when skin slippage or peeling occurred. This pale-coloured underlying tissue could be confused for the actual skin colour of the victim if skin slippage was advanced or complete, potentially misleading investigations. In the descriptions of the Heaton DSS,

green/black discolouration was expected during skin slippage, but pale discolouration was observed in the dark-skinned bodies.



Figure 7.7: Pale appearance of the skin of a dark-skinned cadaver during marbling and wrinkling of the limbs. Picture source: Office Autopsies database/Livelink.

Finally, the Heaton DSS scores the "darkened lips" feature with a FADS of 2; in dark-skinned victims, it was hard to tell whether the lips were darkened post-mortem or if the victim had dark lips naturally. These observations suggest that discolouration, such as pink, yellow, green, and white, does not occur on dark skin during decomposition or is not visible during visual examinations of dark-skinned human remains. The pale appearance of the skin of a dark-skinned victim could be used as a sign to identify decomposition features such as marbling and the wrinkling and skin slippage of the limbs when these discolouration changes expected are not visible.

7.4.4.2 *van Daalen DSS*

Like the Heaton DSS, the van Daalen DSS also described wrinkling of the limbs with a white discolouration, but in dark-skinned victims, the limbs appeared to be grey or a paler colour of their original skin tone. In the descriptions of the van Daalen DSS, grey discolouration was expected during skin slippage, but pale discolouration was observed in the dark-skinned human remains when assessing the extent of decomposition. Unlike the Heaton DSS, the van Daalen DSS does not consider any forms or shades of discolouration in the body's head, neck, and trunk regions before marbling occurs. In dark

skin-toned individuals, it was hard to identify marbling because there were no obvious discolouration signs in the skin; marbling was only visible when the skin was pale, with blood vessels beneath the skin appearing more visible. The pale appearance of the skin of a dark-skinned victim could replace the discolouration changes expected, and it can also be used to identify marbling changes when assessing the extent of decomposition of the body.

7.4.4.3 *Reh DSS*

Unlike the Heaton and van Daalen DSS, the Reh table had no description for the fresh state of a drowned body or "no visible change"; therefore, it was difficult to estimate the PMSI of human remains that had no visible decomposition change. Descriptions such as "marbling" and "discolouration of body" listed in the Reh (1967) table were not specific about the body region they were associated with. Also, in the Reh table, months such as April, May, July, August, and October, had no PMSI estimate for when the only decomposition feature observed in the body was just "wrinkling of hand or feet". It was difficult to estimate the PMSI of such bodies; hence the next expected decomposition feature was used to assign a PMSI estimate. The Reh table could/should be revised to account for the decomposition state of fresh bodies, separate decomposition changes observed in different body regions and consider the different types of discolouration changes observed in both pale and dark-skinned human remains.

7.5 Limitations

A total of 73 human remains were used for this study. The study's sample size was small, which could decrease the study's confidence level, reliability, and statistical power and increase the margin of error and bias in the study (Faber & Fonseca, 2014). In addition, photographs of the human remains were used and not the actual bodies; some vital decomposition changes might have been missed in the pictures, or there may have been inconsistencies in lighting or artefacts from flash/shadow that can confuse colour identification and affect the process of assessing the extent of decomposition in the human remains. The skin tone of the human remains was quantified using the NIS Skin Colour Scale created by Massey & Martin (2003); a limitation of using this scale is that it does not consider the effect of sun-tanning or other light ray effects (Louie & Wilkes, 2018).

In addition, scoring the skin tone and assigning a decomposition score to each body was a subjective choice; there could have been some forms of bias inputted to the study when scoring through visual observations. In future, pictures of the colour change expected in a dark-skinned body could be included with the DSS. The van Daalen *et al*. (2017) study did not provide the prediction model used to estimate the PMSI values from the TADS; therefore, this study had to use a conversion factor to convert the van Daalen TADS to a scale of over 25 (like the Heaton DSS) to substitute the van Daalen TADS into the Heaton *et al*. (2010) regression formula. These converted TADS did not reveal the ideal extent of decomposition of these bodies. For example, a body with no visible decomposition change would be assigned a TADS of 3 when using both the Heaton and van Daalen DSS, but this conversion factor

converts a van Daalen TADS of 3 to a 4.2 for Heaton TADS. A Heaton TADS above 3 indicates that a visible decomposition change has occurred compared to no visible decomposition change for a TADS of 3.

7.6 Conclusion

This study demonstrated that there was no statistically significant difference ($p = 0.612$) between the PMSIs of the three DSS and the case note PMSIs, which were taken as the 'known' PMSIs (Table 7.6). In addition, it also demonstrated that the three DSS were relatively accurate for estimating the PMSI of both dark and pale-skinned bodies in a fresh state of decomposition, while they were not accurate for bodies in advanced decomposition states. The results also showed that the three DSS did not accurately estimate the PMSI of bodies recovered in the cold or winter months. In addition, while assessing the extent of decomposition in dark-skinned bodies, it was challenging to identify the various discolouration changes listed in the DSS descriptions; instead, these dark-skinned bodies appeared paler or lighter. The pale appearance of the skin of a dark-skinned victim could replace these discolouration changes expected when assessing decomposition features such as marbling and the wrinkling and skin slippage of the limbs. Results of this human comparative study have shown that the skin tone and the discolouration changes observed during decomposition play essential roles in assessing the extent of decomposition and the estimation of the PMSI of a body.

Past studies have suggested that colour changes in the human skin can lead to the misidentification of human remains and affect the quantification of decomposition (Cornett *et al*., 2019; Morgan *et al*., 2006). For example, in the Gelderman *et al*. (2018) study, it was challenging to identify the presence of livor mortis in darker skin tone bodies. More knowledge can be gained on the importance of skin tone and the suitability of these DSS for assessing the extent of decomposition and estimating the PMSI of dark skin-toned bodies by carrying out more studies on dark skin-toned victims recovered from various water bodies across the world. Keyes *et al*. (2016) suggested that more decomposition studies should be carried out with human cadavers in regions like Africa. Carrying out these studies in Africa and other areas that predominantly have people with darker skin tones will help improve the suitability of these DSS for bodies with darker skin tones and improve the knowledge of decomposition and PMSI estimations in such regions.

8 PART FOUR: DROWNING CASE DATABASE

8.1 Introduction

As stated earlier, drowning is a process that occurs when a body is immersed in water or other liquid until there is no air access to the lungs; due to the blockage of the airways by the fluid in which the body is immersed (Byard, 2017; Szpilman & Morgan, 2021). In 2014, 372,000 deaths by drowning were recorded worldwide (WHO, 2014). Drowning is one of the top ten causes of death for those under the age of 25, and it is common in low- and middle-income nations (Franklin *et al*., 2020). In a developed country like the U.K., approximately 400-750 deaths are caused by drowning annually (Barwood *et al*., 2011). Globally, drowning results in about 1,000 fatalities daily and many more cases with lifetime morbidity (Tipton & Montgomery, 2022).

These global statistics on drowning are said to be underestimated by about four to five times because of limited documentation of fatal drowning events worldwide (Bierens *et al*., 2016; Lu *et al*., 2010). Therefore, WHO has proposed that efficient means of documenting drowning cases, such as incident reports and databases, should be utilised globally (Hills *et al*., 2021). Such documentation will aid in understanding the epidemiology of drowning, determining drowning frequency and preventive measures to help avoid or reduce the rate of drowning injuries and death (Hills *et al*., 2021; Pearn *et al*., 2008). Over the years, some countries have developed in-depth resourced drowning databases from their coronial and death information systems or registers, including New Zealand, Canada, Australia, and the United Kingdom (Bugeja *et al*., 2016; Peden *et al*., 2019).

The Australian database is referred to as the Royal Life Saving National Fatal Drowning Database, and all cases in this database are obtained from the National Coronial Information System [NCIS] (Royal Life Saving Society, 2021). The NCIS is an online registry of all types of death recorded by coroners, and it is formulated from records such as police, toxicology, autopsy, and coroner reports (Bugeja *et al*., 2016). The Canadian database is known as the Drowning Prevention Research Centre (DPRC) database. The data in the DPRC database is obtained by trained local data collectors that annually review coroner's investigation statements, toxicology reports, death certificates, autopsy reports, police reports and hospital records from different Canadian provinces and territories (Hills *et al*., 2021). The New Zealand database is called the DrownBase™; it was developed by Water Safety New Zealand (WSNZ) in 1994 using Microsoft Access (Water Safety New Zealand, 2022). WSNZ, in conjunction with the Police, New Zealand Health Information Service (NZHIS), and the Coronial Services unit of the Ministry of Justice, collect data from police, media, and coroners' reports (Hills *et al*., 2021).

In 2009, the U.K. National Water Safety Forum (NWSF) and the Royal Society for the Prevention of Accidents (RoSPA) developed the Water Incident Database (WAID). WAID serves as the drowning database for the United Kingdom; details on drowning cases are recorded in WAID by various water safety agencies such as the National Fire Chiefs Council, Royal National Lifeboat Institution, RoSPA,

Her Majesty's Maritime Coastguard Agency and the Royal Lifesaving Society (Hills *et al*., 2021). These details recorded are released as annual reports on the NWSF website. As a guiding principle for ensuring water safety, the NWSF, with the aid of vital information obtained from WAID and the Drowning Prevention Strategy, aims to reduce the drowning rate in the U.K. by 50% by the end of 2026 (National Water Safety Forum, 2019).

Due to ethical restrictions on the use of human bodies for research, this PhD made use of mice and pigs as human analogues, and the previous chapter analysed images of humans. To obtain more data on aquatic decomposition related to human remains and to gain insight into the global occurrence and distribution of drowning, it was decided to create a drowning case database of worldwide cases. The intention was to follow the WHO-required standards on documentation of fatal drowning incidents and set up the database according to how the standard drowning case databases mentioned above were set up. Developing this database focused on collating various data related to drowning to understand the characteristics, epidemiology, demography, frequency, and other variables essential in fatal drowning.

8.1.1 Aim

This study aimed to:

1. Develop a drowning case database that follows the WHO-required standards on documentation of fatal drowning incidents and is comprised of vital information on drowning cases worldwide.
2. Provide insights or establish any trends in the frequency of drowning, demographics, and levels of risk, make meaningful comparisons across various locations and, where possible, suggest prevention and intervention steps for drowning occurrences.

8.1.2 Objectives

To create a drowning case database on an Excel spreadsheet by collating data from numerous sources on the demographic profile of the victim, cause of death, the location of the drowning incident, the type of water body the body was recovered from, circumstances of the drowning event as well as other crucial supplementary information on the drowning incident. These data could then be used to understand the demographics of drowning cases in the U.K. and abroad, identify trends or patterns in drowning cases, and develop meaningful conclusions, comparisons and statistics that are essential for understanding characteristics and processes related to drowning.

8.2 Methodology

All collated data in the database were retrieved from a wide range of sources such as police records and reports, witness reports and statements, autopsy and coroner records, media reports (print, blogs, broadcast, and socials), toxicology reports, academic literature (peer-reviewed journals, systematic reviews, and reports), the Water Decomposition Atlas from Maastricht University/Netherlands Forensic Institute, Office Autopsies database/Livelink from the University of Cape Town and the archive of the Blackpool and Fylde Coroner's Office. Asides from the cases obtained from the Water Decomposition Atlas, Office Autopsies database and the Blackpool Coroner's archive, all other cases were obtained from online sources. The online sources include online newspapers such as the Guardian, Sun, and Daily Mail, news channels or blogs such as BBC, Sky, ITV, Yorkshire Live, and various forensic journals obtained through search engines. Google, Bing, Google Scholar, PubMed, and Scopus search engines were used to search for cases online. A wide range of key search terms was indexed into the search engines to find cases. These search terms are listed in Table 8.1 below.

Table 8.1: List of keywords and search terms indexed into search engines.

Keywords
Examples of "Water; Aquatic; Drown; Drowning; Drowned; Submerged; Immersed; Underwater; River; Canal; Pool; Pond; Bath; Sea; Lake; Stream; Marine; Ocean; Boat; Ship; Shower" cases; Death by drowning; Swimming.

The data recorded in this drowning case database were streamlined according to the data collated in the standardized databases from the U.K., Australia, Canada, and New Zealand (Hills *et al*., 2021; Peden *et al*., 2019). These data include the date of the drowning incident, age (Date of birth or age at death), sex (male or female or not recorded), type of aquatic location or water type (sea, lake, river, pond, pool, and other examples), temperature (body, water and ambient), skin tone or ethnicity, water depth, location of drowning incident (town or city and the country) and details of the source where the case was retrieved.

The skin tone of each drowning case was occasionally mentioned in the various reports used to create this drowning case database. Therefore, this study decided to stratify the cases based on the Massey-Martin (2003) skin colour guide (Figure 7.1). Cases with a skin colour score between 1-3 were tagged as "Pale", while those with a score between 4-10 were tagged as "dark". When pictures of the drowned victims were available, the cases were grouped based on their skin tone into dark and pale categories. For a case to be included in the database, proof of fatal or non-fatal drowning was needed, and cases were excluded when there was no proof of drowning. All forms of identification (except when stated in the online sources) were excluded to keep the victims anonymized. The data retrieved were analysed to draw out patterns related to drowning incidents and frequency of drowning, provide insights on demographics and risk levels, and make meaningful comparisons across various locations.

8.3 Results and Discussion

8.3.1 Breakdown of Data

Figure 8.1 is a schematic of the spreadsheet of the drowning case database developed in this study—[See Appendix 8 for the entire spreadsheet of the database].

	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	Sex	Age	PMSI	Weight (kg)	Height (cm)	Body Temp (°C)	Water temp (°C)	Amb. Temp (°C)	Skin Tone	Water type	Water Depth	Clothing	Last live contact	Date Recovered	Country	Location	Source
2	F	12	4hrs*	-	-	-	-	30*	Dark	River	20 ft	-	27/06/19	27/06/19	England	Bury, Manchester	https://news.sky.com/story/police-hot-weather-warning-as-girl-1
3	M	18	12hrs*	-	-	-	-	-	Dark	Sea	-	-	15/08/20	16/08/20	England	Lancashire coast	https://www.bbc.co.uk/news/uk-england-leeds-
4	M	16							Dark	Sea					England		
5	M	5 mo							-	Bath	13 cm	-	27/01/19	27/01/19	England	Northamptonshire	https://www.northantslive.news/news/little-renzo-died-after-
6	M	19	12hrs*	-	-	-	-	-	-	River	-	-	08/06/14	09/06/14	Scotland	Nairn	https://www.scotsman.com/news/teenager-drowns-highland-river
7	M	36	1hr*	-	-	-	-	-	Pale	Sea	-	-	02/08/20	02/08/20	Wales	Barmouth	https://www.dailypost.co.uk/news/north-wales-news/family-dad-s
8	F	30s	<1hr	-	-	-	-	-	-	Sea	-	-	09/08/20	09/08/20	England	Waxham	https://www.mirror.co.uk/news/uk-news/breaking-mum-drow
9	M	20s	-	-	-	-	-	-	-	Quarry	-	-	08/08/20	08/08/20	England	Norfolk	https://www.mirror.co.uk/news/uk-news/breaking-mum-drow
10	M	61	<1hr	-	-	-	-	-	-	Sea	-	-	06/11/20	06/11/20	England	Studland	https://www.dorsetecho.co.uk/news/18875812.man-drowned-sv
11	F	14	-	-	-	-	-	-	-	Sea	-	-	08/08/19	08/08/19	England	Clacton	https://www.express.co.uk/news/uk/1164342/clacton-sea-drowning-pier-beach-
12	M	34		-	-	-	-	-		Sea	-	-			Wales		
13	M	39	46 days	-	-	-	-	-	Pale	Sea	-	-	27/01/21	14/03/21	Wales	Rhos-on-Sea Harbour	https://www.countytimes.co.uk/news/19161492.bodies-three-men-recove
14	M	20		-	-	-	-	-		Sea	-	-			Wales		
15	M	30s	-	-	-	-	-	-	-	Sea	-	-	22/07/21	22/07/21	England	Crantock Beach, Cornwall	ttps://www.itv.com/news/westcountry/2021-07-23/crantock-death-man-dies-after-
16	M	50s		-	-	-	-	-		River	-	-	18/07/21		England	Water End Bridge, York	https://www.itv.com/news/2021-07-23/warnings-issued-after-at-least-2
17	M	14	4hrs*	-	-	-	-	-	Pale	Lake	-	-	27/07/21	27/07/21	England	Ashby Ville nature, Scunthorpe	https://www.grimsbytelegraph.co.uk/news/local-news/boy-tragicall
18	M	20s	4.5hrs*	-	-	-	-	-		Lake	-	-	18/07/21	18/07/21	England	Crokes Valley Park, Sheffield	https://www.examinerlive.co.uk/news/local-news/triple-drowning-tra
19	M	-	24hrs	-	-	-	-	-		Lake	-	-	18/07/21	19/07/21	England	Pugneys Country Park, Wakefield	https://www.mirror.co.uk/news/uk-news/body-found-hunt-sw
20	M	15	<2hrs	-	-	-	-	-	Pale	Canal	-	-	20/07/21	20/07/21	England	Knottingley canal, Leeds	https://www.examinerlive.co.uk/news/local-news/triple-drowning-tra
21	M	16	12hrs*	-	-	-	-	-	Dark	River	-	-	17/07/21	18/07/21	England	Carlisle, Cumbria	https://www.mirror.co.uk/news/uk-news/body-found-hunt-sw
22	M	19	3hrs	-	-	-	-	-	Dark	Canal	-	-	18/07/21	18/07/21	England	Salford Quays, Manchester	
23	M	19	<1hr	-	-	-	-	28	Pale	River	-	-	22/07/21	22/07/21	England	Wharfe, Grassington	https://www.thesun.co.uk/news/15705803/heartbreaking-tributes-tale
24	M	16	6hrs	-	-	-	-	31	Pale	River	-	shorts	22/07/21	22/07/21	England	Chester, Chesire	
25	M	55	Pronounced dead at hospital							Sea	-	-	22/07/21	22/07/21	England	Reighton Gap	https://www.itv.com/news/calendar/2021-07-23/three-swimmers-rescued-by-rnli-
26	M	14	7 days						Pale	Sea	-	-	15/07/21	22/07/21	England	Headland, Hartlepool	https://www.itv.com/news/2021-07-23/warnings-issued-after-at-least-2
27	M	21	Pronounced dead at hospital						Dark	Sea	-	-	21/07/21	21/07/21	England	Crosby, Merseyside	https://www.itv.com/news/granada/2021-07-21/emergency-services-and-rnli-
28	M	29	5hrs	-	-	-	-	-	Dark	Sea	-	-	17/07/21	17/07/21	England	Fleetwood beach	https://www.itv.com/news/granada/2021-07-27/tributes-paid-to-29-year-old-who-
29	M	16	<24hrs	-	-	-	-	-	-	River	-	-	19/07/21	20/07/21	England	Frodsham, Chesire	https://www.itv.com/news/granada/2021-07-20/body-recovered-from-river-
30	F	13	<1hr	-	-	-	-	-	Pale	Lake	-	-	18/07/21	18/07/21	England	Witney, Oxfordshire	https://www.itv.com/news/meridian/2021-07-21/our-family-is-broken-a-father
31	M	-	4hrs*	-	-	-	-	-	-	Quarry	-	-	18/07/21	18/07/21	England	Dove Holes, Buxton	https://www.manchestereveningnews.co.uk/news/greater-manchester-news/boo
32	M	15	<24hrs	-	-	-	-	-	Dark	River	-	-	20/07/21	21/07/21	England	Swarkestone, Derbyshire	https://www.itv.com/news/central/2021-07-21/searches-continue-a
33	M	50s		-	-	-	-	-	-	River	-	-	27/07/21	27/07/21	England	Derby River Gardens, Derby	https://www.derbytelegraph.co.uk/news/derby-news/body-found-c
34	M	22	<2 days	-	-	-	-	-	Pale	River	-	-	20/07/21	22/07/21	England	River Derwent, Derby	https://www.derbytelegraph.co.uk/news/derby-news/dozens-floral-
35	M	-	12hrs*	-	-	-	-	-	-	Lake	-	-	22/07/21	23/07/21	England	Torside Reservoir, Derby	https://www.derbytelegraph.co.uk/news/local-news/man-dies-aft
36	M	-	2hrs*	-	-	-	-	-	-	Lake	-	-	20/07/21	20/07/21	England	Welwyn Garden City, Hertfordshire	https://www.hertfordshiremercury.co.uk/news/hertfordshire-news/man-c
37	M	49	<24hrs	-	-	-	-	-	-	Lake	-	-	18/07/21	19/07/21	England	Ardingly Reservoir, West Sussex	https://www.itv.com/news/meridian/2021-07-20/body-found-in-search-fo
38	F	61	-	-	-	-	-	-	-	Sea	-	-	17/07/21	17/07/21	England	Croyde beach, North Devon	https://www.itv.com/news/westcountry/2021-07-19/woman-dies-afte
39	At Least 27 Dead After Migrant Boat Capsizes in English Channel														England	English Channel	https://www.nytimes.com/2021/11/24/world/europe/migrants
40	M	60s	Pronounced dead at hospital						-	Canal	-	-	18/11/21	18/11/21	England	Salford Quays, Manchester	https://www.bbc.co.uk/news/uk-england-manchest
41	M	32	-	-	-	-	-	-	-	River	-	-	13/09/21	13/09/21	Scotland	Harpsdale Fishery Park, Halkirk	https://www.dailyrecord.co.uk/news/scottish-news/man-dies-drow
42	M	19	Pronounced dead at hospital							Estuary	-	-	23/07/21	23/07/21	England	St Anne's pier in Lancashire	https://www.theguardian.com/uk-news/2021/jul/24/lancashire-man-drowns
43	M	55	<1hr	-	-	-	-	-	Pale	Lake	-	-	21/07/21	21/07/21	Northern Ireland	Lough Melvin area, Garrison	/www.belfasttelegraph.co.uk/news/northern-ireland/safety-warning-issued-afterm
44	M	13	Pronounced dead at hospital						Pale	Lake	-	-	19/07/21	19/07/21	Northern Ireland	Canal Court, Scarva	lfasttelegraph.co.uk/news/northern-ireland/co-down-schoolboy-jay-moffett-to-be-
45				-	-	-	-	-	-		-	-					

Figure 8.1: A schematic of the drowning case database.

A total of 398 cases were added to the database. The cases were over ten years from 2014–2023 to analyse some of the accessible available data over a span of a decade. The cases in the database were stratified according to the continents where they occurred (Table 8.2).

Table 8.2: Demographic profile and the number of cases in the Drowning Case Database.

Demographics	Category	U.K.	Europe	Asia	Africa	America	Australia	Total
Sex	**Male**	76	43	5	67	14	15	220
	Female	13	6	0	15	2	5	41
	Unrecorded	2	10	92	20	13	0	137
	Total	91	59	97	102	29	20	**398**
Age	**0–17 years**	21	1	1	23	1	4	51
	18–59 years	31	27	3	62	14	11	148
	60 years and older	7	27	1	13	0	2	50
	Unrecorded	32	4	92	4	14	3	149
	Total	91	59	97	102	29	20	**398**
Water Body	**Natural**	88	56	95	76	29	17	361
	Artificial	3	3	2	26	0	3	37
	Unrecorded	0	0	0	0	0	0	0
	Total	91	59	97	102	29	20	**398**
Skin Tone	**Dark**	8	4	1	58	8	0	79
	Pale	29	29	0	20	3	0	81
	Unrecorded	54	26	96	24	18	20	238
	Total	91	59	97	102	29	20	**398**

8.3.1.1 *Sex*

From the total number of cases, 220 (55%) cases were male, 41 (10%) were female, and 137 (34%) cases did not have their sex/gender recorded. For the total number of cases from the U.K., 76 (84%) cases were male, while 13 (14%) were female, and 2 (2%) cases did not have their sex/gender recorded. These statistics follow the general trend that the majority of drowning victims tend to be male (El Sibai *et al*., 2018; Franklin *et al*., 2020; Roberts *et al*., 2021). Results from the Peden *et al*. (2019) study on the drowning database of New Zealand, Canada, and Australia from 2005 to 2014 indicated that the percentage of male victims was 82.4%, 81.3% and 78.2%, respectively. Data from the Water Incident Database for the U.K. shows that between 2010-2013, 81% of the drowned victims were male, while 19% were female (Figure 8.2). The results of the Fortuin *et al*. (2022) study conducted in South Africa also demonstrated that men and boys are more involved in drownings than women and girls; between 2016 and 2021, 5820 drownings cases involved men and boys, while only 1357 drowning cases were women and girls. The Tyler *et al*. (2017) study also shows that in LMICs, 75% of drowning cases are male, while 25% are female.

