Using machine vision and behavioural indicators to monitor cleaner fish welfare in the Atlantic salmon (*Salmo salar*) aquaculture industry

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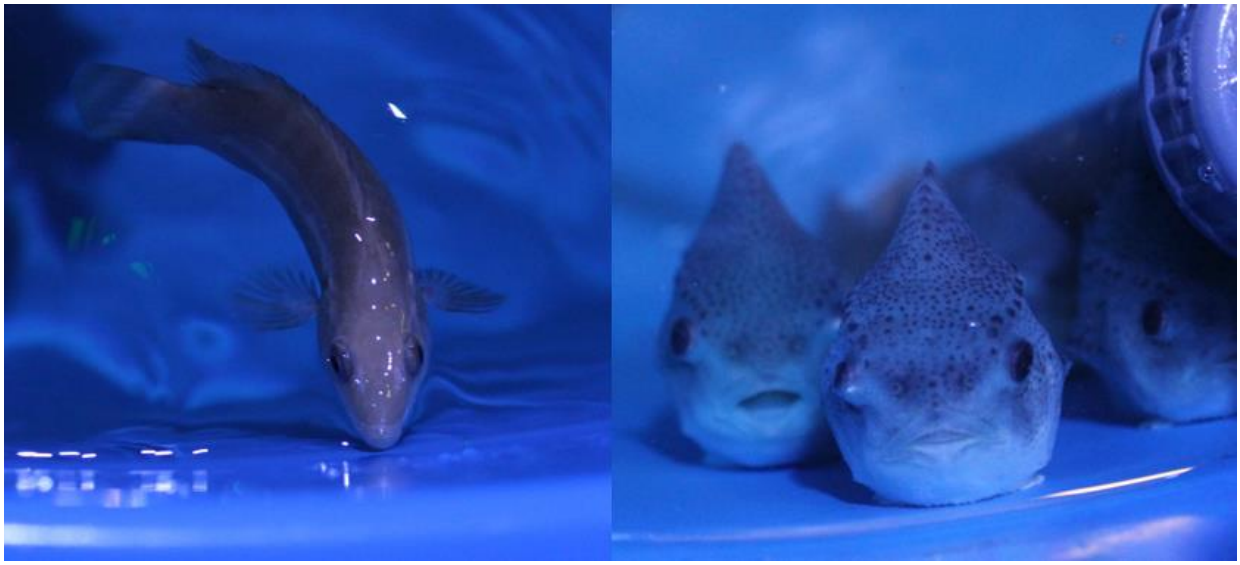

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Summary

The aim of this thesis was to explore how machine vision and behaviour indicators could be used to monitor welfare of Ballan wrasse (*Labrus bergyltus*) and Lumpfish (*Cyclopterus lumpus*), which are used as cleaner fish in the Atlantic salmon industry. This thesis is divided into 4 chapters. Chapter 1 is a systematic literature review on the use of artificial intelligence (AI) in the aquaculture industry. The review looks into examples of different uses for AI in the industry, including intelligent feeding, monitoring of water quality, behaviour and disease, and assessment of body condition. The review also summarises the main benefits, challenges, and limitations of such systems. It is clear from the results that AI as a component of precision farming has the potential to revolutionise practices and non-invasive methods of monitoring. However, continued research and development will help to address the limitations and challenges, including the need for data management and standardisation. By addressing the problems associated with image-based methodologies such as poor video quality and a lack of behavioural and disease monitoring trials using AI in industry, fish welfare could be greatly improved. Chapter 2 tests two image-based methodologies for estimating body mass index (BMI) of lumpfish and ballan wrasse. The first was ImageJ, a software which can be used to measure fish from photographs that include a scalebar. The results from this study showed that measurements obtained from ImageJ could be used to estimate BMI with good accuracy. The second was a new Artificial Intelligence (AI) system that was developed by Visifish, which determined fish measurements from videos. Visifish are a bioinformatics company that use machine vision and deep learning techniques to assess population, growth and behaviour. The AI was only able to provide measurements for 21 out of the 120 fish that were filmed. The results showed that the AI was unable to provide measurements that were accurate enough to determine true BMI. This was likely due to the limited training data set. To validate the AI, more work will need to be carried out to develop it further, including larger sample sizes and more videos. Chapter 3 involves 2 experiments with the goal of determining whether 'personality components' can be identified and potentially used for broodstock selection for the use in the aquaculture industry. The first experiment involved recording individuals of both species and their behavioural responses to different stimuli. Their responses were used to label them with personality components. The second experiment involved recording the same fish' response to artificial Atlantic salmon models with artificial sea lice attached on to their flank. The personality components were then compared with the responses to the salmon models to determine whether the personality

components could relate to good delousing behaviours. The results showed that lumpfish were significantly bolder, more aggressive, more social, and less anxious than the ballan wrasse. The results also showed that bold, social, and not anxious individuals had more interactions with the salmon model carrying a large number of sea lice. This suggests that these personality components could be selected and used to breed potentially good delousing behaviours. Chapter 4 involves using a shuttle-box and machine vision system to determine the temperature preference and thermal niche of ballan wrasse. The temperature preference and thermal niche of lumpfish was not explored in this thesis as it has already been studied using the same system and is awaiting publishing. The results concluded that the preferred thermal niche for ballan wrasse is between 12.1°C and 13.8°C. The optimal temperature preference was 12.8°C. These results could be used to aid decisions about deployment times and locations for ballan wrasse in the aquaculture industry.

Declaration of Statements

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed: 

Date: 10/10/2023

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit reference. A bibliography is appended.

Signed: 

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I hereby give my consent for my work, if relevant and accepted, to be available for photocopying and for inter-library loan after expiry of a bar on access approved by the university.

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Statement of Expenditure

Student name: Isla Monaghan

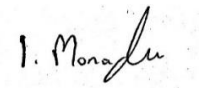
Student number: 

Project Title: Using machine vision and behavioural indicators to monitor cleaner fish welfare in the Atlantic salmon (*Salmo salar*) aquaculture industry.

Category	Item	Description	Cost (Inc VAT & Delivery)
Education	Introduction to Data Analysis in R Course (online) – Eco-explore	Data analysis in R course	£240.00
ICT Equipment	SanDisk Extreme 2TB portable SSD, USB-C	Hard drive for video storage	£199.99
Consumables	Anika 30340 Pack of 4 Self Adhesive Square Mirrors 20cm	Mirrors for experiment tank set up	£20.97
Conference fees	WEEN 2022 Conference	Welsh Ecology and Evolution Network Conference	£60.00
Travel	Hotel, food, and fuel expenses	Trip to Anglesey for experiments conducted at MOWI Hatcheries	£438.65
Total			£959.61

The above expenditures were funded by the students KESS 2 Scholarship.

I hereby certify that the above information is true and correct to the best of my knowledge.

X 

Signature (Student)

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Signature (Supervisor)

Statement of contributions

IM – Isla Monaghan, MSc by Research Candidate

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SCO – Sofia Consuegra de Olmo, Academic supervisor

SW – Sarah Weller, Research Assistant

JVE - Jack Van-Eker, Student

SK – Synne Klute - Student

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Role	**Contributors**
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Data curation	IM, SW, JVE, SK, GW
Formal analysis	IM, CGL
Funding acquisition	CGL, SCO
Methodology	IM, CGL
Project administration	IM, CGL
Software	IM
Supervision	CGL, SCO
Visualisation	IM
Writing – original draft and preparation	IM
Writing – review and editing	IM, CGL, SCO

Contents

i. Acknowledgements

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Mae'r Ysgoloriaeth Sgiliau Economi Gwybodaeth (KESS 2) yn fenter sgiliau lefel uwch Cymru gyfan a arweinir gan Brifysgol Bangor ar ran y sector AU yng Nghymru. Fe'i cyllidir yn rhannol gan raglen cydgyfeirio Cronfa Gymdeithasol Ewropeaidd (ESF) ar gyfer Gorllewin Cymru a'r Cymoedd.

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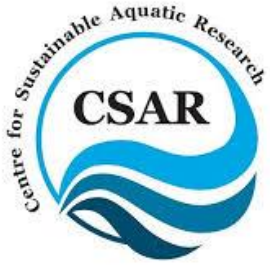

ii. List of figures and tables

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iii. Justification and aims of the thesis

In recent years, there has been a surge in social and political concern regarding animal welfare and management. The idea of the "five freedoms" and the contributions of behavioural science have greatly influenced the perceptions of animal welfare, particularly in Europe and North America (Bayvel and Cross, 2010). Traditionally, the concept of animal welfare has focused on detecting signs of negative welfare (Dawkins, 2008), including stress, pain, boredom, aggression, and abnormal behaviour. Behavioural measures, such as aggressive or repetitive behaviours, serve as crucial indicators of welfare issues. Although such measures are critical indicators of welfare issues, on-farm welfare assessments currently place more emphasis on identifying health problems and less on behavioural indicators (Rushen *et al*., 2012). This is mainly due to increased time and costs associated with collecting behavioural measures during farm visits (Edwards, 2007). Providing farmed animals, including fish, with enough space to exhibit their typical behaviours with minimal pain, stress, and fear is recommended by the Farm Animal Welfare Council (Carter, 2014). However, there is a lack of studies examining what qualifies as normal behaviour, how much space is sufficient, and how to recognise fear in aquaculture environments (Champneys *et al*., 2018). Behavioural indicators can be used as a tool for assessing and improving welfare in many farmed species. Fish, like other animals, have a range of behaviours that are associated with positive welfare, such as feeding, swimming, and exploring their environment. They also have behaviours that are associated with negative welfare, such as lethargy, decreased feeding, and aggressive or abnormal behaviour. Systematic sampling of such quantified behavioural measurements enables monitoring of changes over time (Wolfensohn *et al.*, 2018).

Advanced technologies have the potential to aid in improving animal welfare in farming, as well as increasing sustainability and efficiency, maximising production, profitability and reducing costs (Ferguson, 2014; Saberioon *et al*., 2016; Ellis *et al*., 2020) Due to the rise in demand for farmed products, including fish, meat, dairy and eggs, there is a demand for automated, less invasive, and more accurate tools to improve farm management practices (Hunter *et al*., 2017; Bhat and Huang, 2021; Yaseer & Chen, 2021). Advanced technologies including Artificial Intelligence (AI), machine vision, and image-based methodologies have the potential to improve many farm practices, including welfare, environmental and feed monitoring (Cravero et al., 2022). Using such systems to monitor farmed fish species

throughout a production cycle would aid in health and welfare monitoring, decrease the risk of disease spread and stress incidents (Boglione & Costa, 2011). These systems would reduce the need for traditional techniques for monitoring and assessment, which can be time-consuming, invasive, costly, and sometimes destructive (Saberioon *et al*., 2016).

The aim of this thesis is to examine the use of novel machine vision, image-based methodologies and behavioural indicators for monitoring and improving the welfare of two cleaner fish species within the aquaculture industry. Both ballan wrasse (*Labrus bergyltus*) and lumpfish (*Cyclopterus lumpus*) are commercially farmed for use as 'cleaners' within the industry, consuming parasitic sea lice (*Lepeophthierus salmonis* and *Caligus* species) from the Atlantic salmon (*Salmo salar*) (Øverli *et al*., 2014). The use of cleaner fish as a form of biological control is increasing, despite concerns about their welfare and survival in sea cages (Geitung *et al*., 2020). Due to these problems, the need for better tools for improving and monitoring the welfare of these fish is more important than ever.

This thesis is organised into four chapters. **Chapter 1** provides a systematic literature review on the use of artificial intelligence (AI), including branches of AI such as machine vision and other image-based methodologies, for the assessment of fish welfare in fish farming. It provides a summary of the main benefits, current limitations, and outstanding challenges.

Chapter 2 tests the accuracy of photographic and video images to estimate the body mass index (BMI) of lumpfish and ballan wrasse. The results showed that while body ratio (body height/length and width/length) indices obtained from still photographs could be used to estimate BMI with some precision, video images did not provide accurate results, likely due to the fact that the training data set was too limited. More development work, and larger sample sizes, are required to use videos and AI to estimate BMI on cleaner fish.

In **Chapter 3**, an experimental approach was used to assess individual variation in delousing and exploratory behaviours between lumpfish and ballan wrasse using artificial sea lice and model salmon in an open test arena. The results showed that lumpfish were significantly bolder, more aggressive, more social, and less anxious than wrasse when tested under the same conditions. The results also showed that bold, social, and not anxious individuals had more interactions with the salmon model carrying the most sea lice, suggesting these are potentially good delousing behaviours.

Chapter 4 examines the thermal preferences of a farmed population of ballan wrasse using a shuttle-box and machine vision system. The results indicated that the preferred thermal range

for this population of ballan wrasse lies between 12.1 ºC and 13.8 ºC and that preferred optimal temperature is 12.8 ºC.

A summary of the main findings and the overall conclusions are presented at the end of each chapter.

Chapter 1. The use of AI and machine vision for assessing fish welfare in the aquaculture industry. A systematic review.

1.1. Introduction

The study of 'intelligent machines' dates to the 1950s, when scientists such as Alan Turing first proposed definitions for them (Turing, 2009; Nagy *et al*.,2020). Artificial intelligence (AI) is the simulation of human cognitive abilities in machines, such as interaction, perception, reasoning, and learning (Berente *et al*., 2019). Machine vision is a subfield of AI and describes the capability of a machine to gather information about its surroundings using sensing techniques, and then process that information using computer-based mathematical algorithms for analysis and interpretation (Xiong, 2008). The use of machine learning techniques holds significant promise for enhancing both the scope and precision of marine research (Beyan & Browman, 2020).

Artificial intelligence (AI) and machine vision technologies are increasingly being utilized across the aquaculture industry (Vo *et al*., 2021). The industry currently utilises many different processing technologies and data collection techniques for every-day functions to improve efficiency, productivity, and sustainability (Gladju *et al*., 2022).
Aquaculture is a rapidly advancing industry, playing a vital role in ensuring global food security, providing a significant source of protein, and supporting the livelihoods of people all over the world (Ottinger *et al*., 2018). The aquaculture industry is estimated to bring the global fish supply to 186 million tons by 2030 (Kobayashi *et al.*, 2015). With the increasing demand for fish, there has been a growing concern for fish welfare in aquaculture systems (Turnbull *et al*., 2005). In addition, fish welfare also has an economic impact, since healthy and happy fish grow faster and produce a higher yield (Southgate and Wall, 2001). Moreover, aquaculture industry practices must be more sustainable and ethical in order to meet consumer demand for animal welfare and sustainability (Pieniak *et al*., 2013). In recent years, there has been a growing interest in using technology, such as AI and machine vision, to improve fish welfare in aquaculture systems (Vo *et al*., 2021). Real-time monitoring of fish behaviour, health, and welfare using machine vision systems allows early detection and intervention in case of any problems. This technology has the potential to revolutionise fish welfare management in aquaculture systems. This review explores the current state of

research on the use of AI and machine vision for monitoring fish welfare in aquaculture, as well as the benefits and challenges associated with this technology.

1.2. Materials and methods

1.2.1. Search for publications

Web of Science and Google Scholar were used to investigate the use of machine vision for monitoring welfare in the aquaculture industry. Peer reviewed publications were used as well as some non-peer reviewed items such as government reports. Only documents published between January 2000 and February 2023 were considered. Publications were searched using the following combination of terms: “Artificial Intelligence OR AI OR machine vision AND aquaculture”, “Artificial Intelligence OR machine vision AND fish farming”, “Artificial Intelligence OR AI OR machine vision and fish welfare”, “Artificial Intelligence OR AI OR machine vision OR machine learning AND fish feeding AND aquaculture”, “Artificial Intelligence OR AI OR machine vision AND water quality”, “Artificial Intelligence OR AI OR machine vision AND fish disease”, “Artificial Intelligence OR AI OR machine vision AND fish disease”, “Artificial Intelligence OR AI OR machine vision AND sorting OR grading aquaculture”, “Artificial Intelligence OR machine vision AND fish behaviour”. Articles were included if they met the following criteria: (i) published or translated into English, (ii) available online, (iii) included information on fish species, (iv) were applicable to the aquaculture industry. The searches “welfare AND aquaculture”, “welfare AND fish farming”, were then done after the initial search to screen for any publications that met the criteria but did not appear in the original searches.

The guidelines from Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) (Hutton *et al*., 2015) were used to select the articles for this systematic review (Figure 1.1). Overall, 3,484 articles were found, of which 3,377 were excluded as they did not meet the inclusion criteria. Of the 106 articles retained, 26 articles subsequently excluded as they focused more on machine vision and AI development than on the application to the aquaculture or fish farming industry. A further 15 articles were removed as they focused on DNA or genetics and were not fully related to the review topic. Finally, 6 articles were removed as they focused on ova or milt research, which was outside the aim of this thesis. After filtering, 59 peer-reviewed articles and 4 technical reports produced by commercial companies were retained for analysis.

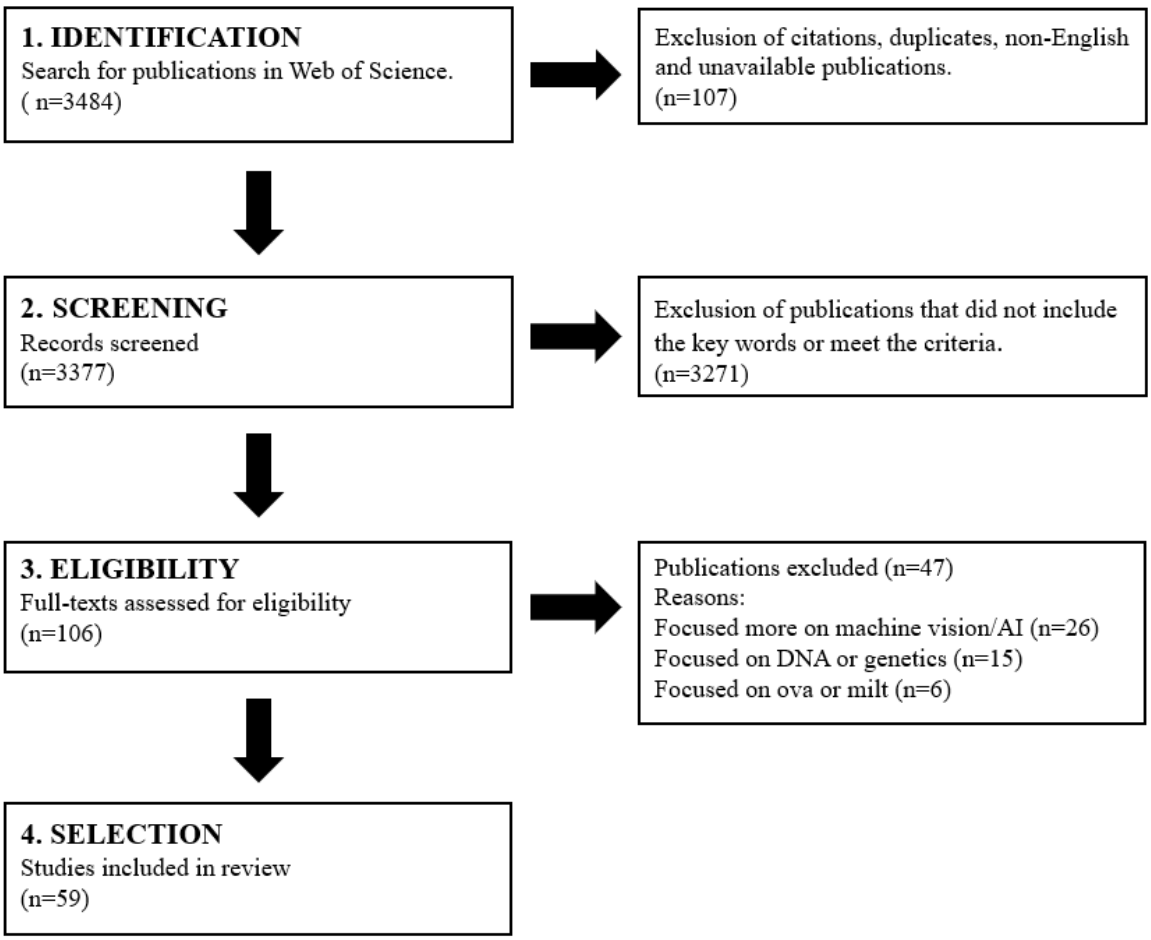

Figure 1.1. The selection of publications following the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA).

1.3. Results and Discussion

1.3.1. Range of applications of AI and machine vision in Aquaculture

The selected publications on the use or potential use of AI and machine vision in the aquaculture industry were split into categories based on their study focus (Figure 1.2). The largest group was categorised as ‘multiple uses’ (n= 20, 34%).

The most common applications of machine vision were for behavioural monitoring, intelligent feeding, and estimation of body condition, followed by use in grading and sorting and disease monitoring. This gives an overview of how AI and machine vision are currently being used in the aquaculture industry and what benefits might be expected from further developments.

Some limitations of AI were noted in the publications, however, namely a shortage of studies under real commercial conditions and an over-reliance on laboratory-based studies (Kato et al., 2004; Kane et al., 2004).

1.3.2. Study species

The reviewed literature focused mostly, but not exclusively on fish species which are farmed in land-based systems, or marine/freshwater pens. A total of 4 technical reports from commercial companies and 59 peer-reviewed publications were used. Of the 59 publications, 18 were focussed on one species (Figure 1.3). The family Salmonidae was the most researched with 6 single species-specific studies (n= 6, 33%).

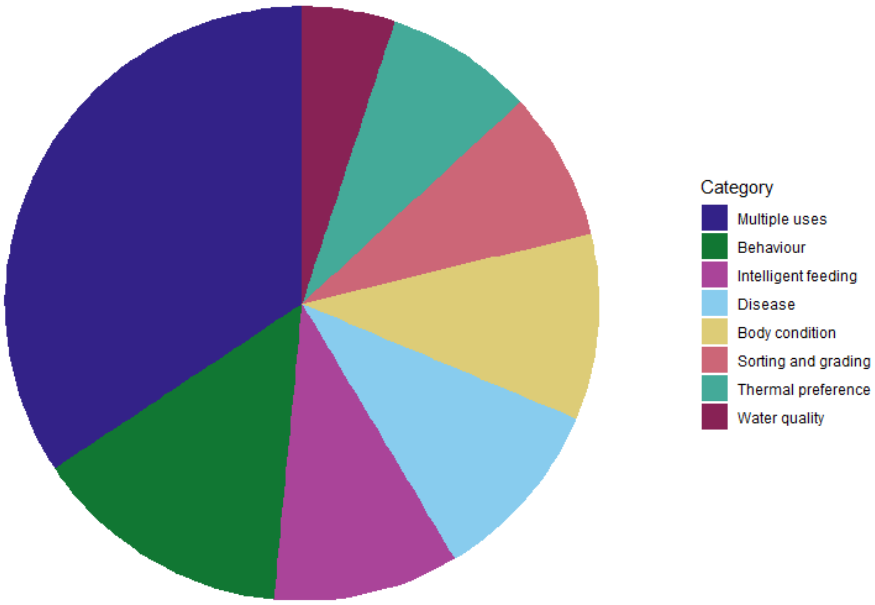

Figure 1.2. Breakdown of AI studies by topic. Most publications fall into the 'multiple uses' category (n= 20, 34%).

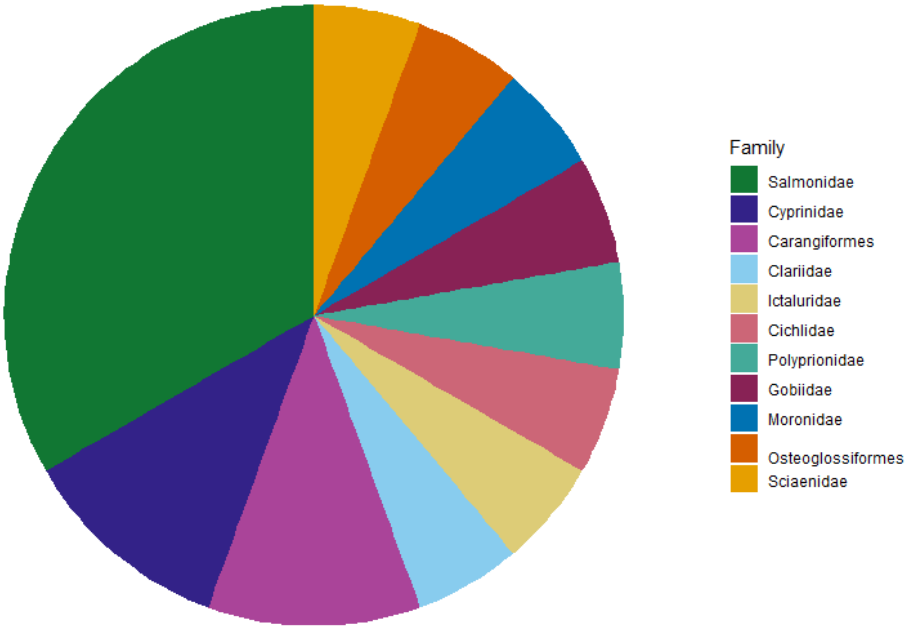

Figure 1.3. Taxonomic breakdown of AI studies that focused on single species. Most studies focused on the family *Salmonidae*.

1.4. AI and machine vision uses in the aquaculture industry

1.4.1. Intelligent feeding systems

With the growth of the aquaculture industry, the utilization of relevant technologies is propelling the development of novel policies aimed at promoting the transformation of the broader industrial landscape, including the conversion into intelligent aquaculture (Hu *et al.*, 2022). Significant economic, ethical, and sustainable gains can be achieved through the optimisation of the fish feeding process in industry (Mia *et al*., 2022; Vo *et al.*, 2021; Zhou *et al.*, 2018). The low cost and non-invasive nature of machine vision technologies have made them a popular choice for analysing and quantifying behavioural parameters during fish feeding processes. Machine vision technology can be employed to detect and count fish, to estimate appetite and automatically dispense feed (Lee et al., 2013). Computer vision systems have been able to achieve 98% feeding decision accuracy (deciding when to continue or stop during the feeding process), which can greatly improve economic efficiency and reduce the environmental impact if applied to the aquaculture industry (Zhou et al., 2018).

The evaluation and analysis of feeding behaviour in species such as Atlantic salmon (*Salmo salar)* often requires the measurement of swimming velocity and position, along with the assessment of population scatter and movement intensity (Liu *et al*., 2014). Atypical behaviours, such as clustering and sudden acceleration exhibited by a school of fish, may convey pertinent information regarding feeding operations and fish appetite (Conrad *et al*., 2011). A viable approach is to analyse a population, comprising a large number of individuals. In intelligent fish feeding research, there has been a growing inclination towards population-based studies (Zhou *et al*., 2018). In industry, commercial video-based feeding systems have been implemented whereby underwater images of feeding fish are wirelessly transmitted through WLAN (wireless local area network) to a feed boat/barge or over 3G (Internet) to any location around the globe (AQ1SYSTEMS, 2017). Through image processing, fish behaviour, size, and quantity can be measured. While these approaches and techniques have the potential to significantly minimize feed wastage, their accuracy still requires improvement to accommodate variations related to complex site and environmental factors. Cameras are widely utilized for monitoring feeding conditions: feeding is halted when the amount of residual feed observed by cameras reaches a certain level. Currently, commercial systems such as AKVA's Observe system (AKVA Group, 2023) are employed in feeding operations within sea cages and integrate underwater cameras and feed particle

sensors as well as other components to enable farmers to monitor feeding conditions and achieve automated feeding. However, some limitations have also been noted in the use of machine vision and AI to optimise feeding in aquaculture, including the small size of the feed targets, interference from fish movements and poor visibility due to murky water conditions (Ghani and Isa, 2015; Hu *et al.*, 2022). Image-based methods have helped develop intelligent feeding control systems, but significant hurdles still remain in the realm of computer vision (Hu *et al*., 2022).

1.4.2. Estimating of body condition

Inaccurate feeding rates can lead to overfeeding, which can result in financial loss, poor water quality, deterioration of surrounding environment, disease, and other disorders (Roh *et al*., 2020; Kamler et al., 2006). On the other hand, underfeeding can lead to starvation, malnutrition, stunted growth, competition and even death (Yogev *et al*., 2020; Attia et al., 2012). Thus, determining accurate feeding rates is essential for ensuring fish welfare, optimizing growth, and reducing waste. Body condition is a crucial measure when determining starvation and malnutrition in farmed fish. Fish body condition is a critical indicator of their health and welfare, and accurate measurements can help farmers adjust feeding rates and improve fish growth and overall health (Gaylord *et al*., 2000; Rikardsen *et al*., 2006; Fernandez-Jover *et al*., 2007). Early research focussed on the use of AI and machine vision for the identification of fish species (Hu *et al*., 2012; Foud *et al*., 2013) but limited progress has been made in relation to the assessment of body condition. The use of machine vision and artificial intelligence would be particularly useful for the estimation of body weight and body condition, as these can reflect poor growth and underfeeding in fish farming (Hvas *et al*., 2019), but is an under-utilised method (explored in Chapter 2). A few companies have now developed AI system that can provide estimates of population weight under commercial conditions (Aquabyte Inc., 2023; Stingray Marine Solutions AS, 2023).

1.4.3. Monitoring of water quality

Water quality is a critical factor impacting the quality and quantity of farmed fish production (Eriegha and Ekokotu, 2017). As such, real-time monitoring of water quality is the initial step towards maintaining good welfare. To ensure successful production, it is vital to monitor essential water parameters. Machine learning provides vast opportunities to evaluate, categorize, and forecast water quality indicators in aquatic studies (Nasir *et al.*, 2022). AI can be used to monitor parameters such as pH, salinity, dissolved oxygen, and temperature, which

are critical for maintaining healthy fish populations (Mustafa *et al.*, 2016). By monitoring these parameters in real-time, farmers can quickly identify and respond to changes that could affect fish health. Several technologies that utilize wireless or WiFi-based systems to monitor water quality have been developed and tested for use in aquaculture. For example, studies utilised wireless sensor networks for real-time monitoring of dissolved oxygen (DO), nitrates, carbonates, ammonia, pH, salinity and temperature (Raju and Varma, 2017; Ma and Ding, 2018; Lu *et al.*, 2022). This kind of monitoring reduces frequent manual testing and allows farmers to take preventative measures before threshold ranges are passed and mortality occurs (Raju and Varma., 2017).

1.4.4. Thermal preference

Thermal monitoring is an essential research tool as temperature can affect consumption, growth, metabolism, and overall health (Oppedal et al., 2007). Temperature gradients in the environment can affect the behaviour and distribution of fish in the wild (Ficke et al., 2007). However, in some aquaculture systems, such as open net cages, maintaining optimal water temperature can be challenging, and inaccurate temperature management can lead to stress, poor cardiac function, and reduced growth rates (Farrell, 2002; Khan et al., 2014). Real-time monitoring of fish behaviour and temperature preferences can be achieved using machine vision and artificial intelligence. Machine vision has been used to track individual fish movements in a shuttle box system equipped with temperature controls to accurately determine thermal preferences (Christensen et al., 2021; Macnaughton et al., 2018). This method is explored further in Chapter 4. Overall, the use of machine vision and AI for determining thermal preferences in fish species has the potential to improve fish welfare, reduce stress and disease, and optimize production in the aquaculture industry. However, further research is needed to validate these technologies in real-world settings, such as sea-cages or farm systems, and ensure their practicality and cost-effectiveness.

1.4.5. Monitoring of disease

Diseases can have devastating effects on both wild and farmed fish populations. They are widely acknowledged as one of the greatest threats to the economic viability of aquaculture worldwide (Ahmed *et al.*, 2022). The high density of fish populations in the confined spaces of land or sea-based farming systems creates the ideal environment for the rapid transmission and spread of infections, which often arise from disruptions in the interactions between pathogens, hosts, and environments (Pérez-Sánchez *et al.*, 2018). In some areas of

aquaculture, such as the Atlantic salmon industry, there has been extensive progress in disease identification, diagnostics, and management. However, many challenges remain, such as those related to sea lice (*Lepeophthierus salmonis* and *Caligus* species) infestation (Stentiford *et al*., 2017). Hence, novel methods to manage and prevent diseases are crucial for the continued growth and progress of the aquaculture industry (Defoirt *et al*., 2011). There is a necessity for AI or computerized systems that can aid farmers, especially those located in rural or remote regions, in detecting initial indications of fish disease (Hu *et al*., 2012). A combination of techniques, including edge detection and machine learning have been used to automatically detect EUS (Epizootic Ulcerative Syndrome) in fish species (Malik *et al.*, 2017). Machine learning-based classification models have been trained to classify Salmonids into 'fresh' or 'infected' categories (Ahmed *et al*., 2022). Treatment optimization, improved welfare and overall survival can all be achieved with such systems for detecting disease in farmed fish. While there are many studies researching the use of AI for disease detection, few are in fish species and fewer still are in farmed fish. This could be due to difficulties with obtaining clear images underwater. However, progress has been made using image processing and machine learning, but the effectiveness is still very variable.

1.4.6. Sorting and grading

Fish grading, particularly size grading, is a common and essential operation in the aquaculture industry and is often performed multiple times throughout the production cycle (Costa *et al.*, 2013). This ensures that fish in the same tank or sea cage are of a similar size, though one often sees significant variation in body weight among fish of the same age (Gjedrem and Olsen, 2005). Grading can help reduce the risk of aggression and competition, can help optimize feed management and is also necessary for the classification of fish into standard sizes prior to processing and sale (Zion *et al.*, 1999; Lima, 2020). Trainable machine learning systems have been developed that can determine the weight of fish based on image measurements (Odone *et al*., 2010). These systems use body shape parameters to predict fish weight and classify fish at high speed with very high accuracy. A machine-vision based technology solution has been developed, for example, for automatic grading and determining the level of "freshness" of large yellow croakers (*Larimichthys crocea)*, based on sensory index characteristics (Wu *et al.*, 2019). This system was deemed accurate and efficient with practical value for use in a production line. Grading throughout a cycle also allows for the removal of damaged or deformed individuals for culling. A combination of techniques including Palinox (Palinox, 2022), Aquadef (Technologia Marina Ximo S.L., 2023) and Vaki

(Vaki Aquaculture Systems, 2023) fish sorting machines can be used for grading out fish with spinal deformities (Costa *et al.*, 2013). They are also able to grade with reliable accuracy, though more research including species-specific testing is needed before use in a commercial setting. Overall, the use of machine vision and artificial intelligence for automated sorting and grading in aquaculture can optimize farmers' operations and increase efficiency and accuracy. Some of the studies reviewed indicate that utilizing advanced imaging systems, data analysis, and automated equipment not only improves productivity and profitability, but it can also enhance fish welfare (Zhou *et al.*, 2018; Hu *et al.*, 2022). While AI assisted grading can offer sorting of size, deformity and damage, mechanical grading bars are still the most commonly used method of size grading in commercial farming (Costa *et al*., 2013). New AI assisted techniques could be introduced in combination with mechanical grading to ensure a productive grade with less error and handling.

1.4.7. Behaviour

Aggression, along with abnormal and stereotypical behaviours of individuals and groups, can reflect the impacts of stressors in aquaculture and may be considered signs of compromised welfare (Martins *et al.*, 2011). Personality profiling, remote detection, and artificial intelligence can help improve fish welfare but are still underutilized (Asher et al., 2009). AI and machine vision systems can use video footage to detect changes in behaviour and swimming speed, which can indicate stress and illness (Papadakis *et al*., 2012). Video-based tracking systems can also accurately assess the schooling behaviour of multiple fish in a quantitative manner and monitor changes in behaviour over extended periods (Kane *et al*., 2004). Such systems can be used to evaluate the impact of sublethal stress and contaminants on fish behaviour and could help farmers quickly identify and address potential health or contamination issues before they become detrimental. However, such systems have only been tested in the laboratory and validation under farm conditions is required to assess their usefulness to the aquaculture industry. Human observations are still relied upon to monitor behaviour on farms (Martins *et al*., 2011). This could be due to a lack of public and industry knowledge on the importance of behaviour in welfare monitoring, resulting in a lack of funding into research.

1.5. Conclusion

The use of AI and machine vision in the aquaculture industry has the potential to revolutionise farming practices. From disease detection to optimising feeding regimes and improving fish welfare, machine vision can reduce environmental impact whilst increasing production efficiency and sustainability (Vo *et al.*, 2021; Mia *et al*., 2022). The application of deep learning algorithms and the development of new imaging technologies have enabled machine vision to become an increasingly reliable and accurate tool for fish farmers (Li & Du, 2022). As with any new technology, there are still limitations and challenges that need to be overcome in aquaculture, for the full potential of machine vision to be truly realised. One of the main challenges is the need for data management and standardisation, as large amounts of high-quality data are required to train and validate machine learning models. The benefits of using machine vision in aquaculture are evident, and continued research and development will help to address these challenges, contributing to ensuring a sustainable and efficient future for the sector.

Chapter 2 - Use of image-based methodologies for the assessment of body condition of cleaner fish lumpfish (*Cyclopterus lumpus*) and ballan wrasse (*Labrus bergylta*)

2.1. Introduction

Cleaner fish are commonly used in Atlantic salmon (*Salmo salar)* farming to control and prevent the spread of parasitic sea lice (*Lepeophthierus salmonis* and *Caligus* species), which pose a significant welfare threat (Øverli *et al*., 2014) and economic impact (Costello, 2006; Erkinharju *et al*., 2020). Sea lice attach to hosts and feed on the mucous layer, skin and tissue (Costello, 1993), leading to skin lesions, typically on or around the head, and severe infestations which frequently result in host mortalities (Finstad *et al.*, 2000). The control of sea lice in aquaculture involves significant treatment costs, decreased efficiency of food conversion, and reduced overall fish growth (Mustafa, 2001; Costello, 2009). In the past, medicinal treatments were regarded as the most dependable means of preventing high sea lice abundance (Aaen *et al.*, 2015) and, while the use of cleaner fish as an alternative method of biological control has been explored since the late 1980s (Bjordal, 1988), it was only in the late 90s/early 2000s that research started gaining momentum, followed by the establishment of organised supply chains (Leclercq et al., 2014; Philis *et al*., 2021).

Several species of cleaner fish are commonly used in aquaculture, including several wrasse species (*Labridea)*, though mainly farmed Ballan wrasse (*Labrus bergyltus*) and lumpfish (*Cyclopterus lumpus)*. Cleaner fish provide a natural and sustainable method of controlling sea lice through removal of external parasites, pathogens, necrotic tissue, bacteria, or detritus from cooperative hosts or 'clients' (Peacock, 2011; Vaughan *et al.*, 2017). In Atlantic salmon aquaculture, cleaner fish create a symbiotic relationship where they gain access to food (sea lice from the clients) and shelter, while clients benefit from reduced sea lice numbers and damage (Costello, 2009). They are more environmentally friendly than chemical treatments, which can harm both the farmed fish and the environment (Imsland, 2023). Additionally, the use of cleaner fish can reduce the need for veterinary medicines, which can lead to drug resistance in lice (Aaen *et al.*, 2015). Drug resistance in sea lice has been described across the global salmon farming industry, with a clear need to research the effects of medicinal compounds on sea lice and possible resistance mechanisms (Aaen *et al*., 2015). The use of non-medicinal methods such as cleaner fish, should be used as a first resort in sea lice control, with medicinal products used if they are unsuccessful in controlling lice numbers (Aaen *et al*., 2015). The use of cleaner fish is not without challenges. The cost of purchasing

and maintaining cleaner fish can be high, although wrasse were found to be a more economical and ecological option than bath or in-feed treatments for lice removal (Lui and Bjelland, 2014).

The effectiveness of cleaner fish may also be influenced by factors such as body condition, water temperature, stocking density, and food availability (Brooker *et al*., 2018; Yuen *et al*., 2019; Geitung *et al*., 2020). Both wrasse and lumpfish are known to be opportunistic feeders, usually relying on multiple food sources in the wild (Imsland *et al*., 2015). Therefore, appropriate supplementary feed in suitable locations in sea cages must be provided to reduce the risk of malnutrition or starvation (Deady & Fives., 1995; Imsland *et al*., 2018).

Assessing fish body condition is a fundamental aspect of fisheries management, aquaculture, and ecological research (Bolger & Connolly, 1989; Cone, 1989). Body condition can provide important information about a fish's overall health, growth, and fitness. Traditionally, body condition assessment in fish has relied on direct measurements such as weight, length, and various morphometric indices (Jones *et al*., 1999). The power of artificial intelligence and machine vision techniques has recently opened new possibilities for accurate and non-invasive body condition assessment in fish (Foud *et al*., 2013; Baretto *et al*., 2022). Body shape, size, and proportion can be characterized by digital images and advanced analytical tools, thereby providing useful information about nutritional status, energy reserves, and overall well-being (Hu *et al*., 2012). Furthermore, image-based approaches are highly valuable in both laboratory, field, and farm settings due to their efficiency, cost-effectiveness, and ability to process large datasets (Li *et al*., 2022). Here, I assessed the value of image-based systems for determining body condition of two species of cleaner fish (ballan wrasse and lumpfish) under hatchery conditions. The first system was ImageJ, a software which can be used to produce subject measurements from photographs that include a scalebar. The other was AI used by Visifish to produce proportions of body measurements from videos of fish from top and lateral views. The proportions were produced using multiple processes carried out by Visifish.

2.2. Materials and Methods

A two-step experiment was carried out to determine if two different image-based methods could be used to assess body condition in fish. The first part of the experiment involved filming and photographing 62 lumpfish and 62 ballan wrasse from 2 different aquaculture locations. The second part of the experiment involved comparing an AI system to generating size ratios of fish from videos. with the measurement of fish images using an image-processing software.

2.2.1. Source and rearing of fish

The ballan wrasse used in this experiment were collected from the MOWI ballan wrasse hatchery in Anglesey, North Wales in November 2022 when they were 10-20g. They were transported from Anglesey to Swansea by road using best practices (Jonassen *et al*., 2018). After arrival, they were held in a RAS (recirculating aquaculture system) at CSAR, Swansea University. The lumpfish used in this experiment were reared at CSAR, Swansea University. They were kept in separate tanks but within the same RAS for water quality and temperature consistency. The water temperature of the system was ~12.5°C and the lumpfish were exposed to a 12/12-hour light-dark cycle. The wrasse were fed twice daily on Otohime S3 feed at 1% of their body weight and the lumpfish were fed twice daily on the same feed at 2% of their body weight. Water quality was tested weekly. After filming, each fish was placed in a new home tank within the same RAS, to avoid repeating the trial on the same fish. The experimental procedure was approved by Swansea University, Animal Welfare Ethical Review Body, permit IP-2122-15.

2.2.2. Experiment

Two plastic, rectangular tanks (32 cm x 45 cm x 50 cm) were used for this experiment. The set up and procedure for the two tanks was the same. Two GoPro Hero 10s (GoPro Inc., 2023) were used in each tank for filming. A frame (60 cm x 85 cm) was built to hold two of the GoPros above the tanks. Colourless Perspex sheets (18 cm x 12.5 cm x 1 cm) were placed in the centre of the tanks to provide a hide/structure for the fish to shelter while maximising their presence in the camera view. Other colours and thicknesses were tested but the colourless Perspex was chosen as it limited the obstruction in the camera view while still providing shelter to the fish. Individual fish labels were printed on waterproof paper and

attached to the side and floor of the tanks to allow for easy identification in the videos after experiments were completed. Each label contained a code which referenced the species, location of experiment, date and fish number. The GoPro cameras were placed in the tanks, one attached to the frame to capture the view from above and one on the floor of the tank in front of the front wall to capture a side view. The water was filled to just above the top of the GoPro (submerging it) (45cm), to prevent any distortion or reflections from the surface of the water affecting the film (see figures 2.1.a and 2.1.b).

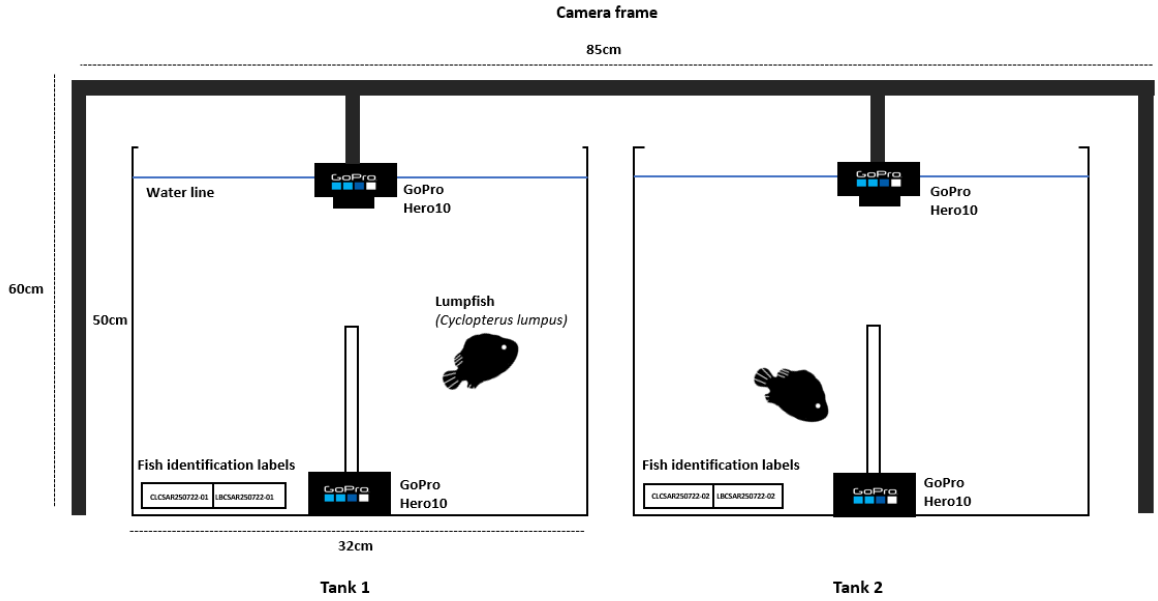

Figure 2.1.a Experiment tank (lateral view).

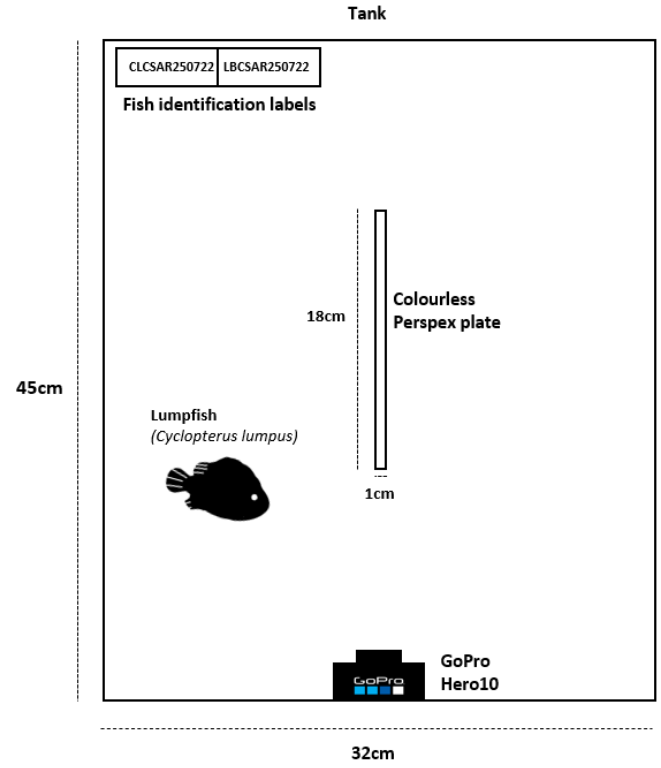

Figure 2.1.b Experiment tank (dorsal view).

2.2.3. Experimental protocol

One lumpfish was placed in each tank and left to acclimatise for 10 minutes before the start of the filming. The fish were filmed for 30 minutes and then euthanized according to the Home Office schedule 1 licence by an overdose of anaesthetic (2-Phenoxyethanol) and exsanguination, by trained CSAR staff in accordance with the Animals Scientific Procedures Act (ASPA).

The fish were then pat-dried using paper towel to remove excess water, measured (total length, standard length), weighed, and photographed. They were then scored for welfare using the operational welfare score index for lumpfish (LOWSI), as per Gutierrez-Rabadan *et al.* (2021). To determine the LOWSI, lumpfish were scored for external body damage, fin damage, eye condition and suction cup deformities using a 3-point scale, 0 being no damage/deformity, 1 being moderate damage/deformity and 2 being severe damage/deformity. They were also weighed, and the BMI was calculated based on relative weight using the life stage-specific length-weight regression coefficients given in Gutierrez-Rabadan *et al*. (2021).

Photographs were taken with a Canon EOS 800D/EFS digital camera fitted with an 18-55mm lens and a ring-flash, mounted on a tripod. Two polystyrene moulds of an oval shape (one for wrasse and one for lumpfish) were made to keep the fish in position during the photographs. Three photographs were taken of each fish to capture the ventral, lateral and dorsal views (Figures 2.2.a and 2.2.b). A ruler and a label were included in the photographs for identification purposes.

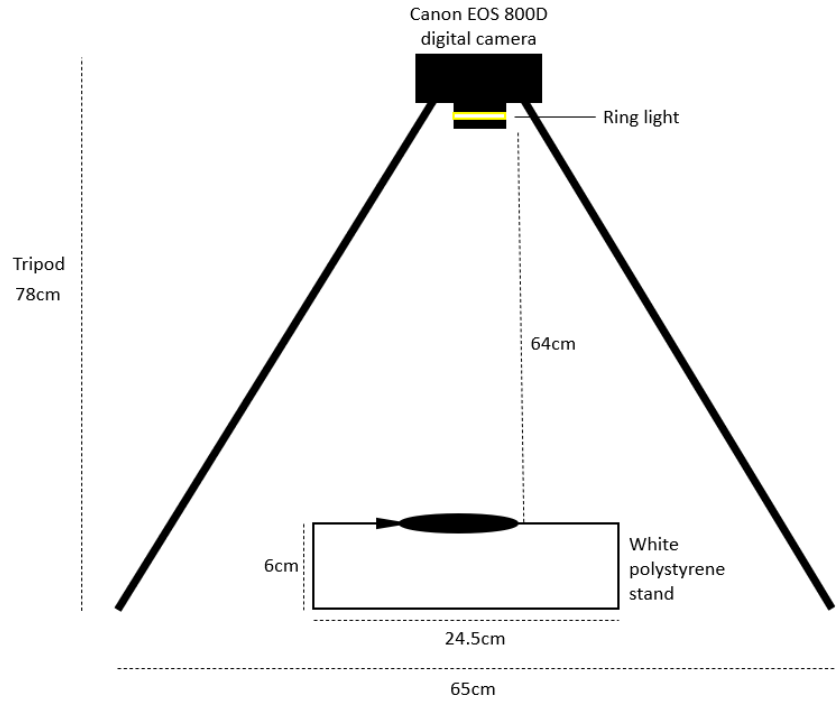

Figure 2.2.a. Photography set-up.

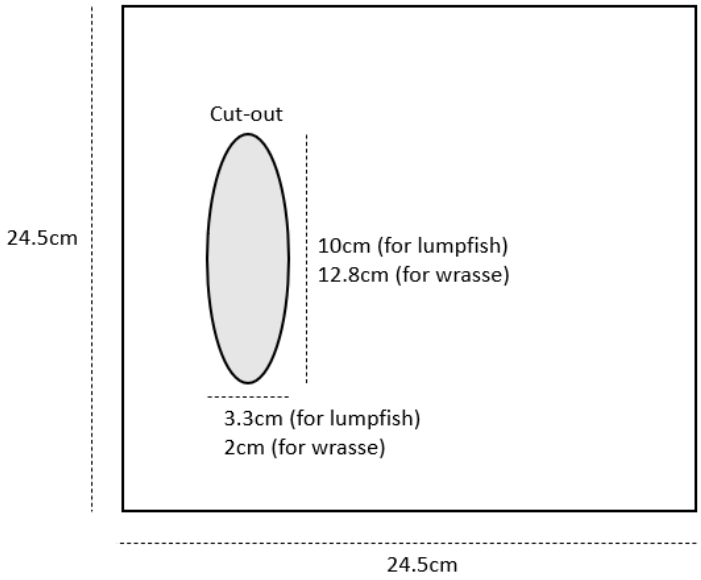

Figure 2.2.b. Square polystyrene stand for positioning fish for photographs.

2.2.4. Landmark recognition and BMI assessment using AI from videos of lumpfish

For each video, the species was detected by Visifish using Detectron2 (Detectron2, 2019) and the area of interest (the lumpfish) was cropped and resized. Detectron2 is an AI system that that uses algorithms to efficiently detect key points and label different body parts from images. It accurately identifies and labels every object within an image, encompassing even background elements (Divya & Peter, 2022). The machine vision system DeepLabCut™ (Mathis Lab, 2023) was then used for point detection and the generation of body size ratios by Visifish. DeepLabCut is a highly effective tool for markerless pose estimation in both 2D and 3D, which utilises transfer learning through deep neural networks (Mathis Lab, 2023). Visifish then processed all of the videos using these systems and measured the pixels between the key points on each fish. Visifish then converted the pixels into measurement ratios, and these used in comparison with known measurements. The same steps were carried out for the top and lateral view videos. The proportion of body measurement calculated from the top view was length/width and the proportion from the lateral view videos was length/height. The following 8 landmarks were used to train the algorithm on top view images: 1. Tip of head/mouth; 2. Head - left side; 3. Head - right side; 4. Left side; 5. Right side; 6. Back; 7. Caudal fin base, and 8. Caudal fin tip. For lateral view images, the following 9 landmarks were used for training: 1. Jaw; 2. Head; 3. Tallest point of hump; 4. Anterior dorsal fin base; 5. Midpoint of caudal peduncle; 6. Caudal fin tip; 7. Anterior anal fin base; 8. Posterior anal fin base; 9. Posterior pelvic fin base (Figure 2.3). They were then used to generate body size ratios by Visifish (Figures 2.4.a and 2.4.b).

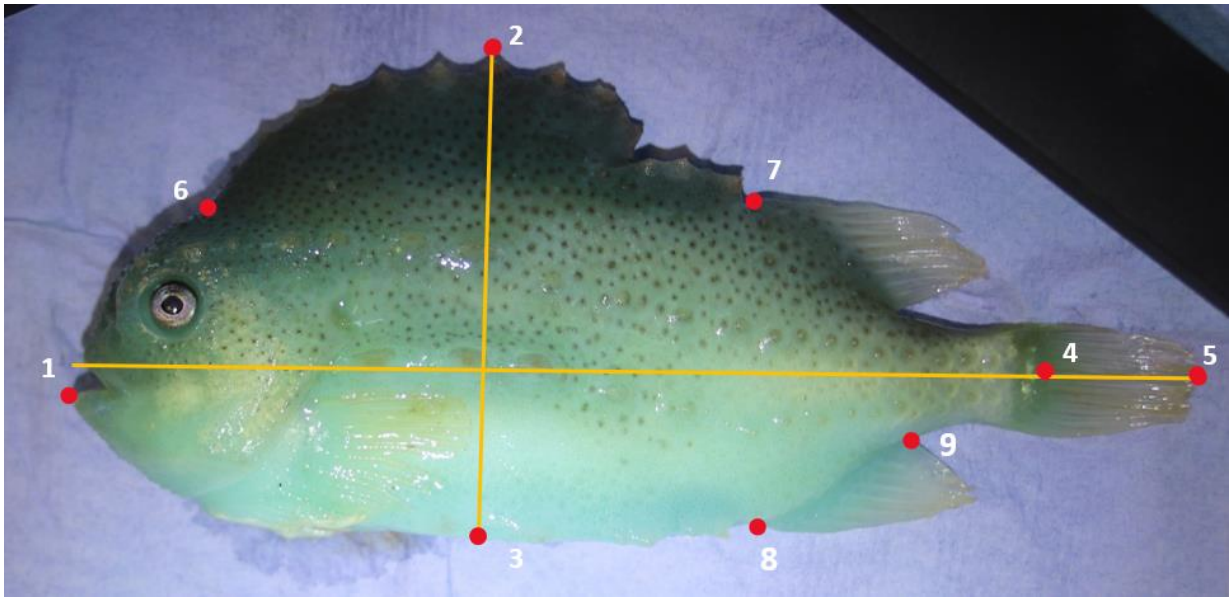

Figure 2.3. Point landmarks used for image recognition and generation of body size ratios in lumpfish using DeepLabCut (lateral view).

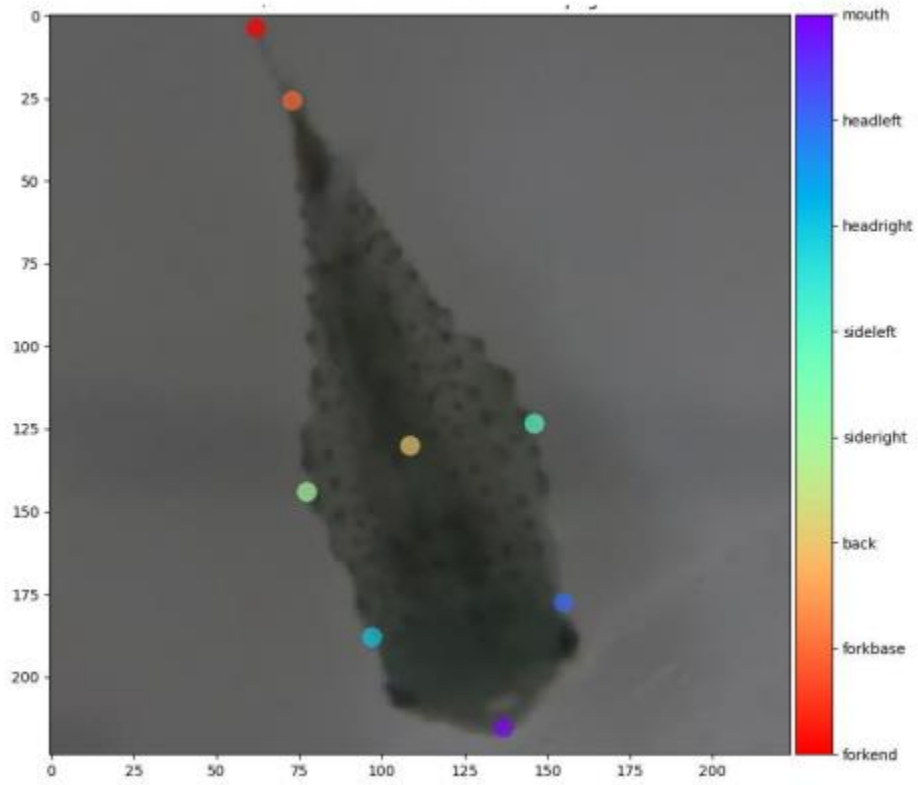

Figure 2.4. Point landmarks used for image recognition and generation of body size ratios in lumpfish using DeepLabCut (dorsal view)

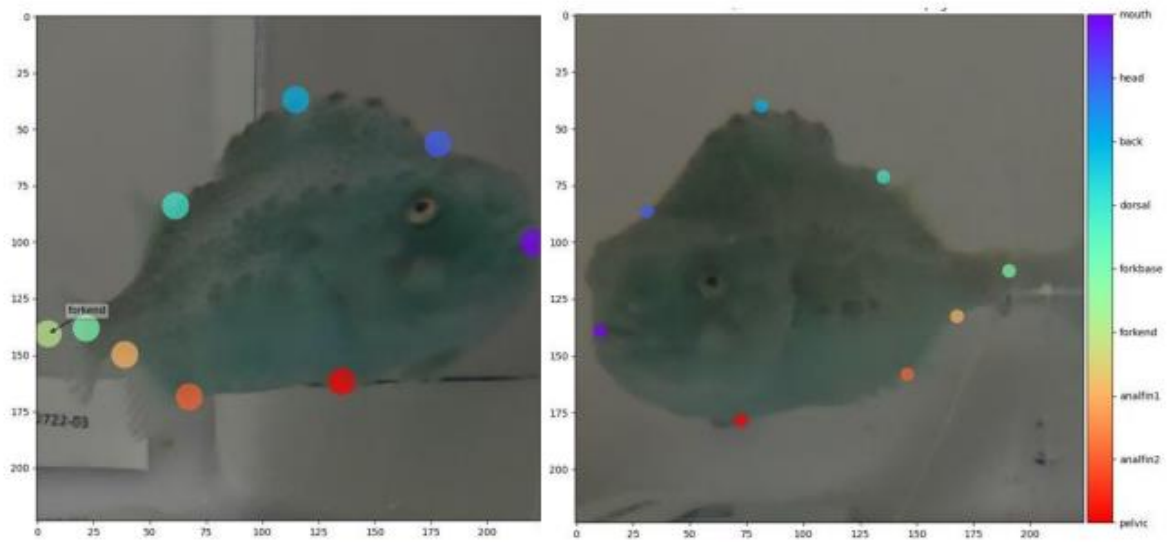

Figure 2.4.b. Image of lumpfish (lateral/frontal view) taken from one of the experiment videos and analysed by DeepLabCut to detect landmarks and generate body size ratios.

The width/length ratio was calculated for the top view videos, and the height/length ratio for the lateral view videos of the same fish. Using the measurements that Visifish generated, the fish were then classified into three BMI categories: normal, underweight, and emaciated using the same cut-off points and protocol as in subsection **2.2.3** (Gutierrez-Rabadan *et al*., 2021).

2.2.5. BMI assessment from still photographs of lumpfish and ballan wrasse using ImageJ

ImageJ (Chang *et al*., 2011; Vargas-Sanchez *et al*., 2017; Andrialovanirina *et al*., 2020) was used to measure 60 ballan wrasse and 60 lumpfish using still photographs of the same individuals that were filmed in section 2.2.4 above. A ruler was included in every photograph to use to set a scale in ImageJ, to ensure accuracy. The scale is set by adding 2 points and drawling a line between them on a known distance on the ruler (i.e. a line marked between 1cm and 2cm on the ruler is known to be a 1cm distance). On ImageJ, key points, as stated in the previous section, were manually marked out and a line drawn in between. The system then produces a measurement for every line (see Figure 2.5). Body height, total length, and standard length were measured using photographs on the lateral view of the fish (Figure 2.5). Width was measured using photographs of the ventral view of the fish. The same body size measurements were obtained directly on the fish with a ruler for comparisons with photographic measurements obtained using ImageJ.