Recent studies have shown that there was an increase in the number of female drowning fatalities when comparing the year 2017 to 2018 in New Zealand and Australia, the number of female drowning victims increased by 2% in both countries despite the number of drowning cases decreasing by 27% in New Zealand and 14% overall in Australia (Mahony *et al*., 2017; Roberts *et al*., 2021; Royal Life Saving Society, 2018). Between 2000 and 2017, the number of women and girls hospitalised because of drowning-related incidents increased in Australia, the United States, and New Zealand (Mahony *et al*., 2017).

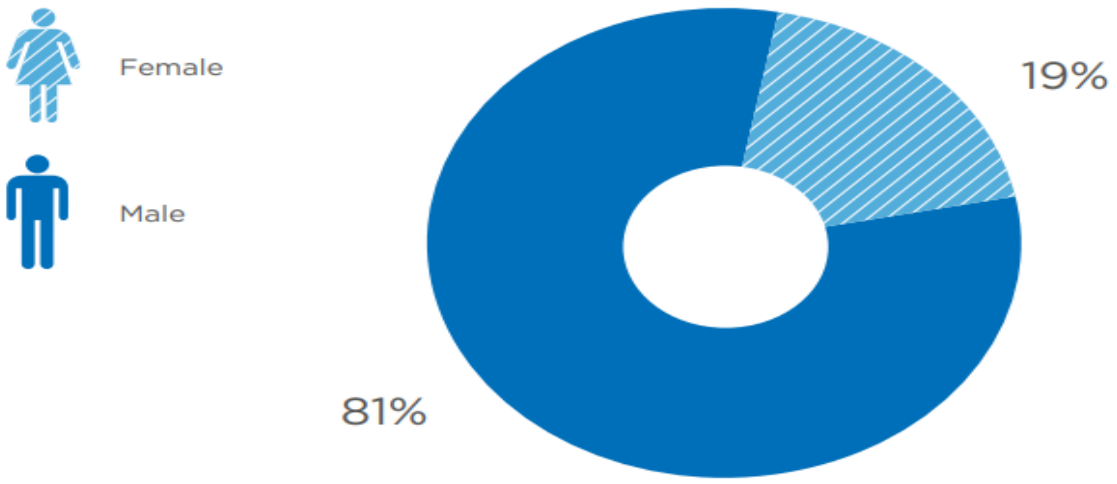


Figure 8.2: Proportion of drowning deaths by sex in the U.K. [2010-2013] (National Water Safety Forum, 2015).

There is no definitive reason why the number of male drowning fatalities is higher than that of female, but it has been suggested that men and boys are known to intentionally partake in more high-risk activities compared to women and girls (Ajibade *et al*., 2013; Peden *et al*., 2018). For example, men and boys are more likely to swim alone, especially in harsh weather conditions, because they are more confident in their swimming skills or are more likely to consume alcohol before a swim (Croft & Button, 2015; Peden *et al*., 2018). The Peden *et al*. (2016) study in Australia suggested that women and girls are more likely to drown compared to men when unintentionally exposed to water bodies, for example, in instances of a flood. These suggested factors are not definite or substantial enough to explain the sex differences in drowning rates; therefore, more studies are required in this field (Hamilton *et al*., 2020).

8.3.1.2 *Age*

The ages of the drowned victims in this database were grouped into categories adapted from the Peden *et al*. (2019) study as follows: 0–17 years, 18–59 years and 60 years and older. 13% (51) of the total cases were aged 0–17 years, 37% (148) were in the 18–59 years category, 13% (50) were 60 years and older, and 37% (149) of the total cases had unrecorded ages. In the U.K., 35% (32) of the total cases had unrecorded ages, the age group of 0–17 years made up 23% (21) of the cases, 34% (31) were in the 18–59 years category, and the 60 years and older group made up 8% (7) of the cases.

The Peden *et al*. (2019) study reviewed cases in the drowning database of New Zealand, Canada, and Australia from 2005 to 2014. In Australia, 17.6% of the cases were aged 0–17 years, 18–64 years made up 44.9%, and the remaining 37.5% were 65 years and older. The same trend was observed in Canada, with the 0–17 years age group making up 12.1% of the cases, while 48.6% of the cases were comprised of 18–64 years, and 39.3% were in the 65+ years category. In New Zealand, victims aged 17 years and under made up 17.0% of the cases, 46.3% of the cases were in the 18–64 age group, while those aged 65 and older made up 36.7% of the cases. The breakdown of the age group in the WAID data for the U.K. differed from that used in the Peden *et al*. (2019) study. The categories used in WAID are illustrated in Table 8.3 below.

Table 8.3: Age categories of the drowned victims (2012-2019) in the Water Incident Database (Hills et al., 2021).

Age Category	0-2 yrs	3-5 yrs	6-12 yrs	13-18 yrs	19-35 yrs	36-60 yrs	Over 60 yrs	N/R
Total cases	39	26	34	189	1138	1807	1181	637

yrs: years, N/R: Not Recorded

The data in Table 8.3 is still analogous to the data obtained from the New Zealand, Canada, and Australia databases, with the age group category of 0–18 years being the least populated with only 5.7% of the cases, the 19–60 years category making up 58.3% of the cases and the 60 years and older age group comprising 23.4% of cases. Also, the Morgan *et al*. (2008) study on the epidemiology of drownings fatalities in Australia indicated that the 20–29 years age group were the most involved in

beach drownings. Fortuin *et al*. (2022) conducted an epidemiological study on fatal drownings in South Africa from 2016 to 2021 and found that the age group with the highest fatal drowning prevalence was the 0–4 age category. In low- and middle-income countries (LMICs), especially in Asian and African countries, children aged 0–20 years are most susceptible to drowning (Tyler *et al*., 2017). The age distribution of this study's drowning case database is similar to those conducted in Australia, New Zealand, Canada, and the U.K., where most drowning cases occurred between 18 to 65 years. In contrast, this database does not follow the South African and LIMCs studies, in which drowning occurs mainly in the age groups below 20 years.

8.3.1.3 *Water Body*

361 (91%) out of the 398 cases in the 'drowning case database' were retrieved from natural water sources defined as seas, rivers, canals, brooks, ponds, estuaries, marshes, streams, caves, and lakes. In comparison, 37 (9%) cases occurred in artificial sources such as dams, baths, bowls, drains, quarries, sewages, and pools. In this PhD research, water sources that were found at home or man-made were defined as artificial sources, while the water sources not found at home or not man-made were classified as natural water sources. In the U.K., 88 (97%) out of the 91 cases were retrieved from natural water sources, while 3 (3%) cases occurred in artificial sources.

These results also follow the trend that drowning occurs more frequently in natural water sources compared to artificial sources (Koon *et al*., 2021). In the Lin *et al*. (2015) study, they investigated unintentional drowning cases in 60 countries and found that in most countries, drowning occurs more in natural water bodies, such as lakes, seas, and ponds, compared to artificial water sources, such as swimming pools and bathtubs. The Fortuin *et al*. (2022) study conducted in South Africa indicated that four times as many drowning cases (4,149) occurred in freshwater and ocean sites than cases that occurred in artificial sources (1,028) such as pools, baths, toilets, and septic tanks.

8.3.1.4 *Skin Tone*

The skin tone of each drowning case was occasionally mentioned in the various reports used to create this drowning case database. Therefore, this study decided to stratify the cases based on the Massey-Martin (2003) skin colour guide (Figure 7.1). Cases with a skin colour score between 1-3 were tagged as "Pale", while those with a score between 4-10 were tagged as "dark". When pictures of the drowned victims were available, the cases were grouped based on their skin tone into dark and pale categories. 79 (20%) of the cases were grouped as dark skin-toned, while 81 (20%) were grouped as pale skin-toned, and 238 (60%) cases had no skin tones assigned. 8 (9%) out of the U.K. cases were classified as dark skin-toned, 29 (32%) were classed as pale skin-toned, and 54 (59%) of the cases had no skin tone assigned.

Africa accounts for the highest frequency of drowning incidents, and LMICs contribute about 90% of the global burden of drowning (Cenderadewi *et al*., 2020; Scarr *et al*., 2022; Tyler *et al*., 2017). It is important to note that these LMICs and Africa are predominantly made up of individuals with dark skin tones. In this study, there was an equal split in the percentage of dark-skinned and pale-skinned drowning victims (20% each), while 60% of the cases did not have their skin tones assigned.

This equal split of dark and pale-skinned victims does not reflect the actual occurrence of worldwide cases because of the bias in the number of cases added to this drowning case database from the "Office Autopsies database/Livelink" (76 cases: 20 Pale-skinned cases and 56 Dark-skinned cases) and the "Maastricht Water Decomposition Atlas" (37 cases: 34 Pale-skinned cases and 3 Dark-skinned cases). The "Office Autopsies database/Livelink" and the "Maastricht Water Decomposition Atlas" contributed 113 cases out of the 160 cases in this study that had their skin tone assigned. In the U.K., there was a higher percentage of pale-skinned drowning fatalities compared to dark-skinned victims.

8.3.2 Drowning Prevention Strategies

Drowning is a global issue that requires preventive strategies but identifying effective strategies and intervention steps is difficult because diverse factors such as health determinants and costs need to be considered, especially in low- and middle-income countries (LMICs) or developing countries in general (Bugeja & Franklin, 2013; Peden *et al*., 2020; WHO, 2017). These LMICs are mostly from Africa, the Caribbean, South America, and Asia; these are places with predominantly dark-skinned individuals. Since part of this PhD focuses on the application of methods to cases involving dark skin individuals, it is essential to learn about drowning strategies that can be applied in these LMICs. In addition, education is needed for forensic investigators and pathologists in LMICs to reduce misidentifications and improve outcomes and for public members to reduce drowning occurrences. Preventing drowning incidents is challenging because every drowning case indicates a reduction in the effectiveness of available prevention and intervention steps; therefore, an evidence-based change over time in the behaviour, culture, policy, environment, and frequency of drowning is required to develop effective preventive measures (Moran *et al*., 2011; Peden *et al*., 2020).

8.3.2.1 *Education*

Numerous prevention strategies are available (Koon *et al*., 2021), but education is the most used strategy (Moran, 2016). The public is educated on how to behave around water through safety tips, thereby increasing their awareness and knowledge of the dangers of drowning (Moran, 2016). An increase in knowledge and awareness does not guarantee safety but evaluating and monitoring the public's behavioural changes and approaches to these safety tips make the education strategy more effective (Hamilton *et al*., 2016; Hatfield *et al*., 2012). An example of a list of safety tips administered to the public is illustrated in Figure 8.3 below.

Keep yourself safe

Learn swimming and water-safety survival skills

Always swim with others

Obey all safety signs and warning flags

Never go in the water after drinking alcohol

Avoid inflatable swimming aids, such as "floaters"; know how and when to use a life jacket

Swim in areas with lifeguards

Know the weather and water conditions before going in the water

Always enter shallow or unfamiliar water feet first

Do not overestimate swimming capability[27]

Know how to stay away from rip currents, which are involved in more than 85% of drowning events at the beach[27]

Keep others safe

Help and encourage others, especially children, to learn swimming and water-safety survival skills

Swim in areas with lifeguards

Set rules for water safety

Always provide close and constant attention to children you are supervising in or near water

Know how and when to use a life jacket, especially for children and weak swimmers

Learn first aid and CPR

Learn safe ways of rescuing others without putting yourself in danger

Obey all safety signs and warning flags

Fence in a pool on four sides and install a self-closing, self-latching gate, measures that reduce the incidence of drowning by 50 to 70%[27]

Provide a warning sign for shallow water in a pool[27]

Figure 8.3: Education guidelines for drowning prevention (Moran et al., 2011)

8.3.2.2 *Lifeguards*

Lifeguards are also a common strategy to prevent drowning (Koon *et al*., 2021). Lifeguards are trained in first aid, can perform CPR when needed and are expected to be good swimmers (Gilchrist & Branche, 2016). The problem of cost could arise in the use of lifeguards; training them could be expensive, and it also must be taken into account that lifeguards cannot be everywhere in the water at all times;

therefore, drownings could still occur in the presence of a lifeguard (Gilchrist & Branche, 2016; Koon *et al*., 2021).

8.3.2.3 *Signage*

Another common preventive strategy is signage; it can be used as an alternative option for lifeguards (Peden *et al*., 2020). It serves as a remote or digital supervision strategy at beaches and rivers (Council Auckland, 2019). However, using signposts and boards could be expensive (Koon *et al*., 2021); for example, California State Parks, USA estimated that over $2,000,000 would be needed to update and renovate signs used in their parks (California State Park, 2019).

8.3.2.4 *Other Strategies*

The Peden *et al*. (2020) study used a modified Delphi process to identify and rank thirteen drowning preventive strategies in order of their effectiveness and suitability across the world. For this study, thirty participants with experience with drowning prevention strategies were selected from twelve countries: USA, Australia, Uganda, England, Bangladesh, Ireland, Canada, Philippines, South Africa, Brazil, Switzerland, and the Netherlands. The study process was divided into four phases.

Phase I: Each participant was told to list 40 strategies. Their listed strategies were compared and evaluated; duplicated strategies in their lists were deleted, and strategies that showed similarities were merged into one. At the end of this phase, the participants came up with a consolidated list comprising 11 categories of drowning prevention strategies (Table 8.4).

Table 8.4: Consolidated list of the 11 strategies identified in Phase I (Peden et al., 2020).

Category	**Strategies (n)**	**Example Strategy**
Life jackets	9	Lifejacket wear for children.
Personal behaviours	17	Do not engage in water recreation in a river alone.
Knowledge	10	Strategies to survive cold water immersion.
Public awareness and advocacy	12	Raise awareness of the dangers of submerged obstacles.
Cardiopulmonary resuscitation (CPR) and rescue	7	Training for all boat personnel in CPR, calling for rescue, search and rescue.
Personal skills	9	Teach self-rescue skills to enable unaided movement to water's edge.
Signage	13	Highly visible signs warning of local hazards at popular swimming destinations.
Engineering	19	Safe and accessible infrastructure, such as bridges, for crossing rivers.

Flooding	18	Establish effective early warning systems for notifying at-risk citizens when rivers are flooded.
Alcohol	11	Restriction of alcohol usage around hire and drive vessels, such as houseboats and party boats.
Other	14	Include river drowning prevention in national and local water safety plans.
Total	139	

Phase II: The strategies in the consolidated list were ranked according to their effectiveness. A 4-level scale with the following options: "very effective", "effective", "neutral", and "not effective" was used to rate the effectiveness of these strategies. New ideas or strategies were also added in this phase and ranked.

Phase III: The proposed strategies that made it to this phase were assessed, along with known evidence, on their effectiveness in low- and middle-income countries (LIMCs) and high-income countries (HICs)

Phase IV: In this phase, the participants were presented with ten scenarios formulated from a combination of real-life drowning cases in Australia (Peden *et al*., 2016). For each scenario, each participant suggested five strategies that would be most suitable and effective for preventing a similar drowning incident in the future. If at least 60% of the participants selected the same strategy for a particular scenario, that strategy was retained on the consolidated list.

At the end of this phase, 13 drowning prevention strategies were selected. These strategies are listed in Table 8.5 in order of their effectiveness. A summary of the steps and phases in this modified Delphi process study is summarised in the flow chart in Figure 8.4 below.

Table 8.5: The final 13 strategies at the end of Phase IV (Peden et al., 2020).

Strategy	**Average Score(%)**	**Effective in both HICs and LIMCs(%)**	**%Aware of evidence supporting strategy**	**Hierarchy of control level**
Barriers between child play areas and rivers	75.0	71.4	71.4	Engineering
River safety education including recognition and awareness of hazards	69.7	92.9	32.1	Education
Raise awareness of the risks of drowning from alcohol	61.9	67.9	50.0	Low-order

Prohibiting/Restricting alcohol use	53.6	50.0	32.1	Elimination
Close flooded roads and/or use physical barriers (such as booms)	50.0	57.1	57.1	Engineering/ Elimination
Swim/recreate/wash/bathe only in designated safe places	40.6	75.0	39.3	Low-order
Build safe and accessible infrastructure such as bridges	40.5	78.6	50.0	Engineering
Do not engage in water recreation alone	38.8	82.1	32.1	Elimination/ Administrative
Wear a life jacket	38.8	57.1	82.1	PPE
Learn to swim with a focus on survival swimming skills	35.3	85.7	78.6	Low-order
Community-wide rescue and resuscitation skills	24.6	82.1	32.1	Low-order
Caregivers maintaining active supervision	22.0	82.1	82.1	Administrative
Designing the urban landscape to improve safety	19.2	60.7	39.3	Engineering

HICs, high-income countries; LMICs, Low-income and middle-income countries; PPE, personal protective equipment.

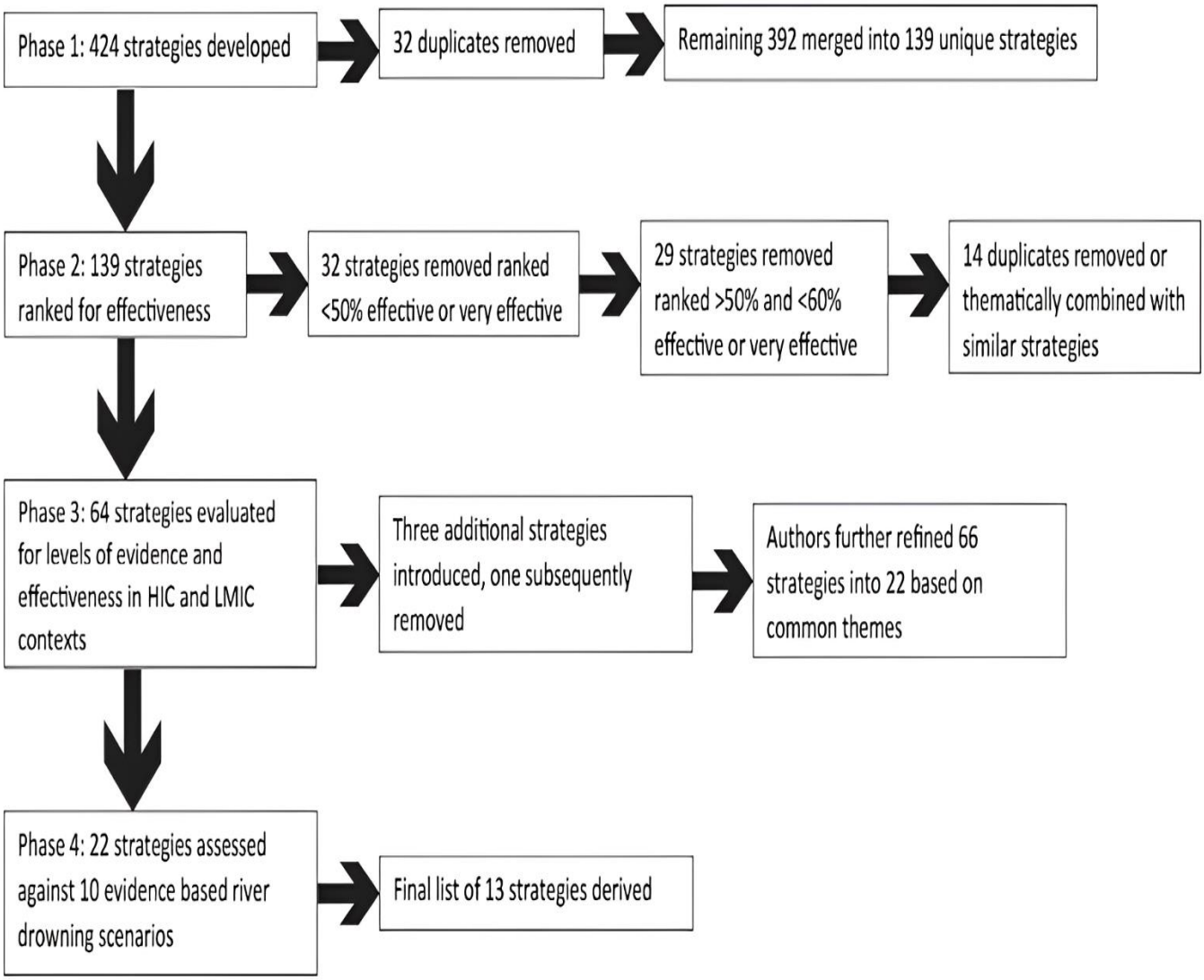


Figure 8.4: Flowchart of the steps in each Phase of the Peden et al. (2020) study.

8.3.3 Limitations

A high volume (52%) of the cases retrieved from online sources were obtained from the United Kingdom or U.K. media sources. This could be because of search engine bias; the search for cases was done in the U.K. using network services connected to U.K. servers. Therefore, the cases from other regions might not be a representative sample of what is out there or the real trend and number of cases. This research section was not validated or peer-reviewed to make it a standardised database. There were limitations to the validity of the results of the online searches. Most of the cases obtained from news sources online offered little to no forensic value; there were no pictures of the victim to analyse, and understandably, for sensitivity reasons, there was barely any mention of the condition of the body or decomposition features that could have been present of the recovered bodies, temperature values, weight, height, and skin colour. This made it difficult to draw correlations between the cases' PMSI, decomposition changes, skin tone and water type. There is also possible bias in what was reported in

these online news cases because the reported cases could have been deemed newsworthy or sensationalist (Reid, 2017). There were different cases of tourists from different countries or cases that would attract readers' empathy, such as drownings where the victim tried to save a family member from drowning.

8.4 Conclusion

This study has demonstrated the severity of the need for proper documentation of fatal and non-fatal drowning cases, as proposed by the WHO (2014) report on preventing drowning, because the epidemiology and frequency of drowning incidents in some regions have been determined through the trends in demographic profiles, skin tone and water type that have been drawn out from this section of the research. From this study, it was demonstrated that more men and boys [220 (55%)] are involved in drowning compared to women and girls [41 (10%)]. This study also indicated that people in their active years (18–59 years; 148 [37%]) account for the highest drowning occurrences. In this study, 361 (91%) cases were retrieved from natural water bodies, while 37 (9%) were retrieved from artificial sources. By documenting fatal and non-fatal drowning incidents, studies have been able to suggest risk factors that contribute to drowning; these factors include sex/gender, lack of education, sociodemographic characteristics, ethnicity, alcohol or substance consumption, weather, flooding, access to water bodies and transport vessels (Cenderadewi *et al*., 2020; Koon *et al*., 2021; Miller *et al*., 2019; Peden *et al*., 2017).

These studies have also been able to suggest diverse types of drowning preventive strategies like signages, life jackets, and lifeguards and raised awareness of the risk factors of drowning (Koon *et al*., 2021; National Water Safety Forum, 2015; Peden *et al*., 2020). These drowning preventive strategies and other crucial information on the diverse aspects of drowning have been developed by retrieving vital information from drowning databases such as the Royal Life Saving National Fatal Drowning Database, Drowning Prevention Research Centre (DPRC) database, DrownBase™, and Water Incident Database. It is of great importance that further studies are carried out to evaluate the currently available drowning prevention strategies and implement new strategies, thereby closing the gaps in knowledge in this field and making water bodies worldwide safer (Koon *et al*., 2021; National Water Safety Forum, 2015; Peden *et al*., 2020).

9 DISCUSSION

This chapter presents an overarching discussion of the four parts of this research: *Laboratory Studies on Water Conditions, Field Studies on Salinity Concentration, Comparative Human Image Analysis* and *Drowning Case Database*; it discusses the similar trends between these four parts in terms of aquatic decomposition, decomposition scoring systems, skin tone and the water conditions studied and their effects on aquatic decomposition and PMSI estimation. Specific discussions for the laboratory studies were given at the end of each water condition study in sections 5.3.1.3, 5.3.2.4, 5.3.3.8 and 5.3.4.8. The discussions for the field studies were given in section 6.4. Specific discussions for the human image analysis and the drowning case database are given in sections 7.4 and 8.3, respectively.

9.1 Decomposition Patterns in Humans and Human Analogues

Due to the challenges in obtaining human cadavers or the ethical restrictions on the use of human bodies for research, particularly in the U.K., animal carcasses have been used as human analogues in many empirical taphonomy and forensic studies to serve as a source of vital information in major research focuses and the development of standard methods and techniques (DeBruyn *et al*., 2021; Matuszewski *et al*., 2020). The animals used in these studies in the last century have ranged from amphibians to mammals of different sizes, including pigs, sheep, rabbits, mice, rats, chickens, cats, dogs, deer, kangaroos, impala, fox, possums, bears, and elephants, as well as birds, such as gulls, and reptiles such as lizards and alligators, as well as fish and squid (Ahmad *et al*., 2011; Barton *et al*., 2013; Martín-Vega *et al*., 2015; Matuszewski *et al*., 2020; Parmenter & MacMahon, 2009; Parry *et al*., 2016; Pérez-Marcos *et al*., 2018; Young *et al*., 2014).