The BMI of lumpfish was calculated as the ratio between the observed and expected weight for a given length using the length-weight regressions detailed in Gutierrez-Rabadan *et al.*, (2021). BMI values based on known lengths and weights were then compared to values derived from body ratios obtained from photographs using ImageJ.

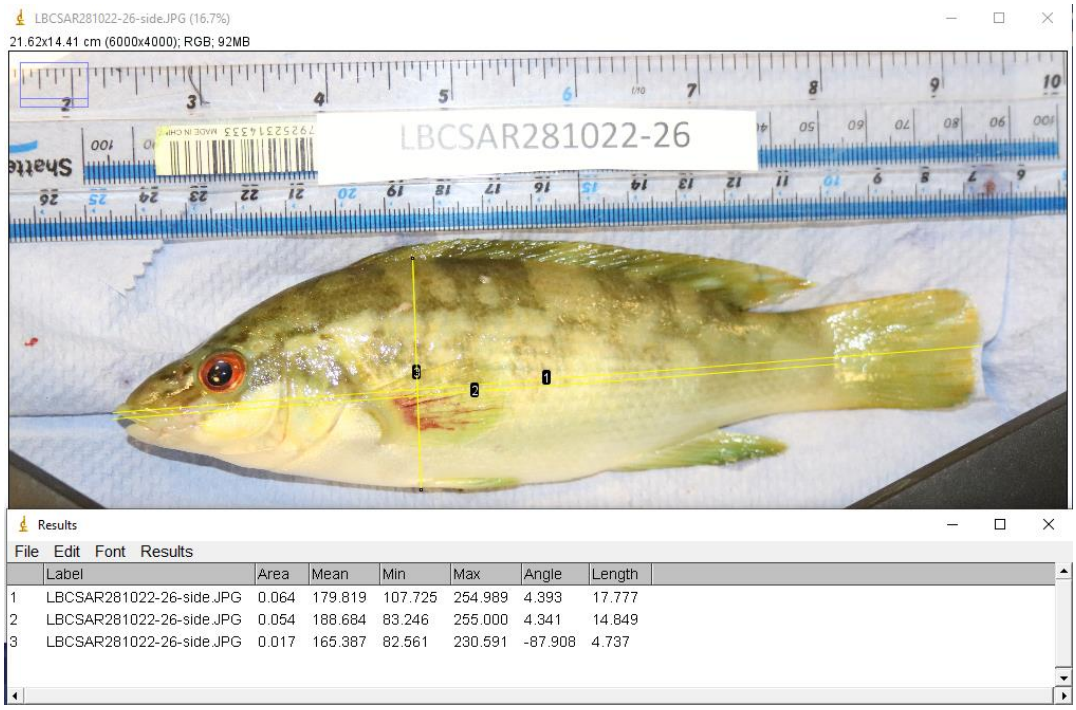

Figure 2.5. Screenshot showing the measurements of a ballan wrasse using ImageJ.

2.3. Data and statistical analysis

Analysis was completed using the statistical computing program R (version 4.2.3). Paired t-tests were used to compare results generated from AI and ImageJ to known body measurements, both in body ratios and predicted BMI. Linear models were used to determine if body ratios (the ratio of height to total length, and of width to total length) could be used to predict BMI using (1) direct body measurements, (2) measurements from still photographs obtained using Image J and (3) AI measurements from videos.

For testing, lumpfish were classified into two simplified BMI categories based on Gutierrez-Rabadan *et al*. (2021): 'underweight' if the BMI was less than 90% or 'normal' if the BMI was greater than 90%.

Measurements for both lumpfish and ballan wrasse were compared with known measurements to determine if ImageJ was an accurate tool for measuring, however, only lumpfish were used for the BMI calculations. The published work by Gutierrez-Rabadan *et al*. (2021) includes an expected weight calculation to be used in BMI calculations for lumpfish, which does not currently exist for ballan wrasse in literature. For the Visifish AI ratios, only lumpfish were used due to time constraints.

2.4. Results

2.4.1 Accuracy of landmark recognition and body ratio estimates using AI

Of 60 lumpfish filmed, body measurements could be generated for 21 fish through the use of AI. No statistically significant difference was found between known body ratios of width/standard length ($p = 0.597$; Figure 2.6) or width/total length ($p= 0.326$; Figure 2.7). Absolute mean errors between true and AI-derived body ratios were 5.40% for width/total length and 6.98%. for width/standard length.

2.4.2 Accuracy of body ratio estimates using still photographs and ImageJ

Body measurements obtained from photographs of ballan wrasse and lumpfish using ImageJ were not statistically different from direct measurements on the same fish (paired t-tests) for body height ($p= 0.74$, mean error = 3.7%; Figure 2.8, standard length ($p= 0.57$, mean error = 3.7%, Figure 2.9), total length $p = 0.57$, mean error = 3.7%, Figure 2.10), or width ($p = 0.57$, mean error = 3.7%, Figure 2.11). All body size ratios obtained from photographs using Image J were significantly correlated with the true values, but photographic measurements were much better predictors of height/total length ($r = 0.97$, $p<0.001$) than of width/total length ($r = 0.40$, $p < 0.001$).

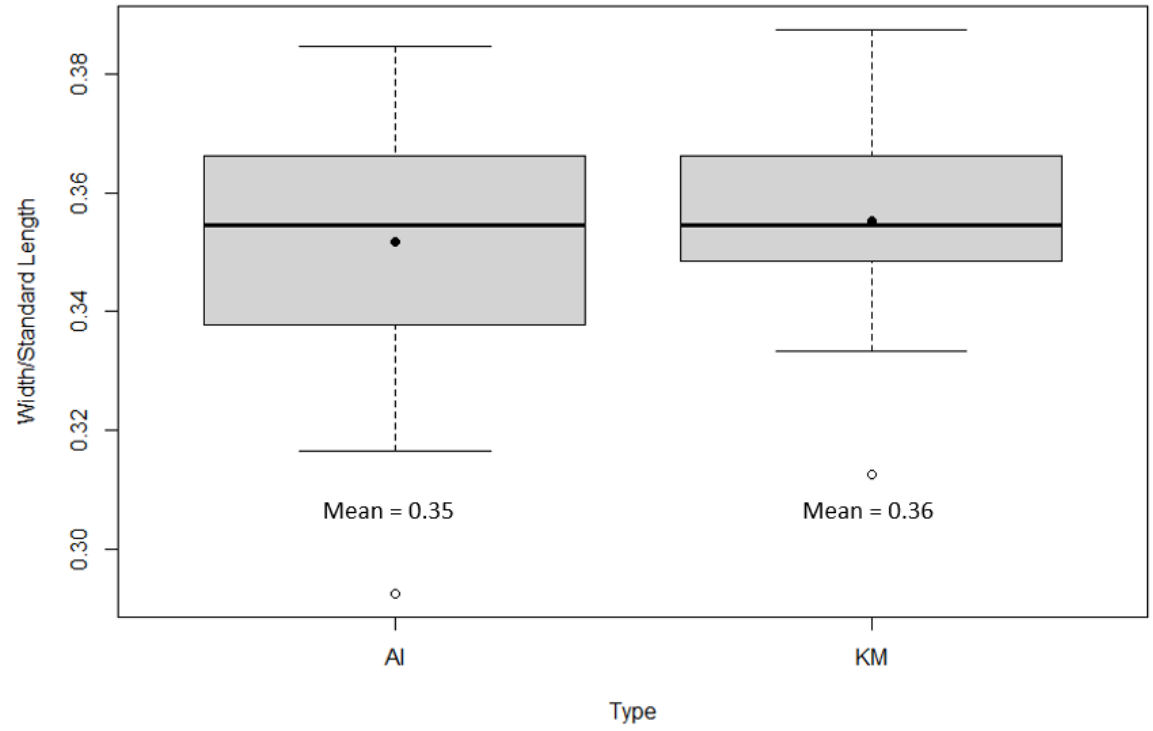

Figure 2.6. Comparison and means of the AI results (AI) and known measurements (KM) of standard length/width (mm) of lumpfish (n= 21).

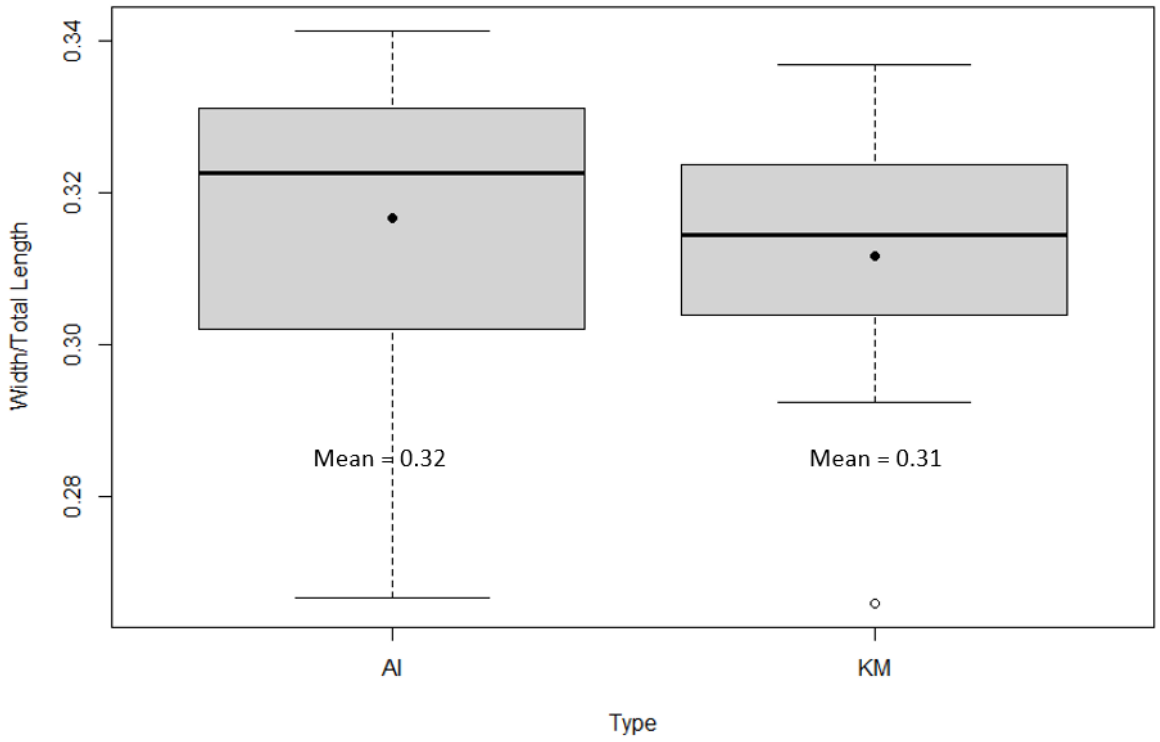

Figure 2.7. Comparison and means of the AI results (AI) and known measurements (KM) of total length/width (mm) of lumpfish (n= 21).

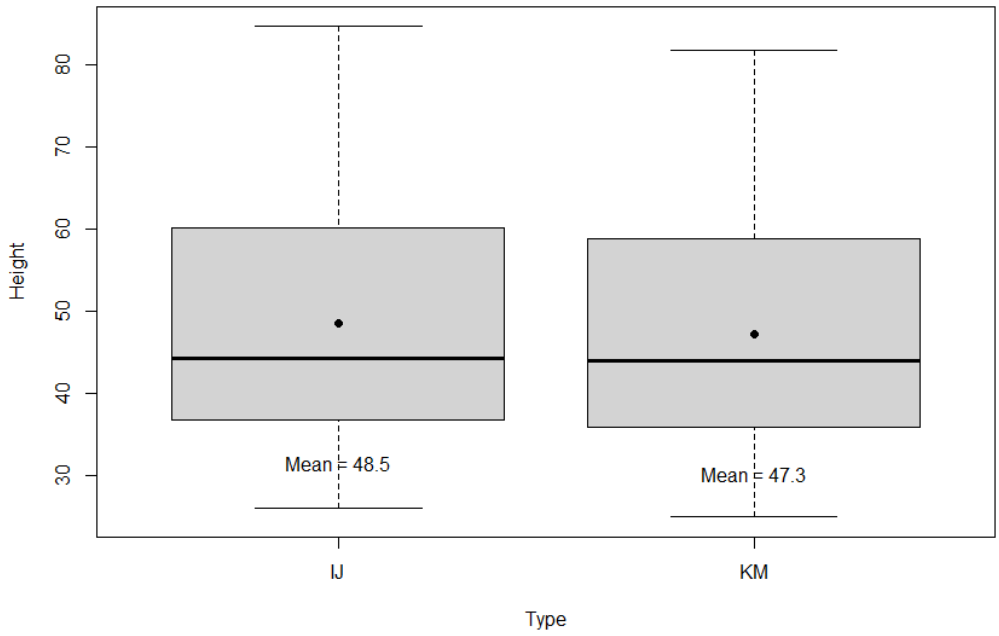

Figure 2.8. Comparison and means of the ImageJ results (IJ) and known measurements (KM) of height (mm) of lumpfish and Ballan wrasse (n= 124).

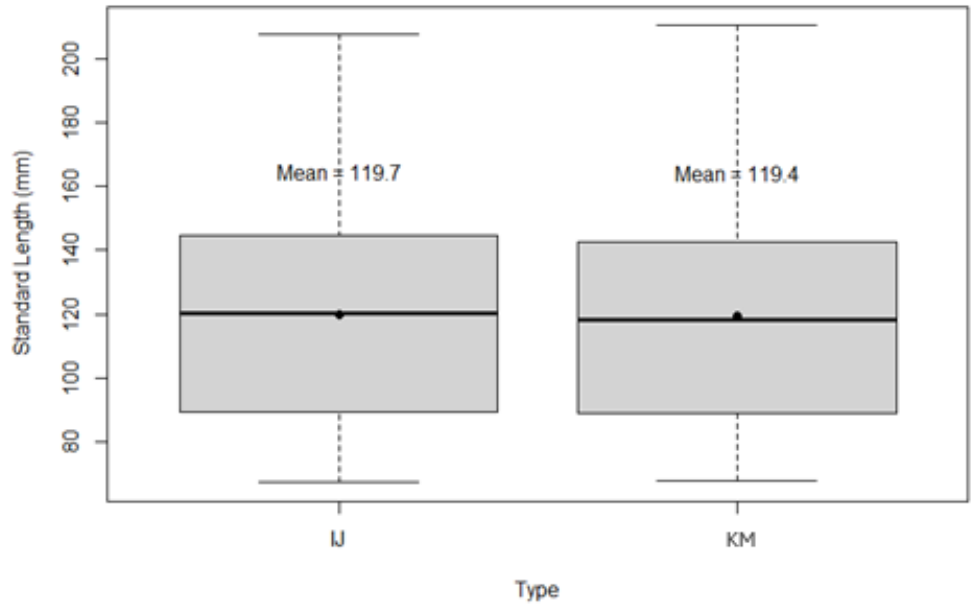

Figure 2.9. Comparison and means of the ImageJ results (IJ) and known measurements (KM) of standard length (mm) of lumpfish and Ballan wrasse (n= 124).

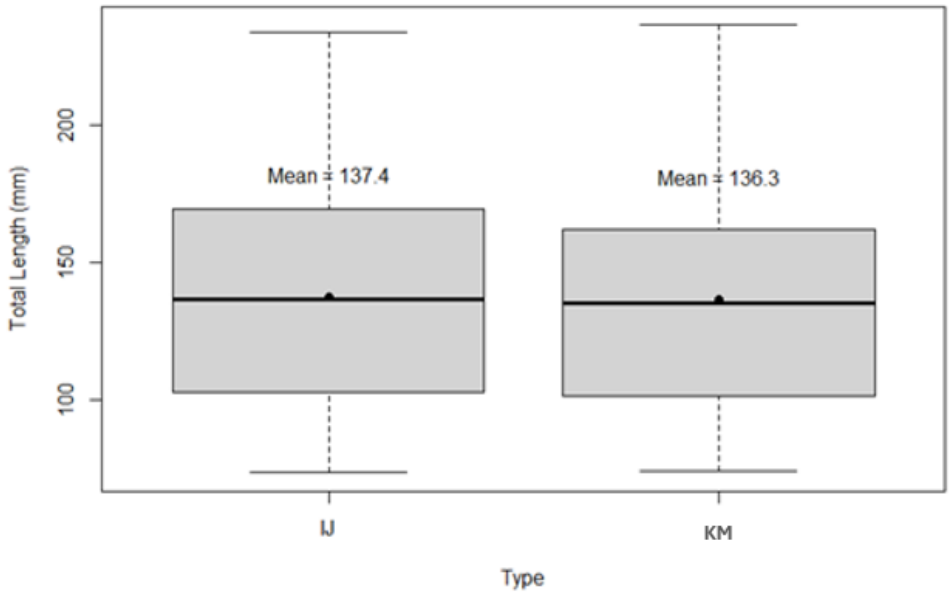

Figure 2.10. Comparison and means of the ImageJ results (IJ) and known measurements (KM) of total length (mm) of lumpfish and ballan wrasse (n= 162).

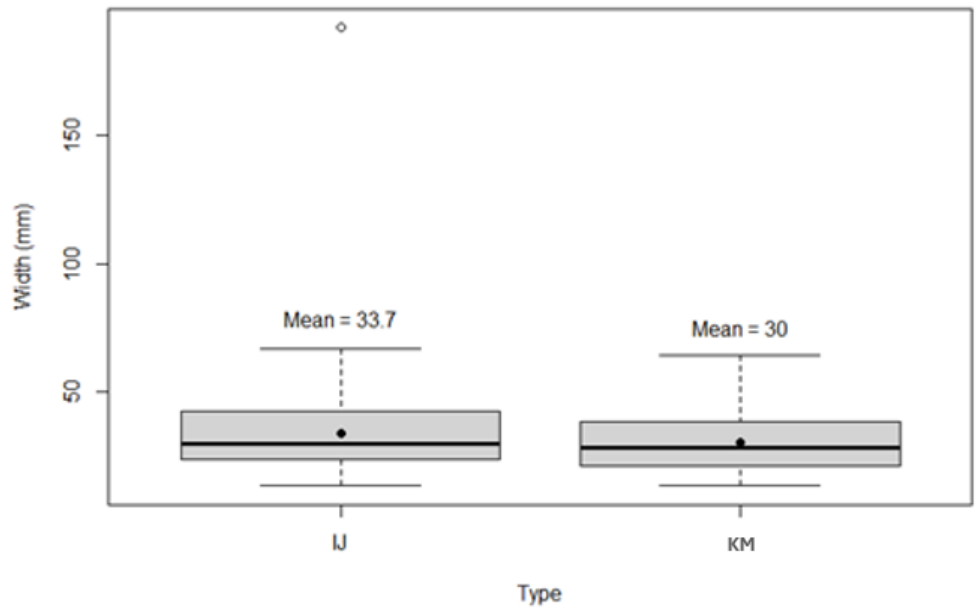

Figure 2.11. Comparison and means of the ImageJ results (IJ) and known measurements (KM) of width (mm) of lumpfish and Ballan wrasse (n= 124).

2.4.3. Accuracy of BMI estimation from body ratios using photographs and videos

Ratios using known (true) measurements of lumpfish were used to predict true BMI. The height/total length ratio predicted BMI with 61% accuracy (adjusted R^2= 0.614, F-statistic= 93.24, df = 57, p<0.001), whereas width/total length predicted true BMI with 59% accuracy (adjusted R^2= 0.586, F-statistic= 83.08, df = 57, p<0.001) (figure 2.12.). The most accurate way to predict true BMI from true body measurements was by using both ratios in the same linear model (height/width and width/total length). This predicted BMI with 73% accuracy (adjusted R^2= 0.731, F-statistic= 78.15, df = 2,56, p<0.001).

This was repeated using the same ratios of ImageJ measurements of lumpfish. The ImageJ height/total length ratio predicted BMI with 42% accuracy (adjusted R^2= 0.432, F-statistic= 45.16, df = 57, p<0.001) (figure 2.14.), whereas width/total length predicted true BMI with 39% accuracy (adjusted R^2= 0.399, F-statistic= 37.77, df = 57) (figure 2.15.). The most accurate way to predict true BMI from ImageJ measurements was by using both ratios in the same linear model (height/width and width/total length). This predicted BMI with only 45% accuracy (adjusted R^2= 0.454, F-statistic= 25.11, df = 2,56, p<0.001). BMI estimates based on body ratios from photographs obtained from Image J were closely related to true BMI values (Figure 2.16).

As the Visifish AI produces ratios of width/total length and width/standard length of lumpfish, these ratios of known (true) measurements were used first to predict BMI. Based on the aforementioned analysis, we know that width/total length can predict true BMI with 59% accuracy (figure 2.13.). The ratio of width/standard length predicted true BMI with 23% accuracy (adjusted R^2= 0.23, F-statistic= 6.96, df = 19, p= 0.01) (figure 2.17.). The most accurate way to predict true BMI from these known measurements was by using both ratios (width/standard length and width/total length). This predicted BMI with 81% accuracy (adjusted R^2= 0.811, F-statistic= 43.97, df = 2,56, p<0.001).

This was repeated using lumpfish measurement ratios produced by the Visifish AI. The AI width/total length ratio cannot predict true BMI (adjusted R^2= 0.117, F-statistic= 3.67, df = 19, p= 0.07). The AI width/standard length ratio cannot predict true BMI (adjusted R^2= 0.063, F-statistic= 2.35, df = 19, p= 0.14). Using both ratios, prediction of BMI was slightly better but not sufficiently accurate (adjusted R^2= 0.15, F-statistic= 2.76, df = 18, p= 0.08).

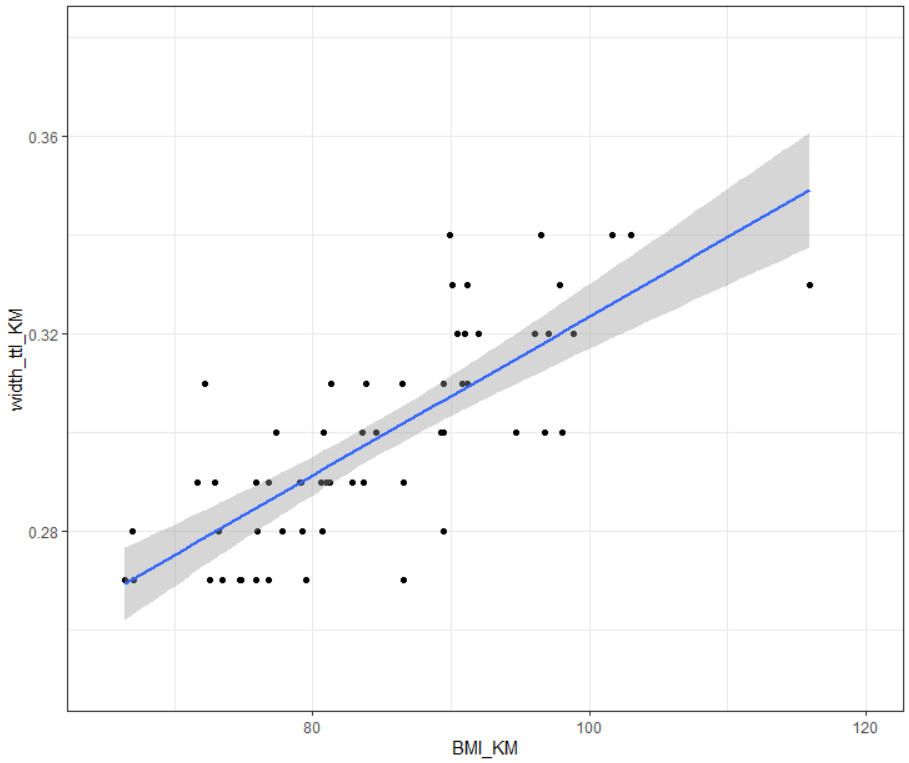

Figure 2.12. The ratios of known width/total length (w_ttl_KM) of lumpfish (n=21) compared with known BMI (BMI_KM).

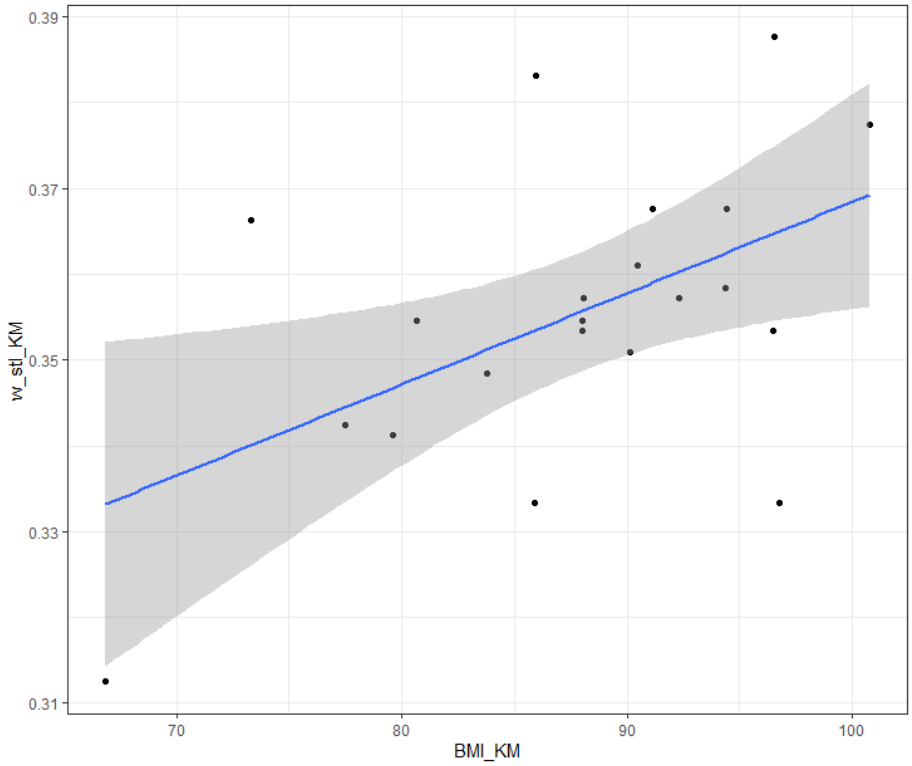

Figure 2.13. The ratios of known width/standard length (w_stl_KM) of lumpfish (n=21) compared with known BMI (BMI_KM).

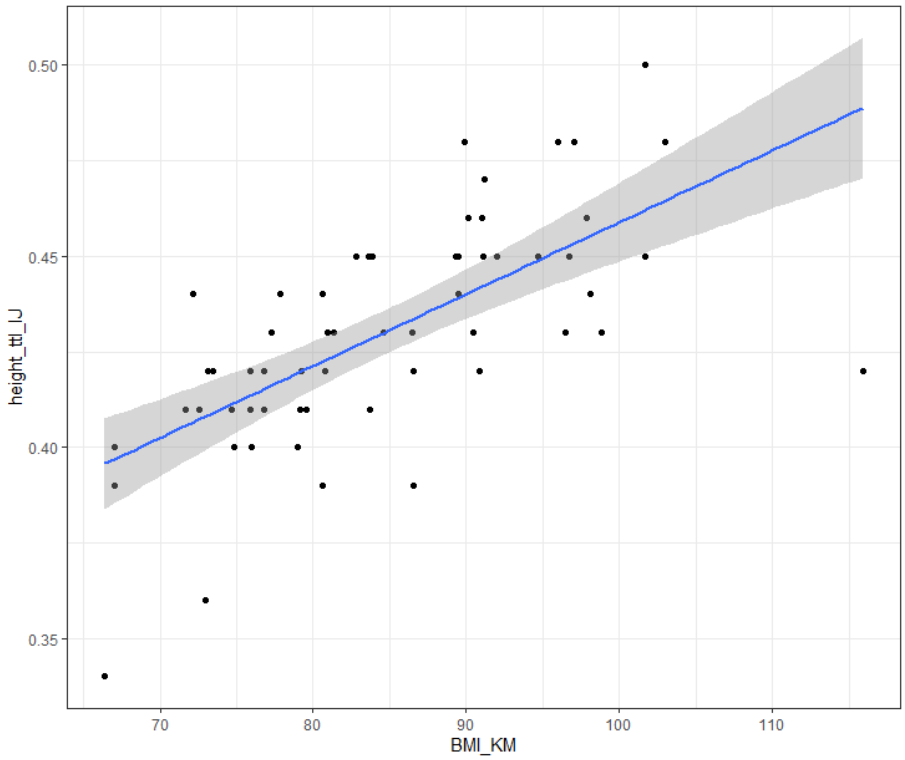

Figure 2.14. The ratios of ImageJ height/total length (height_ttl_IJ) of lumpfish (n=21) compared with known BMI (BMI_KM).

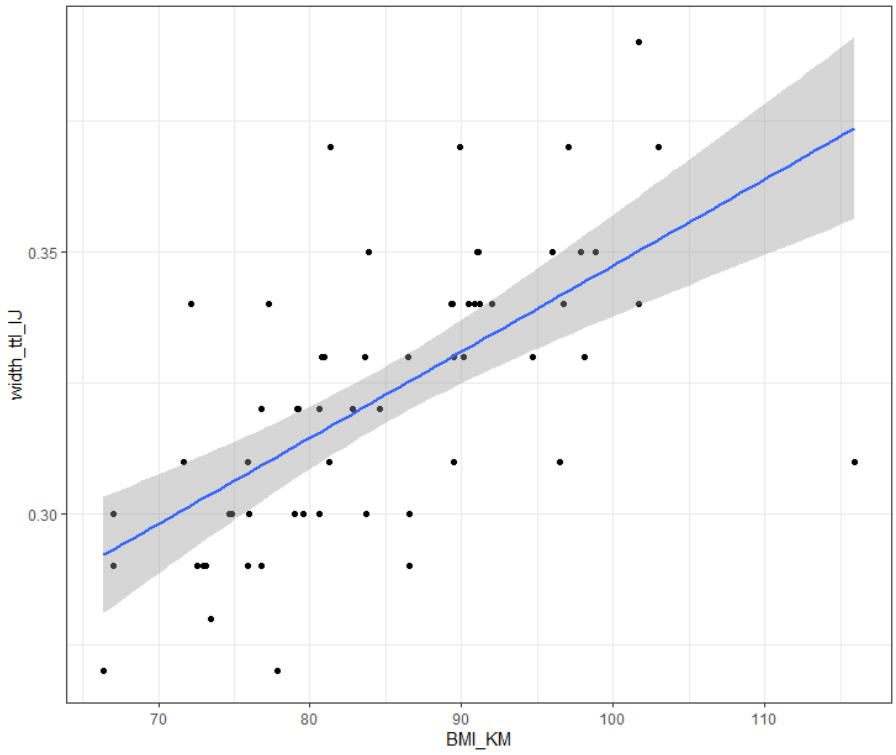

Figure 2.15. The ratios of ImageJ width/total length (width_ttl_IJ) of lumpfish (n=21) compared with known BMI (BMI_KM).

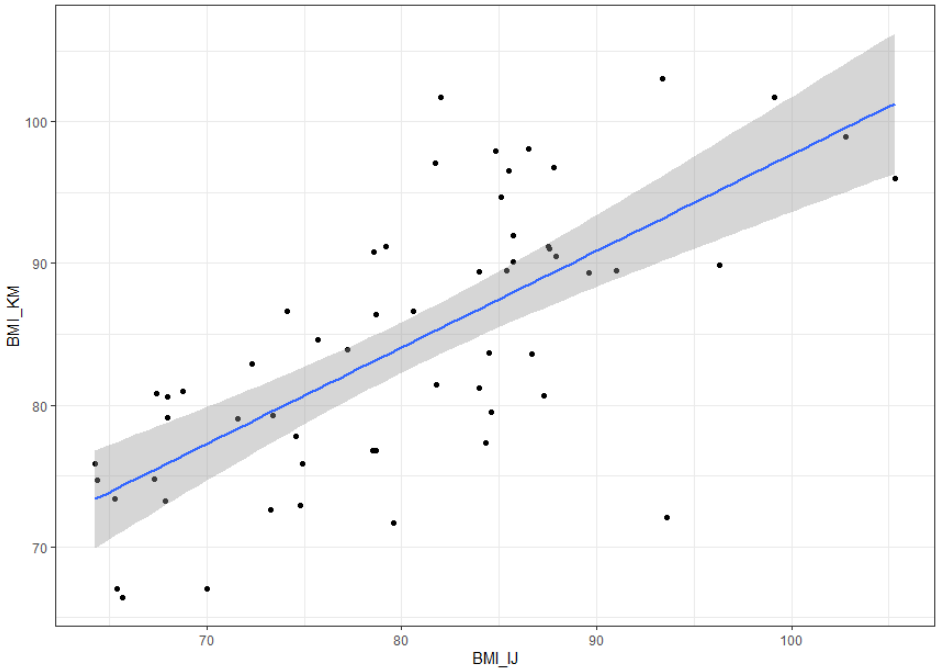

Figure 2.16. The comparison of BMI of lumpfish (n=21) calculated using ImageJ (BMI_IJ) and BMI calculated using known measurements (BMI_KM).

2.4. Discussion

Health and welfare within the aquaculture industry are adversely affected by stress events such as crowding, transportation, confinement, poor water quality, and handling (Huntingford *et al*., 2006). Prolonged stress in farmed fish has been known to cause appetite loss, a reduction in growth, reproductive issues, problems with the immune response and increased cortisol levels (Weyts *et al*., 1999; Braithwaite and Ebbesson, 2014). In addition to affecting metabolism and cell processes, these changes will impact the body's natural immunity, and thus on disease outcomes (Vazzana *et al*., 2002). An increase in stress load and duration can lead to weak primary barrier functions (mucous and epidermal surfaces) and lead to increased risk of disease (Segner *et al*., 2012). The effects of disease on fish farms can reduce reproductive performances and feed conversion efficiency, affecting growth, overall performance, and increasing mortality levels. An effective health management plan for farmed fish should include stress reduction as a primary goal. Reducing stress can help to maintain a healthy fish population and ensure optimal performance. Introducing image-based monitoring for fish would minimize handling, limit stress, and therefore reduce disease and economic losses (Mustafa and Campbell, 2001; Tavares-Dias and Martins, 2017).

While image-based methodologies could be greatly beneficial, there are problems and limitations with this type of technology (Darapaneni *et al*., 2022). Due to the nature of differing fish species, variable conditions, and the difficulty of acquiring high-definition images of fish, image-based methods of disease diagnosis are generally not accurate (Berbedo, 2014). Additionally, such methods rely on clear, high-quality images for detection of disease, which can be difficult or impossible to obtain on a fish farm or hatchery due to exposure to poor lighting or weather conditions (Li *et al*., 2022). This is the case for monitoring other factors underwater, including behaviour, size, and species recognition (Abangan *et al*., 2023). Underwater recognition is inevitably susceptible to factors such as shadow, blur, noise, colour distortion, or illumination which can lead to errors in recognition (Gao *et al*., 2019). However, some image-processing technology can be employed to improve fish image quality (Petit *et al*., 2009).

While AI and image-based monitoring can reduce fish stress, there are further limitations to consider. The validity of automatically collected measures is often evaluated through a comparison with human observations, either through direct observation or from video (Rushen *et al.*, 2012). Automating animal welfare assessments, especially those conducted by

farmers or health professionals, may pose a risk of reducing the amount of time that farmers spend physically observing their animals, therefore risking disease outbreaks, injuries, and other factors. Conversely, automation can also provide farmers with information about their animals that they may not have access to otherwise (Rushen *et al*., 2012).

While ImageJ proved to be an accurate tool for measuring fish lengths, heights and true BMI, it still requires handling and possibly euthanising the fish to obtain good-quality images for analysis. More experiments testing this method with videos rather than images of fish would have to be carried out to determine if this can be used to limit handling and therefore reduce stress and improve welfare. While this method does not reduce stress, it can still be utilised as a useful tool for farmers to obtain accurate measurements if they don't have time to manually collect the measurements.

The Visifish AI system has the potential to provide fish measurements without handling the fish or causing undue stress. The results deemed that the measurement ratios produced by the AI system had no statistically significant difference to the known measurements but could not be used to determine accurate BMI. The system was also only able to produce results for 21 out of the 60 fish. As these fish were filmed in perfect or near perfect laboratory conditions, more results would be expected. More work must be carried out to improve and verify this system for use in the aquaculture industry. The AI system must be further trained using more images of fish of known measurements to produce more accurate results. Testing measurements using different key points could also be explored in further trials. If validated, this system could become an integral part of routine fish health and welfare monitoring, thus limiting disease and generally poor welfare. This could lead to better fish production, improved animal welfare, and increased profits for aquaculture producers.

2.5. Conclusion

This chapter explored the use of image-based methodologies to determine body condition in 2 cleaner fish species. The image processing software ImageJ was used to measure fish using photographs and a machine vision system (AI) developed by Visifish was used to generate measurement ratios from videos. Both methods were compared with known measurements for statistical analysis and validation. ImageJ was determined to be an accurate method of measuring fish using images. The Visifish AI system generated accurate measurement ratios but only for a small percentage of fish from the sample videos. Measurements generated from ImageJ were used to calculate BMI for lumpfish and compared with known BMI results. The measurements generated from ImageJ were found to determine BMI with 45% accuracy if height, width, and total length measurements are combined. Predicted BMIs for each fish calculated using the ImageJ ratios were also found to have no statistically significant difference to known BMIs. The measurements generated by the Visifish AI system were unable to determine true BMI. The Visifish AI must be improved, and samples and analysis done to determine if the system can be employed to measure fish reliably. Determining the body condition of fish in the aquaculture industry from images has the potential to greatly reduce fish handling and stress and therefore reduce mortality levels.

Chapter 3 – Novel behavioural indicators and delousing efficacy

3.1. Introduction

While cleaner fish have been widely used in the aquaculture industry to control sea lice and other parasites, there are several issues that can impact their effectiveness and welfare. Cleaner fish are susceptible to various diseases (Erkinharju *et al.*, 2020) and can become stressed in different farm environments such as sea cages or tanks, which can impact their survival and efficacy. Juvenile lumpfish are usually found around coastal areas, but mature lumpfish can be found in pelagic environments around kelp or floating seaweed (Vandendriessche *et al.*, 2007), which they adhere to using their ventral suction disc. Ballan Wrasse, which are farmed for use in sea cages, are most commonly found in rocky shore areas and kelp beds (Figueiredo *et al.*, 2005; Villegas-Rios *et al*., 2013). To optimise their effectiveness as cleaner fish in the aquaculture industry, it is critical to provide Wrasse species and/or Lumpfish with an environment that is similar to their natural habitat. This can include the presence of real or artificial seaweed or kelp, as well as other types of substrates that provide shelter and hiding places for the fish (Imsland *et al.,* 2015). By creating an environment that is conducive to the natural behaviour and preferences of cleaner fish, farmers can improve their survival, health, and effectiveness.

Mortality rates of cleaner fish can be high, which can result in additional costs for farmers. In 2021, nearly 30 million farmed cleaner fish were sold in Norway (Directorate of Fisheries, 2022), with some reports of high or near total mortality throughout the production cycle (Norwegian Veterinary Institute, 2022). The use of cleaner fish can raise ethical concerns and farmers must take steps to ensure that the welfare of the cleaner fish is considered, and that they are not being subjected to unnecessary stress or harm. While the use of cleaner fish in aquaculture can be an effective and sustainable method of controlling parasites (Bolton-Warberg, 2017), there are several challenges that need to be considered and managed. By addressing issues related to disease, compatibility, availability and cost, welfare, and alternative methods, farmers can optimise the use of cleaner fish to ensure sustainable and effective parasite control.

For many years, animal behaviour has been used to enhance management practices and animal welfare in agriculture (Fraser & Broom, 1997). The behaviour of fish in aquaculture, however, is less understood than the behaviour of those in terrestrial agriculture (Bui *et al*.,

2017). As aquaculture is one of the fastest growing global primary industries, understanding the behaviours of the species that we farm could facilitate more sustainable, optimal husbandry and welfare practices (Martins *et al*. 2012; Teletchea & Fontaine, 2014).

This chapter focuses on the behavioural responses of lumpfish and ballan wrasse in two different experiments. The first is an experiment adapted from Champneys *et al*., 2018 and Whittaker *et al*., 2021 to determine personality components based on the fish reactions to different stimuli, including a novel object and a mirror. Fish exhibit aggressive behaviour towards their own reflection, indicating that they perceive it as a separate individual (Desjardins and Fernald, 2010). Thus, studying fish responses to a mirror is a good measure of aggression in a species. Neophobia, fear of the new, is observed in species responses to novel environments or objects (Champneys *et al*., 2018). Neophobia has been extensively studied in relation to predatory avoidance and new or unfamiliar food (Greenberg, 1990). The second experiment involved recording fish reactions to salmon models, 3D-printed to the measurements and shape of an Atlantic salmon (*Salmo salar*) smolt with differing numbers of latex sea lice attached (zero, one and six), also made to the measurements and shape of a caligus sea lice (*Caligus elongatus*). The lumpfish and ballan wrasse reactions to the different salmon models will be compared with the personality components calculated from the first experiment to link these components with behaviours that could potentially be associated with delousing.

3.2 Materials and Methods

Experiments were carried out to determine novel behavioural indicators in two cleaner fish species and if they influence delousing efficacy. The first experiment involved behavioural observations of 60 Ballan wrasse and 60 lumpfish in a controlled arena. Observations and recordings were taken of the fish' interactions with a novel object (Lego) and a mirror, as well as other variables. The second experiment involved placing half of the fish from the first experiment (randomly selected) in a new tank with a selection of 3D-printed Atlantic salmon models with either six, one or no silicone sea lice attached on to one side. Interactions with the models, as well as other factors, were observed and recorded. The fish behaviours/interactions from the first experiment were categorised into personality components. These personality components were compared with the fish interactions in the second experiment, to determine potentially good delousing behaviours can be identified.

3.2.1. Source and rearing of fish

The source and rearing of the fish were the same as that detailed in section **2.2.1.**

The experimental procedure was approved by Swansea University, Animal Welfare Ethical Review Body, permit IP-2223-07.

3.2.1. Behavioural profiling experiment set-up

This design and protocol for this experiment was adapted from Whittaker *et al*., 2021. A white plastic rectangular tank (L120 cm × W55 cm × D25 cm) was used for this part of the experiment. The tank was divided into four equal zones (~30 cm each – Zone 0, Zone 1, Zone 2, Zone 3). Black lines were drawn to delineate zones and facilitate analysis. Zone 0 was fitted with a removable overhead cover. There was a sliding door separating zone 0 from zone 1 and another separating the mirrors from the rest of the tank. These sliding doors were operated remotely by a pulley system, reducing disruption. The tank was also surrounded by a dark tarpaulin screen to further minimise disruption. The tarpaulin had small holes cut into it to allow viewing of the tank. A small red Lego brick (novel object), which was attached to the centre of zone 2 with marine-grade silicone. A black 10 cm reference circular area was marked around the Lego. The wall of the tank at the end of zone 3 was fitted with a mirror and a black reference line was drawn 10 cm in front of the mirror. The reference line was

drawn using a thin line marker to limit the influence on fish behaviour. As the fish showed no reaction to the line, it was not deemed to influence behavioural responses.

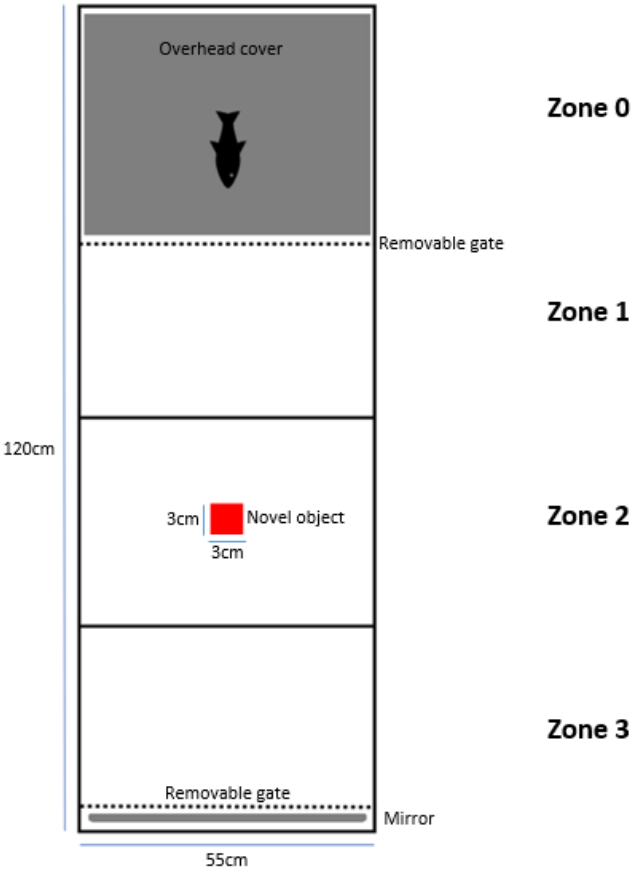

Figure 3.1.a. Behaviour arena (dorsal view) - Personality profiling using novel behavioural indicators experiment set-up.

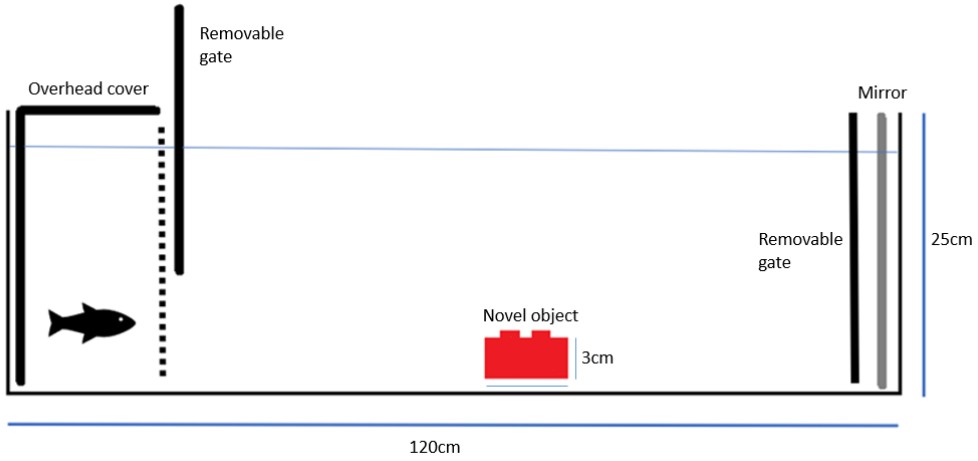

Figure 3.1.b. (lateral view)

3.2.2. Behavioural profiling experiment protocol

The tank was filled with water from the same system that feeds the home tanks (ensuring the same temperature, pH, salinity, and oxygen levels), to avoid any additional stress to the fish. The overhead cover in zone 0 was removed to allow the fish to be placed inside and the sliding doors were pulled down to separate the zones. A ballan wrasse or lumpfish was randomly selected from their home tanks and placed in zone 0 of the experiment tank. The overhead cover was then placed over zone 0 and the fish were left to acclimatise for 10 minutes. A stopwatch was used to keep time. After 10 minutes, the remote pulley system was used to slowly lift the sliding door, allowing the fish to pass through into the next zone. The sliding door wasn't fully lifted out of the water. It was left with the bottom of the door touching the water surface, this was to avoid any water dripping and disturbing the fish.

Once the door was open, the stopwatch was started, and the fish was observed for 10 minutes. The following metrics were recorded:

a. Latency (seconds) to leave the shelter

b. Approaches to novel object (No.) within 10 cm (a reference circle marked on the tank bottom will help determine this)

c. Charges to novel object (No.), i.e., when fish touches the novel object

d. Returns to shelter (No.)

After 10 minutes, the pulley system was used to lift the door to reveal the mirror in zone 3. Once the door was open, the stopwatch was started, and the fish was observed for 10 minutes. The following metrics were recorded:

a. Approaches to mirror (No.) within 10 cm (a reference line marked on the tank bottom may help determine this)

b. Charges at mirror (No.), i.e., when fish touches the mirror

c. Returns to shelter (No.)

After 10 minutes, the fish was removed from the tank and placed in the acclimatisation pen in the second experiment tank. The original tanks were drained, flushed and the water was replaced to avoid a build-up of stress hormone.

3.2.3. Model salmon interactions experiment

A dark blue plastic circular tank (W124cm x D65cm) was used for this experiment. The tank was divided into four equal quarters (Zone 0, Zone 1, Zone 2, Zone 3) using dark red tape to delineate zones and facilitate analysis. There was a circular acclimatisation pen in the centre of the tank, made of white plastic mesh. The tank was surrounded by a dark tarpaulin screen to minimise disruption. The tarpaulin had small holes cut into it to allow viewing of the tank. The tank contained three 3D-printed salmon models (see specifications at the end of the chapter). Each zone contained either one salmon model or it was empty. The empty zone was used as a control zone. One of the quarters contained a plain salmon model with no latex sea lice, the next quarter had a salmon model with one latex sea louse (sea lice mould printed on a 3D printer) stuck to the side with marine-grade silicone, and the third quarter contained a salmon model with 6 latex sea lice stuck to the side (all on the same side). The sea lice were modelled to the same measurements (L3cm x W0.6cm) and aesthetic specifications as a caligus sea lice (*Caligus elongatus*). The final quarter was empty of any models (control quarter). The salmon models were attached to the walls of the tanks with Velcro, to allow them to be removed easily. The salmon models had zero, one and six sea lice on the side of them to see if the fish would react differently to different numbers of novel object on the models i.e. none, a small number, and a higher number.

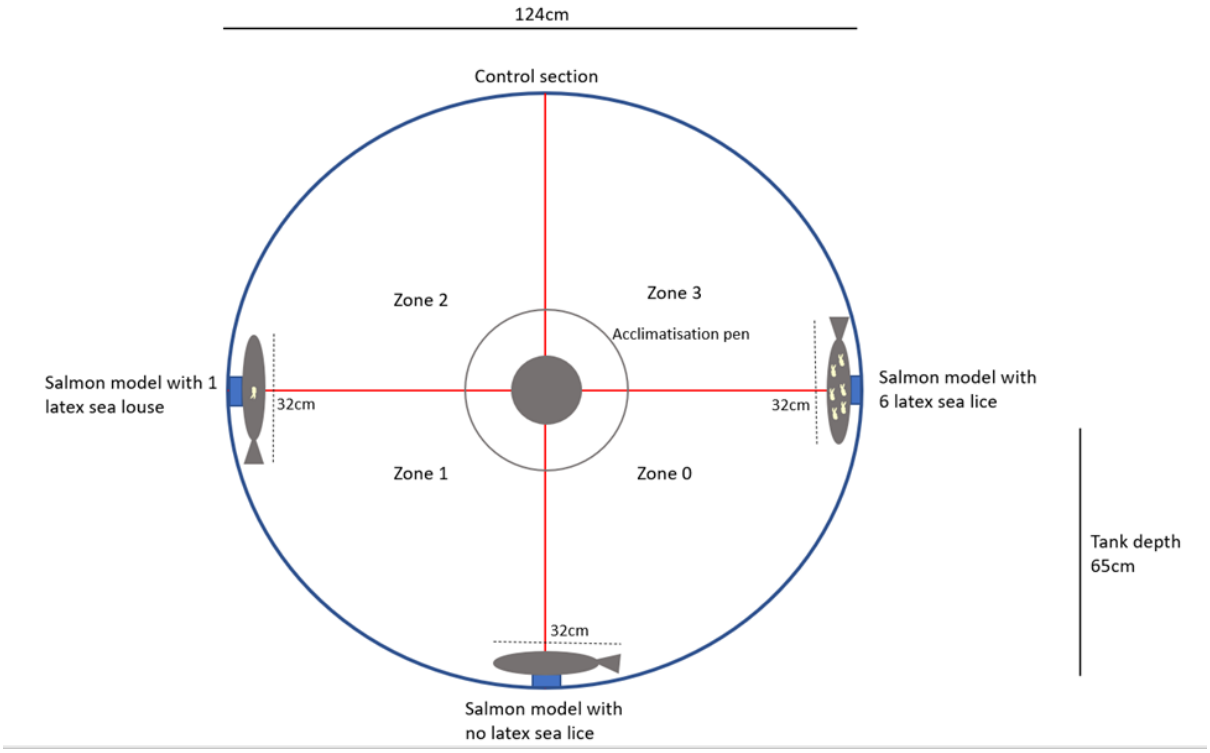

Figure 3.2. Salmon model interactions experiment set-up containing salmon models with latex sea lice (dorsal view).

3.2.4. Model salmon interactions experiment protocol

The tanks were filled with water and the acclimatisation pen and model salmon were placed inside. The fish from the previous experiment was placed into the acclimatisation pen in the centre of the tank. The fish was left to acclimatise for 10 minutes. A stopwatch was used to keep time. After 10 minutes, the acclimatisation pen was lifted out of the tank, leaving the fish to move around freely. The fish was left in this tank for 60 minutes. A stopwatch was used to keep time. The following metrics were recorded:

a. Time taken to first approach any of the salmon models (within 10 cm)

b. No. of approaches to each salmon model (within 10cm)

c. No. of contacts with each salmon model (touches)

d. Time spent within approx. 10cm of each model (seconds)

After 60 minutes, the fish was removed and weighed. The water temperature of the water system used for each experimental tank was recorded, as well as date and time of each experiment. To randomise the position of the models between experiments, the models were detached and placed into different quarters at the end of each experiment. This experiment was repeated for 30 of each species.

3.2.5. Salmon Model Specifications

The salmon post-smolts were printed in PLA (Polylactic acid) on a Fusion 360 3D printer using Prusament PLA Galaxy Silver (Prusa Research, 2023). This colour was chosen as it is the colour closest to that of an Atlantic salmon smolt that was available. However, it is still novel to the wrasse and lumpfish. The sea lice moulds were also printed using the same material. The lice were made with an off-white/yellow latex and attached to the model with colourless marine grade silicone. The models were soaked in water from the same system as the experiment fish for 24 hours to remove as much smell as possible. Though visually the lice look similar to real lice, the colour, smell and movement will be different.

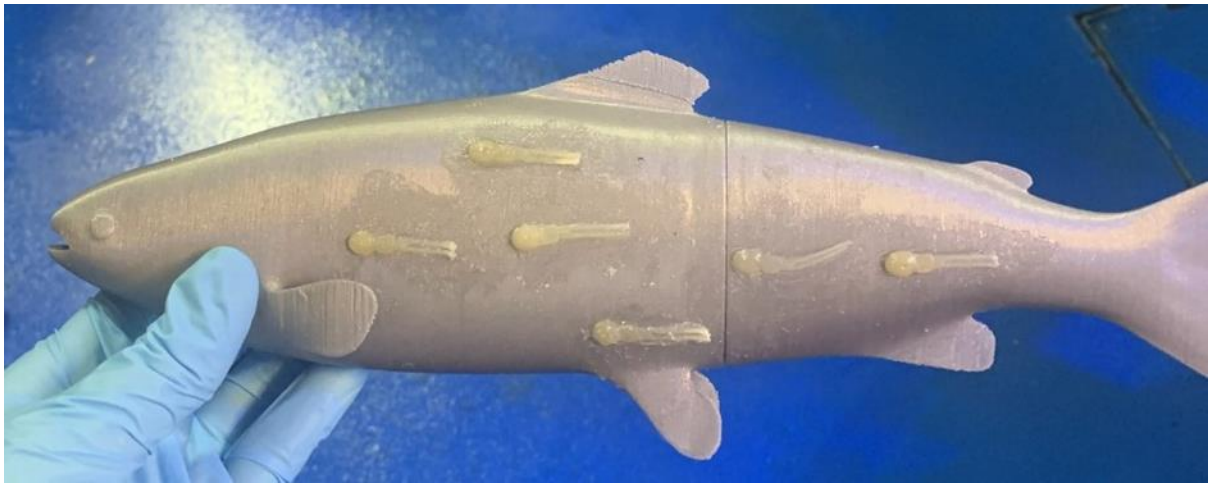

Figure 3.3. 3D-printed salmon model (designed with Atlantic salmon smolt measurements) with latex sea lice attached to side with marine grade silicone.

3.3 Data and statistical analysis

Analysis was completed using the statistical computing program R (version 4.2.3).

Linear models were used to compare data with time (seconds). LMs were used to test if the number of approaches and touches to the novel object were influenced by species and latency to leave the hide; if the time spent within 10cm of each salmon model was influenced by weight, species, and time to first approach a salmon model. For comparing results from experiments 1 and 2, LMs were used for testing variables including 'anxiety' (latency to leave the hide in experiment 1) and time spent within 10cm of each salmon model. GLMs (general linear models) were used to test if the number of approaches and touches to the mirror were influenced by species. GLMs with quasipoisson family were used to compare interactions between the personality components 'boldness', 'sociality', and 'aggression' and the interactions in experiment 2.

Two GLMs were created to see if there is a relationship between the number of touches to the novel object and the number of approaches to the novel object, the number of approaches to the mirror and the number of touches to the mirror. Zero-inflated Poisson models and zero-inflated negative binomial models were tested, and an AIC (Akaike Information Criterion) was used to determine the model that was the best fit. The strength of the evidence for each model is measured using the AIC (Mazerolle, 2006). The zero-inflated Poisson model is used for count data that has a large number of zero counts. The negative binomial distribution model is used for count data with overdispersion. Various models were tested with the data set before analysis was completed using the previously discussed models. They were selected due to their suitability to the specific data sets.

Welch's two-sample T-tests were used to compare species and latency to leave the hide; weight and latency to leave the hide; species and number of touches to the novel object; weight and number of touches to the novel object; species and number of touches and approaches to each salmon model; species and the time taken to first approach a salmon model; and weight and the time taken to first approach a salmon model.

3.4. Results

Behaviours were categorised into personality components based on Whittaker *et al*., 2021 personality profiling experiments.

3.4.1. Experiment 1

Anxiety

Behaviours involving the hide in the first experiment were placed into 'anxiety' personality component.

Species vs latency

The t-test found that species had a statistically significant effect on latency to leave hide ($t= 0.13$, $DF= 117.7$, $p< 0.05$). On average, lumpfish took longer to leave the hide, and had a larger range of latency times than wrasse (see figure 3.4).

Weight vs latency

The t-test found that weight had a highly statistically significant effect on latency to leave hide for both species (wrasse, $t= -4.9$, $DF= 30$, $p<0.001$) (lumpfish, $t= -4.6$, $DF= 29$, $p< 0.001$).

Boldness

Behaviours involving the novel object in the first experiment and the salmon models in the second experiment were placed into 'boldness' personality component.

Species vs number of touches to the novel object

The t-test found that species had no statistically significant effect on number of touches to the novel object ($t= 0.397$, $DF= 116.9$, $p = 0.692$).

Weight vs number of touches to the novel object

The t-test found that weight had a highly statistically significant effect on number of touches to the novel object in both species (wrasse, $t= 24.8$, $DF= 30$, $p< 0.001$) (lumpfish, $t= 7.6$, $DF= 29$, $p < 0.001$). Larger fish of both species tended to be more neophobic (i.e., less likely to approach the novel object) than the smaller ones.

Effect on approaches and touches to novel object

The LM determined that the number of approaches to the novel object was found to be significantly affected by latency (Estimate= 10.08(SD = 0.001, p <0.001) and species (Estimate= 2.09(SD = 1.42, p <0.001). The fish is more likely to approach the novel object, the longer it takes to leave the hide (see figure 3.5). Lumpfish were also more likely to approach the novel object than ballan wrasse. The number of touches to the novel object was not significantly affected by latency (Estimate= $7.3_{x10^{-3}}$(SD= $5.4_{x10^{-5}}$, p= 0.899) or species (Estimate= $5_{x10^{-3}}$(SD= $6.4_{x10^{-2}}$, p= 0.712).

Sociality and aggression

Behaviours involving the mirror in the first experiment were placed into 'sociality' or 'aggression' personality components. Approaches to the mirror are classed as 'sociality', whereas touches to the mirror are classed as 'aggression'.

Number of approaches and touches to the mirror

The LM found the number of approaches to the mirror was significantly affected by latency (Estimate= 12.81(SD = 0.002, p <0.001) and species (Estimate= 2.10(SD = 2.14, p <0.001) (see figure 3.6). The number of touches to the mirror was also found to be significantly affected by latency (Estimate= 27.68(SD= 0.005, p <0.001) and species (Estimate= 11.97(SD= 6.4, p= 0.015) (see figure 3.7).

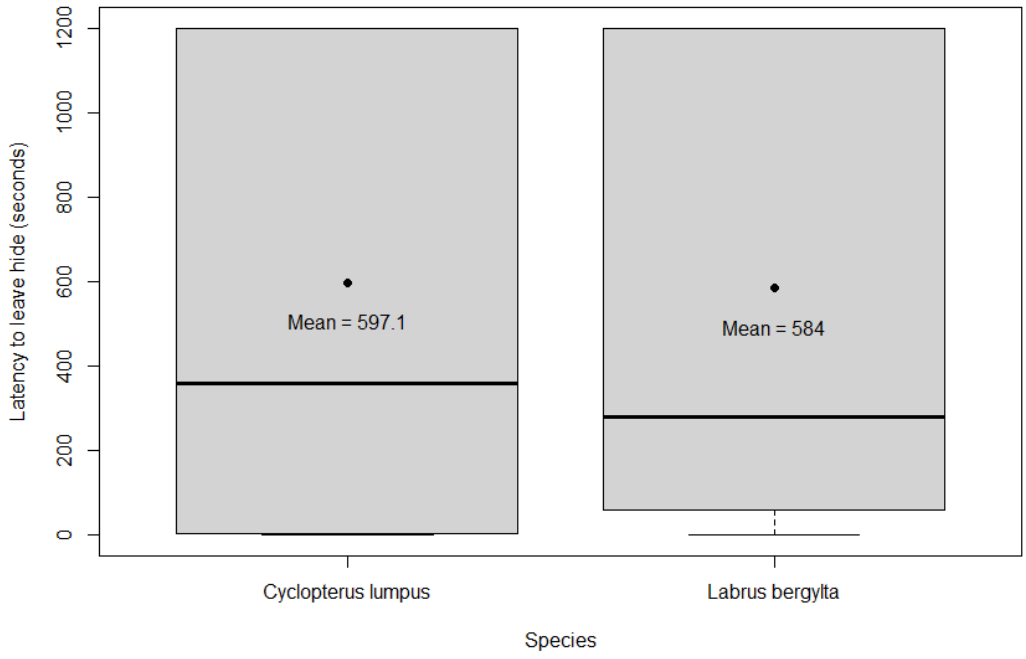

Figure 3.4. Comparison of the two species of cleaner fish used and the time it took for them to leave the hide in the first experiment.