In this PhD research, mice and pigs were used as human analogues to investigate the effect of various water conditions on the decomposition rate, while pictures of human remains recovered from water were used to investigate the effect of various water conditions on the decomposition rate and improve the suitability of the available DSS for individuals with dark skin tones. These broad studies assessed the extent of decomposition in the mice, pigs, and human remains using the DSS. The decomposition changes, scavenging patterns and the rate of the decomposition process varies for different organisms; humans decompose at a different rate and pattern compared to other animals (Dautartas *et al*., 2018; DeBruyn *et al*., 2021; Matuszewski *et al*., 2020; Steadman *et al*., 2018). For example, the Dautartas *et al*. (2018) study investigated if there were differences in the decomposition rate and pattern of human, rabbit and pig remains across three seasons. Their study found that insect colonisation, decomposition rate and patterns, and scavenging patterns, varied significantly in the rabbits, pigs, and human samples.

This variance in the decomposition rate and features was also observed in the mice, pigs and humans used for this PhD research. Figure 9.1 illustrates the decomposition days or range of days during which some of the decomposition features and patterns observed in the mice, pigs and humans first occurred; it shows the similar and different external decomposition features observed between the mice, pigs, and

humans. Similar decomposition changes were observed at different intervals in the three analogues used in these studies.

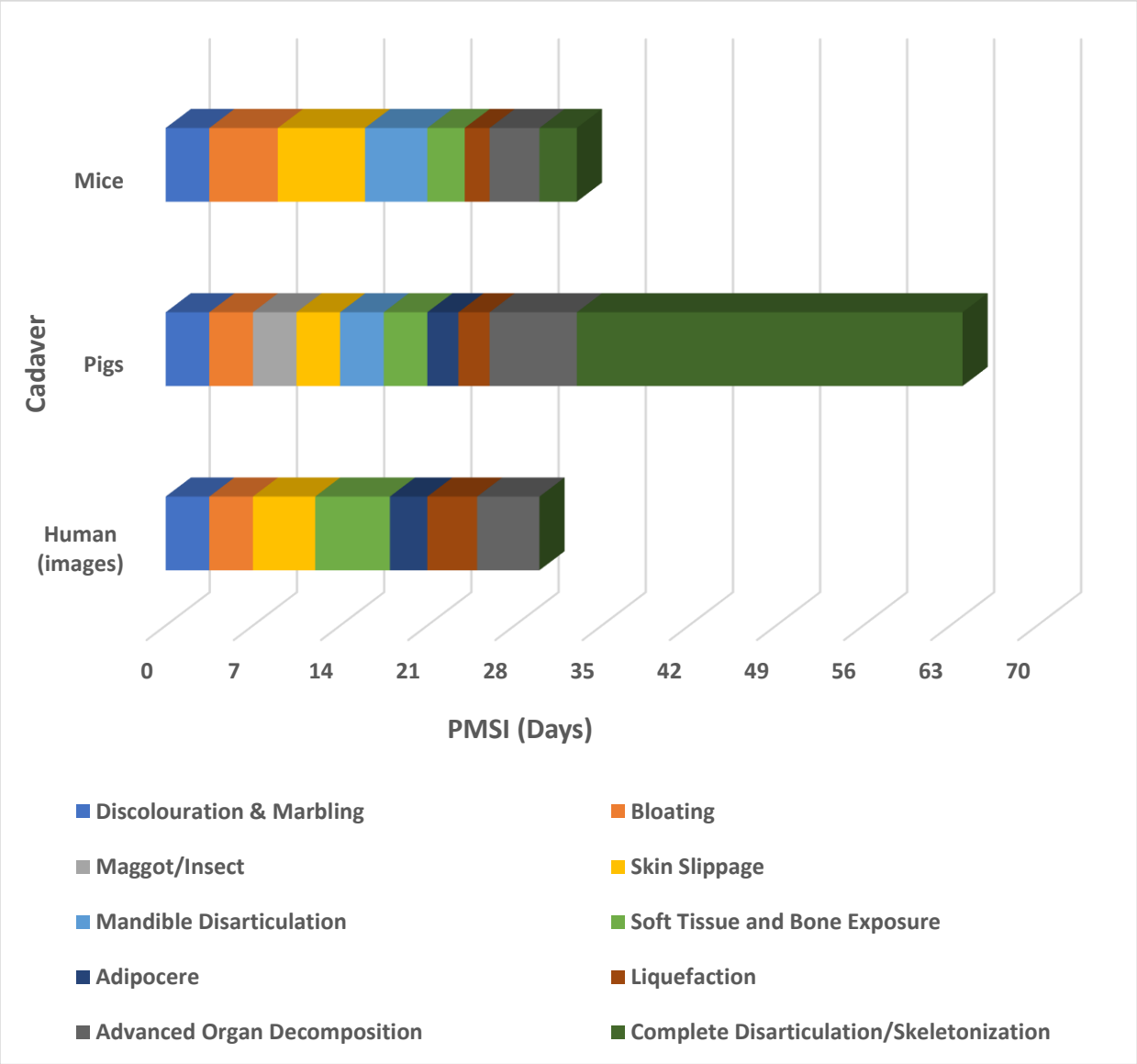


Figure 9.1: Decomposition changes and features observed in the human (images) and human analogues.

Figure 9.1 shows that all carcasses exhibited discolouration or marbling changes for about three days and experienced bloating just before the seven days mark. The bloat stage was longer (approximately five days) for the mice cadavers than the other animals. Skin slippage occurred earlier in the human and mice remains at a PMSI of about 4 and 7 days, respectively. The three carcasses were in the advanced stage of decomposition at a PMSI of approximately 14 days, with signs such as mandible disarticulation and soft tissues or bone exposure. Adipocere was observed in the pigs from a PMSI of 21 days, and it was observed in one of the human samples with a PMSI of 18 days, but there was no trace of adipocere in any of the mice all through the 35 days of decomposition and the duration of the laboratory studies. Maggot or insect activity was observed in the human and pig samples as early as a PMSI of 7 days, but

there was no insect or maggot presence on the mice carcasses. The absence of insects and maggots in the mice studies is due to the experiments conducted in enclosed laboratory settings where insect access was restricted. Algae was also found on some pigs by day 63 of decomposition, but no algae were present in the mice and human remains.

Despite these differences in the decomposition features exhibited on different days in the mice, pigs, and human remains, there were some similarities in the decomposition changes and the order in which these features were observed in the three cadaver types used in the research. For example, marbling and discolouration of body parts were observed in the three samples; other similar features observed in the same order in the mice, pigs, and humans include bloating, skin slippage, bone exposure, advanced soft tissue and advanced internal organ decomposition, and partial disarticulation and skeletonization.

The similarities observed in the decomposition features and the order in which they occurred across the two human analogues and the images of human cases could be due to the water types in which they were submerged because the differences between the decomposition rates in the different animals were over-shadowed by the variable of the water type not the variance in the species of the cadavers. These water parameters may be similar in different geographical or climatic regions and types of water. For example, seawater in different parts of the world has a salinity concentration range of approximately 25–35 ppt (Duncan Seraphin *et al*., 2014), while soft water is considered to have below 60 mg/L of $CaCO_3$, and various grades of hard water are above 60 mg/L (McGowan & Harrison, 2000). Therefore, the results of these experiments and observations with different analogues may be applicable to other regions and water types across the world. However, there is still a need for more studies need to be carried out on different human analogues, human samples, and water types from different regions worldwide to have a better understanding of PMSI estimation and the related conditions of aquatic decomposition.

9.2 Effect of Water Conditions on Decomposition Rate

Factors such as insect activity and weather conditions, which play essential roles in terrestrial decomposition, have limited influence on aquatic decomposition (Macfarlane *et al*., 2020). For example, insects have limited access to a body submerged in water. Most water parameters are easy to measure, relatively inexpensive to monitor, and can serve as crucial tools for estimating PMSI (Macfarlane *et al*., 2020). Past studies have investigated water parameters that influence aquatic decomposition, such as temperature and ADD (Heaton *et al*., 2010; Reh, 1967), and water biota (Anderson & Bell, 2016; 2017; Anderson & Hobischak, 2004; Dickson *et al*., 2011), while the Macfarlane *et al*. (2020) study focused on pH, total organic carbon, dissolved oxygen, conductivity, turbidity, and nitrogen. This PhD focused on different levels or concentrations of some water conditions to investigate the effect of these water conditions on the decomposition rate of mice and pigs; the effect of each condition on the decomposition rate is discussed below.

9.2.1 Temperature

Temperature is the most crucial factor affecting the rate of aquatic decomposition (De Donno *et al*., 2014; Zhou & Byard, 2011). At high temperatures, enzymatic, biological, and chemical reactions occurring during decomposition are increased by at least two-folds because bacteria and other microorganism activities are increased at elevated temperatures (Vass, 2011; Vij, 2014). In this PhD research, the effect of the water temperature on the decomposition rate of the mice in the laboratory studies and the pigs in the field studies was observed by measuring the hourly water temperatures in the mice and pig studies. The effect of temperature could not be investigated in the human image analysis study because most cases did not mention body, water, and ambient temperatures or the average daily temperatures in the case notes.

Results of the laboratory and field studies showed that the decomposition rate increased with an increase in air and water temperatures. In addition, the ADD showed that temperature was the most significant influencer of decomposition rates in the mice and pigs. In the Laboratory current study (See Section 5.3.3), mice that were completely submerged in freshwater during the summer months decomposed at a significantly faster rate ($p < 0.001$) than mice that were completely submerged in freshwater during the spring months despite being placed under similar conditions, where the only difference was the water temperature (See Table 5.24, Table 5.27, and Figure 5.30). In addition, when comparing the Initial salinity study and the Changing-salinity study, the first two weeks of the initial study occurred during a heat wave in the country. By day 21 of the initial study, complete skeletonization was observed in some of the pigs, while no form of skeletonization was observed in any of the pigs in the changing-salinity study by this time (See Figure 6.12 and Figure 6.18). The heat wave during the initial salinity study could have been the only reason for the advanced decomposition rate observed.

9.2.2 Salinity

Most decomposition studies on salinity and aquatic decomposition have only been conducted on human drowning cases or human analogues deposited in freshwater from a lake (Humphreys *et al*., 2012) or rivers (Byard, 2018; Heaton *et al*., 2010; Palazzo *et al*., 2020; Reijnen *et al*., 2018) and drowning cases from seas (Mateus & Vieira, 2014; van Daalen *et al*., 2017). In addition to freshwater and seawater, this PhD research also investigated other types, such as brackish and hypersaline water. The effect of these four salinity levels (fresh, brackish, sea and hypersaline) on the decomposition rate of mice and pigs was investigated. The salinity concentrations of the bodies of water from which the human cadavers were retrieved were not provided in the case notes; therefore, the effect of salinity levels on the decomposition rate of the human cadavers could not be directly investigated in this study.

In the mice and pigs, it was evident that the decomposition rate was significantly faster ($p < 0.05$) in freshwater compared to brackish, sea and hypersaline water (See Table 5.11 and Section 6.3.1.1). Advanced decomposition features and changes were observed in the freshwater groups at earlier PMSI

days compared to the salt-containing groups. The decomposition rate of the mice and pigs in the brackish water was slower than those in freshwater but faster than those submerged in seawater and hypersaline water. These changes were due to the reduced bacterial activity in the salt-containing water because salt inhibits bacterial activity (De Donno *et al*., 2014). Brackish water [~20 ppt] has a lower salt concentration compared to seawater [~35 ppt] and hypersaline water [~50 ppt] (Duncan Seraphin *et al*., 2014); this lower salt content in brackish water could be the reason why mice and pigs submerged in brackish water had the fastest decomposition rates among the salt-containing water groups.

These results from the research have shown that conducting more taphonomy experiments with brackish and hypersaline water can be essential to understanding PMSI estimation, aquatic decomposition, and other related conditions in a forensic context because brackish and hypersaline water are found quite a lot around the world, and there are lots of opportunities for people to be drowned in brackish and hypersaline water. Examples of brackish water sources are estuaries, salt marshes, mangrove swamps, and brackish seas and lakes; some include the Baltic Sea and Lake Turkana (Gray *et al*., 2011; Singh, 2015). Examples of hypersaline water include the Dead Sea and the Great Salt Lake (McGenity & Oren, 2012). Therefore, care must be taken, and salt concentration must be considered when assessing the extent of decomposition or estimating the PMSI of bodies recovered from freshwater, brackish water, seawater, and hypersaline water.

9.2.3 Hardness

Studies on the water hardness of a water body and its relation to or effect on aquatic decomposition in a forensic context have never been published in the available literature. This PhD research investigated the effect of different levels of water hardness [Soft water (< 60 mg/L), Moderately Hard water (90 mg/L), Hard water (150 mg/L), and Very Hard water (210 mg/L)] on the decomposition rate of mice. However, the effect of water hardness was not investigated in the pig studies, and there was no mention of water hardness concentrations or records of water hardness in the human image analysis or the cases used in the drowning case database.

The results of the laboratory study indicated that mice submerged in soft water (< 60 mg/L) decomposed at a significantly faster rate when compared to mice placed in the three harder water groups ($p < 0.05$) [See Section 5.3.2 and Table 5.22]. Cations that cause water hardness react with some anions in the water to form stable salts such as magnesium and calcium bicarbonates and sulphates (Sengupta, 2013). The formation of these stable salts may lead to the inhibition of the activity of bacteria present in the hard water groups. Therefore, decomposition reactions such as those associated with putrefaction are slowed down due to reduced bacterial activity in the presence of salt (De Donno *et al*., 2014). More studies need to be conducted to bridge the gap in knowledge on the effects of water hardness on aquatic decomposition and PMSI estimation because there are a lot of sources of hard water worldwide, such as regions with rocks, mountains, caves, and waterways that flow through chalk, gypsum, and limestone

deposits. For example, human remains have been found in the El Miron and Gough's caves, which are limestone caves (Bello *et al*., 2015; Marín-Arroyo, 2015). The Marín-Arroyo (2015) study revealed that 77% of the skeletal remains recovered from the caves exhibited a high level of preservation. This preservation could have been due to the hardness of the water in the caves.

9.2.4 TDS

Total Dissolved Solids (TDS) is a measure of the combination of dissolved organic and inorganic matter in water; it is a water property used to describe salinity (Rusydi, 2018). Past studies that have investigated the effect of water salinity on the decomposition rate did not consider the measurement of TDS, but in this PhD research, the TDS concentrations of the water types used for the mice and pigs were measured. The weekly TDS levels recorded in the studies are listed in Tables 5.19, 5.29, 5.36 and 6.11. There was no mention of total dissolved solids or records of TDS concentrations in the human image analysis or the cases used in the drowning case database.

The tables for the TDS of the mice studies show that although the TDS levels of all water types remained in their standard ranges throughout the studies, there was an apparent increase in the TDS concentrations as the days of decomposition and extent of decomposition in the mice (TADS) increased. In the pig study, the TDS concentrations for each water type increased as the days of decomposition increased, and the TDS concentration of freshwater increased past the standard range of freshwater (~1.0 ppt) into the seawater range of ~10–30 ppt (Moran, 2018). This increase in TDS may be due to the release of decomposition products in the water, such as putrescent mass and other organic or inorganic matter and may be correlated to the extent of decomposition observed over time. Hence, monitoring the TDS of water over time may help narrow down the submersion interval for a body.

In addition, TDS and conductivity are water parameters that are a measure of salinity (Rusydi, 2018). They are interchangeable and are related by the formula: TDS = Conductivity × Conversion Factor; this conversion factor can range from 0.5–0.75 (Rusydi, 2018). The Macfarlane *et al*. (2020) study monitored conductivity over time in relation to TADS and found that conductivity can be used to narrow down the PMSI as the conductivity of the water increased as the TADS increased. Since TDS and conductivity are related and interchangeable, it can be suggested that the results of the Macfarlane *et al*. (2020) study and this PhD research indicate a correlation between the extent of decomposition and TDS in a closed water system/standing water. Therefore, more studies need to be conducted on TDS and conductivity to have a better understanding of their effect on aquatic decomposition and PMSI estimation.

9.2.5 Current

Water current, turbulence and tide affect the degree of water flow and, in turn, the decomposition rate (Haglund & Sorg, 2002). Water current is crucial in body displacement; when the current exceeds the frictional forces in water, a body moves a distance from its initial point of entry into the water (Mateus

et al., 2013). In this PhD research, laboratory experiments were conducted on mice to investigate the effect of current/water movement on the decomposition rate of mice completely submerged and immobile, mice left to float, and mice placed in water moving under the influence of a turbine. In contrast, field studies were conducted on pigs where water in boxes was changed weekly. These studies were set up in these formats to make them analogous to bodies snagged or trapped underwater, floating bodies, bodies in the water with fast current, and bodies dragged across a distance in the same water or dragged into various water sources. Unfortunately, there was no mention of water current or records of water current in the human image analysis or the cases used in the drowning case database.

Results of the laboratory study indicated that mice placed in moving water (200 RPM or 21 ms^{-1} or 47 mph) decomposed faster than mice left to float and mice completely submerged ($p < 0.05$) [See Section 5.5.1.1 and Table 5.25]. The field study also suggested that pigs placed in changing water (changing group) decompose faster (but not at a significant rate) than pigs completely submerged (constant group) for the entirety of the study (See Section 6.3.2.1 and Table 6.6). The pigs in the Changing groups were exposed to conditions akin to real-life scenarios, such as open seas and rivers with different water sources, while the pigs in the Constant group were exposed to conditions designed to be analogous to real-life scenarios, such as bathtubs, pools, and enclosed water bodies. These studies provide promising preliminary results that suggest that there is more to learn from studies in different water bodies, such as seas, rivers, canals, and lakes with varying flow, current and turbulence, to understand better the effect of water current on aquatic decomposition, body displacement, and PMSI estimation. Examples of studies conducted on different water bodies with varying flow include the Palmer (2020) and Blanco Pampín & López-Abajo Rodríguez (2001) studies.

9.2.6 pH

Temperature is a water parameter typically measured in aquatic decomposition studies (Heaton *et al*., 2010; van Daalen *et al*., 2017). Few studies, such as the Barrios & Wolff (2011) study, have measured pH levels, but these studies have not investigated the role or effect of pH on decomposition rate and PMSI estimation (Christensen & Myers, 2011; Macfarlane *et al*., 2020). In this PhD research, pH levels of water were measured weekly to investigate if the pH levels had any effect on the extent of decomposition or decomposition rate in pigs and mice. There was no mention of pH or records of pH levels in the human image analysis or the cases used in the drowning case database.

The pH of freshwater ranges between 6.5–8.0, while seawater is primarily alkaline, and its pH ranges between 7.0–8.4 (Chester & Jickells, 2012), while that of hypersaline water can range from 6.2–10 (Golan *et al*., 2014; Papadimitriou *et al*., 2016). The pH levels recorded in these PhD studies are listed in Tables 5.18, 5.28, 5.35 and 6.10. The values in these tables show that, although the pH levels of all water types remained in their standard ranges throughout the studies, there was no change/distinction in the change in pH levels for a particular water type over time or between water types; there were

inconsistencies in the change in pH levels between the sample days with some days exhibiting an increase in pH and the next sampling day exhibiting a decrease in pH levels. Also, as the PMSI and TADS increased, there was no distinct increase or decrease in the pH over time. Therefore, these results suggest that the decomposition changes observed cannot be directly linked or attributed to the pH levels and that the other factors were more influential on the extent of decomposition observed in the pigs and mice used in these studies.

These results and conclusions on the effect of pH on decomposition are consistent with the Macfarlane *et al*. (2020) study in which they investigated the value of pH in estimating the PMSI of mice submerged in water. Their study indicated that using pH to estimate PMSI was not overly helpful due to the inconsistent pH levels recorded over time. Also, in the Christensen & Myers (2011) study, the effect of pH on the decomposition of bones placed in freshwater was investigated, and the results of their study indicated that a pH range of 4–10 had little to no effect on bone decomposition. The pH of most natural water types falls in the range observed in the Christensen & Myers (2011) study (Chester & Jickells, 2012; Golan *et al*., 2014; Papadimitriou *et al*., 2016). Despite the corroboration of the results of this research and other studies available in the literature, more studies need to be conducted to investigate the effect of the pH of various water types on aquatic decomposition and PMSI estimation.

9.3 Decomposition Scoring Systems

Decomposition scoring systems (DSS) were extensively applied in this PhD research. The extent of decomposition in mice, pigs and human remains was assessed with the Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS, while the Reh (1967) table was used to assess the extent of decomposition in only the human samples. Further observations on these DSS are discussed in the subsections below.

9.3.1 Differences between the Heaton and van Daalen DSS

The Heaton DSS and van Daalen DSS were used to assess the extent of decomposition in the mice, pigs and human cadavers used in this PhD research, and statistical tests were conducted with the Mann-Whitney U test to investigate if there was any statistically significant difference between the TADS obtained when using the Heaton and van Daalen DSS. All through the field studies, there was no statistically significant difference between the TAD scores of the pig carcasses when using the two DSS. In the laboratory studies on mice, there was a statistically significant difference ($p < 0.05$) between the TADS of the Heaton DSS and van Daalen DSS in the laboratory salinity study (See Table 5.12) and the laboratory skin tone study (See Table 5.33). There was also a statistically significant difference ($p < 0.05$) between the TAD scores of the human cadavers when using the Heaton and van Daalen DSS (See Section 7.33).

It is possible that the different descriptions of the Heaton and van Daalen DSS could have been the reason for these statistical differences observed. For example, descriptions such as “liquefaction” and

"exposure of soft tissues and bones" that were missing from the van Daalen DSS could have been the cause of this statistically significant difference because when these features were observed in some mice, the score of the preceding decomposition feature was assigned to the mice because these features were missing in the van Daalen DSS. For example, a body with "hair slough off", "liquefaction", and "exposure of soft tissues" decomposition changes in the head would have been scored with a FADS of 4, which describes a "Gray, matte discoloration of the skin with a crumbly surface" because that is the closest description in the van Daalen DSS to "liquefaction" and "exposure of soft tissues". In addition, the difficulty in noticing discolouration changes such as red, pink, purple, yellow, green, grey, and black in dark-furred/skinned mice and humans could have led to these statistically significant differences observed between the two DSS; the van Daalen DSS only mentioned grey and black discolouration of the skin, while the Heaton DSS mentioned all the listed discolouration changes.

9.3.2 Skin tone, Decomposition and DSS

Skin tone and its relation to decomposition and the available DSS were investigated in the mice and human studies in this research; see Section 5.3.4 and Chapter 7, respectively. The pig studies also highlighted the importance of colour changes in a decomposing carcass (See Chapter 6). From these studies, it was understood that the colour of the skin plays essential roles in different stages of decomposition. Discolouration changes were observed in the mice, pigs, and human remains during the fresh stage of decomposition. In addition to this, some physical decomposition changes noted in the Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS are described as the discolouration of the skin of different body parts; these colours include pink, red, green, black, grey, yellow, purple, and white.

Pink and red discolouration of the skin of the mice, pigs, and pale-skinned human remains was observed as early as a PMSI of 1 day, while in dark-skinned human remains, the skin tone appeared paler or lighter compared to their actual skin tone. This pink/red discolouration was also accompanied by yellow/green discolouration during marbling and putrefaction or the bloat stage of decomposition. The greenish discolouration observed was that of the gases and by-products of decomposition (Goff, 2009; Rosier *et al*., 2014). Other discolouration changes observed in the three cadavers include purple-red discolouration, which occurred due to the pooling of blood beneath the skin (Hayman & Oxenham, 2016), grey/black/brown discolouration as skin slippage and advanced internal organ decomposition occurred, and brown/black discolouration when the drying of skin occurred (Gennard, 2012; Vass *et al*., 2004; Verheggen *et al*., 2017). Green discolouration of the pigs' skin, soft tissue, and bones also occurred due to the formation and presence of algae.

Another critical observation on skin tone and decomposition was the higher accumulation (but not significant [$p = 1.000$]) of heat energy in dark-furred mice compared to pale-furred mice (See Section 5.5.1.3). Despite being placed in the same laboratory, under the weather conditions, the *Dark: Sea* group had a higher ADD (656.7) than the *Pale: Fresh* group (648.3); this could have occurred because darker

objects absorb all wavelengths of light, which then get converted into heat, while lighter objects reflect all wavelengths of light (Massoud, 2005). This accumulation of heat energy in the dark-skinned human remains was not observed. It is possible that it occurred in the human remains, but it would have only been certain if the average daily water temperature of the human remains' surroundings were recorded; only retrospective temperature data were available for this study.

9.3.3 DSS Limitations

Although these DSS have been validated over the years and passed as reliable through their application in different studies (Franceschetti *et al*., 2021; Humphreys *et al*., 2013; Palazzo *et al*., 2020), they have limitations. These decomposition scoring systems are visual charts assessing physical changes observed in bodies; therefore, they are subjective and depend on the observer's perception. Vague terms such as "heavy maggot activity", "mild wrinkling", "partial skeletonization", "bone exposure", and "exposing large areas of underlying muscles and tendons", along with the various skin colour changes listed in descriptions of these scoring systems, add to the subjectivity and inconsistency of these DSS (Gelderman *et al*., 2018; Nawrocka *et al*., 2016). Furthermore, different stages and decomposition features were observed simultaneously on the same cadaver (Paczkowski & Schütz, 2011; Teo *et al*., 2021). For example, two limbs had different decomposition changes; bone exposure was observed on one limb and marbling on the other. This could have led to a lesser accuracy of the scores when using the scoring systems (Nawrocka *et al*., 2016).

When using the van Daalen DSS, some limitations were encountered because the DSS did not include some decomposition changes observed in the mice, pigs, and human cadavers; some of these changes include liquefaction, adipocere formation, and the exposure and decomposition of soft tissues and organs. The van Daalen system only considered grey and white colours as discolouration, while purple, pink, red, yellow, and green discolouration was observed in the human, pig, and mice cadavers. Despite including skeletonization in the DSS, changes like the disarticulation of body parts and exposure of soft tissues, organs and bones were not included in the DSS description.

When using the Reh (1967) table for human remains, it was challenging to assess decomposition in fresh bodies with no visible decomposition change or feature because it did not mention "no visible changes" or fresh body states of decomposition. The Reh table was formed from a German study that used temperatures recorded in Germany. The warmer months in Germany, especially the summer months, coincide with the winter months in South Africa (May-August). This difference in temperature levels for both countries could be why the Reh table overestimated the PMSIs of bodies recovered in the winter months in South Africa. This overestimation will also be the case with countries or regions with a different climate from Germany. Due to climate changes in the last 40 years, there has been an increase in the water temperature of the river Rhine; therefore, for cases with water temperatures over 20 °C, there is a decrease in the reliability of the Reh table (Madea & Doberentz, 2010). Therefore,

further studies are required to investigate the correlation between these higher temperatures and the decomposition rate (Doberentz & Madea, 2010).

9.3.4 DSS Recommendations

The various decomposition studies conducted on mice and pig samples and human images in this PhD research have highlighted the effectiveness, suitability, flaws, and limitations of the three DSS used. The subsections below talk about recommendations that should be considered by forensic scientists, pathologists, anthropologists, entomologists, and other related professionals when assessing the extent of decomposition or estimating the PMSI of pale-skinned or dark-skinned bodies recovered from water.

9.3.4.1 *Assessing Decomposition Changes*

From the studies conducted in this PhD research, the Heaton *et al*. (2010) DSS was the most suitable DSS among the three DSS used for assessing decomposition changes in human analogues and human images. When comparing the three DSS used in this PhD research, the Heaton DSS is more specific in describing the decomposition changes expected in a body, it uses a lesser number of vague terms to classify the decomposition changes, and it includes more decomposition changes and features. The only decomposition change listed in the van Daalen *et al*. (2017) and Reh (1967) table but missing in the Heaton DSS is the absence or loss of nails in the limbs.

In terms of discolouration changes observed in the decomposition process, the Heaton DSS is more specific and puts more colours into consideration; it notes colours such as pink/white/red (used to describe early decomposition changes in the colour of the skin, wrinkling of limbs and marbling), yellow/green/purple (used to describe marbling and bloating changes) and black (used in describing post-bloating stages and drying of the skin and soft tissues). The van Daalen DSS only considers white (used to describe wrinkling of limbs) and grey (used to describe post-bloating stages) colours. The Reh table makes mention of "discolouration of the body" in its description of decomposition changes, but it does not give any specific colour change or specific body part where the colour change is expected. More colours can be added to the descriptions listed in the van Daalen DSS and Reh table to give more specific descriptions of the decomposition features observed. Therefore, more studies need to be conducted to improve the descriptions listed in these three DSS, while the colours listed in the Heaton DSS can be adapted into the van Daalen DSS and Reh table.

The Heaton DSS mentions decomposition changes such as "soft tissue, tendon, and bone exposure"; it also mentions "extensive disarticulation and skeletonization" before listing "complete disarticulation and skeletonization"; from these descriptions, the Heaton DSS differentiates bone exposure from partial or complete skeletonization. The van Daalen DSS only mentions partial and complete skeletonization; it will be challenging to differentiate between bone exposure and partial skeletonization when using this DSS. In this study, "bone exposure" was scored as "partial skeletonization", and this choice can be

utilised in other studies prior to any review of the van Daalen DSS. The Reh table makes no mention of "soft tissue, tendon, and bone exposure", "extensive disarticulation and skeletonization", and "complete disarticulation and skeletonization". Bone disarticulation is a fundamental decomposition change that can be utilised in PMSI; therefore, more studies need to be conducted to improve the Reh table concerning disarticulation and skeletonization changes, or the Reh table should be used with caution in further studies.

The Heaton DSS description mentions the presence of possible features that can be observed in aquatic decomposition, such as "adipocere" and "animal activity/predation", but these features are not listed in the van Daalen DSS or the Reh table. The van Daalen DSS and Reh table can be reviewed to add these missing features. Also, in cases when scoring two limbs with different decomposition changes leads to difficulties in assigning a score, it has been suggested that each limb is scored, and an average score of the four limbs is calculated as the final LAD score (Dabbs *et al*., 2016).

9.3.4.2 *Suitability for Dark-Skinned Bodies*

It was challenging to assess discolouration changes in the dark-skinned human image cases and the dark-furred mice because fewer colour changes were observed in the dark-skinned bodies, but the variety of colours listed in the Heaton *et al*. (2010) DSS made it the most suitable DSS for assessing the extent of decomposition in dark-skinned cases, most especially in the fresh and bloat stages of the decomposition process.

It was easier to detect one or two of the white/pink/red/yellow/green/purple/black colour changes from the Heaton DSS in dark-skinned bodies compared to the white/grey colour changes in the van Daalen DSS and the "discolouration of the body" from the Reh table. The advanced decomposition stages exhibited more anatomical changes than discolouration changes; therefore, it was easier to use the three DSS to assess the extent of decomposition of a dark-skinned body in an advanced decomposition stage. From the human image study, the skin of dark-skinned individuals did not appear pink, red, yellow, purple, or green but as a lighter or paler shade of their original skin tone. The pale appearance of the skin of a dark-skinned victim could be used as a sign to identify decomposition features such as marbling and the wrinkling and skin slippage of the limbs when these discolouration changes expected are not visible. In addition, pictures of the colour change expected in a dark-skinned body could be included with the DSS.

9.3.4.3 *PMSI Estimation*

The three DSS were relatively accurate for estimating the PMSI of both dark and pale-skinned bodies in a fresh state of decomposition, while the Reh table was the most accurate DSS for estimating the PMSI of bodies in advanced decomposition states. The three DSS overestimated the PMSI of bodies recovered in cold months. It is possible that the low temperatures experienced during the winter months could be the reason for the overestimation of the PMSIs of the cases when using the Heaton and van

Daalen DSS; there were days in which the average daily temperatures recorded were below 0℃, leading to an overestimated PMSI value because temperatures below 0℃ are added as 0 when calculating the ADD but the retrospective days forming the PMSI accumulate. For example, if a body has an extrapolated ADD of 10ADD and the average daily temperature of the past ten days are -1℃, -3℃, 3℃, -2℃, 4℃, 2℃, -2℃, 1℃, -5℃ and 1℃, the average daily temperatures are retrospectively added till they reach 10℃. Therefore, by replacing negative temperature values with 0, we have:

$$0 + 0 + 3 + 0 + 4 + 2 + 0 + 1 = 10$$

Thus, the PMSI of that body is 8 days; but in a case where the average daily temperatures are not below 0℃, the ADD of 10 would have been reached before 8 days.

The Heaton and van Daalen DSS overestimated the PMSI of bodies in advanced decomposition states. These overestimated PMSIs could be because advanced decomposition states led to a high TADS value, resulting in a high extrapolated ADD from the regression formula. Therefore, a lot of average daily temperatures were needed to add up to the high ADD values, thereby increasing the days of the estimated PMSI. The Reh table is also not suitable for estimating the PMSI of bodies recovered from regions or countries with a different climate or weather pattern from Germany, especially when there is a significant difference in the monthly temperatures of the regions. It will be necessary to make temperature adjustments to the table to make it suitable for other regions. More studies need to be conducted on both pale and dark-skinned bodies in different regions with varying climates across the world to improve the estimation of the PMSI of a body using these DSS.

9.4 Study Limitations

The use of animal carcasses as human analogues is cheap and makes replicate studies easy to conduct (Troutman *et al*., 2014), but studies comparing human remains and animal carcasses have shown that the animals decompose and affect the environment differently to human cadavers (Dautartas *et al*., 2018; DeBruyn *et al*., 2021). This PhD research faced limitations because it used mice, pigs, and photographs of human corpses to conduct decomposition studies. The results showed that animal carcasses are not entirely suitable as human analogues because there were differences in decomposition patterns and effects of water conditions on these carcasses. The Williams *et al*. (2019) study argues that it is risky to conclude that the knowledge obtained from studies on animal carcasses can be directly applied to human decomposition and PMSI estimation.

Therefore, more studies should be conducted in Human Taphonomy Facilities (HTFs) to study specific human lifestyle behaviours, choices and conditions that could affect the decomposition process and rate (Madea & Doberentz, 2017; Williams *et al*., 2019). The Heaton and van Daalen DSS were designed for human beings; adapting the two systems to be used on mice and pig cadavers was a limitation of the study because the size and composition of the human body and the mice and pigs are not alike. It was

difficult to observe some changes, such as marbling, wrinkling and discolouration in the limbs of the mice and pigs because of their small size, limb morphology, and the presence of fur. Observer bias was also a limitation because only one person assessed the extent of decomposition when using the three DSS; equivalent results would be expected because there is a tendency for the observer not to see the differences in the DSS but instead see the similarities they expect or want to see.

9.5 Further Research

Further studies should be conducted on the water conditions explored in this PhD research, such as salinity, hardness, current, pH and TDS. These studies will improve the current knowledge of the effect of these water conditions on decomposition rate and PMSI estimation. Future research would benefit from measuring the salinity, hardness, pH, and TDS of the water on sampling days to track if the concentrations and levels of these water parameters change as decomposition advances. The salinity of water in the hardness studies can also be measured using a hydrometer to investigate the salinity in the hard water. In addition, a microbial or DNA analysis could be done on the sampling days to identify the presence or growth of any bacteria in the water. The presence of bacteria could indicate they are linked explicitly to the water type or decomposition stage when they were identified.

Further human analysis should be conducted on cadavers with varying skin tones to validate these DSS' suitability for PMSI estimation and assess the extent of decomposition in bodies with dark skin tones. Further studies can utilise photographs of decomposing drowned cadavers like this PhD research did, but the ideal sample for such studies will be actual human cadavers. Establishing HTFs in the U.K. and other regions worldwide would enable such human analysis, PMSI estimation experiments, essential drowning studies, and further crucial forensic experiments that will contribute immensely to the field of forensic science. In addition, the existing HTFs could expand their facilities/capabilities to include systems for undertaking aquatic taphonomy, such as tanks, moving water systems, pools and ponds to enable analyses of human aquatic decomposition.

10 CONCLUSIONS

10.1 Key Results

The aims and objectives have been achieved, and the research questions of this PhD research have been answered.

The results of the laboratory studies on mice and the field studies on pigs demonstrated that:

1. The Heaton *et al*. (2010) and van Daalen *et al*. (2017) DSS are suitable and effective when adapted for assessing the extent of decomposition in mice and pigs that have been submerged in water. Therefore, the alternative hypotheses for the suitability and effectiveness of these DSS for assessing the extent of decomposition in human analogues that have been submerged in water (given in Section 1.4.1) can be accepted.
2. Water conditions such as temperature, salinity, hardness, and current significantly affect the decomposition rate of these human analogues submerged in water. Therefore, the alternative hypotheses for these conditions (given in Sections 1.4.1, 5.1.1 and 6.1.1) can be accepted. Key results from the two studies concerning these water conditions include:
   - Decomposition is faster in freshwater compared to the different types of saltwater: Fresh *vs* Brackish ($p = 0.029$), Sea ($p = 0.003$), and Hypersaline ($p < 0.001$).
   - Decomposition is faster in soft water compared to the different types of hard water: Soft Water *vs* Moderately Hard Water ($p < 0.001$), Hard Water ($p < 0.001$), and Very Hard Water ($p < 0.001$).
   - Decomposition is faster in fast-moving/flowing water compared to slow-moving/non-flowing water: Moving *vs* Submerged groups ($p < 0.001$); Floating ($p < 0.001$).
   - Decomposition is faster in water with frequently renewed water salinity compared to water with constant salinity.

The human image analysis demonstrated that:

1. The Heaton *et al*. (2010), van Daalen *et al*. (2017), and Reh (1967) DSS are relatively accurate for estimating the PMSI of both dark and pale-skinned bodies in a fresh state of decomposition.
2. These three DSS are inaccurate for estimating the PMSI of dark and pale-skinned bodies in advanced decomposition states or recovered in the cold or winter months.
3. It is challenging to identify the various discolouration changes listed in the DSS descriptions; instead, these dark-skinned bodies appeared paler or lighter.
4. The pale appearance of the skin of a dark-skinned victim could be used as a sign to identify decomposition features such as marbling and the wrinkling and skin slippage of the limbs when these discolouration changes expected are not visible. In addition, pictures of the colour change expected in a dark-skinned body could be included with the DSS.

Future studies and practical cases in real-life scenarios will benefit from the following observations:

1. Water salinity should always be considered when estimating the PMSI of bodies recovered from seawater and marine environments.
2. Extra care must be taken when estimating the PMSI of victims recovered from water bodies containing rocks made up of chalk and limestone or other water bodies that get their source from underground aquifers. This is because rocks and aquifers are made up of soluble minerals (such as calcium) that contribute to water hardness. High water hardness will retard the decomposition process; therefore, not taking water hardness into account would lead to an incorrectly short PMSI.
3. This research has provided preliminary data that suggests that current, water flow and turbulence influence decomposition rate. Therefore, forensic investigations could benefit from more research on how such factors affect aquatic decomposition, body displacement, and PMSI estimation.
4. This research has shown that there is potential for forensic investigations of dark-skinned drowning victims to be compromised and PMSI estimations inaccurate due to inadequate visual decomposition scoring systems, as many of the colour change features on which they rely are difficult to discern in dark skin. The understanding of decomposition processes in dark-skinned individuals is vital for making PMSI estimation techniques applicable across the world. Carrying out these studies in Africa and other areas that predominantly have people with darker skin tones will help improve the suitability of these DSS for bodies with darker skin tones and improve the knowledge of decomposition and PMSI estimations in such regions.

10.2 Implications

Existing research on aquatic decomposition has broadly been based on how various factors influence decomposition processes in different seasons and the comparison of their influence in freshwater and seawater. This PhD research took a novel approach that involved more water types, such as brackish and hypersaline water and investigated the effect of water conditions such as temperature, salinity, hardness, current, pH and TDS. It has been shown here that these water conditions significantly alter the aquatic decomposition process, and they need to be considered in forensic or real-life practical cases when assessing the extent of decomposition in remains recovered from different water types and when estimating the PMSI of these remains to avoid obtaining incorrect or inaccurate PMSI estimates. In addition, this PhD research has demonstrated the insufficiency of validated DSS such as the Heaton *et al*. (2010), van Daalen *et al*. (2017) and the Reh (1967) DSS in assessing decomposition changes associated with discolouration in dark-skinned human remains. This suggests that forensic cases involving dark-skinned bodies recovered from water could be seriously compromised. There is a dire need for more inclusive methods of PMI and PMSI estimation that consider the differences that skin tone can have on recognising post-mortem features, to improve forensic outcomes for all victims and

their families. This research has also shown that the application of these DSS when estimating the PMSI of bodies in advanced decomposition states or bodies recovered during cold months needs to be reviewed because they could lead to inaccurate estimates of the PMSI of these bodies. This PhD research has highlighted these issues for the first time, and in so doing, acts as the foundation for future studies to build on to improve DSS and PMSI estimation of both pale and dark-skinned victims recovered from different water types worldwide.

REFERENCES

Adlam, R., & Simmons, T. (2007). The effect of repeated physical disturbance on soft tissue decomposition—are taphonomic studies an accurate reflection of decomposition?. *Journal of Forensic Sciences*, *52*(5), 1007-1014.

Adserias-Garriga, J., Quijada, N., Hernandez, M., Rodríguez Lázaro, D., Steadman, D., & Garcia-Gil, L. (2017). Dynamics of the oral microbiota as a tool to estimate time since death. *Molecular Oral Microbiology*, *32*(6), 511–516.

Agarwal, S., & Krishnamurthy, K. (2022). Histology, Skin. [Internet]. Treasure Island (FL): StatPearls Publishing. PMID: 30726010.

Ahmad, A., Ahmad, A., Dieng, H., Satho, T., Ahmad, H., Aziz, A., & Boots, M. (2011). Cadaver wrapping and arrival performance of adult flies in an oil palm plantation in northern Peninsular Malaysia. *Journal of Medical Entomology*, *48*(6), 1236–1246.

Ajibade, I., McBean, G., & Bezner-Kerr, R. (2013). Urban flooding in Lagos, nigeria: Patterns of vulnerability and resilience among women. *Global Environmental Change*, *23*(6), 1714–1725.

Alexander, R., & Jentzen, J. (2011). Neck and scleral haemorrhage in drowning. *Journal of Forensic Sciences*, *56*(2), 522–525.

Alfsdotter, C., & Petaros, A. (2021). Outdoor human decomposition in Sweden: A retrospective quantitative study of forensic-taphonomic changes and postmortem interval in terrestrial and aquatic settings. *Journal of Forensic Sciences*, *66*(4), 1348–1363.

Alkilani, A., McCrudden, M., & Donnelly, R. (2015). Transdermal Drug Delivery: Innovative Pharmaceutical Developments Based on Disruption of the Barrier Properties of the Stratum Corneum. *Pharmaceutics*, *7*(4), 438-470.

Amendt, J., Richards, C., Campobasso, C., Zehner, R., & Hall, M. (2011). Forensic entomology: Applications and limitations. *Forensic Science, Medicine, and Pathology*, *7*(4), 379–392.

Anders, S., Kunz, M., Gehl, A., Sehner, S., Raupach, T., & Beck-Bornholdt, H. (2013). Estimation of the time since death—reconsidering the re-establishment of Rigor Mortis. *International Journal of Legal Medicine*, *127*(1), 127–130.

Anderson, G. (2008). Investigation into the effects of oceanic submergence on carrion decomposition and faunal colonisation using a baited camera. *Part I. (No. TR-10-2008). Canadian Police Research Centre, Ottawa, ON*.

Anderson, G. (2009). Decomposition and invertebrate colonization of cadavers in coastal marine environments. In *Current concepts in forensic entomology* (pp. 223-272). Dordrecht: Springer.

Anderson, G., & Bell, L. (2014). Deep coastal marine taphonomy: investigation into carcass decomposition in the Saanich Inlet, British Columbia using a baited camera. *PLoS One*, *9*(10), 1–25.

Anderson, G., & Bell, L. (2016). Impact of marine submergence and season on faunal colonization and decomposition of pig carcasses in the Salish Sea. *PLoS One*, *11*(3), 1-20.

Anderson, G., & Bell, L. (2017). Comparison of Faunal Scavenging of Submerged Carrion in Two Seasons at a Depth of 170 m, in the Strait of Georgia, British Columbia. *Insects*, *8*(1), 33.

Anderson, G., & Hobischak, N. (2004). Decomposition of carrion in the marine environment in British Columbia, Canada. *International Journal of Legal Medicine*, *118*(4), 206-209.

Aptel, I., Cance-Rouzaud, A., & Grandjean, H. (1999). Association Between Calcium Ingested from Drinking Water and Femoral Bone Density in Elderly Women: Evidence from the EPIDOS Cohort. *Journal Of Bone And Mineral Research*, *14*(5), 829-833.

August, D., Hitchcock, I., Tangney, J., Ray, R., Kandasamy, Y., & New, K. (2019). Graduated colour tape measure: Development and demonstration of this tool in a case series of neonatal skin injuries. *Journal Of Tissue Viability*, *28*(3), 133-138.

Bajerlein, D., Taberski, D., & Matuszewski, S. (2018). Estimation of postmortem interval (PMI) based on Empty Puparia of Phormia regina (Meigen) (Diptera: Calliphoridae) and third larval stage of Necrodes littoralis (L.) (Coleoptera: Silphidae) – advantages of using different PMI indicators. *Journal of Forensic and Legal Medicine*, *55*(1), 95–98.

Banks, D., & Boyce, A. (2023). Dissolved sulfate δ34S and the origin of sulfate in coal mine waters; NE England. *Quarterly Journal of Engineering Geology and Hydrogeology*, *56*(2), 1–12. https://doi.org/10.1144/qjegh2022-106.

Barat, J., Grau, R., Ibáñez, J., & Fito, P. (2005). Post-salting studies in Spanish cured ham manufacturing. Time reduction by using brine thawing–salting. *Meat Science*, *69*(2), 201-208.

Barrios, M., & Wolff, M. (2011). Initial study of arthropods succession and pig carrion decomposition in two freshwater ecosystems in the Colombian andes. *Forensic Science International*, *212*(1-3), 164–172.

Barton, P., Cunningham, S., Macdonald, B., McIntyre, S., Lindenmayer, D., & Manning, A. (2013). Species traits predict assemblage dynamics at ephemeral resource patches created by Carrion. *PLoS ONE*, *8*(1), 1–9.

Barwood, M., Bates, V., Long, G., & Tipton, M. (2011). “Float first:” trapped air between clothing layers significantly improves buoyancy on water after immersion. *International Journal of Aquatic Research and Education*, *5*(2), 147–163.

Becker, R., Nordby, S., & Jon, J. (2013). *Underwater forensic investigation* (2nd ed.). Boca Raton: CRC Press.

Beerman, S., Bierens, J., Clemens, T., Meddings, D., Rahman, A., & Szpilman, D. (2018, August). Clarification and Categorization of Non-fatal Drowning A draft Position Statement for review and input by the global drowning community Prepared by the Working Group on Non-fatal Drowning. In *Proceedings of the Workshop on Non-Fatal Drowning, Toronto, ON, Canada* (pp. 8-9).

Bello, S., Saladié, P., Cáceres, I., Rodríguez-Hidalgo, A., & Parfitt, S. (2015). Upper Palaeolithic ritualistic cannibalism at Gough's Cave (Somerset, UK): The human remains from head to Toe. *Journal of Human Evolution*, *82*(1), 170–189.

Benbow, M., Pechal, J., Lang, J., Erb, R., & Wallace, J. (2015). The Potential of High-throughput Metagenomic Sequencing of Aquatic Bacterial Communities to Estimate the Post-mortem Submersion Interval. *Journal of Forensic Sciences*, *60*(6), 1500-1510.

Bernitz, H., van Staden, P., Rossouw, S., & Jordaan, J. (2018). Tongue position and its relation to the cause of death and sequential stages of body decomposition observed during 608 forensic post-mortems. *International Journal of Legal Medicine*, *133*(4), 1279–1283.

Best, P., & Photopoulou, T. (2016). Identifying the “demon whale-biter”: Patterns of scarring on large whales attributed to a cookie-cutter shark Isistius sp. *PLOS ONE*, *11*(4), e0152643.

Betts, J.G., Young, K.A., Wise, J.A., Johnson, E., Poe, B., Kruse, D.H., Korol, O., Johnson, J.E., Womble, M., & DeSaix, P. (2013). The integumentary system. In OpenStax. (Ed.), *Anatomy and physiology*. Houston, Texas: OpenStax.

Bickler, P., Feiner, J., & Severinghaus, J. (2005). Effects of skin pigmentation on pulse oximeter accuracy at low saturation. *The Journal of the American Society of Anesthesiologists*, *102*(4), 715-719.

Bierens, J., Knape, J., & Gelissen, H. (2002). Drowning. *Current opinion in critical care*, *8*(6), 578-586.

Bierens, J., Lunetta, P., Tipton, M., & Warner, D. (2016). Physiology Of Drowning: A Review. *Physiology*, *31*(2), 147-166.

Blanco Pampín, J, & López-Abajo Rodríguez B. (2001). Surprising drifting of bodies along the coast of Portugal and Spain. *Legal Medicine*, *3*(3), 177–182.

Bonzon, J., Schön, C., Schwendener, N., Zech, W.-D., Kara, L., Persson, A., & Jackowski, C. (2015). Rigor mortis at the myocardium investigated by post-mortem magnetic resonance imaging. *Forensic Science International*, *257*(1), 93–97.

Bortolotti, F., Del Balzo, G., Calza, R., Valerio, F., & Tagliaro, F. (2011). Testing the specificity of the diatom test: Search for false-positives. *Medicine, Science and the Law*, *51*(1), 7–10.