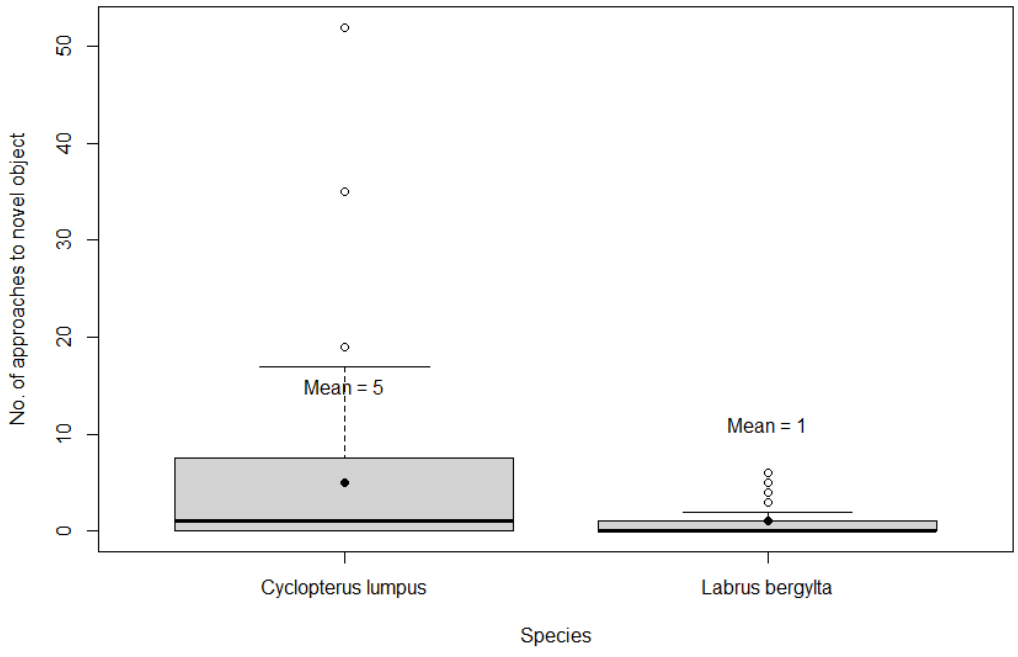

Figure 3.5. Comparison of the two species and the number of approaches to the novel object.

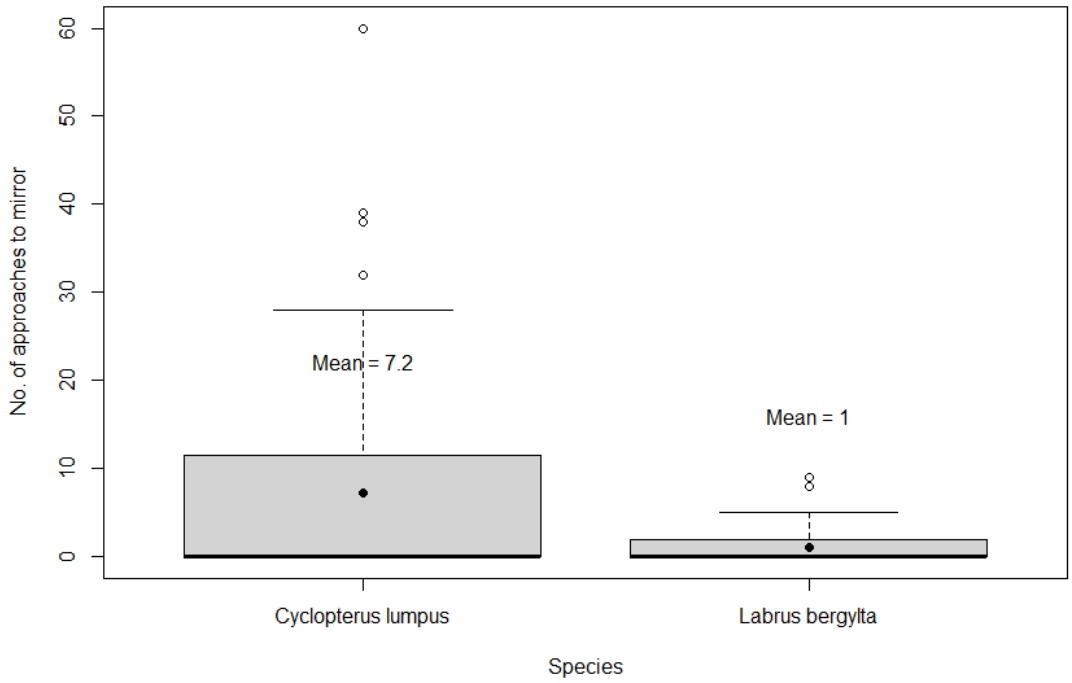

Figure 3.6. Comparison of the two species and the number of approaches to the mirror.

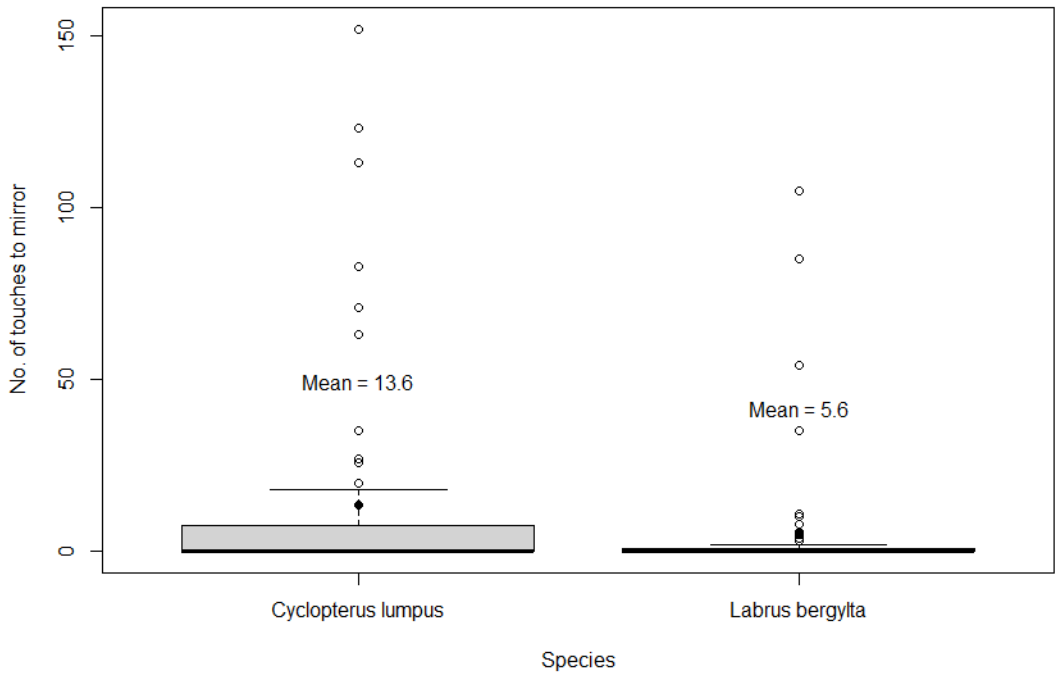

Figure 3.7. Comparison of the two species and the number of touches to the mirror.

3.4.2. Experiment 2

Number of approaches, touches and time spent around each salmon model

On average, the model with 6 sea lice attached had the highest number of approaches, touches and time spent within 10cm of the model (see figures 3.8, 3.9 and 3.10).

T tests found that species had a highly statistically significant effect on the number of approaches to the salmon model with 0 sea lice (t= 4.7, DF= 43.5, p < 0.001), the model with 1 sea lice (t= 4.1, DF= 43.1, p< 0.001), and the model with 6 sea lice (t= 4.2, DF= 37.2, p< 0.001). Species also had a significant effect on the number of touches to the model with 0 sea lice (t= 2.9, DF= 30.9, p= 0.007), but not on the model with 1 sea lice (t= 1.4, DF= 39.7, p= 0.184) or 6 sea lice (t= 1.5, DF= 32.5, p= 0.131). The average number of lumpfish to approach and touch the salmon models was much higher than the average number of ballan wrasse to do the same (see figures 3.8 and 3.9).

The LM found that latency to approach the first model, weight and species were found to have no significant effect on the time spent within 10cm of the salmon model with 0 lice (species Estimate= -205.2(SD= 153.8, p= 0.06) (weight Estimate= 5.4(SD= 0.86, p= 0.386) (latency Estimate= 80.6(SD= 0.037, p= 0.747), or the salmon model with 1 sea lice (species Estimate= -318.8(SD= 293.6, p= 0.135) (weight Estimate= 125.1(SD= 1.64, p= 0.431) (latency Estimate= 126.4(SD= 0.07, p= 0.834), or the salmon model with 6 sea lice (species Estimate= -2.7(SD= 130.3, p= 0.244) (weight Estimate= 150.8(SD= 0.73, p= 0.882) (latency Estimate= 150.8(SD= 0.032, p= 0.109).

Latency to approach salmon models

This was recorded as time taken to first approach any of the models as an initial test of neophobia. Once they overcome the fear of the salmon model (a novel object), time spent and number of approaches were recorded for the separate models.

Species had a statistically significant effect on the time taken to first approach a salmon model (t= -2.7, DF= 110.3, p= 0.007). On average, the lumpfish were a lot quicker to first approach a salmon model, however, wrasse had a larger range of time than lumpfish in this experiment (see figure 3.10).

Weight vs first approach to salmon model

Weight had a positive effect on the time taken to first approach a salmon model ($t = -8.8$, $DF = 120$, $p < 0.001$). On average, fish weighing above 71g were quickest to first approach a model in experiment 2.

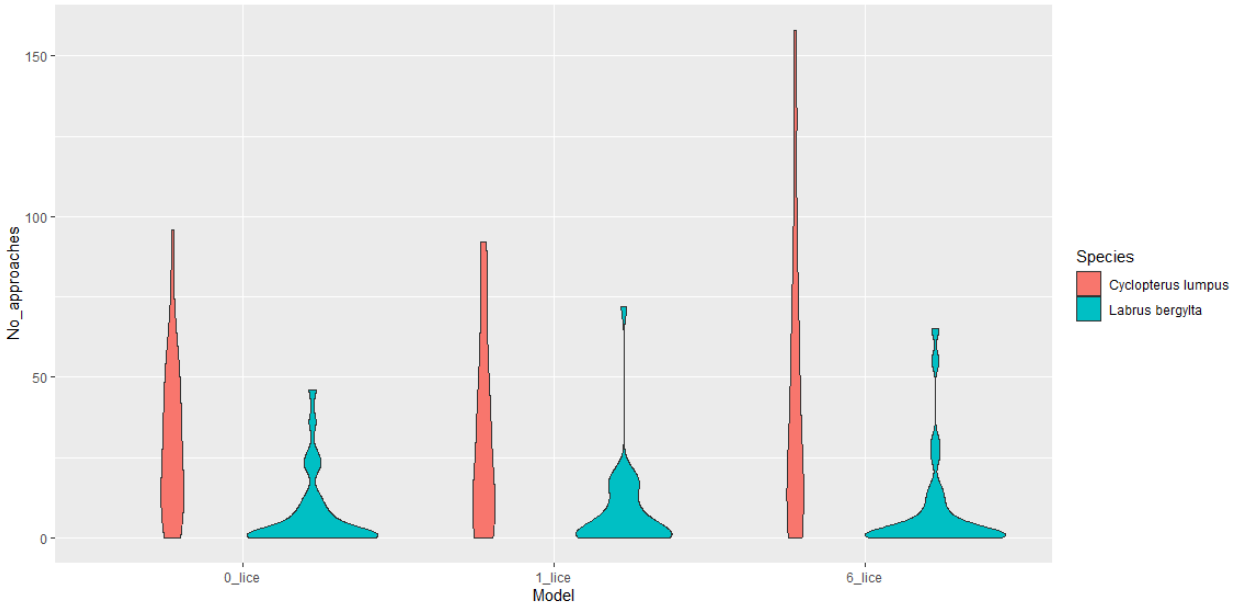

Figure 3.8. The model with 6 sea lice attached had the highest average number of approaches at 26.

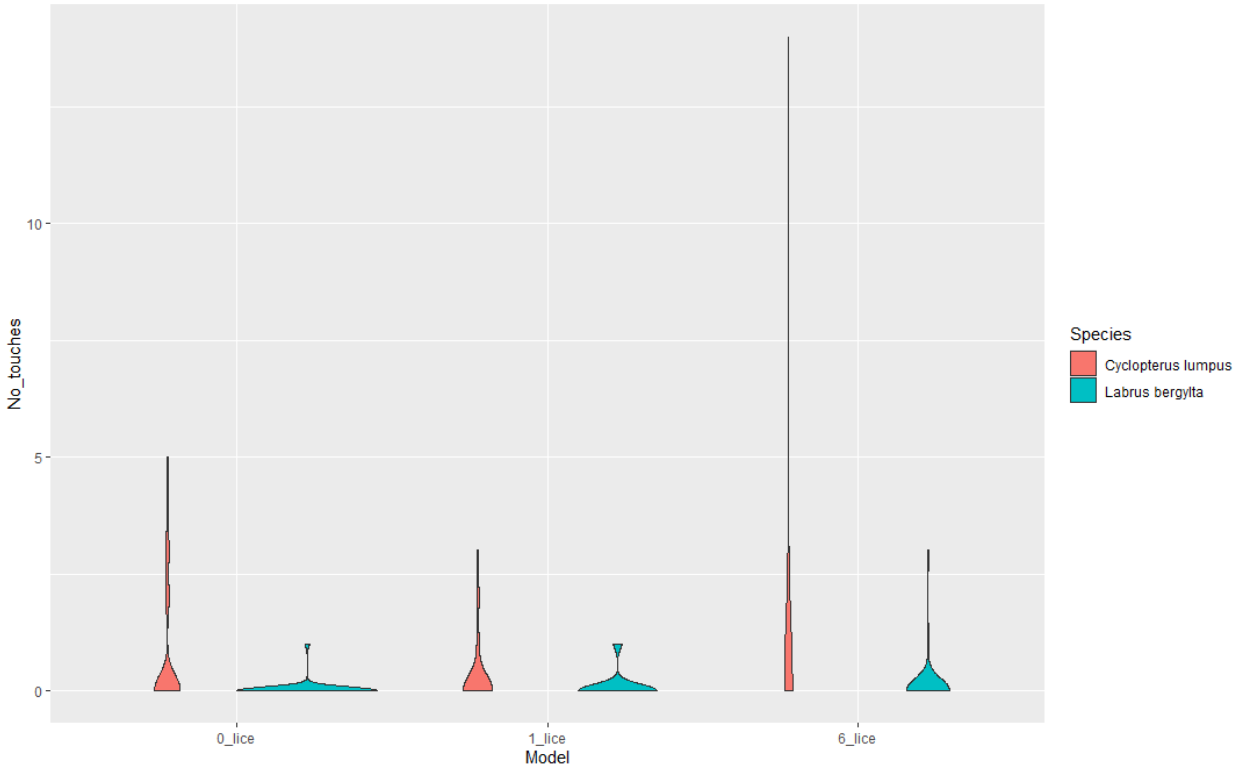

Figure 3.9. The model with 6 sea lice attached had the highest average number of touches at 0.5.

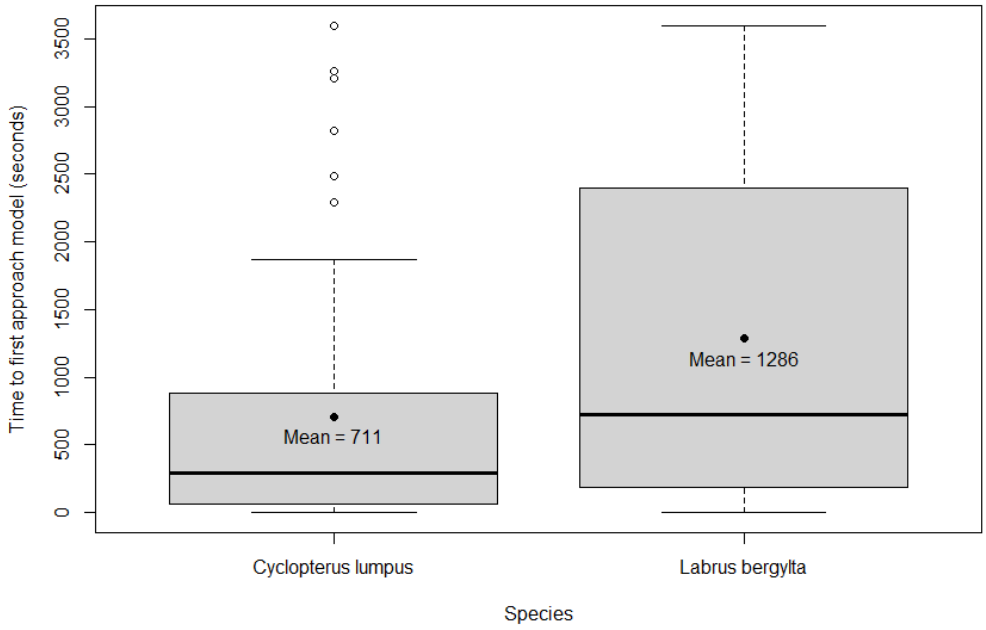

Figure 3.10. Comparison of the two species of cleaner fish used and the time it took for them to first approach a salmon model in the second experiment.

3.4.3. Is there a relationship between the personality components and the fish reactions to the salmon models?

To determine if there is a relationship between personality components and reactions, the fish were split into 2 sections based on their personality component. Those above the median time taken to leave the hide were deemed 'anxious' and those that look less time than the median to leave the hide were deemed 'not anxious'. For boldness, those who approached the novel object more often than the median were deemed 'bold' and those below the median were deemed 'shy'. For sociality, those who approached the mirror more often than the median were deemed 'social' and those below the median were deemed 'antisocial'. For aggression, those who touched the mirror more often than the median were deemed 'aggressive' and those who touched it less times than the median were deemed 'not aggressive'.

Anxiety

Anxiety had a statistically significant effect on the time taken to first approach a salmon model in experiment 2 (Estimate= 533(SD= 300.5, p= 0.016). As discussed previously, lumpfish were quicker on average to first approach a salmon model than the wrasse. Wrasse had a larger range of times and behaved as expected – anxious wrasse were slower to approach the salmon model than the not anxious ones. In contrast, anxious lumpfish were quicker than expected to first approach a salmon model, but still slower than the not anxious ones (see figure 3.11).

Anxiety had no statistically significant effect on the number of approaches to the salmon model with 0 sea lice (Estimate= 22.9(SD= 4.8, p= 0.299), or the model with 1 sea lice (Estimate= 19(SD= 5.7, p= 0.143). Anxiety did have a significant effect on the number of approaches to the salmon model with 6 sea lice (Estimate= 15.9(SD= 8.4, p= 0.027). As discussed in the previous section, lumpfish had significantly higher number of approaches to the salmon model with 6 sea lice than the wrasse. As expected, anxious lumpfish and wrasse approached the salmon model much less than the not anxious individuals (see figure 3.12).

Anxiety had no statistically significant effect on the number of contacts with the salmon model with 0 sea lice (Estimate= 0.78(SD= 0.27, p= 0.92), or the model with 1 sea lice (Estimate= -0.04(SD= 0.15, p= 0.125), or the model with 6 sea lice (Estimate= -0.06(SD= 0.51, p= 0.21).

Anxiety had no statistically significant effect on the time spent within 10cm of the salmon model with 0 sea lice (Estimate= 101.4(SD= 32.8, p= 0.479), or the model with 1 sea lice (Estimate= 57.73(SD= 38, p= 0.388), or the model with 6 sea lice (Estimate= -27.3(SD= 69, p= 0.07). However, the model with 6 sea lice had a p-value that was very close to being significant, therefore, with a larger sample size, it could have been significant.

Boldness

Boldness had a statistically significant effect on the time taken to first approach a salmon model in experiment 2 (Estimate= -241.2(SD= 296, p= 0.012). Again, the wrasse behaved as expected – bold wrasse were quicker to approach the salmon model than the shy ones. As before, the shy lumpfish were quicker than expected to first approach a salmon model, but still slower than the bold ones (see figure 3.13).

Boldness had no statistically significant effect on approaches to the salmon model with 0 sea lice (Estimate= 3.3(SD= 0.226, p= 0.397), or the model with 1 sea lice (Estimate= 3.21(SD= 0.28, p= 0.14). However, boldness did have a significant effect on the number of approaches to the salmon model with 6 sea lice (Estimate= 3.34(SD= 0.3, p= 0.013). As discussed in the previous section, the number of approaches to this model was much higher for lumpfish. For wrasse, both bold and shy individuals had few approaches compared to lumpfish. Bold wrasse approached the model more than shy, as expected. For lumpfish, the bold individuals had a higher number of approaches than the shy (see figure 3.14).

Boldness had no statistically significant effect on the number of contacts with the salmon model with 0 sea lice (Estimate= -0.28(SD= 0.54, p= 0.94), or the model with 1 sea lice (Estimate= -2.31(SD= 0.9, p= 0.08), or the model with 6 sea lice (Estimate= -1.2(SD= 0.93, p= 0.09).

Boldness had no statistically significant effect on the time spent within 10cm of the salmon model with 0 sea lice (Estimate= 126(SD= 32.4, p= 0.518), or the model with 1 sea lice (Estimate= 85.4(SD= 37.4, p= 0.253). Boldness did have a statistically significant effect on the time spend within 10cm of the salmon model with 6 sea lice (Estimate= 90.2(SD= 67.5, p= 0.032). Bold fish of both species spend more time near the model than the shy individuals (see figure 3.15).

Sociality

Sociality had no statistically significant effect on time taken to first approach a salmon model (Estimate= 551.2(SD= 335.3, p= 0.543).

Sociality had no statistically significant effect on approaches to the salmon model with 0 sea lice (Estimate= 2.65(SD= 0.27, p= 0.07), or the model with 1 sea lice (Estimate= 2.97(SD= 0.3, p= 0.3). As with boldness, sociality had a statistically significant effect on the number of approaches to the salmon model with 6 sea lice (Estimate= 2.49(SD= 0.32, p= 0.012). For both lumpfish and wrasse, the social individuals had a higher number of approaches to the model with 6 sea lice (see figure 3.16).

Sociality had no statistically significant effect on the number of contacts with the salmon model with 0 sea lice (Estimate= -1.2(SD= 0.57, p= 0.35), or the model with 1 sea lice (Estimate= -3.47(SD= 0.92, p= 0.14), or the model with 6 sea lice (Estimate= -1.7(SD= 0.97, p= 0.11).

Sociality had no statistically significant effect on the time spent within 10cm of the salmon model with 0 sea lice (Estimate= 83.4(SD= 35.8, p= 0.32), or the model with 1 sea lice (Estimate= 43(SD= 41.5, p= 0.298), or the model with 6 sea lice (Estimate= -33(SD= 75.8, p= 0.08).

Aggression

Aggression had a statistically significant effect on the time taken to first approach a salmon model (Estimate= -228.9(SD= 323, p= 0.036). As with anxiety and boldness, wrasse behaved as expected – aggressive wrasse were quicker to approach the salmon model than the not aggressive ones. Again, the not aggressive lumpfish were quicker than expected to approach a salmon model but still slower than the aggressive ones (see figure 3.17).

There was no statistically significant effect of aggression on any of the interactions with the individual salmon models ($p > 0.05$).

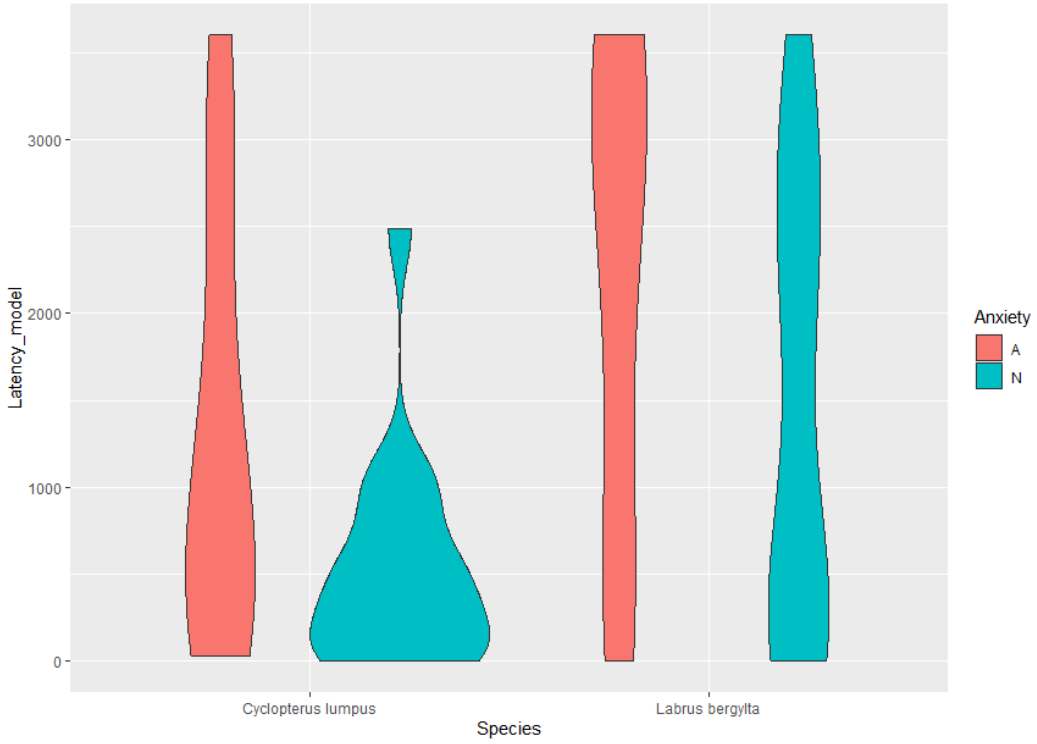

Figure 3.11. Anxious (A) lumpfish and ballan wrasse took longer to first approach a salmon model in the second experiment than the not anxious (N) counterparts.

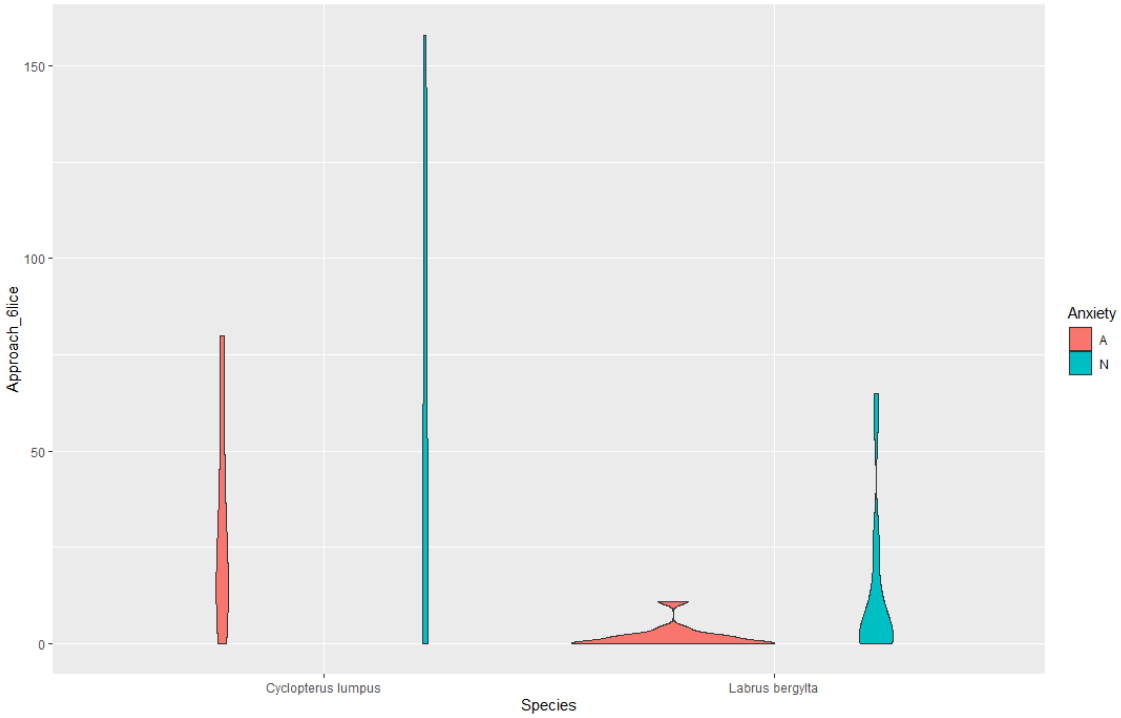

Figure 3.12. Anxious (A) lumpfish and ballan wrasse had less approaches to the salmon model with 6 sea lice than the not anxious (N) counterparts.

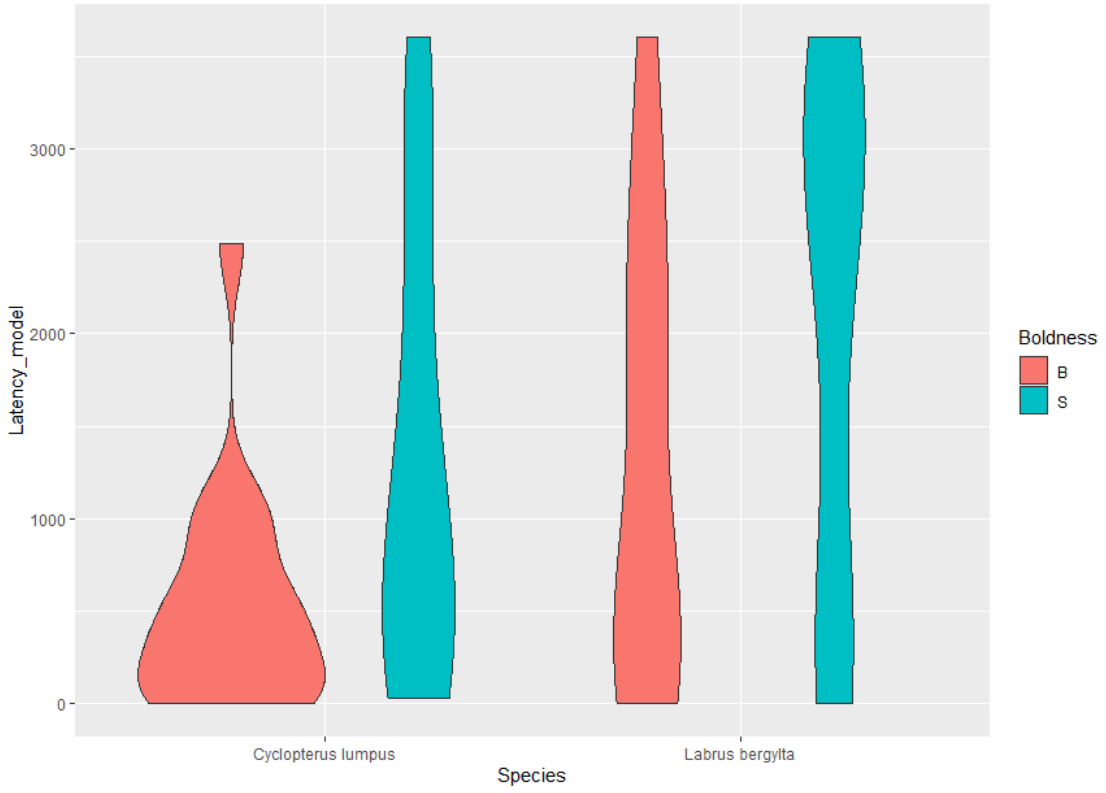

Figure 3.13. Shy (S) lumpfish and ballan wrasse took longer to first approach a salmon model than the bold (B) counterparts.