Boumba, V., Ziavrou, K., & Vougiouklakis, T. (2008). Biochemical pathways generating post-mortem volatile compounds co-detected during forensic ethanol analyses. *Forensic Science International*, *174*(2-3), 133-151.

Boxberger, M., Cenizo, V., Cassir, N., & La Scola, B. (2021). Challenges in exploring and manipulating the human skin microbiome. *Microbiome*, *9*(1), 1-14.

Boyle, S., Galloway, A., & Mason, R.T. (1997). Human Aquatic Taphonomy in the Monterey Bay Area. In W.D. Haglund, & M.H. Sorg (Eds.) *Forensic Taphonomy: The Post-mortem Fate of Human Remains*. (pp. 605-613). Boca Raton: CRC Press.

Brainard, D. & Stockman, A. (2010). Colorimetry. *Osa Handbook of Optics*. (3rd ed). New York: McGraw-Hill.

Bright, F., Winskog, C., Tsokos, M., Walker, M., & Byard, R. (2014). Issues in the diagnosis of hypothermia: A comparison of two geographically separate populations. *Journal Of Forensic And Legal Medicine*, *22*(1), 30-32.

Bryda, E. (2013). The Mighty Mouse: The Impact of Rodents on Advances in Biomedical Research. *Missouri Medicine*, *110*(3), 207-211.

Bugeja, L., & Franklin, R. (2013). An analysis of stratagems to reduce drowning deaths of young children in private swimming pools and spas in Victoria, Australia. *International Journal of Injury Control and Safety Promotion*, *20*(3), 282-294.

Bugeja, L., Ibrahim, J., Ferrah, N., Murphy, B., Willoughby, M., & Ranson, D. (2016). The utility of Medico-legal databases for Public Health Research: A systematic review of peer-reviewed publications using the National Coronial Information System. *Health Research Policy and Systems*, *14*(1), 1–10.

Butler, J. (2005). *Forensic DNA typing: biology, technology, and genetics of STR markers* (2nd ed.). Burlington: Elsevier.

Byard, R. (2013). Commercial fishing industry deaths – forensic issues. *Journal of Forensic and Legal Medicine*, *20*(3), 129–132.

Byard, R. (2015). Immersion deaths and drowning: issues arising in the investigation of bodies recovered from water. *Forensic Science, Medicine, And Pathology*, *11*(3), 323-325.

Byard, R. (2016). Adipocere—the fat of graveyards. *The American journal of forensic medicine and pathology*, *37*(3), 208-210.

Byard, R. (2017). Drowning deaths in rivers. *Forensic Science, Medicine and Pathology*, *13*(3), 388-389.

Byard, R. (2018). Putrefaction: An Additional Complicating Factor in the Assessment of Freshwater Drownings in Rivers. *Journal Of Forensic Sciences*, *63*(3), 899-901.

Byard, R. (2020). Estimation of the time since death in the early postmortem period (24–48 hours). In J. Hayman & M. Oxenham (Eds.) *Estimation of the Time since Death: Current Research and Future Trends*. (pp. 11-27). London: Academic Press.

Byard, R., Both, K., & Simpson, E. (2008). The Identification of Submerged Skeletonized Remains. *American Journal Of Forensic Medicine &Amp; Pathology*, *29*(1), 69-71.

California State Park (2019). California Department of Parks and Recreation Budget Change Proposal; State Beaches and Parks Smoking Ban (SB9), No. 3790-070-BCP-2019-GB. Sacramento: Department of Parks and Recreation. Retrieved November 23, 2022, from https://esd.dof.ca.gov/Documents/bcp/2021/FY2021_ORG3790_BCP3388.pdf.

Cameron, A., & Oxenham, M. (2012). Disarticulation sequences and scattering patterns in temperate southeastern Australia. *Australian Journal of Forensic Sciences*, *44*(2), 197-211.

Campero, M., Serra, J., & Ochoa, J. (1996). C-polymodal nociceptors activated by noxious low temperature in human skin. *The Journal Of Physiology*, *497*(2), 565-572.

Campobasso, C., Di Vella, G., & Introna, F. (2001). Factors affecting decomposition and Diptera colonisation. *Forensic Science International*, *120*(1-2), 18-27.

Carballeira, R., Vieira, D., Febrero-Bande, M., & Muñoz Barús, J. (2018). A valid method to determine the site of drowning. *International Journal of Legal Medicine*, *132*(2), 487–497.

Card, A., Cross, P., Moffatt, C., & Simmons, T. (2015). The effect of clothing on the rate of decomposition and Diptera colonization on Sus scrofa carcasses. *Journal of forensic sciences*, *60*(4), 979-982.

Carter, D., & Tibbett, M. (2008). Cadaver decomposition and soil: processes. In *Soil analysis in forensic taphonomy* (pp. 41-64). Boca Raton: CRC Press.

Carter, D., Metcalf, J., Bibat, A., & Knight, R. (2015). Seasonal variation of post-mortem microbial communities. *Forensic Science, Medicine, and Pathology*, *11*(2), 202-207.

Caruso, J. (2016). Decomposition Changes in Bodies Recovered from Water. *Academic Forensic Pathology*, *6*(1), 19-27.

Casamatta, D., & Verb, R. (2000). Algal Colonization of Submerged Carcasses in a Mid-Order Woodland Stream. *Journal of Forensic Sciences*, *45*(6), 14880J.

Celata, E. (2015). Postmortem intervals in mice submerged in aqueous environments at 20°C. *Journal of Forensic Sciences*, *60*(6), 1495–1499.

Cenderadewi, M., Franklin, R., Peden, A., & Devine, S. (2020). Fatal intentional drowning in Australia: A systematic literature review of rates and risk factors. *PLOS ONE*, *15*(5), 1–20.

Chahal, H., Wu, W., Ransohoff, K., Yang, L., Hedlin, H., Desai, M., Lin, Y., Dai, H.-J., Qureshi, A., Li, W.-Q., Kraft, P., Hinds, D., Tang, J., Han, J., & Sarin, K. (2016). Genome-wide association study identifies 14 novel risk alleles associated with basal cell carcinoma. *Nature Communications*, *7*(1), 1–10.

Champaneri, N. (2006). *Decomposition in Canal Water: The Effects of Varying Temperature* (Dissertation). University of Central Lancashire, Preston.

Chanda, A. (2018). Biomechanical Modeling of Human Skin Tissue Surrogates. *Biomimetics*, *3*(3), 1-18.

Chandra, A., Goswami, H., & Sengupta, P. (2012). Dietary calcium induced cytological and biochemical changes in thyroid. *Environmental Toxicology And Pharmacology*, *34*(2), 454-465.

Charabidze, D., Colard, T., Vincent, B., Pasquerault, T., & Hedouin, V. (2013). Involvement of larder beetles (Coleoptera: Dermestidae) on human cadavers: a review of 81 forensic cases. *International Journal of Legal Medicine*, *128*(6), 1021-1030.

Chen, Y. (2017). State of the art in post-mortem forensic imaging in China. *Forensic Sciences Research*, *2*(2), 75–84.

Chen, Y., Branicki, W., Walsh, S., Nothnagel, M., Kayser, M., & Liu, F. (2021). The impact of correlations between pigmentation phenotypes and underlying genotypes on genetic prediction of pigmentation traits. *Forensic Science International: Genetics*, *50*(1), 1-9.

Chester, R., & Jickells, T. (2012). *Marine geochemistry*. Wiley-Blackwell.

Christe, A., Aghayev, E., Jackowski, C., Thali, M. J., & Vock, P. (2008). Drowning—post-mortem imaging findings by computed tomography. *European Radiology*, *18*(2), 283–290.

Christensen, A., & Myers, S. (2011). Macroscopic Observations of the Effects of Varying Fresh Water pH on Bone. *Journal of Forensic Sciences*, *56*(2), 475-479.

Clemens, T., Moreland, B., & Lee, R. (2021). Persistent racial/ethnic disparities in fatal unintentional drowning rates among persons aged ≤29 years — United States, 1999–2019. *MMWR. Morbidity and Mortality Weekly Report*, *70*(24), 869–874.

Cobaugh, K., Schaeffer, S., & DeBruyn, J. (2015). Functional and structural succession of soil microbial communities below decomposing human cadavers. *PLOS ONE*, *10*(6), 1–20.

Cockle, D., & Bell, L. (2015). Human decomposition and the reliability of a 'Universal' model for post-mortem interval estimations. *Forensic Science International*, *253*(1), 136.e1-136.e9.

Cohen, A. (2019, February 2). *What Are The Stages Of Human Decomposition?* 5 Stages of Human Decomposition | Bio Socal.com. Retrieved November 1, 2022, from https://biosocal.com/what-are-the-stages-of-human-decomposition/

Cornett, D.C., Bolme, D.S., Steadman, D.W., Sauerwein, K.A., & Saul, T.B. (2019). Effects of postmortem decomposition on face recognition. In *2019 IEEE 10th International Conference on Biometrics Theory, Applications and Systems (BTAS)* (pp. 1-8). IEEE.

Costi, D., Calcaterra, P., Iori, N., Vourna, S., Nappi, G., & Passeri, M. (1999). Importance of bioavailable calcium drinking water for the maintenance of bone mass in post-menopausal women. *Journal Of Endocrinological Investigation*, *22*(11), 852-856.

Council Auckland (2019). *Safeswim switches on digital signs at local beaches*. Retrieved November 23, 2022 from https://ourauckland.aucklandcouncil.govt.nz/articles/news/2019/08/safeswimswitches-on-digital-signs-at-local-beaches/

Croft, J., & Button, C. (2015). Interacting factors associated with adult male drowning in New Zealand. *PLOS ONE*, *10*(6), 1–13.

Dabbs, G., Connor, M., & Bytheway, J. (2016). Interobserver reliability of the total body score system for quantifying human decomposition. *Journal of Forensic Sciences*, *61*(2), 445–451.

Dalgaard, L. (2015). Comparison of minipig, dog, monkey and human drug metabolism and disposition. *Journal of Pharmacological and Toxicological Methods*, *74*(1), 80–92.

Damann, F., Williams, D., & Layton, A. (2015). Potential use of bacterial community succession in decaying human bone for estimating postmortem interval. *Journal of Forensic Sciences*, *60*(4), 844–850.

Dash, H., & Das, S. (2020). Thanatomicrobiome and epinecrotic community signatures for estimation of post-mortem time interval in human cadaver. *Applied Microbiology and Biotechnology*, *104*(22), 9497-9512.

Dautartas, A., Kenyhercz, M., Vidoli, G., Meadows Jantz, L., Mundorff, A., & Steadman, D. (2018). Differential Decomposition Among Pig, Rabbit, and Human Remains. *Journal Of Forensic Sciences*, *63*(6), 1673-1683.

Davidson, P., Taylor, T., & Schmidt, S. (2012). Chemical Preservatives and Natural Antimicrobial Compounds. In M.P. Doyle, & R.L. Buchanan (Eds.), *Food Microbiology: Fundamentals and Frontiers* (4th ed., pp. 765-801). ASM Press.

Davis, J., & Goff, M. (2000). Decomposition Patterns in Terrestrial and Intertidal Habitats on Oahu Island and Coconut Island, Hawaii. *Journal of Forensic Sciences*, *45*(4), 14780J.

De Donno, A., Campobasso, C., Santoro, V., Leonardi, S., Tafuri, S., & Introna, F. (2014). Bodies in sequestered and non-sequestered aquatic environments: A comparative taphonomic study using decompositional scoring system. *Science & Justice*, *54*(6), 439-446.

DeBruyn, J., & Hauther, K. (2017). Postmortem succession of gut microbial communities in deceased human subjects. *PeerJ*, *5*(1), 1–14.

DeBruyn, J., Hoeland, K., Taylor, L., Stevens, J., Moats, M., Bandopadhyay, S., Dearth, S., Castro, H., Hewitt, K., Campagna, S., Dautartas, A., Vidoli, G., Mundorff, A., & Steadman, D. (2021). Comparative Decomposition of Humans and Pigs: Soil Biogeochemistry, Microbial Activity and Metabolomic Profiles. *Frontiers In Microbiology*, *11*(1), 1-16.

Dekeirsschieter, J., Stefanuto, P., Brasseur, C., Haubruge, E., & Focant, J. (2012). Enhanced Characterisation of the Smell of Death by Comprehensive Two-Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry (GCxGC-TOFMS). *Plos ONE*, *7*(6), e39005.

Dekeirsschieter, J., Verheggen, F. J., Gohy, M., Hubrecht, F., Bourguignon, L., Lognay, G., & Haubruge, E. (2009). Cadaveric volatile organic compounds released by decaying pig carcasses (Sus domesticus L.) in different biotopes. *Forensic Science International*, *189*(1-3), 46-53.

Del Bino, S., & Bernerd, F. (2013). Variations in skin colour and the biological consequences of ultraviolet radiation exposure. *British Journal Of Dermatology*, *169*(3), 33-40.

Dent, B., Forbes, S., & Stuart, B. (2004). Review of human decomposition processes in soil. *Environmental Geology*, *45*(4), 576-585.

Derrick, B., White, P., & Toher, D. (2020). Parametric and non-parametric tests for the comparison of two samples which both include paired and unpaired observations. *Journal of Modern Applied Statistical Methods*, *18*(1), 2–23.

Di Giancamillo, A., Giudici, E., Andreola, S., Porta, D., Gibelli, D., Domeneghini, C., Grandi, M., & Cattaneo, C. (2010). Immersion of piglet carcasses in water–the applicability of microscopic analysis and limits of diatom testing on an animal model. *Legal Medicine*, *12*(1), 13-18.

Di Maio, V., & Di Maio, D. (2001). *Forensic Pathology* (2nd ed.). Boca Raton (FL): CRC Press.

Dickson, G., Poulter, R., Maas, E., Probert, P., & Kieser, J. (2011). Marine bacterial succession as a potential indicator of post-mortem submersion interval. *Forensic Science International*, *209*(1-3), 1-10.

DiGiancamillo, A., Domeneghini, C., Gibelli, D., & Cattaneo, C. (2011). Diatom extraction with hcl from animal tissues: A technical note. *Legal Medicine*, *13*(5), 268–271.

Doberentz, E., & Madea, B. (2010). Estimating the time of immersion of bodies found in water - an evaluation of a common method to estimate the minimum time interval of immersion. *Revista Española De Medicina Legal*, *36*(2), 51–61.

Dominiczak, M., & Woodward, M. (2007). *Flesh and Bones of Metabolism*. Mosby: Elsevier Health Sciences.

Dua, K., Surapaneni, S., Kuribayashi, S., Hafeezullah, M., & Shaker, R. (2011). Pharyngeal airway protective reflexes are triggered before the maximum volume of fluid that the hypopharynx can safely hold is exceeded. *American Journal Of Physiology-Gastrointestinal And Liver Physiology*, *301*(2), G197-G202.

Dumser, T., & Türkay, M. (2008). Postmortem changes of human bodies on the Bathyal Sea floor—two cases of aircraft accidents above the open sea. *Journal of Forensic Sciences*, *53*(5), 1049-1052.

Duncan Seraphin, K., Philippoff, J., Pottenger, F., Kaupp, L., Lurie, M., Lin, D., & Baumgartner, E. (2014). Practices of Science: Making Simulated Seawater. Retrieved October 2 2020, from https://manoa.hawaii.edu/exploringourfluidearth/physical/density-effects/density-temperature-and-salinity/practices-science-making-simulated-seawater

Dye, S., Berx, B., Opher, J., Tinker, J., & Renshaw, R. (2020). Climate change and salinity of the coastal and marine environment around the UK. *MCCIP Science Review*, *1*(1), 76-102.

Dyer, O. (2020). Pulse oximetry may underestimate hypoxaemia in black patients, study finds. *BMJ*, *371*(1), m4926.

Eilers, S., Bach, D., Gaber, R., Blatt, H., Guevara, Y., Nitsche, K., Kundu, R., & Robinson, J. (2013). Accuracy of Self-report in Assessing Fitzpatrick Skin Phototypes I Through VI. *JAMA Dermatology*, *149*(11), 1289-1294.

El Sibai, R., Bachir, R., & El Sayed, M. (2018). Submersion injuries in the United States: Patients characteristics and predictors of mortality and morbidity. *Injury*, *49*(3), 543-548.

Ellingham, S., Perich, P., & Tidball-Binz, M. (2017). The fate of human remains in a maritime context and feasibility for forensic humanitarian action to assist in their recovery and identification. *Forensic Science International*, *279*(1), 229–234.

Eriksson, N., Macpherson, J., Tung, J., Hon, L., Naughton, B., Saxonov, S., Avey, L., Wojcicki, A., Pe'er, I., & Mountain, J. (2010). Web-based, participant-driven studies yield novel genetic associations for common traits. *PLoS Genetics*, *6*(6), 1–20.

Erskine, K., & Armstrong, E. (2021). *Water-related Death Investigation: Practical Methods and Forensic Applications* (2nd ed.). Boca Raton: CRC Press.

Ertekin, C. (2011). Voluntary Versus Spontaneous Swallowing in Man. *Dysphagia*, *26*(2), 183-192.

Everett, J., Budescu, M., & Sommers, M. (2012). Making Sense of Skin Color in Clinical Care. *Clinical Nursing Research*, *21*(4), 495-516.

Faber, J., & Fonseca, L. (2014). How sample size influences research outcomes. *Dental Press Journal of Orthodontics*, *19*(4), 27–29.

Fancher, J., Aitkenhead-Peterson, J., Farris, T., Mix, K., Schwab, A., Wescott, D., & Hamilton, M. (2017). An evaluation of soil chemistry in human cadaver decomposition islands: Potential for estimating post-mortem interval (PMI). *Forensic Science International*, *279*(1), 130-139.

Fiedler, S., Buegger, F., Klaubert, B., Zipp, K., Dohrmann, R., Witteyer, M., Zarei, M. & Graw, M. (2009). Adipocere withstands 1600 years of fluctuating groundwater levels in soil. *Journal of Archaeological Science*, *36*(7), 1328-1333.

Findlay, H. S., Artoli, Y., Birchenough, S. N. R., Hartman, S., León, P., & Stiasny, M. (2022). Climate change impacts on ocean acidification relevant to the UK and Ireland. *MCCIP Science Review*, *1*(1), 1-24.

Finley, S., Pechal, J., Benbow, M., Robertson, B., & Javan, G. (2016). Microbial signatures of cadaver gravesoil during decomposition. *Microbial Ecology*, *71*(3), 524–529.

Fisher, B. (2007). *Techniques of Crime Scene Investigation* (7th ed.). New York: CRC Press.

Fitzpatrick, T. (1988). The Validity and Practicality of Sun-Reactive Skin Types I Through VI. *Archives Of Dermatology*, *124*(6), 869-71.

Forbes, S., Dent, B., & Stuart, B. (2005). The effect of soil type on adipocere formation. *Forensic Science International*, *154*(1), 35–43.

Forbes, S., Perrault, K., & Comstock, J. (2017). Microscopic Post-Mortem Changes: The Chemistry of Decomposition. In E.M. Schotsmans, N. Márquez-Grant, & S.L. Forbes (Eds.) *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment*. (pp. 26-38). Oxford: John Wiley & Sons Ltd.

Forbes, S., Perrault, K., Stefanuto, P., Nizio, K., & Focant, J. (2014). Comparison of the decomposition VOC profile during winter and summer in a moist, mid-latitude (CFB) climate. *PLoS ONE*, *9*(11), 1–11.

Förster, M., Bolzinger, M., Fessi, H., & Briançon, S. (2009). Topical delivery of cosmetics and drugs. Molecular aspects of percutaneous absorption and delivery. *European Journal Of Dermatology*, *19*(4), 309-323.

Fortuin, J., Karangwa, I., Mahlalela, N., & Robertson, C. (2022). A South African epidemiological study of fatal drownings: 2016–2021. *International Journal of Environmental Research and Public Health*, *19*(22), 1–10.

Franceschetti, L., Palamenghi, A., Mazzarelli, D., Cappella, A., Gibelli, D., De Angelis, D., Verzeletti, A., & Cattaneo, C. (2021). Taphonomic study on drowned victims in a non-sequestered aquatic environment in the Mediterranean Sea. *International Journal of Legal Medicine*, *136*(3), 887–895.

Franklin, R., Peden, A., Hamilton, E., Bisignano, C., Castle, C., Dingels, Z., ... & James, S. L. (2020). The burden of unintentional drowning: global, regional and national estimates of mortality from the Global Burden of Disease 2017 Study. *Injury prevention*, *26*(Suppl 2), i83-i95.

Fredericks, J., Brown, K., Williams, A., & Bennett, P. (2013). DNA analysis of skeletal tissue recovered from the English Channel. *Journal of Forensic and Legal Medicine*, *20*(6), 757-759.

Fucci, N., Campobasso, C. P., Mastrogiuseppe, L., Puccinelli, C., Marcheggiani, S., Mancini, L., Marino, L., & Pascali, V. L. (2017). Diatoms in drowning cases in forensic veterinary context: A preliminary study. *International Journal of Legal Medicine*, *131*(6), 1573–1580.

Gallo, R. (2017). Human Skin Is the Largest Epithelial Surface for Interaction with Microbes. *Journal Of Investigative Dermatology*, *137*(6), 1213-1214.

Galloway, A., Birkby, W. H., Jones, A. M., Henry, T. E., & Parks, B. O. (1989). Decay rates of human remains in an arid environment. *Journal of Forensic Science*, *34*(3), 607-616.

Ge, T., Chen, C.-Y., Neale, B., Sabuncu, M., & Smoller, J. (2017). Phenome-wide heritability analysis of the UK Biobank. *PLOS Genetics*, *13*(4), 1–21.

Gelderman, H., Boer, L., Naujocks, T., IJzermans, A., & Duijst, W. (2018). The development of a post-mortem interval estimation for human remains found on land in the Netherlands. *International Journal Of Legal Medicine*, *132*(3), 863-873.

Gennard, D. (2012). *Forensic Entomology: An Introduction* (2nd ed.). Chichester: Wiley-Blackwell.

Gerstenblith, M., Shi, J., & Landi, M. (2010). Genome-wide association studies of pigmentation and skin cancer: A review and meta-analysis. *Pigment Cell & Melanoma Research*, *23*(5), 587–606.

Gilchrist, J., & Branche, C. (2016). Lifeguard Effectiveness. In M. Tipton, & A. Wooler (Eds.) *The Science of Beach Lifeguarding*. (pp. 29-36). Boca Raton: CRC Press, Taylor & Francis Group.

Goff, M. (2009). Early post-mortem changes and stages of decomposition in exposed cadavers. *Experimental and Applied Acarology*, *49*(1-2), 21-36.

Golan, R., Gavrieli, I., Lazar, B., & Ganor, J. (2014). The determination of ph in hypersaline lakes with a conventional combination glass electrode. *Limnology and Oceanography: Methods*, *12*(11), 810–815.

Goodwin, W., McFarlane, J., & Roberts, J. (2003). The identification of a US serviceman recovered from the Holy Loch, Scotland. *Science & Justice: Journal of the Forensic Science Society*, *43*(1), 45-47.

Gray, S., Semiat, R., Duke, M., Rahardianto, A., & Cohen, Y. (2011). Seawater use and desalination technology. *Treatise on Water Science*, *4*(1), 73–109.

Grmec, Š., Strnad, M., & Podgoršek, D. (2009). Comparison of the characteristics and outcome among patients suffering from out-of-hospital primary cardiac arrest and drowning victims in cardiac arrest. *International Journal Of Emergency Medicine*, *2*(1), 7-12.

Grossman, S., Johnston, A., Vanezis, P., & Perrett, D. (2011). Can we assess the age of bruises? An attempt to develop an objective technique. *Medicine, Science And The Law*, *51*(3), 170-176.

Gubelit, Y., & Berezina, N. (2010). The causes and consequences of algal blooms: The Cladophora Glomerata Bloom and the Neva Estuary (Eastern Baltic Sea). *Marine Pollution Bulletin*, *61*(4-6), 183–188.

Gutierrez, K., Dicks, N., Glanzner, W., Agellon, L., & Bordignon, V. (2015). Efficacy of the porcine species in biomedical research. *Frontiers in Genetics*, *6*(1), 1–9.

Ha, S., Lee, M., Lee, O., Lee, G., Kim, J., Moon, J., Kim, M., & Oh, C. (2009). A study of a method for distribution analysis of skin color. *Skin Research And Technology*, *15*(2), 200-213.

Hadley, J.A., & Fowler, D.R. (2003). Organ weight effects of drowning and asphyxiation on the lungs, liver, brain, heart, kidneys, and spleen. *Forensic Science International*, *133*(3), 190–196.

Haefner, J., Wallace, J., & Merritt, R. (2004). Pig Decomposition in Lotic Aquatic Systems: The Potential Use of Algal Growth in Establishing a Post-mortem Submersion Interval (PMSI). *Journal of Forensic Sciences*, *49*(2), 330-336.