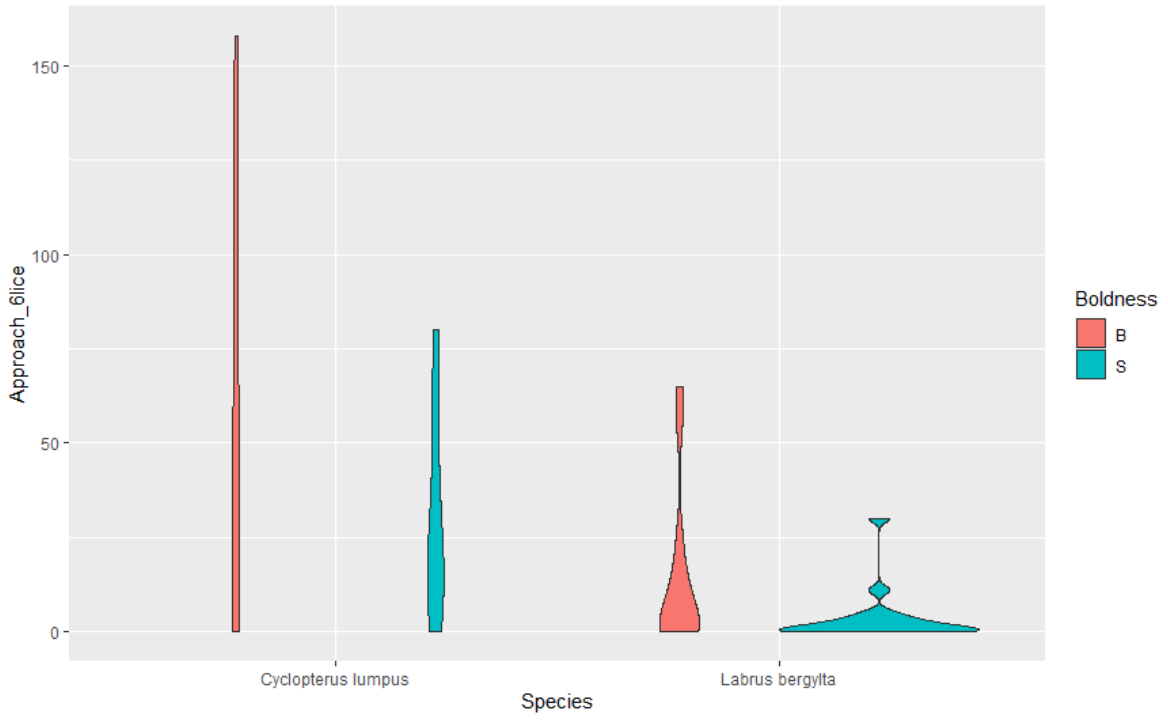

Figure 3.14. Shy (S) lumpfish and ballan wrasse approached the salmon model with 6 sea lice less than the bold (B) counterparts.

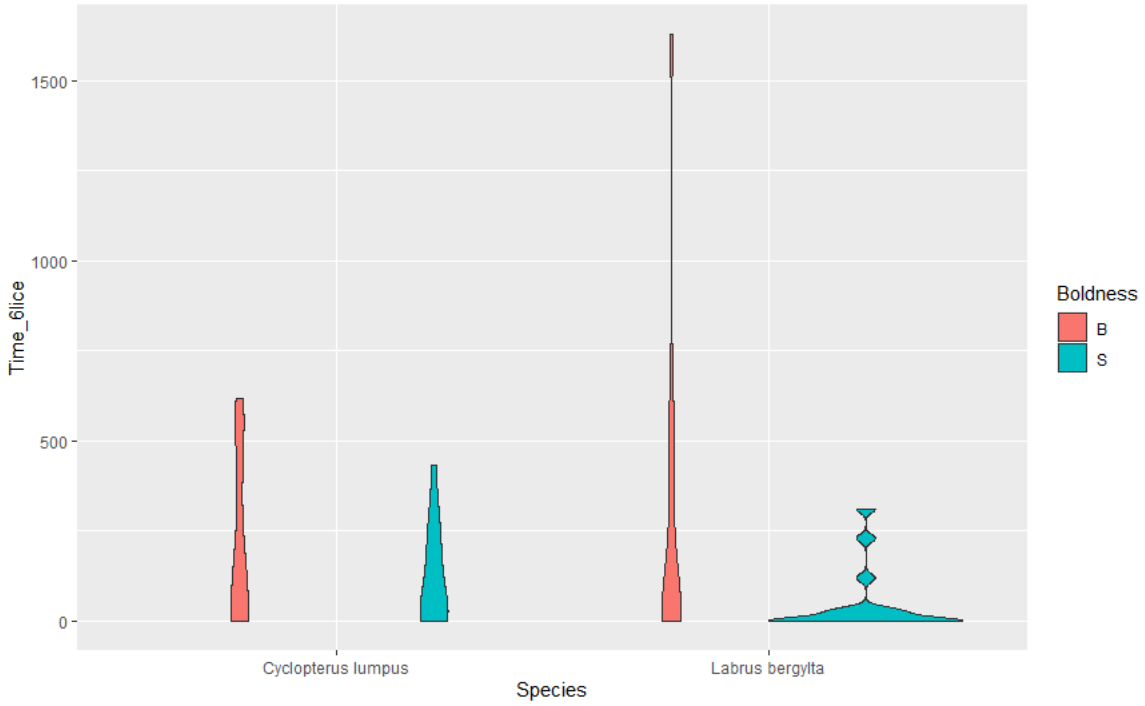

Figure 3.15. Bold (B) lumpfish and ballan wrasse spend more time within 10cm of the salmon model with 6 sea lice than the shy (S) counterparts.

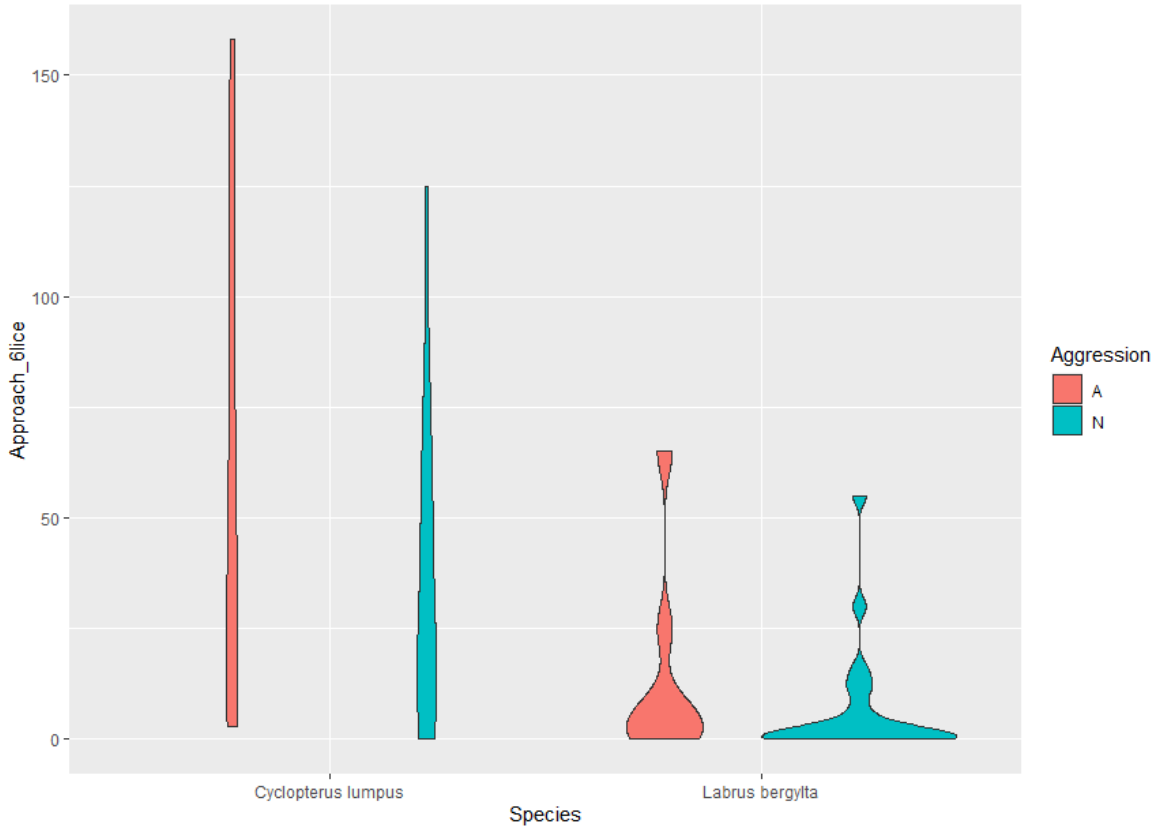

Figure 3.16. Aggressive (A) lumpfish and ballan wrasse approached the salmon model with 6 sea lice more than the not aggressive (N) counterparts.

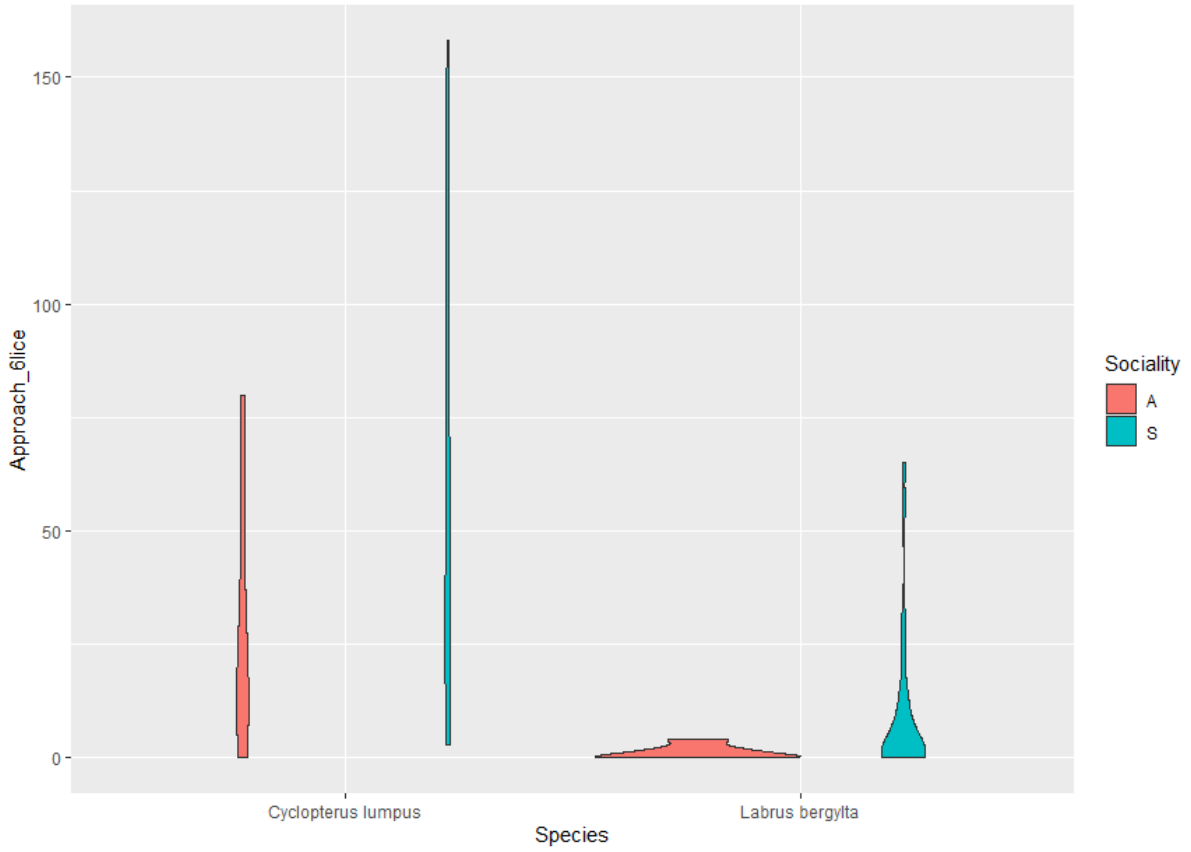

Figure 3.17. Social (S) lumpfish and wrasse approached the salmon model with 6 sea lice more than the antisocial (A) counterparts.

3.5. Discussion

This chapter gives an insight into how behavioural indicators could relate to delousing traits of cleaner fish in aquaculture. With more research and experiments, this could help with selection of brood stock for farmed cleaner fish and therefore, improve efficacy and welfare in sea cages. Measuring more metrics such as the time spent around the mirror could provide more insight into the behavioural differences between individuals as well as the two species. Though it is impossible to directly compare the two separate species, behavioural differences were noted. The lumpfish proved to be a very active species in these experiments, displaying more 'bold' and 'aggressive' traits and less 'anxious' traits. This was different for the ballan wrasse, who displayed more shy, not aggressive, and anxious traits. As sea lice offer a potential food source for both cleaner fish species (Leclercq et al., 2014; Powell et al., 2018), one would hope they would interact (approach, touch or spend time near) a novel object such as salmon model with sea lice attached. Though delousing is a learned behaviour, approaches to a novel object is promising as it could indicate that they might show interest and approach real lice on salmon. One would also hope those that displayed 'bold', 'social' or 'aggressive' personalities in the first experiment to interact with these 2 models (Whittaker et al., 2021). ‘Anxiety’, ‘boldness’ and ‘aggression’ all proved to have a significant effect on the time taken to first approach a salmon model in the second experiment. Not anxious, bold, and aggressive fish were quicker to first approach a salmon model than their anxious, shy and not aggressive counterparts (this was true for both species). There was also much significance with the salmon model with 6 sea lice. Anxiety had a significant effect on approaches to this model, with not anxious fish approaching more than anxious fish. Boldness also had a significant effect on approaches to this model and also time spent within 10cm of this model. Bold fish approached the model more and also spend more time around it than shy fish. Sociality also had a significant effect, with social fish approaching the model with 6 sea lice more than antisocial fish. Though the interest in the salmon models with artificial sea lice cannot be directly linked to delousing behaviour, their interest in the novel objects could support the theory that personality components were significant and could aid in identifying potential delousing behaviours in further studies. By selecting fish for broodstock that were not anxious, bold, and social, the resulting offspring could, in theory, carry these traits and become good cleaners when deployed to salmon farms.

Although behavioural indicators have the potential to optimize aquaculture practices, brood stock selection and improve fish welfare, there are both positive and negative aspects to consider (Martins et al., 2011; Colson et al., 2013). One such positive aspect means that welfare can be evaluated using behavioural indicators without involving unnecessary stressors such as physical handling or invasive procedures (Dawkins, 2006; Barreto et al., 2021). In densely populated aquaculture systems, this non-invasive method is crucial for reducing stress-related health issues and preventing disease spread. Behavioural indicators allow for real-time monitoring of fish responses to other fish, stressors, and changes in their environment (Dawkins, 2003). It is possible to monitor fish well-being and potential stress responses by observing feed-related activity, swimming patterns, and interactions with conspecifics and variables (Barreto et al., 2021). These can be used to adjust management practices and necessary interventions. Changes in behaviour and coping styles are often among the first signs of stress or discomfort in animals (Koolhaas et al., 1999). Monitoring alterations in behaviours, such as reduced activity or altered swimming patterns, can provide early indications of suboptimal conditions, allowing for prompt intervention before more severe health issues arise (Ashley, 2007; Matthews et al., 2016).

However, fish behaviour is highly context-dependent, with different species responding in different ways and influenced by factors such as the specific environment, social interactions, and the presence of predators or competitors (Brown et al., 2007). Understanding such contextual factors is crucial for accurately interpreting behavioural indicators (Mendl, 1999). Laboratory experiments are necessary when developing baseline behaviour and welfare indicators, but such tools must be tested and validated within an industry setting, such as RAS units or sea cages (Dawkins, 2003). Different fish species exhibit varied behavioural responses, therefore, what might be considered a stress response in one species could be considered normal behaviour in another (Wendelaar Bonga, 1997). This is an important factor that should be explored further in future studies. Additionally, there are differences in preference between individuals within a species regarding which behaviour, response, or coping style they display (Brick and Jakobsson, 2002; Martins et al., 2011) Behavioural observations can be influenced by the observer's subjectivity, skill, and potential biases (Martins et al., 2011; Colson et al., 2019). Implementing standardized protocols and automated monitoring systems can help mitigate these issues, ensuring consistent and objective data collection. Analysing behavioural indicators can be complex due to the many potential responses and their different meanings. Additionally, other indicators are often

required when making and validating welfare assessments, such as physical, pathological, and psychological health, as well as environmental indicators (Huntingford et al., 2006; Cañon Jones et al., 2010; Bergqvist and Gunnarsson, 2013).

This study cannot be relied upon to accurately determine cleaner fish behavioural responses to real 'delousing behaviours' due to the use of plastic models of salmon and silicone sea lice. Further experimentation using real salmon and lice is essential in concluding whether behavioural indicators can be used to determine delousing efficacy (Whittaker et al., 2021). Live fish have long been used in experiments for a range of scientific purposes, resulting in advancements in many scientific fields (Gerlai, 2020; Ajuwon et al., 2023). Many studies involve fish' behavioural responses to novel objects in their environments (Castanheira et al., 2013; Champneys et al., 2018; Villegas-Ríos et al., 2018) but few have examined whether responses to fake models of fish can relate to responses to real fish. A study looking into fish response to on screen-video stimuli found that fish were able to identify that the stimuli were not real, theorising a number of reasons for this, including the fact that the image would not interact with the fish, thus creating a limitation for behavioural research (D'eath, 1997). This might have also been the case in this study as well, due to the fact that the models did not move and were fixed to the side of the tanks.

3.6. Conclusion

This study determined that there was a significant difference in behaviour between the 2 cleaner fish species that are bred for use in the aquaculture industry, with lumpfish displaying stronger behaviours that may be indicative of delousing behaviour. The results also determined that not anxious, bold, and social fish of both species showed indications of potentially good delousing behaviours - due to their approaches and time spend around the salmon model with a high number of sea lice attached to the flank. These results could be used in broodstock selection. However, repeating the experiment with a larger sample size and with real sea lice-infected salmon would provide a more accurate and reliable way to predict delousing behaviours. The utilization of behavioural indicators for aquaculture species does offer a valuable means of assessing welfare and optimizing farming practices. While there are many challenges involved in the use of behavioural indicators, such as context dependency and species-specific variation, the benefits of real-time, non-invasive monitoring, and early stress and disease detection make behavioural indicators a powerful tool for improving farming practices and fish welfare. By incorporating tools such as standardized protocols, automated monitoring systems, and expert interpretation, the aquaculture industry can harness the potential of behavioural indicators to promote sustainable and ethical practices.

Chapter 4 - Determining the thermal preference of ballan wrasse (*Labrus bergylta*) using a Shuttle-Box machine vision system.

4.1. Introduction

Thermal niche is defined as the range of water temperatures in which organisms can tolerate, grow, and thrive in (Fry, 1971; Angilletta Jr *et al*., 2002). Most fish are poikilothermic, meaning their body temperature varies with the water temperature they inhabit (Elliot and Elliot, 2010). It is thus likely that thermal preference windows evolved to be somewhat narrow to reduce maintenance expenditure for fish, which lead to functional distinctions among species and even populations of species in different climates (Stillman, 2003; Pörtner *et al*., 2008). Each fish species has a unique thermal niche, which is influenced by a variety of factors such as geographic origin, life stage, and genetics (Pörtner and Farrel, 2008). Knowledge of the thermal niche of farmed fish is critical for welfare as water temperature can have a significant impact on fish physiological processes, metabolism, and behaviour (Pörtner and Peck, 2010; Kir, 2020).

The optimal temperature range for farmed fish varies greatly depending on the species. Although thermal preference has conventionally been regarded as one optimal temperature for growth, the variability in preferred temperatures observed within fish species suggests that it may be more suitable to view thermal preference as a range of temperatures rather than a single fixed temperature (Johansson *et al*., 2009). Some studies indicate that the optimal temperature for growth of Atlantic salmon (*Salmo salar*) lies between 16-20°C (Jonsson *et al*., 2001), but they can survive extreme temperatures as low as -0.8°C and as high as 28°C, albeit briefly (Reddin *et al*., 2004; Elliot and Elliot, 2010). The large yellow croaker, one of China's most economically important fish, can tolerate a much broader range of temperatures, between 5.3°C and 36.6°C (Wu et al., 2022), though this depends on acclimation temperatures. In general, studies have shown that fish can develop acclimatory responses to long-term temperature changes (Kir, 2020). Maintaining the appropriate water temperature is critical for aquaculture production (Roessig *et al*., 2004).

The concept of thermal niche is of particular importance in intensive aquaculture systems where fish are reared under controlled recirculating aquaculture systems (RAS). In these systems, water temperature can be manipulated to optimize growth and health (Ahmed and

Turchini, 2021). Significant deviations from the thermal niche can be avoided to reduce stress.

Traditionally, temperature preference has been studied by manually observing fish behaviour, measuring body temperature or biochemical indicators (Cai *et al*., 2020). However, these methods are time-consuming, labour-intensive, and may not provide accurate or comprehensive data (Macaulay et al., 2021). Advancements in machine vision and AI offer new opportunities for monitoring and analysing fish behaviour and temperature preferences in fish (Gutiérrez-Estrada *et al*., 2008; Andrewartha *et al*., 2015; Zach *et al*., 2021). By providing real-time and accurate data on fish behaviour and temperature preferences, machine vision and AI can help optimize feeding, thermal management, and overall fish welfare in aquaculture (Terayama *et al*., 2019; Yang *et al*., 2021).

A shuttle box system can be used to determine optimal temperature preference and range. For example, Christensen et al., (2021) determined the preference of round gobys (*Neogobius melanostomus*) and Macnaughton *et al*., (2020) determined the same for westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) using the Loligo machine vision shuttle box system (Loligo® Systems, 2023).

This study used a Loligo shuttlebox to examine the temperature preference of ballan wrasse (*Labrus bergylta*). Ballan wrasse are one of four species of wrasse used as 'cleaner fish' in the Atlantic salmon farming industry, though they are the only species of wrasse that is farmed for this purpose and most used (Skiftesvik *et al*., 2014). The natural habitat range of the ballan wrasse spans from northern Norway to Morrocco (Wheeler and Du Heaume, 1969; Leclercq *et al*., 2018). While they are native to UK seas (Almada et al., 2017), they are known to become inactive at low temperatures during the winter, thus decreasing their 'cleaning' efficacy (Treasurer, 2002; Imsland, 2016). Farmers can choose to stock wrasse in spring, when the temperatures are warmer (Treasurer, 2002), however, better knowledge of ballan wrasse's preferred temperature would help make more informed decisions on stocking times and optimise their welfare, delousing efficacy, and survival. The objective of this study was to determine the optimal temperature and thermal niche of ballan wrasse. Ballan wrasse were selected for this study as lumpfish had already been studied using the same system and method. This information will be critical for farmers when making decisions on location and time of deployment in Atlantic salmon farms.

4.2. Materials and Methods

4.2.1. Source and rearing of fish

A total of 30 wrasse were used in this trial. The source and rearing of the fish were the same as that detailed in section **2.2.1.**

The experimental procedure was approved by Swansea University, Animal Welfare Ethical Review Body, permit IP-2223-05.

4.2.2. Experiment set-up

The Shuttle Box, a machine vision system developed by Loligo (Loligo® Systems, 2023) was used to determine the thermal preference of wrasse (n = 30, mean weight = 35.7g) that were acclimated to 12°C. The shuttle box contained 2 (40 cm wide), circular chambers interconnected by a 10 cm×7 cm central passage. Each chamber is filled to 12cm depth or approx. 23.5 litres of water from the home tank system. The chambers were connected to a 10-litre mixing tank where the water was aerated. Temperature, oxygen, ammonia and pH were measured with a Seneye sensor (Seneye, 2023). The water temperature in the 2 chambers was regulated by pumping water from the mixing tanks through heat exchanger coils in either a heating or cooling bath (see figure 4.1.). Control and activation of the temperature regulation pumps was managed by the ShuttleSoft 3 software using a mechanical relay and USB interface (DAQ-M). The software also tracked the fish's position and activity in real time using an overhead USB camera and recorded the temperature in the two mixing tanks through the DAQ-M unit. The setup was monitored by an infrared-sensitive USB 2.0 camera. A tarpaulin was hung up around the experiment to minimise disturbance and only essential work was carried out in the laboratory. A black mesh was placed over each chamber to prevent the fish from jumping out. The experiments began on the 23rd of November 2022 and finished on the 2nd of February 2023. Each individual test lasted 24 hours with a 15-minute acclimatisation period before the experiment began.

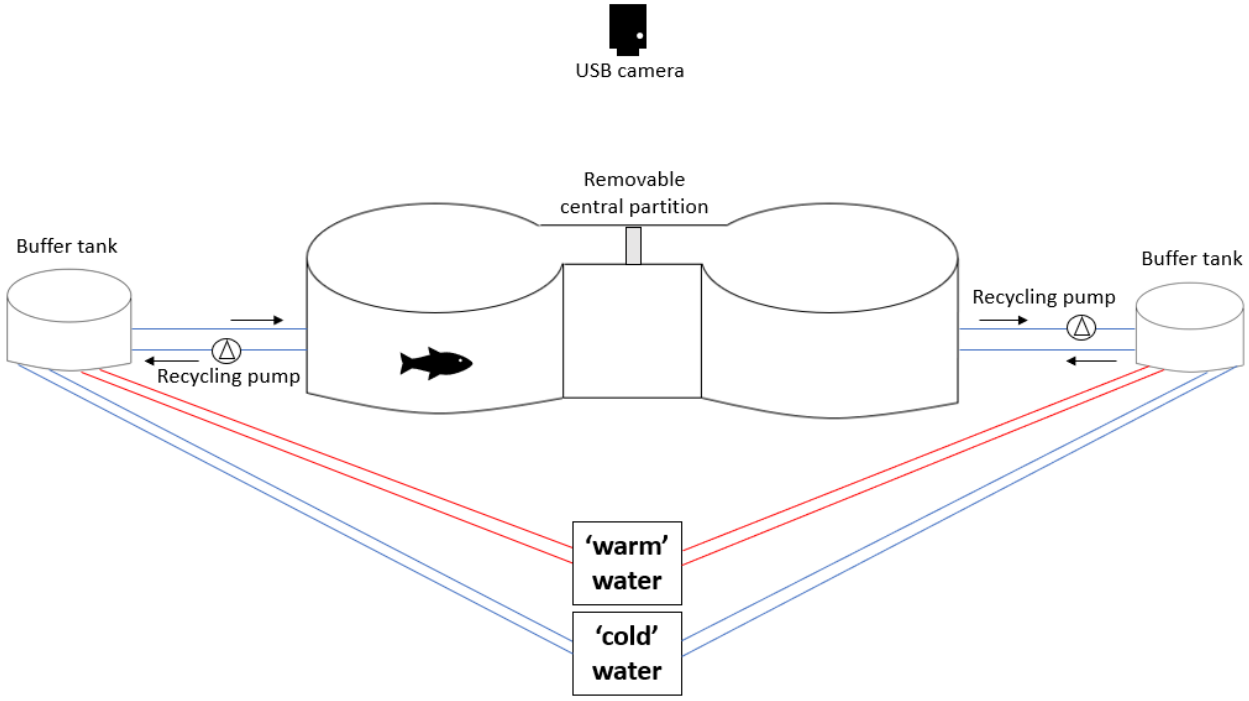

Figure 4.1. Schematic detailing the shuttle-box with two chambers ("warm" and "cold") and other components.

4.2.3. Experiment protocol

The video-tracking software ShuttleSoft 3 (Loligo Systems) was used to prepare the shuttle box system, determining the minimum, maximum and acclimatisation period temperatures. Individual fish were placed on one side of the shuttle box, using a coin toss website (RANDOM.ORG - Coin Flipper) to ensure randomisation each time. Once in the shuttle box, the wrasse was left to acclimatise for 15 minutes, with the central partition in place to stop the fish moving between chambers. After this period, the central partition was lifted, and the fish were able to travel between the two chambers. Once the fish had moved through to the opposite chamber, ShuttleSoft 3, was set to 'dynamic' and the logging begins. The temperature was dynamically regulated according to the fish's position. The presence of the fish on one side of the shuttle tank initiates an increase in the overall temperature of the system. In contrast, the presence of the fish on the other side initiates a decrease in temperature. Temperature changes occurred at 0.1°C rates, with a constant difference of 2.0°C between the two compartments. The known temperature range of ballan wrasse was investigated, and a minimum and maximum temperature range was agreed upon (10-18°C) based on studies by Sayer et al., 1996, Cavrois-Rogacki et al., 2019 and Yuen *et al*., 2019. The dynamic temperature experiment was run for 24 hours before the fish is removed, weighed (0.1g), and placed in a new home tank (within the same RAS).

4.2.4. Data and statistical analysis

The shuttle box experiment was carried out with 1 fish at a time, a total of 30 fish were tested. Preferred temperature for each fish was determined by the Shuttlesoft software as the cumulative median occupied temperature (measured throughout the course of the trial). Statistical analysis was carried out using R (version 3.02.1, R Development Core Team, 2015). Thermal niche was determined as the upper and lower quartiles of the temperature preference data. The optimal temperature was determined as the mean of the temperature preference data. The relationship between fish weight and temperature preference was investigated using a linear model to determine if there is significance. The distance travelled in each of the zones was also investigated using a Welch's t-test for significance.

4.3. Results

4.3.1. Optimal temperature preference and thermal niche

The results from this experiment revealed individual variations in the movement and therefore preferred temperatures among the ballan wrasse (see figure 4.2.). The results showed a 5.6°C difference between the lowest and highest preferred temperature. Although the majority of wrasse opted for temperatures higher than the temperature at the beginning of the experiment (77%), there were a few that preferred lower temperatures (23%).