Haglund, W. (1993). Disappearance of soft tissue and the disarticulation of human remains from aqueous environments. *Journal of Forensic Science*, *38*(4), 806-815.

Haglund, W., & Sorg, M. (1997). *Forensic Taphonomy: The Post-mortem Fate of Human Remains*. Boca Raton: CRC Press.

Haglund, W., & Sorg, M. (2002). Human Remains in Water Environments. In W.D. Haglund, & M.H. Sorg (Eds.) *Advances in Forensic Taphonomy: Method, Theory and Archaelogical Perspectives*. (pp. 201-218). Boca Raton: CRC Press.

Hamilton, K., Demant, D., Peden, A., & Hagger, M. (2020). A systematic review of human behaviour in and around floodwater. *International Journal of Disaster Risk Reduction*, *47*(1), 1–22.

Hamilton, K., White, K., Wihardjo, K., & Hyde, M. (2016). Targets to promote swimming between the flags among Australian beachgoers. *Health Promotion International*, *31*(4), 908-914.

Hamilton, S., & Green, M. (2017). Gross Post-Mortem Changes in the Human Body. In E.M. Schotsmans, N. Márquez-Grant, & S.L. Forbes (Eds.) *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment*. (pp. 11-25). Oxford: John Wiley & Sons Ltd.

Han, J., Kraft, P., Nan, H., Guo, Q., Chen, C., Qureshi, A., Hankinson, S., Hu, F., Duffy, D., Zhao, Z., Martin, N., Montgomery, G., Hayward, N., Thomas, G., Hoover, R., Chanock, S., & Hunter, D. (2008). A genome-wide association study identifies novel alleles associated with hair color and skin pigmentation. *PLoS Genetics*, *4*(5), 1–11.

Hannon, L., & DeFina, R. (2016). Reliability Concerns in Measuring Respondent Skin Tone by Interviewer Observation. *Public Opinion Quarterly*, *80*(2), 534-541.

Hatfield, J., Williamson, A., Sherker, S., Brander, R., & Hayen, A. (2012). Development and evaluation of an intervention to reduce rip current related beach drowning. *Accident Analysis & Prevention*, *46*(1), 45-51.

Hauther, K., Cobaugh, K., Jantz, L., Sparer, T., & DeBruyn, J. (2015). Estimating time since death from postmortem human gut microbial communities. *Journal of forensic sciences*, *60*(5), 1234-1240.

Hayman, J., & Oxenham, M. (2016). *Human body decomposition* (1st ed.). London: Academic Press.

He, J., Guo, J., Fu, X., & Cai, J. (2019). Potential use of high-throughput sequencing of bacterial communities for post-mortem submersion interval estimation. *Brazilian Journal of Microbiology*, *50*(4), 999-1010.

Healy, C., Schultz, J., Parker, K., & Lowers, B. (2015). Detecting Submerged Bodies: Controlled Research Using Side-Scan Sonar to Detect Submerged Proxy Cadavers. *Journal of forensic sciences*, *60*(3), 743-752.

Heaton, V., Lagden, A., Moffatt, C., & Simmons, T. (2010). Predicting the Postmortem Submersion Interval for Human Remains Recovered from U.K. Waterways. *Journal Of Forensic Sciences*, *55*(2), 302-307.

Henssge, C. (1988). Death time estimation in case work. I. The rectal temperature time of death nomogram. *Forensic science international*, *38*(3-4), 209-236.

Henssge, C. (1992). Rectal temperature time of death nomogram: dependence of corrective factors on the body weight under stronger thermic insulation conditions. *Forensic science international*, *54*(1), 51-66.

Henssge, C., & Madea, B. (2004). Estimation of the time since death in the early post-mortem period. *Forensic Science International*, *144*(2-3), 167-175.

Henssge, C., & Madea, B. (2007). Estimation of the time since death. *Forensic Science International*, *165*(2-3), 182-184.

Higgs, N., & Pokines, J. (2013). Marine environmental alterations to bone. In J.T. Pokines, & S. A. Symes (Eds.) *Manual of Forensic Taphonomy*. (pp. 143-180). Boca Raton: CRC Press.

Hills, S., Hobbs, M., Tipton, M., & Barwood, M. (2021). The Water Incident Database (WAID) 2012 to 2019: A systematic evaluation of the documenting of UK drownings. *BMC Public Health*, *21*(1), 1–12.

Hobischak, N., & Anderson, G. (1999). Freshwater-Related Death Investigations in British Columbia in 1995–1996. A Review of Coroners Cases. *Canadian Society of Forensic Science Journal*, *32*(2-3), 97-106.

Hobischak, N., & Anderson, G. (2002). Time of Submergence Using Aquatic Invertebrate Succession and Decompositional Changes. *Journal of Forensic Sciences*, *47*(1), 142-151.

Holmlund, G., Wetterling, G., Formisto, T., & Kauppila, R. (2008). Identification, of fourcrewmembers of the douglas DC-3 79001 hugin by DNA analysis, 52 years postmortem. *Scandinavian Journal of Forensic Science*, *1*(1-36), 5-9.

Hopkins, S., Dettori, J., & Chapman, J. (2018). Parametric and nonparametric tests in spine research: Why do they matter? *Global Spine Journal*, *8*(6), 652–654.

Hughes, V., Ellis, P., & Langlois, N. (2004). The perception of yellow in bruises. *Journal Of Clinical Forensic Medicine*, *11*(5), 257-259.

Human Tissue Authority (2004). *Human Tissue Act 2004*. Human Tissue Authority. Retrieved May 8, 2023, from https://www.hta.gov.uk/guidance-professionals/hta-legislation/human-tissue-act-2004

Humphreys, M., Panacek, E., Green, W., & Albers, E. (2013). Comparison of Protocols for Measuring and Calculating Postmortem Submersion Intervals for Human Analogs in Fresh Water. *Journal Of Forensic Sciences*, *58*(2), 513-517.

Hürlimann, J., Feer, P., Elber, F., Niederberger, K., Dirnhofer, R., & Wyler, D. (2000). Diatom detection in the diagnosis of death by drowning. *International journal of legal medicine*, *114*(1), 6-14.

Hyde, E., Haarmann, D., Petrosino, J., Lynne, A., & Bucheli, S. (2015). Initial insights into bacterial succession during human decomposition. *International Journal of Legal Medicine*, *129*(3), 661–671.

Hyodoh, H., Terashima, R., Rokukawa, M., Shimizu, J., Okazaki, S., Mizuo, K., & Watanabe, S. (2016). Experimental drowning lung images on postmortem CT – difference between sea water and fresh water. *Legal Medicine*, *19*(1), 11–15.

Iancu, L., Carter, D., Junkins, E., & Purcarea, C. (2015). Using bacterial and necrophagous insect dynamics for post-mortem interval estimation during cold season: Novel case study in Romania. *Forensic Science International*, *254*, 106-117.

Iancu, L., Junkins, E. N., Necula-Petrareanu, G., & Purcarea, C. (2018). Characterizing forensically important insect and microbial community colonization patterns in buried remains. *Scientific Reports*, *8*(1), 1–16.

Iqbal, M., Nizio, K., Ueland, M., & Forbes, S. (2017). Forensic decomposition odour profiling: A review of experimental designs and analytical techniques. *Trends in Analytical Chemistry*, *91*(3), 112-124.

Irish, L., Rennie, S. R., Parkes, G. M. B., & Williams, A. (2019). Identification of decomposition volatile organic compounds from surface-deposited and submerged porcine remains. *Science & Justice*, *59*(5), 503-515.

Jabbar-Lopez, Z., Ung, C., Alexander, H., Gurung, N., Chalmers, J., Danby, S., Cork, M., Peacock, J., & Flohr, C. (2020). The effect of water hardness on atopic eczema, skin barrier function: A systematic review, meta-analysis. *Clinical & Experimental Allergy*, *51*(3), 430–451.

Jacobs, L., Hamer, M., Gunn, D., Deelen, J., Lall, J., van Heemst, D., Uh, H.-W., Hofman, A., Uitterlinden, A., Griffiths, C., Beekman, M., Slagboom, P., Kayser, M., Liu, F., & Nijsten, T. (2015). A genome-wide association study identifies the skin color genes IRF4, MC1R, ASIP, and BNC2 influencing facial pigmented spots. *Journal of Investigative Dermatology*, *135*(7), 1735–1742.

Javan, G., Finley, S., Can, I., Wilkinson, J., Hanson, J., & Tarone, A. (2016). Human thanatomicrobiome succession and time since death. *Scientific Reports*, *6*(1), 1–9.

Jean, A. (2001). Brain Stem Control of Swallowing: Neuronal Network and Cellular Mechanisms. *Physiological Reviews*, *81*(2), 929-969.

Jiang, S., Wu, X., Du, S., Wang, Q., & Han, D. (2022). Are UK rivers getting saltier and more alkaline? *Water*, *14*(18), 1–16. https://doi.org/10.3390/w14182813.

Johnson, H., Trinidad, D., Guzman, S., Khan, Z., Parziale, J., DeBruyn, J., & Lents, N. (2016). A machine learning approach for using the postmortem skin microbiome to estimate the postmortem interval. *PLOS ONE*, *11*(12), 1–23.

Jonkers, A., & Sharkey, K. (2016). The differential warming response of Britain's rivers (1982–2011). *PLOS ONE*, *11*(11), 1–23. https://doi.org/10.1371/journal.pone.0166247.

Joodaki, H., & Panzer, M. (2018). Skin mechanical properties and modeling: A review. *Proceedings Of The Institution Of Mechanical Engineers, Part H: Journal Of Engineering In Medicine*, *232*(4), 323-343.

Jothishankar, B., & Stein, S. (2019). Impact of skin color and ethnicity. *Clinics In Dermatology*, *37*(5), 418-429.

Kahana, T., Almog, J., Levy, J., Shmeltzer, E., Spier, Y., & Hiss, J. (1999). Marine Taphonomy: Adipocere Formation in a Series of Bodies Recovered from a Single Shipwreck. *Journal of Forensic Sciences*, *44*(5), 897-901.

Kakizaki, E., & Yukawa, N. (2015). Simple protocol for extracting diatoms from lung tissues of suspected drowning cases within 3 h: First practical application. *Forensic Science International*, *251*(1), 179–185.

Kakizaki, E., Sonoda, A., Shinkawa, N., & Yukawa, N. (2019). A new enzymatic method for extracting diatoms from organs of suspected drowning cases using papain: Optimal digestion and first practical application. *Forensic Science International*, *297*(1), 204–216.

Karhunen, P., Goebeler, S., Winberg, O., & Tuominen, M. (2008). Time of death of victims found in cold water environment. *Forensic science international*, *176*(2-3), e17-e22.

Karnieli-Miller, O., Vu, T., Frankel, R., Holtman, M., Clyman, S., Hui, S., & Inui, T. (2011). Which Experiences in the Hidden Curriculum Teach Students About Professionalism? *Academic Medicine*, *86*(3), 369-377.

Kaszubinski, S., Receveur, J., Wydra, B., Smiles, K., Wallace, J., Babcock, N., Weatherbee, C., & Benbow, M. (2020). Cold Case Experiment Demonstrates the Potential Utility of Aquatic Microbial Community Assembly in Estimating a Postmortem Submersion Interval. *Journal Of Forensic Sciences*, *65*(4), 1210-1220.

Kawasumi, Y., Kawabata, T., Sugai, Y., Usui, A., Hosokai, Y., Sato, M., Saito, H., Ishibashi, T., Hayashizaki, Y., & Funayama, M. (2013). Diagnosis of drowning using post-mortem computed tomography based on the volume and density of fluid accumulation in the maxillary and sphenoid sinuses. *European Journal of Radiology*, *82*(10), e562–e566.

Kawasumi, Y., Kawabata, T., Sugai, Y., Usui, A., Hosokai, Y., Sato, M., Saito, H., Ishibashi, T., Hayashizaki, Y., & Funayama, M. (2012). Assessment of the relationship between drowning and fluid accumulation in the paranasal sinuses on post-mortem computed tomography. *European Journal of Radiology*, *81*(12), 3953–3955.

Kayser, M. (2015). Forensic DNA Phenotyping: Predicting human appearance from crime scene material for investigative purposes. *Forensic Science International: Genetics*, *18*(1), 33-48.

Kayser, M., & de Knijff, P. (2011). Improving human forensics through advances in genetics, genomics and molecular biology. *Nature Reviews Genetics*, *12*(3), 179-192.

Keiper, J., Chapman, E., & Foote, B. (1997). Midge larvae (Diptera: Chironomidae) as indicators of post-mortem submersion interval of carcasses in a woodland stream: a preliminary report. *Journal of Forensic Science*, *42*(6), 1074-1079.

Kelly, J., van der Linde, T., & Anderson, G. (2009). The influence of clothing and wrapping on carcass decomposition and arthropod succession during the warmer seasons in central South Africa. *Journal of Forensic Sciences*, *54*(5), 1105–1112.

Keyes, C., Hill, L., & Gordon, G. (2016). An Appraisal of Decomposition Cases Received at the Johannesburg Forensic Pathology Service Medico-legal Mortuary During 2010-2011. *Journal Of Forensic Sciences*, *61*(2), 452-457.

Koon, W., Peden, A., Lawes, J., & Brander, R. (2021). Coastal drowning: A scoping review of burden, risk factors, and prevention strategies. *PLOS ONE*, *16*(2), 1–22.

Krstic, S., Duma, A., Janevska, B., Levkov, Z., Nikolova, K., & Noveska, M. (2002). Diatoms in forensic expertise of drowning—a Macedonian experience. *Forensic science international*, *127*(3), 198-203.

Lang, I. (2009). Brain Stem Control of the Phases of Swallowing. *Dysphagia*, *24*(3), 333-348.

Lang, J., Erb, R., Pechal, J., Wallace, J., McEwan, R., & Benbow, M. (2016). Microbial Biofilm Community Variation in Flowing Habitats: Potential Utility as Bioindicators of Post-mortem Submersion Intervals. *Microorganisms*, *4*(1), 1.

Laurent, A., Mistretta, F., Bottigioli, D., Dahel, K., Goujon, C., Nicolas, J., Hennino, A., & Laurent, P. (2007). Echographic measurement of skin thickness in adults by high frequency ultrasound to assess the appropriate microneedle length for intradermal delivery of vaccines. *Vaccine*, *25*(34), 6423-6430.

Law, M., Bishop, D., Lee, J., Brossard, M., Martin, N., Moses, E., Song, F., Barrett, J., Kumar, R., Easton, D., Pharoah, P., Swerdlow, A., Kypreou, K., Taylor, J., Harland, M., Randerson-Moor, J., Akslen, L., Andresen, P., Avril, M., Azizi, E., Scarra, G., Brown, K., ... Iles, M. (2015). Genome-wide meta-analysis identifies five new susceptibility loci for cutaneous malignant melanoma. *Nature genetics*, *47*(9), 987-995.

Layon, A., Modell, J., Warner, D., & Warner, M. (2009). Drowning: update 2009. *The Journal of the American Society of Anesthesiologists*, *110*(6), 1390-1401.

Leavy, J., Crawford, G., Leaversuch, F., Nimmo, L., McCausland, K., & Jancey, J. (2016). A review of drowning prevention interventions for children and young people in high, low and middle income countries. *Journal of Community Health*, *41*(2), 424–441.

Lei, G., Liu, F., Liu, P., Zhou, Y., Jiao, T., & Dang, Y.-H. (2019). A bibliometric analysis of forensic entomology trends and perspectives worldwide over the last two decades (1998–2017). *Forensic Science International*, *295*(1), 72–82.

Lima, S., & Reis, S. (2018). Nanotechnological approaches in drug absorption through skin topical delivery. In *Nanoparticles in Life Sciences and Biomedicine* (pp. 171-194). New York: Jenny Stanford Publishing.

Lin, C., Wang, Y., Lu, T., & Kawach, I. (2015). Unintentional drowning mortality, by age and body of water: An analysis of 60 countries. *Injury Prevention*, *21*(e1), 43–50.

Lindholm, C., Sterner, E., Romanelli, M., Pina, E., Torray Bou, J., Hietanen, H., Iivanainen, A., Gunningberg, L., Hommel, A., Klang, B., & Dealey, C. (2008). Hip fracture and pressure ulcers – the Pan-European Pressure Ulcer Study – intrinsic and extrinsic risk factors. *International Wound Journal*, *5*(2), 315-328.

Liu, F., Visser, M., Duffy, D., Hysi, P., Jacobs, L., Lao, O., Zhong, K., Walsh, S., Chaitanya, L., Wollstein, A., Zhu, G., Montgomery, G., Henders, A., Mangino, M., Glass, D., Bataille, V., Sturm, R., Rivadeneira, F., Hofman, A., … Kayser, M. (2015). Genetics of skin color variation in Europeans: Genome-wide association studies with functional follow-up. *Human Genetics*, *134*(8), 823–835.

Louie, P., & Wilkes, R. (2018). Representations of race and skin tone in medical textbook imagery. *Social Science & Medicine*, *202*(1), 38-42.

Lu, T.-H., Lunetta, P., & Walker, S. (2010). Quality of cause-of-death reporting using ICD-10 drowning codes: A descriptive study of 69 countries. *BMC Medical Research Methodology*, *10*(1), 1–6.

Lucas, J., Goldfeder, L., & Gill, J. (2002). Bodies Found in the Waterways of New York City. *Journal of Forensic Sciences*, *47*(1), 15214J.

Ludes, B., Coste, M., North, N., Doray, S., Tracqui, A., & Kintz, P. (1999). Diatom analysis in victim's tissues as an indicator of the site of drowning. *International Journal of Legal Medicine*, *112*(3), 163–166.

Ludes, B., Siegel, J., Saukko, P., & Houck, M. (2013). In *Encyclopedia of Forensic Sciences* (2nd ed., pp. 33–38). Waltham: Academic Press.

Lunetta, P. (2016). Autopsy Findings: Drowning and Submersion Deaths. In: J Payne-James, & R.W. Byard (Eds) *Encyclopedia of Forensic and Legal Medicine*. (2nd ed., pp. 315–322). Oxford: Academic Press.

Lunetta, P., Miettinen, A., Spilling, K., & Sajantila, A. (2013). False-positive diatom test: a real challenge? A post-mortem study using standardized protocols. *Legal Medicine*, *15*(5), 229-234.

Ly, B., Dyer, E., Feig, J., Chien, A., & Del Bino, S. (2020). Research Techniques Made Simple: Cutaneous Colorimetry: A Reliable Technique for Objective Skin Color Measurement. *Journal Of Investigative Dermatology*, *140*(1), 3-12.

Lyman, R. (2010). What Taphonomy Is, What it Isn't, and Why Taphonomists Should Care about the Difference. *Journal of Taphonomy*, *8*(1), 1-16.

Macfarlane, D., Nelson, F., Hudson, M. B., & Murphy, H. (2020). Using measures of water quality to narrow down the postmortem submersion interval (PMSI) in water. *Environmental Earth Sciences*, *79*(1), 1-9.

Madea, B. (2015). *Estimation of the time since death* (3rd ed., pp. 19-196). CRC Press.

Madea, B., & Doberentz, E. (2010). Commentary on: Heaton V, Lagden A, Moffatt C, Simmons T. predicting the postmortem submersion interval for human remains recovered from U.K. waterways. J forensic sci 2010;55(2):302-7. *Journal of Forensic Sciences*, *55*(6), 1666–1667.

Madea, B., & Doberentz, E. (2017). Body farms. *Forensic Science, Medicine and Pathology*, *13*(4), 480–481.

Madea, B., Dettmeyer, R., & Schmidt, P. (2007). Thanatologie. In *Praxis Rechtsmedizin* (pp. 7-82)., Berlin, Heidelberg: Springer.

Madea, B., Henssge, C., Reibe, S., Tsokos, M., & Kernbach-Wighton, G. (2014). Post-mortem changes and time since death. In B. Madea (Ed.) *Handbook of Forensic Medicine*. (pp. 75-133). Hoboken, NJ: Wiley-Blackwell.

Magni, P., Venn, C., Aquila, I., Pepe, F., Ricci, P., & Di Nunzio, C. *et al*. (2015). Evaluation of the floating time of a corpse found in a marine environment using the barnacle Lepas anatifera L. (Crustacea: Cirripedia: Pedunculata). *Forensic Science International*, *247*(1), e6-e10.

Mahony, A., Barnsley, P., Peden, A., & Scarr, J. (2017). A thirteen year national study of non-fatal drowning in Australia: data challenges, hidden impacts and social costs. *Sydney: Royal Life Saving Society Australia*.

Mañas-Jordá, S., León-Cortés, J., García-García, M., Caballero, U., & Infante, F. (2018). Dipteran diversity and ecological succession on dead pigs in contrasting mountain habitats of Chiapas, Mexico. *Journal of Medical Entomology*, *55*(1), 59–68.

Mangione-Smith, R., Elliott, M., Stivers, T., McDonald, L., Heritage, J., & McGlynn, E. (2004). Racial/Ethnic Variation in Parent Expectations for Antibiotics: Implications for Public Health Campaigns. *PEDIATRICS*, *113*(5), e385-e394.

Mao, S., Fu, G., Seese, R., & Wang, Z. (2013). Estimation of PMI depends on the changes in ATP and its degradation products. *Legal Medicine*, *15*(5), 235-238.

Marín-Arroyo, A. (2015). Taphonomic study of the human remains from the Magdalenian burial in El Mirón Cave (Cantabria, Spain). *Journal of Archaeological Science*, *60*(1), 57–65.

Maroñas, O., Phillips, C., Söchtig, J., Gomez-Tato, A., Cruz, R., Alvarez-Dios, J., de Cal, M., Ruiz, Y., Fondevila, M., Carracedo, Á., & Lareu, M. (2014). Development of a forensic skin colour predictive test. *Forensic Science International: Genetics*, *13*(1), 34-44.

Martín-Vega, D., Cifrián, B., Díaz-Aranda, L., & Baz, A. (2015). Necrophilous histerid beetle communities (Coleoptera: Histeridae) in central Spain: Species composition and habitat preferences. *Environmental Entomology*, *44*(4), 966–974.

Martlin, B., Anderson, G., & Bell, L. (2022). A review of human decomposition in Marine Environments. *Canadian Society of Forensic Science Journal*, *1*(1), 1–30.

Mashaly, A., Mahmoud, A., & Ebaid, H. (2019). Influence of clothing on decomposition and presence of insects on rabbit carcasses. *Journal of medical entomology*, *56*(4), 921-926.

Massey, D., & Martin, J. (2003). The NIS skin color scale. *Office of Population Research, Princeton University*.

Massoud, M. (2005). *Engineering Thermofluids: Thermodynamics, Fluid Mechanics, and Heat Transfer* (p. 568). Berlin: Springer.

Mateus, M., & Vieira, V. (2014). Study on the postmortem submersion interval and accumulated degree days for a multiple drowning accident. *Forensic Science International*, *238*(1), e15-e19.

Mateus, M., de Pablo, H., & Vaz, N. (2013). An investigation on body displacement after two drowning accidents. *Forensic Science International*, *229*(1-3), e6–e12.

Matsuo, K., & Palmer, J. (2008). Anatomy and Physiology of Feeding and Swallowing: Normal and Abnormal. *Physical Medicine And Rehabilitation Clinics Of North America*, *19*(4), 691-707.

Matuszewski, S. (2021). Post-mortem interval estimation based on insect evidence: Current challenges. *Insects*, *12*(4), 1–21.

Matuszewski, S., Bajerlein, D., Konwerski, S., & Szpila, K. (2010). Insect succession and carrion decomposition in selected forests of Central Europe. Part 1: Pattern and rate of decomposition. *Forensic Science International*, *194*(1-3), 85-93.

Matuszewski, S., Frątczak, K., Konwerski, S., Bajerlein, D., Szpila, K., Jarmusz, M., Szafałowicz, M., Grzywacz, A., & Mądra, A. (2016). Effect of body mass and clothing on Carrion Entomofauna. *International Journal of Legal Medicine*, *130*(1), 221–232.

Matuszewski, S., Hall, M., Moreau, G., Schoenly, K., Tarone, A., & Villet, M. (2020). Pigs vs people: the use of pigs as analogues for humans in forensic entomology and taphonomy research. *International Journal of Legal Medicine*, *134*(2), 793-810.

Matuszewski, S., Konwerski, S., Frątczak, K., & Szafałowicz, M. (2014). Effect of body mass and clothing on decomposition of pig carcasses. *International Journal of Legal Medicine*, *128*(6), 1039-1048.

Mayer, R. (2012). *Embalming: History, Theory, and Practice* (5th ed.). New York: McGraw-Hill Education.

McGenity, T., & Oren, A. (2012). Hypersaline Environments. In E. Bell (Ed.), *Life at Extremes: Environments, Organisms and Strategies for Survival* (pp. 402–437). Wallingford: CABI.