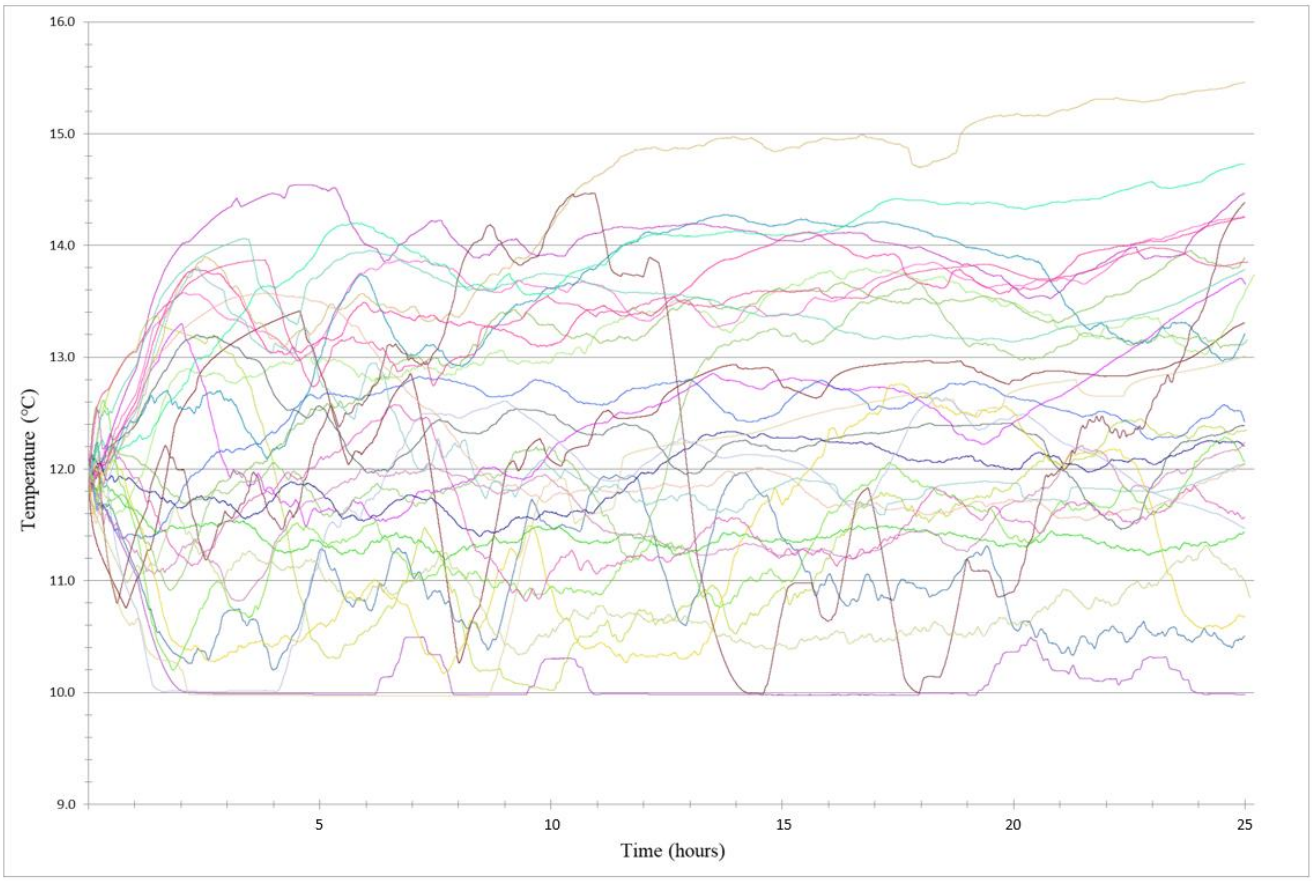

Figure 4.2. The range of temperatures over the course of the 24-hour experiment, with each trace corresponding to 1 fish. This graph clearly shows some wrasse spending time at the lowest temperature limit, suggesting that they might have preferred a lower range.

The optimal temperature for ballan wrasse was determined to be 12.8°C (mean temperature of thermal preferences). The thermal niche was determined to be between 13.8°C (upper quartile) and 12.1°C (lower quartile). The maximum preference temperature was 15.5°C and the lowest was 9.9°C (see figure 4.3.). As the system was set to reduce the temperature to no lower than 10°C, there must have been a small margin of error with the system to allow the temperature to go to 9.9°C. Over the course of the experiment, there was a range of 5.6°C between the lowest and highest preference temperature (9.9°C and 15.5°C) (see figure 4.2.). Overall, most fish preferred temperatures between 13°C and 14°C (n= 9, 30%) while a minority preferred temperatures between 9°C and 10°C (n= 1, 3%) and 15°C and 16°C (n= 1, 3%) (see figure 4.3.). Weight was found to have no significant effect on thermal preference in ballan wrasse (Estimate (SE) = -0.021(0.036), p = 0.5).

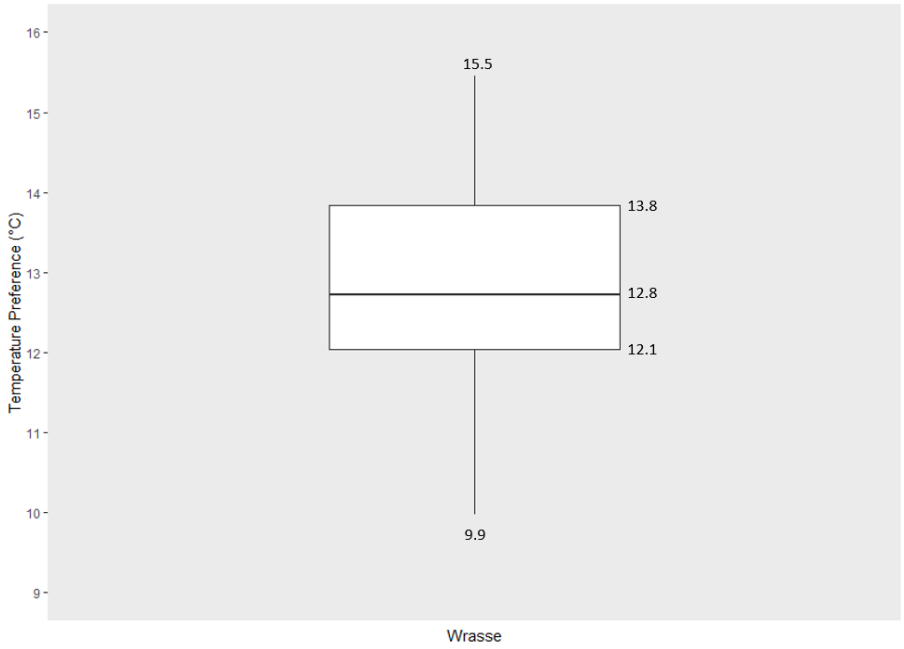

Figure 4.3. The thermal niche of ballan wrasse is between 12.1°C and 13.8°C, with the optimal temperature being 12.8°C.

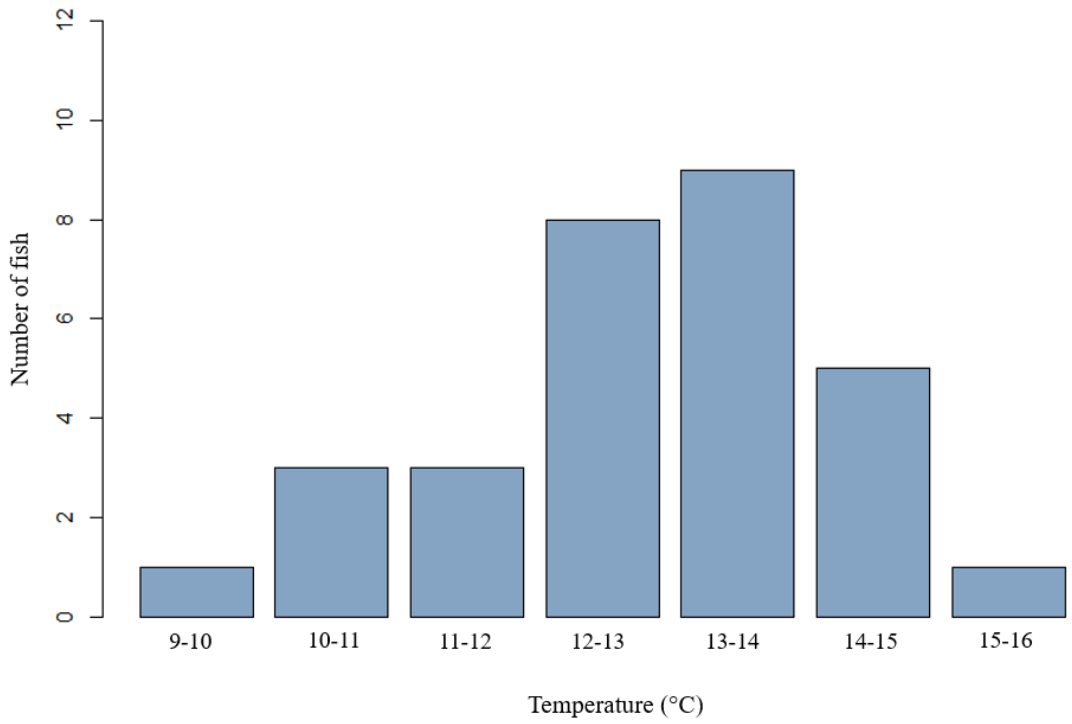

Figure 4.4. The largest number of fish (n = 9, 30%) preferred temperatures between 13°C and 14°C.

4.3.3. Distance travelled in each zone

The distance travelled by the wrasse in the ‘warm zone’ was found to be much significantly greater than the distance travelled in the ‘cold zone’. The average distance travelled in the ‘warm zone’ was 96018.6cm (SE= 53299.7cm) and the average distance travelled in the ‘cold zone’ was 33507.9cm (SE= 14094.1cm). A t-test determined that there was a significant difference (p= <0.001) between the distance travelled between the high zone and low zones.

Table 4.1. The distances travelled by the ballan wrasse in the two zones of the shuttle box.

Zone	Minimum distance travelled (cm)	Maximum distance travelled (cm)	Average distance travelled (cm)	Standard error (cm)
Warm	8418.8	377210.8	96018.6	53299.7
Cold	7385.1	91763.9	33507.9	14094.1

4.4. Discussion

This experiment allowed for complete choice by providing the fish with the unrestricted ability to move between the chambers and therefore regulate temperature as per their preference. Animals likely select ambient temperatures according to their core body temperature, which is influenced by their recent thermal conditions (Christensen *et al*., 2021). However, there has been some debate on temperature differences in shuttle boxes. Some research states that a temperature change of 2°C would result in more accurate behavioural thermoregulation than 1°C, whereas others theorise that a higher temperature difference may deter movement between chambers (Neill and Magnuson, 1974; Neill *et al*., 1972; Christensen *et al*., 2021). Individual choice is important to note and variety between individuals can greatly affect results. Thus, monitoring throughout the experiment is essential. The continuous recording throughout the 24-hour period of this experiment allowed for accurate, real-time thermal preference choices and the results provided accurate information on thermal niche and optimal temperature for ballan wrasse, crucial for deciding deployment times and farm location when they are being utilised in Atlantic salmon aquaculture and reducing thermal stress.

Due to ongoing anthropogenic greenhouse gas emissions, it is projected that global temperatures will undergo significant shifts, resulting in ocean warming and potential thermal stress among fish species (Yao and Somero, 2014; Bilbao *et al*., 2019). Thus, it is important to understand the potential implications of thermal stress for the aquaculture industry. Anticipated shifts in climate are likely to change current environments, potentially resulting in the localized depletion of organisms and their corresponding roles within ecosystem (Freitas *et al*., 2021). Changes in behaviour within species could help them adapt to thermally challenging environments, enabling them to uphold their ecological functions (Fey *et al*., 2019; Wolff *et al*., 2020; Freitas *et al*., 2021). In the case of ballan wrasse, increased temperatures in norther regions (Scotland, Norway, Iceland and Faroe Islands) as a result of climate change could be beneficial, as improved locomotory and aerobic performance is possible beyond 25°C and delousing efficacy was best in warmer temperatures (Penghan *et al*., 2014; Claësson *et al*., 2016; Yuen *et al*., 2019).

Thermal stress, resulting from fluctuations or extreme temperatures in aquatic environments, poses significant challenges to fish welfare in both natural habitats and aquaculture settings (Verleih *et al*., 2015; Vakili *et al*., 2021). Water temperature changes can have profound

effects on their physiological processes, behaviours, and overall health (Overgaard *et al*., 2004; Mendonça and Gamperl, 2010). High water temperatures can increase metabolic rates, oxygen demand, and respiratory stress, for example (Filice *et al*., 2021). Conversely, exposure to low water temperatures can cause reduced metabolic rates, compromised immune systems, and decreased feeding activity, leading to malnutrition and a weakened overall condition (Clarke and Johnston, 1999). Furthermore, fish exhibit behavioural adaptations to cope with thermal stress, including altered feeding and foraging behaviours, interactions with other fish, and changes in swimming patterns (White *et al*., 2019). A change in their behaviours can negatively affect their energy balance, reproduction, social interactions, and overall welfare (Olla *et al*., 1975; Kirby *et al*., 2000). Thermal stress can also affect fish reproduction and development. Higher water temperatures can disrupt spawning behaviour, resulting in lower reproductive success and offspring survival (Ramee *et al*., 2020). In some species, elevated temperatures may even induce sex reversal or impact offspring sex ratio (Ramee *et al*., 2020). To avoid thermal stress among fish species in aquaculture, it is critical to understand their thermal niche and optimal temperature.

Mortality rates of cleaner fish can be high, which can result in additional costs for farmers. In 2021, nearly 30 million farmed cleaner fish were sold in Norway (Directorate of Fisheries, 2022), with some reports of high or near total mortality throughout the production cycle (Norwegian Veterinary Institute, 2022). The use of cleaner fish has raised ethical concerns, such as the welfare of the cleaner fish and the potential impact on wild fish populations. Farmers must take steps to ensure that the welfare of the cleaner fish is considered, and that they are not being subjected to unnecessary stress or harm. While the use of cleaner fish in aquaculture can be an effective and sustainable method of controlling parasites (Bolton-Warberg, 2017), there are several challenges that need to be considered and managed. By addressing issues related to welfare, farmers can optimise the use of cleaner fish to ensure sustainable and effective parasite control.

It is important to note that thermal niche varies significantly between ballan wrasse and lumpfish, which are also used as cleaner fish in the Atlantic salmon industry. While ballan wrasse are known to become inactive at low temperatures, lumpfish are well-suited to cold water conditions and are active below 10 °C (Mortensen *et al*., 2020; Remen *et al*., 2022; Rodríguez-Rey & Whittaker, 2023). However, the lumpfish cannot survive for long periods in warmer waters of 18 °C (Hvas *et al*., 2019). Using ballan wrasse and lumpfish in combination has the potential to greatly improve a production cycle by utilizing each species

according to their optimal temperature preferences and location. The most appropriate match for optimal temperatures are determined by the season and location. For example, ballan wrasse are more suitable during the summer and autumn, while lumpfish are more efficient during the winter-spring seasons where water temperatures are low or throughout the year in higher latitude areas such as northern Norway (Yuen *et al*., 2019).

The lower distance travelled in the 'cold zone' compared to the 'warm zone' supports the theory that ballan wrasse have a lower metabolic rate in colder temperatures, causing them to become inactive (Imsland, 2016; Yuen et al., 2019), thus potentially reducing their delousing efficacy in Atlantic salmon sea-cages.

With the lowest recorded preference temperature being 9.9°C and 23% of the wrasse being below the thermal niche, this suggests that some individuals may prefer psychrophilic conditions (temperatures lower than thermal niche). It is recommended that further studies be carried out with a lower minimum temperature, to allow more data to support and further explore this theory.

The Loligo shuttle box system has proved to be an accurate and reliable tool for non-invasive and non-destructive method of determining thermal niche. Additionally, the system offers the possibility to determine preferences for various other environmental factors, such as water turbidity, salinity, pH and oxygen saturation. With this enhancement, the system can be used as a more versatile tool for analysing fish responses to multiple environmental stressors. Assessing these factors in ballan wrasse would add to the knowledge and understanding of the species and assist with deployment decisions and welfare monitoring.

4.5. Conclusion

By using a non-destructive and stress-limiting machine vision shuttle box system, this study provided an accurate thermal niche and optimal temperature for ballan wrasse. The data indicates that there are significant differences between ballan wrasse and Atlantic salmon in thermal niche, a critical biological characteristic. Atlantic salmon are known to have a very large thermal niche, compared to the results from this study on wrasse (Lacroix, 2013; Anttila *et al*., 2014; Hvas *et al*., 2017). Learning from these fundamental differences is essential to optimize the use of ballan wrasse as cleaner fish and ensure proper animal welfare. Taking thermal niche into account when making decisions on use of wrasse as a cleaner fish in the salmon farming industry has the potential to improve welfare, as well as improve cleaning efficacy. The effects of cohabitation of ballan wrasse and lumpfish in sea cages should be further explored and trialled, due to their differing thermal niches, thus differing seasons that they will be most effective at delousing.

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ETHICS STATEMENT AND APPROVALS

Daily routine checks and observations were conducted and documented by CSAR technicians to ensure the health and normal behaviour of all Lumpfish and Wrasse. This included monitoring for indicators of poor health, such as fin or skin damage, deformed suction discs, emaciation, pop-eye or eye darkening. Additionally, these experiments relied heavily on observations, making it easy to identify any unhealthy or stressed fish. If a significant number of fish showed signs of poor health, the experiments would have either been terminated or a new stock of fish used, and appropriate action taken to safeguard the welfare of the fish. However, the ethical approval obtained for the study (presented in the appendices) showed that such intervention was not necessary.

Appendix

Ethics approval

Chapter 2

Initial Proposal to AWERB Group

Reference Number: STU_BIOL_209494_110522154922_1

Status: Approved Proposal :AWERB Group DECISION Details

Submitted By: Carlos Garcia De Leaniz

Submitted Date: 12 May 2022

Please note form is opened as read-only.

Please provide the information requested in sections 1-16 below. Use lay terms where possible and avoid confidential material.

1. Title of Research Project/teaching activity involving live animals:

Use of machine vision for improving welfare in cleaner fish

2. College:

Science

3. Staff/students undertaking research:

Isla Monaghan

4. Primary staff contact detail (Name, E-mail, Phone):

Carlos Garcia De Leaniz - c.garciadeleaniz@swansea.ac.uk

5.A. Proposed start date of project/activity:

25.04.2022

5.B. Duration:

1 year

6. Location(s) where the project/activity will take place: **(Please note, any work on Swansea University grounds that involves disturbance to live animals must have prior approval from estates and/or the biodiversity team)**

CSAR, Swansea University, Singleton Campus.

7. Partner bodies / organisations:

(i) their full, official name(s) / title(s);

(ii) details of the work to be carried out (a) at the partner(s) and (b) at the University;

(iii) details of the relevant ethical approval(s) from the partner(s), including reference numbers.

Visifish Ltd.
Staff at Visifish will receive video footage of cleaner fish of known sizes (filmed by myself). The company will use the videos to train machine learning algorithms to calculate BMI.

8. Please state or tick, as appropriate, the following questions relating to your project:(tick any that apply during the progression of an experiment)

a) species and taxon: Ballan Wrasse (Labrus bergylta) & Lumpfish (Cyclopterus

b) approximate number: 30 of each species (60 fish in total)

c) life stage:

Juvenile/Adults ☑

Mammal, bird or reptile embryo beyond halfway through incubation/gestation period ☐

Amphibian, cephalopod or fish larvae capable of independent feeding ☐

Strictly only gametes/very early developmental stages of embryos ☐

9.A. Does the proposed project/activity involve a Schedule 1 killing method (as defined by ASPA 1986) being carried out by members of this University's staff or by it's student?

◉ Yes ○ No

9.B. If yes, please list the individuals involved and provide training details:

All fish used in this experiment with be euthanized immediately after they have been filmed (for 5-10 minutes) in a tank via an overdose of anaesthetic as per HO schedule 1. Training will be provided by technician Craig Pooley. After training, I will be supervised until deemed competent.

10. Provide a **brief** scientific background for the work, and describe any pilot work undertaken:

This study will attempt to determine if AI/machine vision technology can be used to calculate BMI (i.e. Body Mass Index) from still images and/or videos, as part of a welfare assessment. No pilot work has been undertaken.

11. Please provide a clear methodology for the work to be undertaken:

1 Ballan Wrasse and 1 Lumpfish (< 30g) will be placed in a 30 litre tank and filmed for 5-10 minutes. Oxygen levels will be maintained at 100% and will be monitored throughout the trial. After filming, both fish will be removed and euthanized by an overdose of anaesthesia. They will then be weighed, measured and photographed for morphometric analysis.

12. Provide a **brief** statement of how science will advance or people or animals will benefit from this project:

This will help to improve the monitoring of cleaner fish health across the Aquaculture industry. It will also provide a non-invasive/non-destructive method of monitoring body condition, which is a key aspect of cleaner fish welfare.

13. Why do animals have to be used in this study? Explain your choice of species, and justify the number of subjects to be used with a power analysis where appropriate.

Ballan Wrasse and Lumpfish are two species of fish that are commonly used as cleaner fish in Aquaculture (Atlantic Salmon farming mostly). We need to use live fish for this study to calibrate the AI/machine vision technology. The reason for using 30 fish per species is to get a variety of sizes and weights to help calibrate for BMI.

14. What effects will your research have on the study organisms, and how suffering will be kept to a minimum?

Fish will be euthanized immediately after 5-10 minutes of filming. Suffering will be kept to a minimum by ensuring that the tanks used for filming are monitored and maintained at optimal water quality conditions (water temperature, Oxygen level, ammonia) similar to the home tanks. The low density (2 fish per tank) and short duration of filming (5-10min) ensures there will be no loss of water quality and minimum stress caused to the fish

15. How will you dispose of carcasses/animals (tick any that apply):

- Landfill ☐
- Sampled/analysis/other destruction of biomass ☑
- Released ☐
- Sent live to external organisation ☐

16. Will your study **make use** of genetic resources, traditional knowledge associated with genetic resources, or benefit from the **utilization** of such genetic resources and associated traditional knowledge? See guidance here and in the UK gov site here. A self-assessment tool is available in the UK gov site. Note that this applies to 'USE' and 'UTILIZATION'. Simply generating knowledge is not 'USE'.

○ Yes ○ No

17. Please provide details of any animal-related training required by staff / students as part of this project / activity. How will their competency be assured?

Husbandry training will be provided by technician Craig Pooley. After training, I will be supervised until deemed competent.

DECLARATION 1

I confirm that the information provided in this proposal is accurate and as complete as possible. ☑

DECLARATION 2

I also confirm that I understand that all projects and activities involving live animals shall be undertaken in accordance with relevant external and internal policies, regulations, codes of practice and other requirements, and that further information on these is available from University and College research and teaching support services. (Further information on the University's AWERB is available via erp@swansea.ac.uk). ☑

College Ethics Committee/AWERB Group DECISION on Ethical Review

Application Details

Project Title: Use of machine vision for improving welfare in cleaner fish

Applicant Name: Isla Monaghan

Submitted by: Carlos Garcia De Leaniz

Full application details can be found in AWERB Review Form .

Having examined the information included in the above application with Reference No. STU_BIOL_209494_110522154922_1, this Committee has decided to:

☑ **Approve this application**

with the following reputation risk to the University

☑ Low Risk ☐ Moderate Risk ☐ High Risk

Any amendments to approved proposals should be emailed to College Ethics Committee for review: cosethics@swan.ac.uk

AWERB IP Reference Number: IP-2122-15

Swansea University

AWERB – Initial Proposal – Approval Notification

The above Initial Proposal has been subject to ethical and welfare review by the University's AWERB and has been **APPROVED** to proceed in accordance with the terms and conditions specified below.

The following reference should be noted and should be used in all communications concerning this proposal: **IP-2122-15**

AWERB approval is subject to the following terms and conditions:-

1. This approval is on the basis of the information which has been provided to AWERB. If the project is modified from the Initial Proposal considered by AWERB then a full amendment shall be submitted to that Body for review and approval before the modified work is undertaken. (Please mark the submission as an IP Amendment and quote the above IP reference number.)
2. Approved work is to commence within 12 months from the date of this notice and shall be for the duration specified in the

The following reference should be noted and should be used in all communications concerning this proposal: **IP-2122-15**

AWERB approval is subject to the following terms and conditions:-

1. This approval is on the basis of the information which has been provided to AWERB. If the project is modified from the Initial Proposal considered by AWERB then a full amendment shall be submitted to that Body for review and approval before the modified work is undertaken. (Please mark the submission as an IP Amendment and quote the above IP reference number.)
2. Approved work is to commence within 12 months from the date of this notice and shall be for the duration specified in the Initial Proposal. This period may be extended by AWERB upon consideration of a request submitted before the end of the current approval period. (Please mark the submission as an IP Extension and quote the above IP reference number.)
3. This project shall be subject to periodic review by AWERB and the approval of that Body may be varied or rescinded at any time for good reason.
4. This project shall be undertaken in accordance with relevant external and internal requirements and regulations.

Date of AWERB Approval: 16 May 2022

Notification issued by: AWERB committee

☐ **Reject this application and allow for resubmission provided the ethical issues raised by the College Ethics Committee/AWERB Group below are addressed**

☐ **Return for minor amendment/clarification (please resubmit using the 'Resubmit minor amendment' option for a quick turnaround for approval)**

Comments:

The AWERB committee approve this proposal (16/05/2022):

R1 - No concerns from myself

R2 - No queries from me on this work

R3 - No concerns

Last Updated Date: 16 May 2022

Chapter 3

Initial Proposal to AWERB Group

Click to view/update Committee DECISION Form

Reference Number: STU_BIOL_225099_211022093538_1

Status: Approved Proposal :AWERB Group DECISION Details

Submitted By: Carlos Garcia De Leaniz

Submitted Date: 31 Oct 2022

Please note form is opened as read-only.

Please provide the information requested in sections 1-16 below. Use lay terms where possible and avoid confidential material.

1. Title of Research Project/teaching activity involving live animals:

Screening of delousing behaviour in cleaner fish

2. College:

Science

3. Staff/students undertaking research:

Isla Monaghan
Synne Klute
Gemma Winslade

4. Primary staff contact detail (Name, E-mail, Phone):

Carlos Garcia de Leaniz - c.garciadeleaniz@swansea.ac.uk

Swansea University

AWERB – Initial Proposal – Approval Notification

The above Initial Proposal has been subject to ethical and welfare review by the University's AWERB and has been **APPROVED** to proceed in accordance with the terms and conditions specified below.

The following reference should be noted and should be used in all communications concerning this proposal: **IP-2223-07**

AWERB approval is subject to the following terms and conditions:-

1. This approval is on the basis of the information which has been provided to AWERB. If the project is modified from the Initial Proposal considered by AWERB then a full amendment shall be submitted to that Body for review and approval before the modified work is undertaken. (Please mark the submission as an IP Amendment and quote the above IP reference number.)
2. Approved work is to commence within 12 months from the date of this notice and shall be for the duration specified in the Initial Proposal. This period may be extended by AWERB upon consideration of a request submitted before the end of the current approval period. (Please mark the submission as an IP Extension and quote the above IP reference number.)
3. This project shall be subject to periodic review by AWERB and the approval of that Body may be varied or rescinded at any time for good reason.
4. This project shall be undertaken in accordance with relevant external and internal requirements and regulations.

Date of AWERB Approval: 16 Jan 2023

Notification issued by: AWERB committee

☐ **Reject this application and allow for resubmission provided the ethical issues raised by the College Ethics Committee/AWERB Group below are addressed**

☐ **Return for minor amendment/clarification (please resubmit using the 'Resubmit minor amendment' option for a quick turnaround for approval)**

8. Please state or tick, as appropriate, the following questions relating to your project:(tick any that apply during the progression of an experiment)

a) species and taxon: Ballan Wrasse (Labrus bergylta) and Lumpfish (Cyclopteru

b) approximate number: up to 90 of each species (up to 180 fish in total)

c) life stage:

Juvenile/Adults ☑

Mammal, bird or reptile embryo beyond halfway through incubation/gestation period ☐

Amphibian, cephalopod or fish larvae capable of independent feeding ☐

Strictly only gametes/very early developmental stages of embryos ☐

9.A. Does the proposed project/activity involve a Schedule 1 killing method (as defined by ASPA 1986) being carried out by members of this University's staff or by it's student?

◉ Yes ○ No

9.B. If yes, please list the individuals involved and provide training details:

CSAR technicians

10. Provide a **brief** scientific background for the work, and describe any pilot work undertaken:

Building on a recent study on lumpfish (Whittaker et al 2021. Personality profiling may help select better cleaner fish for sea-lice control in salmon farming. Applied Animal Behaviour Science 243), approved by AWERB with permit IP1617-27, we wish to extend this to ballan wrasse (Labrus bergylta) and test if personality profiling can be used to predict delousing behaviour using model sea-lice.

11. Please provide a clear methodology for the work to be undertaken:

The study consists of two phases (personality profiling and delousing behaviour)

1. Personality profiling
This will follow a simplified version of the approved protocol used in Whittaker et al 2021. A test arena (L120 cm x W55 cm x D25 cm) divided into four equal zones and fitted with a removable overhead shelter and a sliding door is used. An air stone in the start-box ensures dissolved oxygen is kept at 100% saturation. The arena is surrounded by a black tarpaulin screen to minimise disruption and is fitted with a small red lego brick glued to the bottom of the central section and a mirror (W55 cm x D25 cm) opposite the shelter. A camera is positioned above the tank. A a single cleaner fish (lumpfish or wrasse determined at random) is introduced into the start-box, and acclimatised for 10 min with the sliding door closed; The door is slowly lifted and the time taken to leave the shelter is recorded, as well as the number of approaches to the novel object during the next 10 minutes. After 10 min, the mirror is uncovered and the number of approaches to the mirror is recorded for another 10 mins. In total the test lasts for 30 min (10+10+10). The fish is then removed placed in a zip-lock bag with water, quickly measured and transferred to the delousing test tank (see below). The test tank is drained and refilled with clean water to avoid a build-up of stress hormone and readied for the next fish. This was approved for lumpfish under IP1617-27 and we do not anticipate any problems extending this to wrasse (we are bypassing two phases, reducing handling and dispensing with the need to collect body mass, by simply measuring the fish inside a ziplock bag instead). This will be done for 40 lumpfish and 40 wrasse

2. Delousing behaviour
After screening (above) each fish will be placed in a circular (RAS) tank (similar to the home tank) surrounded by black tarpaulin to minimise disruption and fitted with an overhead camera. The fish will be left to acclimatise for 10 min after which three fish models, located in one side of the tank and previously shielded from view behind a screen, will be uncovered. The fish models will be fixed in place with a frame and consist of two 3D plastic salmon models, one realistically painted and one without any paint (grey colour), while model 3 is a half-pipe of the same size and a colour as the two fish models. The time spent and number of approaches to the models will be recorded for 1 hr and related to the behavioural screening. This will be done for half of the fish (20 lumpfish and 20 wrasse). For the other half of the fish (20 lumpfish, 20 wrasse) instead of three different models we will present them with three half-pipes fitted with realistic 3D dummy models of sea lice (one with no sea lice, one with 1 sea-lice, one with 6 sea lice). These are currently made of silicone, as used by U of Stirling. A pilot trial is under way to ensure these are safe, should a fish accidentally ingest one (we think this is unlikely as they are expected to spit them). We have tried agar for the 3D mould are and also sourcing additional jelly-like, edible materials.