McGowan, W., & Harrison, J. (2000). Water processing: residential, commercial, light-industrial. *Lisle, IL. Water Quality Association*.

McKemy, D. (2005). How Cold is It? TRPM8 and TRPA1 in the Molecular Logic of Cold Sensation. *Molecular Pain*, *1*(1), 1744-8069.

Megyesi, M., Nawrocki, S., & Haskell, N. (2005). Using Accumulated Degree-Days to Estimate the Post-mortem Interval from Decomposed Human Remains. *Journal of Forensic Sciences*, *50*(3), 1-9.

Merritt, R. W., & Wallace, J. R. (2010). The Role of Aquatic Insects in Forensic Investigations. In J.H. Byrd, & J.L. Castner (Eds.) *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. (2nd ed., pp. 272-313). Boca Raton: CRC Press.

Metcalf, J. (2019). Estimating the postmortem interval using microbes: Knowledge gaps and a path to technology adoption. *Forensic Science International: Genetics*, *38*(1), 211–218.

Miller, A. (2008). The neurobiology of swallowing and dysphagia. *Developmental Disabilities Research Reviews*, *14*(2), 77-86.

Miller, L., Alele, F., Emeto, T., & Franklin, R. (2019). Epidemiology, risk factors and measures for preventing drowning in Africa: A systematic review. *Medicina*, *55*(10), 1–25.

Ming, M., Meng, X., & Wang, E. (2007). Evaluation of four digestive methods for extracting diatoms. *Forensic Science International*, *170*(1), 29–34.

Moffatt, C., Simmons, T., & Lynch-Aird, J. (2016). An Improved Equation for TBS and ADD: Establishing a Reliable Post-mortem Interval Framework for Casework and Experimental Studies. *Journal of Forensic Sciences*, *61*(1), S201-S207.

Moniz, T., Costa Lima, S., & Reis, S. (2020). Human skin models: From healthy to disease-mimetic systems; characteristics and applications. *British Journal Of Pharmacology*, *177*(19), 4314–4329.

Moraitis, K., & Spiliopoulou, C. (2010). Forensic implications of carnivore scavenging on human remains recovered from outdoor locations in Greece. *Journal of Forensic and Legal Medicine*, *17*(6), 298–303.

Moran, K. (2016). Beach Safety Education. In M. Tipton, & A. Wooler (Eds.) *The Science of Beach Lifeguarding*. (pp. 245-252). Boca Raton: CRC Press, Taylor & Francis Group.

Moran, K., Quan, L., Franklin, R., & Bennett, E. (2011). Where the evidence and expert opinion meet: A review of open-water recreational safety messages. *International Journal of Aquatic Research and Education*, *5*(3), 251–270.

Moran, S. (2018). *An applied guide to water and effluent treatment plant design* (pp. 61-67). Elsevier.

Moreland, B., Ortmann, N., & Clemens, T. (2022). Increased unintentional drowning deaths in 2020 by age, race/ethnicity, sex, and location, United States. *Journal of Safety Research*, *82*(1), 463–468.

Morgan, D., Ozanne-Smith, J., & Triggs, T. (2008). Descriptive epidemiology of drowning deaths in a surf beach swimmer and surfer population. *Injury Prevention*, *14*(1), 62–65.

Morgan, O., Sribanditmongkol, P., Perera, C., Sulasmi, Y., Van Alphen, D., & Sondorp, E. (2006). Mass Fatality Management following the South Asian Tsunami Disaster: Case Studies in Thailand, Indonesia, and Sri Lanka. *Plos Medicine*, *3*(6), 809-815.

Moses, R. (2012). Experimental Adipocere Formation: Implications for Adipocere Formation on buried bone*,†. *Journal of Forensic Sciences*, *57*(3), 589–595.

Mott, T., & Latimer, K. (2016). Prevention and treatment of drowning. *American Family Physician*, *93*(7), 576–582.

Myburgh, J., L'Abbé, E. N., Steyn, M., & Becker, P. J. (2013). Estimating the postmortem interval (PMI) using accumulated degree-days (ADD) in a temperate region of South Africa. *Forensic Science International*, *229*(1-3), e1–e6.

Naik, P., & Farrukh, S. (2022). Influence of Ethnicities and Skin Color Variations in Different Populations: A Review. *Skin Pharmacology And Physiology*, *35*(2), 65-76.

Nan, H., Kraft, P., Qureshi, A., Guo, Q., Chen, C., Hankinson, S., Hu, F., Thomas, G., Hoover, R., Chanock, S., Hunter, D., & Han, J. (2009). Genome-wide association study of tanning phenotype in a population of European ancestry. *Journal of Investigative Dermatology*, *129*(9), 2250–2257.

National Geographic Society. (2022). *Current*. National Geographic. Retrieved 20 June 2022, from https://education.nationalgeographic.org/resource/current.

National Oceanic and Atmospheric Administration. (2021). *What is a current?*. Ocean Service NOAA. Retrieved 20 June 2022, from https://oceanservice.noaa.gov/facts/current.html.

National Water Safety Forum (2015). *A future without drowning: The UK drowning prevention strategy 2016-2026*. nationalwatersafety.org.uk. Retrieved November 23, 2022, from https://www.nationalwatersafety.org.uk/media/1005/uk-drowning-prevention-strategy.pdf

National Water Safety Forum. (2019, October). *National Water Safety Forum Coordinating Group terms of reference*. https://www.nationalwatersafety.org.uk/. Retrieved November 21, 2022, from https://nationalwatersafety.org.uk/media/1322/nwsf-terms-of-reference-march22.pdf

Nawrocka, M., Frątczak, K., & Matuszewski, S. (2016). Inter-rater reliability of total body score-a scale for quantification of corpse decomposition. *Journal of Forensic Sciences*, *61*(3), 798–802.

Ng, K., & Lau, W. (2015). Skin Deep: The Basics of Human Skin Structure and Drug Penetration. In N. Dragicevic & H.I. Maibach (Eds.), *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement*: *Drug Manipulation Strategies and Vehicle Effects* (pp. 3-11). Berlin, Heidelberg: Springer.

Nishiyama, C., Iwami, T., Nichol, G., Kitamura, T., Hiraide, A., Nishiuchi, T., Hayashi, Y., Nonogi, H., & Kawamura, T. (2011). Association of out-of-hospital cardiac arrest with prior activity and ambient temperature. *Resuscitation*, *82*(8), 1008–1012.

Noor, N., Nubli, A., Mohemad, R., & Bakar, Z. (2018). Hybrid algorithm in estimating underwater postmortem interval: Idepis. *Journal of Forensic Sciences & Criminal Investigation*, *9*(4), 1–8.

Noor, P. (2020). Can we trust ai not to further embed racial bias and Prejudice? *BMJ*, *368*(1), 1–3.

Notter, S., & Stuart, B. (2011). The Effect of Body Coverings on the Formation of Adipocere in an Aqueous Environment. *Journal of Forensic Sciences*, *57*(1), 120-125.

Nushida, H., Adachi, J., Takeuchi, A., Asano, M., & Ueno, Y. (2008). Adipocere formation via hydrogenation of linoleic acid in a victim kept under dry concealment. *Forensic Science International*, *175*(2-3), 160-165.

O'Brien, T. (1997). Movement of bodies in Lake Ontario. In W.D. Haglund, & M.H. Sorg (Eds.) *Forensic Taphonomy: The Post-mortem Fate of Human Remains*. (pp. 559-565). Boca Raton: CRC Press.

O'Brien, T., & Kuehner, A. (2007). Waxing Grave About Adipocere: Soft Tissue Change in an Aquatic Context. *Journal of Forensic Sciences*, *52*(2), 294-301.

Osterkamp, T. (2011). K9 Water Searches: Scent and Scent Transport Considerations. *Journal of Forensic Sciences*, *56*(4), 907-912.

Owings, C., Spiegelman, C., Tarone, A., & Tomberlin, J. (2014). Developmental variation among Cochliomyia Macellaria Fabricius (Diptera: Calliphoridae) populations from three ecoregions of Texas, USA. *International Journal of Legal Medicine*, *128*(4), 709–717.

Paczkowski, S., & Schütz, S. (2011). Post-mortem volatiles of vertebrate tissue. *Applied Microbiology and Biotechnology*, *91*(4), 917-935.

Pakosh, C., & Rogers, T. (2009). Soft Tissue Decomposition of Submerged, Dismembered Pig Limbs Enclosed in Plastic Bags. *Journal of Forensic Sciences*, *54*(6), 1223-1228.

Palazzo, C., Pelletti, G., Fais, P., Boscolo-Berto, R., Fersini, F., Gaudio, R., & Pelotti, S. (2020). Post-mortem submersion interval in human bodies recovered from fresh water in an area of Mediterranean climate. Application and comparison of preexisting models. *Forensic Science International*, *306*(1), 110051.

Palazzo, C., Pelletti, G., Fais, P., Giorgetti, A., Boscolo-Berto, R., Gaudio, R., Pirani, F., Tagliabracci, A., & Pelotti, S. (2021). Application of aquatic decomposition scores for the determination of the post mortem submersion interval on human bodies recovered from the northern Adriatic Sea. *Forensic Science International*, *318*(1), 1–8.

Palmer, C. (2020). Estimating the Impact of Laminar Flow on the Pattern and Rate of Decomposition in Aquatic Environments—Is There a Better Way of Modeling Decomposition? *Journal Of Forensic Sciences*, *65*(5), 1601-1609.

Palmiere, C., Tettamanti, C., Scarpelli, M. P., & Tse, R. (2019). The forensic spleen: Morphological, radiological, and toxicological investigations. *Forensic Science International*, *297*(1), 384–387.

Papadimitriou, S., Loucaides, S., Rérolle, V., Achterberg, E., Dickson, A., Mowlem, M., & Kennedy, H. (2016). The measurement of ph in Saline and hypersaline media at sub-zero temperatures: Characterization of tris buffers. *Marine Chemistry*, *184*(1), 11–20.

Papadodima, S., Athanaselis, S., Skliros, E., & Spiliopoulou, C. (2010). Forensic investigation of submersion deaths. *International Journal of Clinical Practice*, *64*(1), 75-83.

Parker, R., Ruffell, A., Hughes, D., & Pringle, J. (2010). Geophysics and the search of Freshwater Bodies: A Review. *Science & Justice*, *50*(3), 141–149.

Parks, C. (2011). A study of the human decomposition sequence in central Texas*. *Journal of Forensic Sciences*, *56*(1), 19–22.

Parmenter, R., & MacMahon, J. (2009). Carrion decomposition and nutrient cycling in a semiarid shrub–steppe ecosystem. *Ecological Monographs*, *79*(4), 637–661.

Parry, N., Mansell, M., & Weldon, C. (2016). Seasonal, locality, and habitat variation in assemblages of carrion-associated Diptera in Gauteng Province, South Africa. *Journal of Medical Entomology*, *53*(6), 1322–1329.

Pearn, J., Nixon, J. W., Franklin, R. C., & Wallis, B. (2008). Safety legislation, public health policy and drowning prevention. *International Journal of Injury Control and Safety Promotion*, *15*(2), 122-123.

Pechal, J., Schmidt, C., Jordan, H., & Benbow, M. (2018). A large-scale survey of the postmortem human microbiome, and its potential to provide insight into the living health condition. *Scientific Reports*, *8*(1), 1–15.

Peden, A. E., Franklin, R. C., & Leggat, P. A. (2020). Developing drowning prevention strategies for rivers through the use of a modified Delphi process. *Injury prevention*, *26*(3), 240-247.

Peden, A., Demant, D., Hagger, M., & Hamilton, K. (2018). Personal, social, and environmental factors associated with lifejacket wear in adults and children: A systematic literature review. *PLOS ONE*, *13*(5), 1–15.

Peden, A., Franklin, R., & Clemens, T. (2019). Exploring the burden of fatal drowning and data characteristics in three high income countries: Australia, Canada and New Zealand. *BMC Public Health*, *19*(1), 1-12.

Peden, A., Franklin, R., & Leggat, P. (2016). The hidden tragedy of rivers: A decade of unintentional fatal drowning in Australia. *PLOS ONE*, *11*(8), 1–15.

Peden, A., Franklin, R., & Leggat, P. (2017). Alcohol and its contributory role in fatal drowning in Australian rivers, 2002–2012. *Accident Analysis & Prevention*, *98*(1), 259-265.

Pereira, D., Afonso, A., & Medeiros, F. (2015). Overview of Friedman's test and Post Hoc analysis. *Communications in Statistics - Simulation and Computation*, *44*(10), 2636–2653.

Pérez-Marcos, M., Arnaldos, M., López-Gallego, E., Luna, A., Khedre, A., & García, M. (2018). An approach for identifying the influence of carcass type and environmental features on sarcosaprophagous Diptera Communities. *Annales De La Société Entomologique De France (N.S.)*, *54*(4), 367–380.

Perrault, K., Nizio, K., & Forbes, S. (2015). A Comparison of One-Dimensional and Comprehensive Two-Dimensional Gas Chromatography for Decomposition Odour Profiling Using Inter-Year Replicate Field Trials. *Chromatographia*, *78*(15-16), 1057-1070.

Pfeiffer, S., Milne, S., & Stevenson, R. (1998). The Natural Decomposition of Adipocere. *Journal of Forensic Sciences*, *43*(2), 16147J.

Pichon, L., Landrine, H., Corral, I., Hao, Y., Mayer, J., & Hoerster, K. (2010). Measuring skin cancer risk in African Americans: is the Fitzpatrick Skin Type Classification Scale culturally sensitive. *Ethn Dis*, *20*(2), 174-179.

Pinheiro, J. (2006). Decay Process of a Cadaver. In A. Schmitt, E. Cunha, & J. Pinheiro (Eds.), *Forensic Anthropology and Medicine: Complementary Sciences From Recovery to Cause of Death* (pp. 85–116). Totowa, NJ: Humana Press Inc.

Plets, R., Dix, J., Adams, J., Bull, J., Henstock, T., Gutowski, M., & Best, A. (2009). The use of a high-resolution 3D Chirp sub-bottom profiler for the reconstruction of the shallow water archaeological site of the Grace Dieu (1439), River Hamble, UK. *Journal of Archaeological Science*, *36*(2), 408-418.

Prahlow, J., & Byard, R. (2012). *Atlas of forensic pathology: for police, forensic scientists, attorneys, and death investigators*. New York: Springer Publishers.

Prausnitz, M, Elias, P., Franz, T., Schmuth, M., Tsai, J., Menon, G., Holleran, W., & Feingold, K. (2012). Skin Barrier and Transdermal Drug Delivery. In J. Bolognia, J.L. Jorizzo, & J.V. Schaffer (Eds.), *Dermatology* (pp. 2065–2073). Philadelphia: Elsevier Saunders.

Pringle, J., Cassella, J., & Jervis, J. (2010). Preliminary Soilwater conductivity analysis to date clandestine burials of homicide victims. *Forensic Science International*, *198*(1-3), 126–133. https://doi.org/10.1016/j.forsciint.2010.02.005

Rácz, E., Könczöl, F., Tóth, D., Patonai, Z., Porpáczy, Z., Kozma, Z., Poór, V. S., & Sipos, K. (2016). PCR-based identification of drowning: Four case reports. *International Journal of Legal Medicine*, *130*(5), 1303–1307.

Rahmstorf, S. (2003). Thermohaline circulation: The current climate. *Nature*, *421*(6924), 699-699.

Rana, A., & Manhas, S. (2018). Significance of diatoms in diagnosis of drowning deaths: A Review. *Peer Reviewed Journal of Forensic & Genetic Sciences*, *1*(5), 77–81.

Raux, C., Saval, F., Rouge, D., Telmon, N., & Dedouit, F. (2014). Diagnosis of drowning using post-mortem computed tomography – state of the art. *Archives of Forensic Medicine and Criminology*, *2*(1), 59–75.

Rawlings, A. (2006). Ethnic skin types: are there differences in skin structure and function?. *International Journal Of Cosmetic Science*, *28*(2), 79-93.

Rebmann, A., David, E., Sorg, M., Koenig, M. (2000). *Cadaver Dog Handbook: Forensic Training and Tactics for the Recovery of Human Remains*. Boca Raton: CRC Press.

Reh, H. (1967). Anhaltspunkte für die Bestimmung der Wasserzeit. *Deutsche Zeitschrift Für Die Gesamte Gerichtliche Medizin*, *59*(2-3), 235-245.

Reh, H. (1969). Diagnostik des Ertrinkungstodes und Bestimmung der Wasserliegezeit. *Triltsch: Düsseldorf*.

Reh, H., Haarhoff, K., & Vogt, C. (1977). Die Schätzung der Todeszeit bei Wasserleichen. *Zeitschrift Fur Rechtsmedizin*, *79*(4), 261-266.

Reid, C. (2017). *The Global Epidemiology of Tourist Fatalities* (dissertation).

Reijnen, G., Buster, M.C., Vos, P.J.E., & Reijnders, U.J.L. (2017). External foam and the post-mortem period in freshwater drowning; results from a retrospective study in Amsterdam, The Netherlands. *Journal of Forensic and Legal Medicine*, *52*(1), 1–4.

Reijnen, G., Gelderman, H., Oude Grotebevelsborg, B., Reijnders, U., & Duijst, W. (2018). The correlation between the Aquatic Decomposition Score (ADS) and the post-mortem submersion interval measured in Accumulated Degree Days (ADD) in bodies recovered from fresh water. *Forensic Science, Medicine and Pathology*, *14*(3), 301-306.

Reijnen, G., Vos, P., Buster, M., & Reijnders, U. (2019). Can pulmonary foam arise after postmortem submersion in water? an Animal Experimental Pilot Study. *Journal of Forensic and Legal Medicine*, *61*(1), 40–44.

Reisdorf, A.G., Bux, R., Wyler, D., Benecke, M., Klug, C., Maisch, M.W., Fornaro, P., & Wetzel, A. (2012). Float, explode or sink: Postmortem fate of lung-breathing marine vertebrates. *Palaeobiodiversity and Palaeoenvironments*, *92*(1), 67–81.

Ribéreau-Gayon, A., Carter, D., & Regan, S. (2018). New evidence of predation on humans by cookiecutter sharks in Kauai, Hawaii. *International Journal of Legal Medicine*, *132*(5), 1381-1387.

Ribéreau-Gayon, A., Rando, C., Schuliar, Y., Chapenoire, S., Crema, E., Claes, J., Seret, B., Maleret, V., & Morgan, R. (2017). Extensive unusual lesions on a large number of immersed human victims found to be from cookiecutter sharks (Isistius spp.): an examination of the Yemenia plane crash. *International Journal Of Legal Medicine*, *131*(2), 423-432.

Roberts, K., Thom, O., Devine, S., Leggat, P., Peden, A., & Franklin, R. (2021). A scoping review of female drowning: An underexplored issue in five high-income countries. *BMC Public Health*, *21*(1), 1–13.

Roberts, L., & Dabbs, G. (2015). A Taphonomic Study Exploring the Differences in Decomposition Rate and Manner between Frozen and Never Frozen Domestic Pigs (Sus scrofa). *Journal Of Forensic Sciences*, *60*(3), 588-594.

Roberts, L., Spencer, J., & Dabbs, G. (2017). The Effect of Body Mass on Outdoor Adult Human Decomposition. *Journal of Forensic Sciences*, *62*(5), 1145-1150.

Rodriguez, W. (1997). Decomposition of Buried and Submerged Bodies. In W.D. Haglund, & M.H. Sorg (Eds.), *Forensic Taphonomy, the Post-mortem Fate of Human Remains* (pp. 459-467). Boca Raton (FL): CRC Press.

Roger, M., Fullard, N., Costello, L., Bradbury, S., Markiewicz, E., O'Reilly, S., Darling, N., Ritchie, P., Määttä, A., Karakesisoglou, I., Nelson, G., von Zglinicki, T., Dicolandrea, T., Isfort, R., Bascom, C., & Przyborski, S**.** (2019). Bioengineering the microanatomy of human skin. *Journal Of Anatomy*, *234*(4), 438-455.

Rosier, E., Cuypers, E., Dekens, M., Verplaetse, R., Develter, W., Van de Voorde, W., Maes, D., & Tytgat, J. (2014). Development and validation of a new TD-GC/MS method and its applicability in the search for human and animal decomposition products. *Analytical and Bioanalytical Chemistry*, *406*(15), 3611-3619.

Royal Life Saving Society (2018). Drowning Report. *Sydney*: *Royal Life Saving Society Australia*.

Royal Life Saving Society. (2021, March 18). *National Fatal Drowning Database*. Royal Life Saving Society - Australia. Retrieved November 21, 2022, from https://www.royallifesaving.com.au/research-and-policy/drowning-research/national-fatal-drowning-database

Ruffell, A. (2006). Under-water scene investigation using ground penetrating radar (GPR) in the search for a sunken jet ski, Northern Ireland. *Science & Justice*, *46*(4), 221–230.

Ruffell, A., Pringle, J., Cassella, J., Morgan, R., Ferguson, M., Heaton, V., Hope, C., & McKinley, J. (2017). The use of geoscience methods for aquatic forensic searches. *Earth-Science Reviews*, *171*(1), 323–337.

Rusydi, A. (2018). Correlation between conductivity and total dissolved solid in various type of water: A Review. *IOP Conference Series: Earth and Environmental Science*, *118*(1), 1–6.

Rutty, G., Johnson, C., Amoroso, J., Robinson, C., Bradley, C.., & Morgan, B. (2016). Post-mortem computed tomography coaxial cutting needle biopsy to facilitate the detection of bacterioplankton using PCR probes as a diagnostic indicator for drowning. *International Journal of Legal Medicine*, *131*(1), 211–216.

Sachdeva, S. (2009). Fitzpatrick skin typing: Applications in dermatology. *Indian journal of dermatology, venereology and leprology*, *75*(1), 93-96.

Sakuma, A., Saitoh, H., Ishii, N., & Iwase, H. (2015). The effects of racemization rate for age estimation of pink teeth. *Journal of forensic sciences*, *60*(2), 450-452.

Salomez, F., & Vincent, J. (2004). Drowning: a review of epidemiology, pathophysiology, treatment and prevention. *Resuscitation*, *63*(3), 261-268.

Sangild, P., Ney, D., Sigalet, D., Vegge, A., & Burrin, D. (2014). Animal models of gastrointestinal and liver diseases. animal models of infant short bowel syndrome: Translational Relevance and challenges. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *307*(12), 1147–1168.

Saukko, P., & Knight, B. (2015). *Knight's Forensic Pathology* (4th ed.). Boca Raton: CRC Press.

Scafide, K., Sheridan, D., Campbell, J., DeLeon, V., & Hayat, M. (2013). Evaluating change in bruise colorimetry and the effect of subject characteristics over time. *Forensic Science, Medicine, And Pathology*, *9*(3), 367-376.

Scarr, J., Buse, K., Norton, R., Meddings, D., & Jagnoor, J. (2022). Tracing the emergence of drowning prevention on the Global Health and Development Agenda: A policy analysis. *The Lancet Global Health*, *10*(7), 1058–1066.

Schmid, J., Morger, C., Noveanu, M., Binder, R., Anderegg, M., & Saner, H. (2009). Haemodynamic and arrhythmic effects of moderately cold (22°C) water immersion and swimming in patients with stable coronary artery disease and heart failure. *European Journal Of Heart Failure*, *11*(9), 903-909.

Schneider, P., Prainsack, B., & Kayser, M. (2019). The Use of Forensic DNA Phenotyping in Predicting Appearance and Biogeographic Ancestry. *Deutsches Ärzteblatt International*, *116*(51-52), 873-880.

Schotsmans, E.M., Márquez-Grant, N. & Forbes, S.L. (2017). *Taphonomy of Human Remains*: *Forensic Analysis of the Dead and the Depositional Environment*. Oxford: John Wiley & Sons Ltd.

Schuliar, Y., & Perich, P. (2012). French gendarmerie disaster victim identification team crash of the air france flight airbus AF 447 Rio de Janeiro - Paris (1st of june 2009) recovery process in the Atlantic Ocean. Taphonomical and medico-legal considerations, 22nd Congress of the International Academy of the International Academy of Legal Medicine, July; Istanbul.

Schuliar, Y., Chapenoire, S., Miras, A., Contrand, B., & Lagarde, E. (2014). A New Tool for Coding and Interpreting Injuries in Fatal Airplane Crashes: The Crash Injury Pattern Assessment Tool Application to the Air France Flight AF 447 Disaster (Rio de Janeiro–Paris), 1st of June 2009. *Journal of Forensic Sciences*, *59*(5), 1263-1270.

Schultz, J., Healy, C., Parker, K., & Lowers, B. (2013). Detecting submerged objects: The application of side scan sonar to forensic contexts. *Forensic Science International*, *231*(1-3), 306–316.

Scott, K., Morgan, R., Jones, V., & Cameron, N. (2014). The transferability of diatoms to clothing and the methods appropriate for their collection and analysis in Forensic Geoscience. *Forensic Science International*, *241*(1), 127–137.

Sengupta, P. (2013). Potential health impacts of hard water. *International journal of preventive medicine*, *4*(8), 866-875.

Shattock, M., & Tipton, M. (2012). 'Autonomic conflict': a different way to die during cold water immersion?. *The Journal Of Physiology*, *590*(14), 3219-3230.

Shelef, L. A., & Seiter, J. (2005). Indirect and Miscellaneous Antimicrobials. In P . M. Davidson, J . N. Sofos, & B . A. Larry (Eds.), *Antimocrobials in Food* (3rd ed., pp. 573-598). Boca Raton, FL: Taylor and Francis.

Shrestha, R., Kanchan, T., & Krishan, K. (2022). Methods Of Estimation Of Time Since Death. [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK549867/?report.

Simmons, T. (2017). Post-Mortem Interval Estimation: An Overview of Techniques. In E.M. Schotsmans, N. Márquez-Grant, & S.L. Forbes (Eds.) *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment* (pp. 134-142). Oxford: John Wiley & Sons Ltd.

Simmons, T., & Cross, P. (2013). Forensic taphonomy. *Encyclopedia of Forensic Sciences,1*(1), 12-17.

Simmons, T., & Heaton, V. (2013). Postmortem Interval: Submerged Bodies. In A. Jamieson (Ed.) *Wiley Encyclopedia of Forensic Science*. (pp. 1-10). Wiley Online Library.

Simmons, T., Adlam, R., & Moffatt, C. (2010). Debugging decomposition data—comparative taphonomic studies and the influence of insects and carcass size on decomposition rate. *Journal of Forensic Sciences*, *55*(1), 8-13.

Singh, R. (2015). Hybrid Membrane Systems – Applications and Case Studies. In *Membrane Technology and Engineering for Water Purification: Application, Systems Design and Operation.* (2nd ed., pp. 179–281). Oxford: Butterworth-Heinemann.

Sitek, A., Rosset, I., Żądzińska, E., Kasielska-Trojan, A., Neskoromna-Jędrzejczak, A., & Antoszewski, B. (2016). Skin color parameters and Fitzpatrick phototypes in estimating the risk of skin cancer: A case-control study in the Polish population. *Journal Of The American Academy Of Dermatology*, *74*(4), 716-723.

Sjoding, M., Dickson, R., Iwashyna, T., Gay, S., & Valley, T. (2020). Racial bias in pulse oximetry measurement. *New England Journal of Medicine*, *383*(25), 2477-2478.

Smith, D.H., Ehrett, C., Weisensee, K., & Tica, C. (2022). Commentary on: Megyesi MS, Nawrocki SP, Haskell NH. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. J Forensic Sci. 2005; 50 (3): 618–26. doi: 10.1520/JFS2004017; and Moffatt C, Simmons T, Lynch-Aird J. An improved equation for TBS and ADD: Establishing a reliable postmortem interval framework for casework and experimental studies. J Forensic Sci. 2016; 61 (Suppl 1): S201–S207. doi: 10.1111/1556-4029.12931. *Journal of Forensic Sciences*, *68*(1), 355–358.

Sonoda, A., Kakizaki, E., Shinkawa, N., Matsuda, H., & Yukawa, N. (2022). Conventional diatom testing using strong acid: (II) number and types of diatoms detected in closed organs and lungs of 80 autopsy cases using only New Kjeldahl flasks. *Forensic Science International*, *341*(1), 1–17.

Spichenok, O., Budimlija, Z., Mitchell, A., Jenny, A., Kovacevic, L., Marjanovic, D., Caragine, T., Prinz, M., & Wurmbach, E. (2011). Prediction of eye and skin color in diverse populations using seven SNPs. *Forensic Science International: Genetics*, *5*(5), 472–478.

Spickett, C. (2007). Chlorinated lipids and fatty acids: an emerging role in pathology. *Pharmacology & Therapeutics*, *115*(3), 400-409.

Steadman, D., Dautartas, A., Kenyhercz, M., Jantz, L., Mundorff, A., & Vidoli, G. (2018). Differential scavenging among pig, rabbit, and human subjects. *Journal of Forensic Sciences*, *63*(6), 1684–1691.

Steele, C., & Miller, A. (2010). Sensory Input Pathways and Mechanisms in Swallowing: A Review. *Dysphagia*, *25*(4), 323-333.

Stephenson, L., Van den Heuvel, C., & Byard, R. (2019). The persistent problem of drowning - A difficult diagnosis with inconclusive tests. *Journal Of Forensic And Legal Medicine*, *66*(1), 79-85.

Stock, M., Winburn, A., & Burgess, G. (2017). Skeletal Indicators of Shark Feeding on Human Remains: Evidence from Florida Forensic Anthropology Cases. *Journal Of Forensic Sciences*, *62*(6), 1647-1654.

Stuart, B. (2013). Decomposition Chemistry: Overview, Analysis, and Interpretation. In J.A. Siegel, P.J. Saukko, & M.M. Houck (Eds.) *Encyclopedia of Forensic Sciences*. (2nd ed., pp. 11-15). Cambridge: Elsevier Ltd.

Stuart, B., & Ueland, M. (2017). Decomposition in Aquatic Environments. In E.M. Schotsmans, N. Márquez-Grant, & S.L. Forbes (Eds.) *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment* (pp. 235-250). Oxford: John Wiley & Sons Ltd.

Stuart, B., Notter, S., Dent, B., Selvalatchmanan, J., & Fu, S. (2016). The formation of adipocere in model aquatic environments. *International Journal of Legal Medicine, 130*(1), 281-286.

Sugimura, T., Kashiwagi, M., Matsusue, A., Hara, K., Kageura, M., & Kubo, S. (2010). Application of the drowning index to actual drowning cases. *Legal Medicine*, *12*(2), 68–72.

Sulem, P., Gudbjartsson, D., Stacey, S., Helgason, A., Rafnar, T., Magnusson, K., Manolescu, A., Karason, A., Palsson, A., Thorleifsson, G., Jakobsdottir, M., Steinberg, S., Pálsson, S., Jonasson, F., Sigurgeirsson, B., Thorisdottir, K., Ragnarsson, R., Benediktsdottir, K., Aben, K., Kiemeney, L., Olafsson, J., Gulcher, J., Kong, A., Thorsteinsdottir, U., & Stefansson, K. (2007). Genetic determinants of hair, eye and skin pigmentation in Europeans. *Nature Genetics*, *39*(12), 1443–1452.

Sulem, P., Gudbjartsson, D., Stacey, S., Helgason, A., Rafnar, T., Jakobsdottir, M., Steinberg, S., Gudjonsson, S., Palsson, A., Thorleifsson, G., Pálsson, S., Sigurgeirsson, B., Thorisdottir, K., Ragnarsson, R., Benediktsdottir, K., Aben, K., Vermeulen, S., Goldstein, A., Tucker, M., Kiemeney, L., Olafsson, J., Gulcher, J., Kong, A., Thorsteinsdottir, U., & Stefansson, K. (2008). Two newly identified genetic determinants of pigmentation in Europeans. *Nature Genetics*, *40*(7), 835–837.

Suzuki, M., Ikaga, T., & Hori, S. (2017). Relationship between bath-related deaths and low air temperature. *Internal Medicine, 56*(23), 3173-3177.

Suzuki, M., Shimbo, T., Ikaga, T., & Hori, S. (2019). Incidence and characteristics of bath-related accidents. *Internal Medicine, 58*(1), 53–62. -18

Swann, L., Forbes, S., & Lewis, S. (2010). Analytical separations of mammalian decomposition products for forensic science: A review. *Analytica Chimica Acta*, *682*(1-2), 9-22.

Szpilman, D., & Morgan, P. (2021). Management for the Drowning Patient. *Chest, 159*(4), 1473-1483.

Szpilman, D., Bierens, J. J., Handley, A. J., & Orlowski, J. P. (2012). Drowning. *New England Journal of Medicine, 366*(22), 2102-2110.

Taylor, S. (2002). Skin of color: Biology, structure, function, and implications for dermatologic disease. *Journal Of The American Academy Of Dermatology*, *46*(2), S41-S62.

Teather, R. (2013). *Encyclopedia of Underwater Investigations* (2nd ed.). Flagstaff, AZ: Best Publishing Company.

Teo, C., Hing, H., Hamzah, N., & Albakri Amir Hamzah, S. (2021). The effect of different coverings on total body score development of buried carcasses. *Malaysian Journal of Medical Sciences*, *28*(4), 103–112.

The Global Historical Weather and Climate. (n.d.). *Climate zone finder*. Global Historical Weather and Climate Data | Weather and Climate. Retrieved December 28, 2022, from https://tcktcktck.org/

Tinker, J. P., Howes, E. L., Wakelin, S., Menary, M., Kent, E., & Berry, D. (2020). The impacts of climate change on temperature (air and sea), relevant to the coastal and marine environment around the UK. *MCCIP Science Review*, *1*(1), 1-3.

Tipton, M., & Golden, F. (2011). A proposed decision-making guide for the search, rescue and resuscitation of submersion (head under) victims based on expert opinion. *Resuscitation*, *82*(7), 819-824.

Tipton, M., & Golden, F. (2014). The physiology of cooling in cold water. In *Drowning* (pp. 843-848). Berlin, Heidelberg: Springer.

Tipton, M., & Montgomery, H. (2022). The experience of drowning. *Medico-Legal Journal*, *90*(1), 17-26.

Tipton, M., Gibbs, P., Brooks, C., Roiz de Sa, D., & Reilly, T. (2010). ECG during Helicopter Underwater Escape Training. *Aviation, Space, and Environmental Medicine*, *81*(4), 399–404.

Tipton, M., Stubbs, D., & Elliott, D. (1991). Human initial responses to immersion in cold water at three temperatures and after hyperventilation. *Journal Of Applied Physiology*, *70*(1), 317-322.

Tozzo, P., Amico, I., Delicati, A., Toselli, F., & Caenazzo, L. (2022). Post-mortem interval and microbiome analysis through 16S rrna analysis: A systematic review. *Diagnostics*, *12*(11), 1–19.

Troutman, L., Moffatt, C., & Simmons, T. (2014). A preliminary examination of differential decomposition patterns in mass graves. *Journal of Forensic Sciences*, *59*(3), 621–626.

Tse, R., Garland, J., Kesha, K., Morrow, P., Lam, L., Elstub, H., Cala, A., Spark, A., Palmiere, C., & Stables, S. (2018a). The potential diagnostic accuracy of autopsy lung weights, lung-heart ratio, and lung-body ratio in drowning deaths. *American Journal of Forensic Medicine & Pathology*, *39*(3), 223–228.

Tse, R., Garland, J., Kesha, K., Morrow, P., Lam, L., Elstub, H., Cala, A., Spark, A., Palmiere, C., & Stables, S. (2018b). Combining postmortem vitreous sodium and chloride and lung-body ratio in aiding the diagnosing saltwater drowning. *American Journal of Forensic Medicine & Pathology*, *39*(3), 229–235.

Tsokos, M. (2005). Post-mortem changes and artifacts occurring during the early post-mortem interval. In M. Tsokos (Ed.) *Forensic Pathology Reviews, Vol. 3*. (pp. 183-237). Totowa: Humana Press.

Tsokos, M., & Byard, R. (2016). Postmortem Changes: Overview. *Encyclopedia Of Forensic And Legal Medicine*, *4*(1), 10-31.

Tumram, N., Bardale, R., & Dongre, A. (2011). Postmortem analysis of synovial fluid and vitreous humour for determination of death interval: A comparative study. *Forensic Science International*, *204*(1-3), 186–190.

Turner, C.G., Ovodov, N.D., & Pavlova, O.V. (2013). *Animal Teeth and Human Tools: A Taphonomic Odyssey in Ice Age Siberia*. Cambridge: Cambridge University Press.

Tyler, M., Richards, D., Reske-Nielsen, C., Saghafi, O., Morse, E., Carey, R., & Jacquet, G. (2017). The epidemiology of drowning in low- and middle-income countries: A systematic review. *BMC Public Health*, *17*(1), 1–7.

U.S. Geological Survey. (2018, June 11). *Saline water and salinity completed*. Saline Water and Salinity | U.S. Geological Survey. Retrieved November 8, 2022, from https://www.usgs.gov/special-topics/water-science-school/science/saline-water-and-salinity?qt-science_center_objects=0#qt-science_center_objects

Ubelaker, D., & Zarenko, K. (2011). Adipocere: What is known after over two centuries of research. *Forensic Science International*, *208*(1-3), 167-172.

Ueland, M., Breton, H., & Forbes, S. (2013). Bacterial populations associated with early-stage adipocere formation in lacustrine waters. *International Journal Of Legal Medicine*, *128*(2), 379-387.

United Utilities. (2023). *Home*. Water Quality Search Results | United Utilities. https://www.unitedutilities.com/help-and-support/your-water-supply/your-water/water-quality/water-quality-search-results/

USGS. Hardness of Water. Retrieved 2 October 2020, from https://www.usgs.gov/special-topic/water-science-school/science/hardness-water?qt-science_center_objects=0#qt-science_center_objects

Valenzuela, R., Henderson, M., Walsh, M., Garrison, N., Kelch, J., Cohen-Barak, O., Erickson, D., John Meaney, F., Bruce Walsh, J., Cheng, K., Ito, S., Wakamatsu, K., Frudakis, T., Thomas, M.,

& Brilliant, M. (2010). Predicting phenotype from genotype: Normal pigmentation. *Journal of Forensic Sciences*, *55*(2), 315–322.

van Daalen, M., de Kat, D., Oude Grotebevelsborg, B., de Leeuwe, R., Warnaar, J., Oostra, R., & M Duijst-Heesters, W. (2017). An Aquatic Decomposition Scoring Method to Potentially Predict the Post-mortem Submersion Interval of Bodies Recovered from the North Sea. *Journal of Forensic Sciences*, *62*(2), 369-373.

van der Ploeg, G., Goslings, J., Walpoth, B., & Bierens, J. (2010). Accidental hypothermia: Rewarming treatments, complications and outcomes from one university medical centre. *Resuscitation*, *81*(11), 1550-1555.

van Hoyweghen, A.J., Jacobs, W., Op de Beeck, B., & Parizel, P.M. (2014). Can post-mortem CT reliably distinguish between drowning and non-drowning asphyxiation? *International Journal of Legal Medicine*, *129*(1), 159–164.

Vander Plaetsen, S., De Letter, E., Piette, M., Van Parys, G., Casselman, J.W., & Verstraete, K. (2015). Post-mortem evaluation of drowning with whole body CT. *Forensic Science International*, *249*(1), 35–41.

Vass, A. (2011). The elusive universal post-mortem interval formula. *Forensic Science International*, *204*(1-3), 34-40.

Vass, A., Bass, W., Wolt, J., Foss, J., & Ammons, J. (1992). Time since Death Determinations of Human Cadavers Using Soil Solution. *Journal of Forensic Sciences*, *37*(5), 1236-1253.

Vass, A., Smith, R., Thompson, C., Burnett, M., Wolf, D., Synstelien, J., Dulgerian, N., & Eckenrode, B. (2004). Decompositional odor analysis database. *Journal of forensic sciences*, *49*(4), 760–769.

Venus, M., Waterman, J., & McNab, I. (2010). Basic physiology of the skin. *Surgery (Oxford)*, *28*(10), 469–472.

Verheggen, F., Perrault, K., Megido, R., Dubois, L., Francis, F., Haubruge, E., Forbes, S., Focant, J., & Stefanuto, P. (2017). The Odor of Death: An Overview of Current Knowledge on Characterisation and Applications. *Bioscience*, *67*(7), 600-613.

Vij, K. (2014). *Textbook of forensic medicine and toxicology* (6th ed., pp 112-134). New Delhi: Elsevier Ltd.

Villet, M., & Amendt, J. (2011). Advances in Entomological Methods for Death Time Estimation. In E.E. Turk (Ed.) *Forensic Pathology Reviews*. (pp. 213-237). Berlin: Humana Press.

Visconti, A., Duffy, D., Liu, F., Zhu, G., Wu, W., Chen, Y., Hysi, P., Zeng, C., Sanna, M., Iles, M., Kanetsky, P., Demenais, F., Hamer, M., Uitterlinden, A., Ikram, M., Nijsten, T., Martin, N.,

Kayser, M., Spector, T., … Falchi, M. (2018). Genome-wide association study in 176,678 Europeans reveals genetic loci for tanning response to Sun Exposure. *Nature Communications*, *9*(1), 1–7.

Vos, P., De Kleine, M., & Rutten, G. (2012). Efficient Stepped Approach to Site Investigation for Underwater Archaeological Studies. *The Leading Edge*, *31*(8), 940-944.

Voss, S.C., Cook, D.F., & Dadour, I.R. (2011). Decomposition and insect succession of clothed and unclothed carcasses in Western Australia. *Forensic Science International*, *211*(1-3), 67-75.

Wallace, J., Merritt, R., Kimbirauskas, R., Benbow, M., & McIntosh, M. (2008). Caddisflies Assist with Homicide Case: Determining a Post-mortem Submersion Interval Using Aquatic Insects. *Journal of Forensic Sciences*, *53*(1), 219-221.

Walsh, S., Chaitanya, L., Breslin, K., Muralidharan, C., Bronikowska, A., Pospiech, E., Koller, J., Kovatsi, L., Wollstein, A., Branicki, W., Liu, F., & Kayser, M. (2017). Global skin colour prediction from DNA. *Human Genetics*, *136*(7), 847-863.

Wang, L., Wang, X., Jin, X., Xu, J., Zhang, H., Yu, J., Sun, Q., Gao, C., & Wang, L. (2017). Analysis of algae growth mechanism and water bloom prediction under the effect of multi-affecting factor. *Saudi Journal of Biological Sciences*, *24*(3), 556–562.

Wang, Y., Wang, M., Xu, W., Wang, Y., Zhang, Y., & Wang, J. (2022). Estimating the postmortem interval of carcasses in the water using the carrion insect, brain tissue RNA, bacterial biofilm, and algae. *Frontiers in Microbiology*, *12*(1), 1–12.

Wang, Z., Ma, K., Zou, D., Liu, N., Li, Z., Shao, Y., & Chen, Y. (2020). Diagnosis of drowning using postmortem computed tomography combined with endoscopic autopsy. *Medicine*, *99*(11), 1–6.

Wardak, K. S., Buchsbaum, R. M., & Walyzada, F. (2014). The drowning index: Implementation in drowning, mechanical asphyxia, and acute myocardial infarct cases. *Journal of Forensic Sciences*, *59*(2), 399–403.

Warther, S., Sehner, S., Raupach, T., Püschel, K., & Anders, S. (2012). Estimation of the time since death: post-mortem contractions of human skeletal muscles following mechanical stimulation (idiomuscular contraction). *International Journal of Legal Medicine*, *126*(3), 399-405.

Water Safety New Zealand. (2022, June 27). *Drowning stats*. Water Safety New Zealand. Retrieved November 21, 2022, from https://watersafety.org.nz/drowning%20statistics

Weather Underground. (n.d.). *Local weather forecast, news and Conditions*. Weather Underground. Retrieved November 15, 2022, from https://www.wunderground.com/

Weenink, R., & Wingelaar, T. (2021). The circulatory effects of increased hydrostatic pressure due to immersion and submersion. *Frontiers in Physiology*, *12*(1), 1–4.

Wells, J. (2019). A forensic entomological analysis can yield an estimate of postmortem interval, and not just a minimum postmortem interval: An explanation and illustration using a case. *Journal of Forensic Sciences*, *64*(2), 634–637.

Wescott, D.J. (2018). Recent advances in forensic anthropology: decomposition research. *Forensic Sciences Research*, *3*(4), 278-293.

Widya, M., Moffatt, C., & Simmons, T. (2012). The Formation of Early Stage Adipocere in Submerged Remains: A Preliminary Experimental Study*. *Journal of Forensic Sciences*, *57*(2), 328-333.

Wilk, L., Hoveling, R., Edelman, G., Hardy, H., van Schouwen, S., van Venrooij, H., & Aalders, M. (2020). Reconstructing the time since death using noninvasive thermometry and numerical analysis. *Science Advances*, *6*(22), 1–7.

Williams, A., Rogers, C., & Cassella, J. (2019). Why does the UK need a human taphonomy facility? *Forensic Science International*, *296*(1), 74–79.

Wilmshurst, P. (2019). Immersion Pulmonary Oedema: A cardiological perspective. *Diving and Hyperbaric Medicine Journal*, *49*(1), 30–40.

Winskog, C. (2012). Underwater disaster victim identification: The process and the problems. *Forensic Science, Medicine, and Pathology*, *8*(2), 174–178.

World Health Organization (2011). *Hardness in drinking-water: Background document for development of WHO guidelines for drinking-water quality* (No. WHO/HSE/WSH/10.01/10). World Health Organization.

World Health Organization. (2014). *Global report on drowning: Preventing a leading killer*. World Health Organization. Retrieved November 21, 2022, from https://www.who.int/publications-detail-redirect/global-report-on-drowning-preventing-a-leading-killer

World Health Organization. (2017). *Preventing drowning: an implementation guide*. World Health Organization.

Xiao, C., Xu, Q., Li, H., Zhu, X., Yu, Z., Zhao, J., Li, Y., Liu, H., Shi, H., & Liu, C. (2021). Development and application of a multiplex PCR system for drowning diagnosis. *Electrophoresis*, *42*(11), 1270–1278.

Yapo Etté, H., Grill, S., Botti, K., Djodjo, M., Telmon, N., & Rougé, D. (2006). Interest of a method for assessing post-mortem lividity in black skin. *Journal de Medecine Legale Droit Medical*, *49*(5), 149-153.

Yorulmaz, C., Arican, N., Afacan, I., Dokgoz, H., & Asirdizer, M. (2003). Pleural effusion in bodies recovered from water. *Forensic Science International*, *136*(1-3), 16-21.

Young, A., Stillman, R., Smith, M., & Korstjens, A. (2014). An experimental study of vertebrate scavenging behavior in a northwest European woodland context. *Journal of Forensic Sciences, 59*(5), 1333–1342.

Zhang, M., Song, F., Liang, L., Nan, H., Zhang, J., Liu, H., Wang, L., Wei, Q., Lee, J., Amos, C., Kraft, P., Qureshi, A., & Han, J. (2013). Genome-wide association studies identify several new loci associated with pigmentation traits and skin cancer risk in European Americans. *Human Molecular Genetics*, *22*(14), 2948–2959.

Zhao, J., Liu, C., Bardeesi, A.S., Wu, Y., Ma, Y., Hu, S., Shi, H., & Cheng, J. (2017). The diagnostic value of Quantitative Assessment of diatom test for drowning: An analysis of 128 water-related death cases using microwave digestion-vacuum filtration-automated scanning electron microscopy. *Journal of Forensic Sciences*, *62*(6), 1638–1642.

Zhao, J., Liu, C., Hu, S., He, S., & Lu, S. (2013). Microwave digestion—vacuum filtration-automated scanning electron microscopy as a sensitive method for forensic diatom test. *International Journal of Legal Medicine*, *127*(2), 459–463.

Zhou, C., & Byard, R.W. (2011). Factors and processes causing accelerated decomposition in human cadavers – An overview. *Journal of Forensic and Legal Medicine*, *18*(1), 6-9.

Zhou, Y., Cao, Y., Huang, J., Deng, K., Ma, K., Zhang, T., Chen, L., Zhang, J., & Huang, P. (2020). Research advances in forensic diatom testing. *Forensic Sciences Research*, *5*(2), 98–105.

Zhou, Y., Zhang, J., Huang, J., Deng, K., Zhang, J., Qin, Z., Wang, Z., Zhang, X., Tuo, Y., Chen, L., Chen, Y., & Huang, P. (2019). Digital whole-slide image analysis for automated diatom test in forensic cases of drowning using a convolutional neural network algorithm. *Forensic Science International*, *302*(1), 1–9.

Zhu, B.L., Quan, L., Li, D.-R., Taniguchi, M., Kamikodai, Y., Tsuda, K., Fujita, M. Q., Nishi, K., Tsuji, T., & Maeda, H. (2003). Postmortem lung weight in drownings: A comparison with acute asphyxiation and cardiac death. *Legal Medicine*, *5*(1), 20–26.

Zilg, B., Bernard, S., Alkass, K., Berg, S., & Druid, H. (2015). A new model for the estimation of time of death from vitreous potassium levels corrected for age and temperature. *Forensic Science International*, *254*(1), 158-166.

Zimmerman, K., & Wallace, J. (2008). The Potential to Determine a Post-mortem Submersion Interval Based on Algal Diatom Diversity on Decomposing Mammalian Carcasses in Brackish Ponds in Delaware. *Journal of Forensic Sciences, 53*(4), 935-941.

APPENDIX

SEE APPENDICES DOCUMENT