After the trial, the fish will be returned to a separate home tank.

12. Provide a **brief** statement of how science will advance or people or animals will benefit from this project:

Using live sea-lice to examine delousing behaviour is problematic as it has welfare implications for the fish host and it is difficult to replicate in the laboratory. The use of use of dummy sea-lice and fish models may overcome these difficulties without affecting fish welfare.

13. Why do animals have to be used in this study? Explain your choice of species, and justify the number of subjects to be used with a power analysis where appropriate.

We can only use live fish to improve the delousing behaviour of cleaner fish. Ballan wrasse and lumpfish are the two species most commonly used as cleaner fish in salmon farming

14. What effects will your research have on the study organisms, and how suffering will be kept to a minimum?

Suffering will be kept to a minimum by ensuring that the tanks used for filming are monitored and maintained at optimal water quality conditions (temperature, oxygen level, ammonia similar to their home tanks. The water in the tanks will also be replaced after every fish to avoid a build-up of stress hormone. The low density (1 per tank) and short duration of the trial (30+70 minutes) ensures that there will be no loss of water quality and minimum stress caused to the fish. The fish will be monitored continuously and immediate action will be taken should any problem occur. We have never had any problem using similar procedures in other cleaner fish student projects approved by AWERB.

15. How will you dispose of carcasses/animals (tick any that apply):

Landfill ☐

Sampled/analysis/other destruction of biomass ☑

Released ☐

Sent live to external organisation ☐

16. Will your study **make use** of genetic resources, traditional knowledge associated with genetic resources, or benefit from the **utilization** of such genetic resources and associated traditional knowledge? See guidance here and in the UK gov site here. A self-assessment tool is available in the UK gov site. Note that this applies to 'USE' and 'UTILIZATION'. Simply generating knowledge is not 'USE'.

○ Yes ◉ No

17. Please provide details of any animal-related training required by staff / students as part of this project / activity. How will their competency be assured?

Husbandry training was provided by senior technician Craig Pooley.

DECLARATION 1

I confirm that the information provided in this proposal is accurate and as complete as possible. ☑

DECLARATION 2

I also confirm that I understand that all projects and activities involving live animals shall be undertaken in accordance with relevant external and internal policies, regulations, codes of practice and other requirements, and that further information on these is available from University and College research and teaching support services. (Further information on the University's AWERB is available via erp@swansea.ac.uk). ☑

Applicants comments to the issues/concerns raised for previous submission:

Queries from AWERB:
R1 - No concerns with this one once there is clarity that either the latex lice pose no harm to the fish or an edible material is sourced.

R2 - Yes, I agree no concerns with the work in general but could they provide details of the pilot trial that is being undertaken to ensure safety.

RESPONSE

In response to the queries from AWERB please see below the details of the pilot trial undertaken this week.

A pilot trial was carried out to determine if the sea lice models made from latex could be removed (bitten off/swallowed) by any of the fish used in the pilot.
4 tests were carried out with 6 fish per tank. Ballan Wrasse and Lumpfish were filmed for this trial (not mixed in the tanks). 4 latex sea lice were attached to the sides of a 3D printed Atlantic Salmon model (using marine grade silicone). The 6 fish were placed in the tank and left to acclimatise for 10 minutes. After 10 minutes, the salmon model was lowered into the tank. A GoPro was also placed under the salmon model and set to film for one hour. The tanks were checked approximately every 10 minutes to see if any lice had been removed. After the hour of filming, the model was removed, and the fish were placed back in their home tanks. The footage captured on the GoPros was then analyzed.

The lumpfish showed no interest in the salmon model or the latex sea lice.
The Ballan Wrasse showed some interest (i.e. swam close to the latex sea lice and circled) but did not nibble or bite them.

Different materials were also trialed for the sea lice to see if there was an option that was not harmful if ingested. Agar, gelatin and edible glue were all tested several times (at different concentrations) but were deemed unusable.

##########
RESPONSE (31/10/2022)

Please note

1. Yes the proposed experiment involves ONLY visual clues (no ingestion)
2. The sea-lice are GLUED firmly to the salmon model, they cannot be dislodged by the cleaner fish
3. The use of the same artificial sea-lice as visual clues has previously been approved by the AWERB ctee of our project partners at Stirling University

College Ethics Committee/AWERB Group DECISION on Ethical Review

Application Details

Project Title: Use of machine vision for improving welfare in cleaner fish

Applicant Name: Isla Monaghan

Submitted by: Carlos Garcia De Leaniz

Full application details can be found in AWERB Review Form .

Having examined the information included in the above application with Reference No. STU_BIOL_225099_211022093538_1, this Committee has decided to:

☑ **Approve this application**

with the following reputation risk to the University

☑ Low Risk ☐ Moderate Risk ☐ High Risk

Any amendments to approved proposals should be emailed to College Ethics Committee for review: cosethics@swan.ac.uk

AWERB IP Reference Number: IP-2223-07

Swansea University

AWERB – Initial Proposal – Approval Notification

The above Initial Proposal has been subject to ethical and welfare review by the University's AWERB and has been **APPROVED** to proceed in accordance with the terms and conditions specified below.

The following reference should be noted and should be used in all communications concerning this proposal: **IP-2223-07**

AWERB approval is subject to the following terms and conditions:-

1. This approval is on the basis of the information which has been provided to AWERB. If the project is modified from the Initial Proposal considered by AWERB then a full amendment shall be submitted to that Body for review and approval before the modified work is undertaken. (Please mark the submission as an IP Amendment and quote the above IP reference number.)
2. Approved work is to commence within 12 months from the date of this notice and shall be for the duration specified in the Initial Proposal. This period may be extended by AWERB upon consideration of a request submitted before the end of the current approval period. (Please mark the submission as an IP Extension and quote the above IP reference number.)
3. This project shall be subject to periodic review by AWERB and the approval of that Body may be varied or rescinded at any time for good reason.
4. This project shall be undertaken in accordance with relevant external and internal requirements and regulations.

Date of AWERB Approval: 16 Jan 2023

Notification issued by: AWERB committee

☐ **Reject this application and allow for resubmission provided the ethical issues raised by the College Ethics Committee/AWERB Group below are addressed**

☐ **Return for minor amendment/clarification (please resubmit using the 'Resubmit minor amendment' option for a quick turnaround for approval)**

Comments:

NOTE - Title on approval does not match that of the application. This is an error that I can't correct due to the form now being locked but I can confirm that this is the approval to go with the project: Screening of delousing behaviour in cleaner fish - Becky Stringwell

The AWERB committee have no further concerns (31/10/2022)

R1 - That's fine with me

R2 - No further concerns

##########

The AWERB committee still have concerns regarding the latex sealice. Is it possible to consider fixing them securly so that no parts can be pulled off and ingested? See comment from reviewer 1 (31/10/2022):

R1 - Unfortunately it doesn't help assessment of the suitability of latex sealice as they weren't eaten. My general view would be they shouldn't be ingested as they won't be digestible and with their size we don't have evidence they can be passed safely without causing an obstruction. If they could be used as a visual cue to check for interest from cleanerfish I feel that's fine but without evidence to the contrary they shouldn't have access to be able to eat them.

##########
#

The AWERB require further information regarding the pilot trial prior to approval being given (26/10/2022):

R1 - No concerns with this one once there is clarity that either the latex lice pose no harm to the fish or an edible material is sourced.

R2 - Yes, I agree no concerns with the work in general but could they provide details of the pilot trial that is being undertaken to ensure safety.

##########
Response, please note

1. Yes the proposed experiment involves ONLY visual clues (no ingestion)
2. The sea-lice are GLUED firmly to the salmon model, they cannot be dislodged by the cleaner fish
3. The use of the same artificial sea-lice as visual clues has previously been approved by the AWERB ctee of our project partners at Stirling University

Chapter 4

Initial Proposal to AWERB Group

Reference Number: STAFF_BIOL_224593_171022155204_1

Status: Approved Proposal :AWERB Group DECISION Details

Submitted By: Rebecca Stringwell

Submitted Date: 28 Oct 2022

Please note form is opened as read-only.

Please provide the information requested in sections 1-16 below. Use lay terms where possible and avoid confidential material.

1. Title of Research Project/teaching activity involving live animals:

Shuttle box experiments to determine the temperature preference of ballan wrasse

2. College:

Science

3. Staff/students undertaking research:

Sarah Weller, Jack Van-Eker, Ben Overland, Isla Monaghan

4. Primary staff contact detail (Name, E-mail, Phone):

Sarah Weller - sarah.weller@swansea.ac.uk

5.A. Proposed start date of project/activity:

17/10/2022

5.B. Duration:

6 months

6. Location(s) where the project/activity will take place: **(Please note, any work on Swansea University grounds that involves disturbance to live animals must have prior approval from estates and/or the biodiversity team)**

Centre for Sustainable Aquatic Research (CSAR)

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7. Partner bodies / organisations:

(i) their full, official name(s) / title(s);

(ii) details of the work to be carried out (a) at the partner(s) and (b) at the University;

(iii) details of the relevant ethical approval(s) from the partner(s), including reference numbers.

N/A

8. Please state or tick, as appropriate, the following questions relating to your project:(tick any that apply during the progression of an experiment)

a) species and taxon: Labrus bergylta

b) approximate number: 30

c) life stage:

Juvenile/Adults ☑

Mammal, bird or reptile embryo beyond halfway through incubation/gestation period ☐

Amphibian, cephalopod or fish larvae capable of independent feeding ☐

Strictly only gametes/very early developmental stages of embryos ☐

9.A. Does the proposed project/activity involve a Schedule 1 killing method (as defined by ASPA 1986) being carried out by members of this University's staff or by it's student?

○ Yes ◉ No

9.B. If yes, please list the individuals involved and provide training details:

N/A

N/A

10. Provide a **brief** scientific background for the work, and describe any pilot work undertaken:

Temperature is a key factor determining physiological function in fish and influences habitat usage as fish actively seek out suitable thermal zones. Determining species temperature preferences is essential to predict how future climate change is likely to influence species distributions. Traditional approaches have involved the use of chambered setups allowing the fish to seek out their temperature preference. However, these fixed temperature experiments are often confounded by heterogeneous conditions of light intensity, cover, water depth etc (McCauley et al., 1977; Kwain and McCauley, 1978; Myrick et al., 2004).

We propose to test the temperature preference of ballan wrasse (Labrus bergylta) using a Loligo shuttle box setup (Neill et al., 1972; Nay et al., 2020; Christensen et al., 2021), which allows automated precision control of water temperature. The setup allows fish to shuttle back and forth between the two sides of the shuttle box, thereby adjusting their ambient conditions, allowing for more accurate measurement of temperature preference. Ballan wrasse are an important commercial species used as cleaner fish to control sea lice infestations in salmonid aquaculture but temperature preference data for this species is currently lacking. The temperature preference data obtained will be fed into species distribution models to indicate where wrasse can be used as cleaner fish and also to predict the future impact of climate change on wild wrasse distributions. The underlying mechanisms driving individual differences in temperature preference are largely unknown.

McCauley, R.W., 1977. Laboratory methods for determining temperature preference. Journal of the Fisheries board of Canada, 34(5), pp.749-752.

Kwain, W.H. and McCauley, R.W., 1978. Effects of age and overhead illumination on temperatures preferred by underyearling rainbow trout, Salmo gairdneri, in a vertical temperature gradient. Journal of the Fisheries Board of Canada, 35(11), pp.1430-1433.

A Myrick, C. and Cech, J.J., 2004. Temperature effects on juvenile anadromous salmonids in California's central valley: what don't we know?. Reviews in Fish Biology and Fisheries, 14(1), pp.113-123.

Neill, W.H., Magnuson, J.J. and Chipman, G.G., 1972. Behavioral thermoregulation by fishes: a new experimental approach. Science, 176(4042), pp.1443-1445.

Nay, T.J., Johansen, J.L., Rummer, J.L., Steffensen, J.F., Pratchett, M.S. and Hoey, A.S., 2020. Habitat complexity influences selection of thermal environment in a common coral reef fish. Conservation Physiology, 8(1), p.70.

Christensen, E.A., Andersen, L.E., Bergsson, H., Steffensen, J.F. and Killen, S.S., 2021. Shuttle-box systems for studying preferred environmental ranges by aquatic animals. Conservation physiology, 9(1), p.28.

11. Please provide a clear methodology for the work to be undertaken:

Thirty juvenile ballan wrasse (approx. 93-136 mm total length; approx. 14-48 g each) will be collected from Ocean Matters in Anglesey. Once 30 ballan wrasse have been collected, they will be transported back to CSAR.
The wrasse will be housed in cylindrical housing tanks [1.2 m (D) * 0.6 m (H); approx. 500 L] connected to a recirculating aquaculture system (total volume approx. 5000L) incorporating mechanical and biological filtration, as well as UV treatment. The system will be filled with seawater (source: Swansea Bay) and temperature maintained at 15°C as confirmed by CSAR technicians. Biological filters will be primed in advance. Water quality parameters (nitrite, nitrate, ammonia, pH) will be checked weekly. Tanks are equipped with alarm systems should water quality (DO,temp, pH) deviate substantially from the parameters set. No more than 20 fish will be housed in each 500L tank ensuring a low biomass:water volume ratio. Ballan wrasse will be fed daily to satiation on marine pellet food. Tanks will be checked and cleaned if necessary on a daily basis.

11. Please provide a clear methodology for the work to be undertaken:

Thirty juvenile ballan wrasse (approx. 93-136 mm total length; approx. 14-48 g each) will be collected from Ocean Matters in Anglesey. Once 30 ballan wrasse have been collected, they will be transported back to CSAR.
The wrasse will be housed in cylindrical housing tanks [1.2 m (D) * 0.6 m (H); approx. 500 L] connected to a recirculating aquaculture system (total volume approx. 5000L) incorporating mechanical and biological filtration, as well as UV treatment. The system will be filled with seawater (source: Swansea Bay) and temperature maintained at 15°C as confirmed by CSAR technicians. Biological filters will be primed in advance. Water quality parameters (nitrite, nitrate, ammonia, pH) will be checked weekly. Tanks are equipped with alarm systems should water quality (DO,temp, pH) deviate substantially from the parameters set. No more than 20 fish will be housed in each 500L tank ensuring a low biomass:water volume ratio. Ballan wrasse will be fed daily to satiation on marine pellet food. Tanks will be checked and cleaned if necessary on a daily basis.

The shuttle box setup used to test temperature preference consists of two circular tanks (50 cm diameter, 25 cm deep) connected by a
10 cm (L) * 7.5 cm (W) * 25 cm (D) channel. A red-tinted acrylic cover will be placed over the top of the entire shuttle box whilst leaving a gap for the removable gate, to make the fish feel safe and hidden whilst in the tank. The setup will be filled with housing water to 12 cm depth. A continuous circular current will be maintained in each chamber by pumping water into two raised buffer tanks placed beside the shuttlebox and allowing water to flow gravitationally back into chambers. An overhead camera will monitor fish movements within the setup, relaying the location of the fish in chambers, and triggering an increase or decrease in system's water temperature based on the fish's position in the increasing or decreasing chamber. The specialised camera can detect fish movements, which are input into the software.
Water temperature will be controlled with a series of pumps connected to a relay device instrument that will turn them on and off as directed by automation software. These drive water through heat exchange coils located in warm (25°C) and cold (3°C) baths (Figure 1). Water temperature in each chamber will therefore be independently controlled by the software, relaying information between the temperature sensors, the relay device, and a series of pumps, maintaining desired temperatures between chambers. The thermal limits in the shuttle box will be set at 10°C and 25°C: the thermal limits of ballan wrasse (Brooker et al., 2018; Treasurer et al., 2018; Yuen et al., 2019). However, because fish always have the choice to enter warmer or colder water (temperature difference between two sides of the shuttle box kept within 2°C and not allowed to change more than 2°C/h) it is highly unlikely that they will choose to occupy temperatures close to their thermal limits. During the first few trials we will carefully monitor experiments to ensure software is working correctly and the fish are never exposed to unsuitable temperatures. There will also be a Seneye probe in the shuttle box to measure the oxygen levels within the tank to ensure the correct levels are maintained throughout the experiments. The Seneye will notify us through an alarm if the oxygen levels become too low, allowing us to rectify any issues immediately, before any harm is caused to the fish.
For each experiment, a single ballan wrasse will be netted from housing tanks and transferred to one side (randomly selected) of the shuttle box, both sides set to the same temperature as the water in their housing tanks which will be between 11°C and 15°C. Experiments will be started at 9am daily with the first 4 fish being closely monitored throughout the initial 6 hours of the experiments to ensure the system is working as it should, with no significant temperature fluctuations. The minimum and maximum temperatures used in the software will be set to the thermal preference for ballan wrasse (10-25 °C) and therefore any changes in temperature will be within this range, not being able to change outside of these values. This will be left to run overnight, and fish will be removed at 9am the next morning. The fish will not require observation overnight due to the reliability of the system with both of the pumps needing to fail at the same time for there to be an issue, which is very unlikely. The fish will be observed for any signs of stress e.g. abnormal swimming behaviour. If any fish shows signs of prolonged stress, they will be removed from the shuttlebox and returned to housing tanks. We will work closely with the NACWO while undertaking experiments to ensure welfare standards are maintained.
After 24hrs the fish will be removed from the shuttlebox and slowly acclimatised in a separate tank also covered by a red-tinted acrylic sheet back to the temperature of their housing tanks and will be returned to these straight after their acclimation. This will allow a complete water change of the shuttle box to be undertaken following each experiment.

Brooker, A. J., Papadopoulou, A., Gutierrez, C., Rey, S., Davie, A., & Migaud, H., 2018. Sustainable production and use of cleaner fish for the biological control of sea lice: recent advances and current challenges. Veterinary Record, 183

PALCI EBSCO books & Treasurer, J. W. (2018). Cleaner fish biology and aquaculture applications. 5m Publishing Ltd.

Yuen, J.W., Dempster, T., Oppedal, F. and Hvas, M., 2019. Physiological performance of ballan wrasse (Labrus bergylta) at different temperatures and its implication for cleaner fish usage in salmon aquaculture. Biological Control, 135, pp.117-123.

12. Provide a **brief** statement of how science will advance or people or animals will benefit from this project:

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The project will help identify the underlying mechanisms determining individual differences in temperature preference. The temperature preference data will help us determine where ballan wrasse can be used in salmonid aquaculture and predict future changes in the distribution of wild populations with climate change using species distribution models.

13. Why do animals have to be used in this study? Explain your choice of species, and justify the number of subjects to be used with a power analysis where appropriate.

There is no viable alternative to using animals because this study looks at the behaviour of fish.
Ballan wrasse are used as cleaner fish in salmonid aquaculture and it is important to determine their temperature preference to inform the industry to improve welfare.

14. What effects will your research have on the study organisms, and how suffering will be kept to a minimum?

The shuttle box allows the fish to control their ambient temperature according to their preference and the fish experience minimal stress. The maximum temperature range (10-25 degrees C) proposed is within the habitat preferences of the species (Brooker et al., 2018; Treasurer et al., 2018; Yuen et al., 2019). Based on the guidance in Hawkins et al. (2011) the procedure described above would fall under the 'subthreshold' category.
Hawkins et al., (2011) include an example: "Manipulations of temperature within temperature ranges experienced by the species in its natural habitat where the speed of change is such that the animals can adapt without significant physiological stress." which is similar to what we propose. In the shuttle box the fish will always have the choice to adjust their ambient conditions according to their preferences. The fish will not be fed while in the shuttle box but this should not cause any suffering over the timeframe proposed (24hrs). Hawkins et al. (2011) also describe this as a subthreshold procedure: "Withdrawal of food for a short interval relative to normal food intake at that stage of the life cycle, for example food withdrawal in adult salmonids for up to 48h.". Suffering will be kept to a minimum by handling fish as little as possible while transferring them between housing tanks and the experimental apparatus.
In addition, this shuttle box has already been used for 60 experiments across seabass and lumpfish and the system has been running as it should with no issues for a number of weeks. Therefore, we are confident the shuttle box will be able to maintain the desired conditions without putting the fish under any unnecessary stress. In addition, this protocol has already been approved for both seabass and lumpfish.

Brooker, A. J., Papadopoulou, A., Gutierrez, C., Rey, S., Davie, A., & Migaud, H., 2018. Sustainable production and use of cleaner fish for the biological control of sea lice: recent advances and current challenges. Veterinary Record, 183

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15. How will you dispose of carcasses/animals (tick any that apply):

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Landfill ☑

Sampled/analysis/other destruction of biomass ☑

Released ☐

Sent live to external organisation ☐

16. Will your study **make use** of genetic resources, traditional knowledge associated with genetic resources, or benefit from the **utilization** of such genetic resources and associated traditional knowledge? See guidance here and in the UK gov site here. A self-assessment tool is available in the UK gov site. Note that this applies to 'USE' and 'UTILIZATION'. Simply generating knowledge is not 'USE'.

○ Yes ◉ No

17. Please provide details of any animal-related training required by staff / students as part of this project / activity. How will their competency be assured?

Staff and students working on this project will undergo fish husbandry training before the project begins, to ensure stress and suffering inflicted on the fish is kept to a minimum.

DECLARATION 1

I confirm that the information provided in this proposal is accurate and as complete as possible. ☑

DECLARATION 2

I also confirm that I understand that all projects and activities involving live animals shall be undertaken in accordance with relevant external and internal policies, regulations, codes of practice and other requirements, and that further information on these is available from University and College research and teaching support services. (Further information on the University's AWERB is available via erp@swansea.ac.uk). ☑

Applicants comments to the issues/concerns raised for previous submission:

The following text addresses the comments made by reviewers 1 and 2.

The invalid typo of 'Collections will be undertaken under permit: fisheries exemptions from the Welsh Government have been applied for and NRW have been informed of the work' has been removed from the methods.

The temperature within the shuttle box will be set to the same temperature as the housing tanks the fish have come from which CSAR staff have confirmed will be between 11°C and 15°C.

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The temperature within the shuttle box will be set to the same temperature as the housing tanks the fish have come from which CSAR staff have confirmed will be between 11°C and 15°C.

A red-tinted acrylic cover will be placed over the top of the entire shuttle box, whilst leaving a gap for the removable gate to ensure the fish feel safe and hidden whilst in the tank.

The timings of the experiments will begin at 9am and the first 4 fish will be closely monitored throughout the initial 6 hours of the experiments to ensure the system is working as it should with no significant temperature fluctuations. The minimum and maximum temperatures used in the software will be set to the thermal preference for ballan wrasse (10-25 °C) and therefore any changes in temperature will be within this range, not being able to change outside of these values. Also, the acclimation is never sudden, slowly bringing the fish back to the temperature of their housing tanks before being returned.

The Seneye will notify us through an alarm if the oxygen levels become too low, allowing us to rectify any issues immediately, before any harm is caused to the fish.

In addition, the following procedure has already been approved for both sea bass and lumpfish.

Amendment 1 IP-2223-05.pdf

Amendment 2_shuttlebox salmon.pdf

Risk Assessments

COS Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #	Title: Routine Husbandry Duties in RAS A
Associated Protocols #.....	**Description: Carrying out routine daily duties to feed and care for the livestock held within the facility**

Location: RAS A, Centre for Sustainable Aquaculture Research, Llyr Building

circle which COS Local Rules apply –

~~**Boat Field Genetic Manipulation**~~ **Laboratory** ~~**Office/Facility Radioisotope**~~

Identify here risks and control measures for work in this environment, additional to Local Rules:
Slips, trips and falls- this room sometimes has wet floors, there is grey pipe at or just above floor level – suitable footwear to be worn in this area, floors to be dried after water spillages, high visibility tape to be applied to potential trip hazards.
Manual handling- There are some items that weigh in excess of 20kg ie, bag of fish feed, salt. Staff involved in heavy lifting should attend a manual handling course. Loads, where possible, should be distributed into smaller sizes or lifting equipment used.
Use of vaccines, therapeutics and disinfectant. See separate risk assessments.

Chemicals	Quantity	Hazards	Category (A,B,C,D)*	Exp. Score

Hazard Category (known or potential)
A (e.g. carcinogen/teratogen/mutagen)
B (e.g. v.toxic/toxic/explosive/pyrophoric)
C (e.g. harmful/irritant/corrosive/high flammable/oxidising)
D (e.g. non classified)

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

Low **Medium** **High**

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- n/a

Storage conditions and maximum duration :- n/a

Secondary containment (of protocol) open bench/fume hood/special (state) :- n/a

Disposal e.g. autoclaving of biohazard, UWS chemical disposal

Identify other control measures (circle or delete) - ~~latex/nitrile/heavy gloves; screens; full face mask; dust mask~~; protective shoes; ~~spillage tray; ear defenders; other (state)~~

Justification and controls for any work outside normal hours It may be necessary to work out of hours as animal husbandry can stretch into evenings and the weekends. Where possible when working out of hours should be done in pairs, if working alone then signing in and out using the CSAR in/out board and log book (Wallace Foyer) is imperative.

Emergency procedures (e.g. spillage clearance; communication methods) Nearest telephone is located in adjacent office, nearest first aid box located in CSAR changing room.

Supervision/training for worker (circle)

None required ~~Already trained~~ ~~Training required~~ ~~Supervised always~~

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker: Isla Monaghan

Name & counter-signature of supervisor: Carlos Garcia de Leaniz

COS Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #	**Title: Experiments at MOWI Anglesey**
Associated Protocols #.....	**Description: Filming, photographing and measuring in a laboratory in MOWI wrasse and lumpfish hatcheries in Anglesey, North Wales.**

Location: MOWI Wrasse and Lumpfish Hatcheries, Anglesey, North Wales.

circle which COS Local Rules apply –

~~**Boat Field Genetic Manipulation**~~ **Laboratory** ~~**Office/Facility Radioisotope**~~

Identify here risks and control measures for work in this environment, additional to Local Rules:
Slips, trips and falls- this room sometimes has wet floors – suitable footwear to be worn in this area, floors to be dried after water spillages, high visibility tape to be applied to potential trip hazards.

Chemicals	**Quantity**	**Hazards**	**Category (A,B,C,D)***	**Exp. Score**

Hazard Category (known or potential)
A (e.g. carcinogen/teratogen/mutagen)
B (e.g. v.toxic/toxic/explosive/pyrophoric)
C (e.g. harmful/irritant/corrosive/high flammable/oxidising)
D (e.g. non classified)

Exposure Potential Circle the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

Low **Medium** **High**

Primary containment (of product) sealed flask/bottle/glass/plastic/other (state) :- n/a

Storage conditions and maximum duration :- n/a

Secondary containment (of protocol) open bench/fume hood/special (state) :- n/a

Disposal e.g. autoclaving of biohazard, UWS chemical disposal

Identify other control measures (circle or delete) - ~~latex/nitrile/heavy gloves; screens; full face mask; dust mask~~; protective shoes; ~~spillage tray; ear defenders; other (state)~~

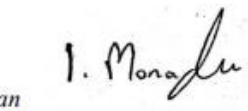
Justification and controls for any work outside normal hours n/a

Emergency procedures as per MOWI guidelines

Supervision/training for worker (circle)

None required ~~Already trained~~ ~~Training required~~ ~~Supervised always~~

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.



Name & signature of worker: Isla Monaghan

Name & counter-signature of supervisor Carlos Garcia de Leaniz

Date 01/10/2022.

Date of first reassessment	Frequency of reassessments

Raw Data

Chapter 1

Family	No_studies	Place	Percent
Salmonidae	6	1	33
Cyprinidae	2	2	11
Carangiformes	2	3	11
Clariidae	1	4	6
Ictaluridae	1	5	6
Cichlidae	1	6	6
Polyprionidae	1	7	6
Gobiidae	1	8	6
Moronidae	1	9	6
Osteoglossiformes	1	10	6
Sciaenidae	1	11	6

	LUMPFISH												
Number	Home Tank	Filming Tank	Date	Fish ID	Height_mm	St_length_mm	Total_length_mm	Width_mm	Weight_g		Skin Damage	Caudal Fin Damage	Eye Condition	Suction Disc
1	A7	1	05.07.2022	CLCSAR050722-01	65	135	148	45	142		0	0	0	0
2	A7	2	05.07.2022	CLCSAR050722-02	51	108	123	39	74		0	1	0	0
3	A7	1	05.07.2022	CLCSAR050722-03	61	125	140	46	112		0	1	0	0
4	A7	2	05.07.2022	CLCSAR050722-04	56	122	140	38	92		0	0	0	0
5	A7	1	05.07.2022	CLCSAR050722-05	64	132	151	46	140		0	1	0	0
6	A7	2	05.07.2022	CLCSAR050722-06	58	125	143	64	134		0	1	0	0
7	A7	1	06.07.2022	CLCSAR060722-01	67	142	162	47	158		0	0	0	0
8	A7	2	06.07.2022	CLCSAR060722-02	67	136	156	48	146		0	0	0	0
9	A7	1	08.07.2022	CLCSAR070822-01	63	131	151	42	124		0	0	0	0
10	A7	2	08.07.2022	CLCSAR070822-02	60	124	140	44	112		0	0	0	0
11	A7	1	08.07.2022	CLCSAR070822-03	65	143	162	44	146		0	1	0	0
12	A7	2	08.07.2022	CLCSAR070822-04	72	150	167	47	168		0	1	0	0
13	A7	1	11.07.2022	CLCSAR110722-01	77	151	169	57	228		0	1	0	0
14	A7	2	11.07.2022	CLCSAR110722-02	68	134	153	47	149		0	0	0	0
15	A7	1	11.07.2022	CLCSAR110722-03	67	139	160	49	153		0	0	0	0
16	A7	2	11.07.2022	CLCSAR110722-04	63	128	143	47	129		0	1	0	0
17	A7	1	13.07.2022	CLCSAR130722-01	61	129	146	46	129		0	1	0	0
18	A7	2	13.07.2022	CLCSAR130722-02	59	124	140	44	106		0	1	0	0
19	A7	1	13.07.2022	CLCSAR130722-03	60	126	138	42	111		0	2	0	0
20	A7	2	13.07.2022	CLCSAR130722-04	58	126	145	43	106		0	0	0	0
21	A7	1	14.07.2022	CLCSAR140722-01	69	139	158	54	162		0	0	0	0
22	A7	2	14.07.2022	CLCSAR140722-02	62	126	141	45	115		1	0	0	0
23	A7	1	14.07.2022	CLCSAR140722-03	57	116	130	39	82		0	1	0	0
24	A7	2	14.07.2022	CLCSAR140722-04	63	127	147	43	119		0	0	0	0
25	A7	1	18.07.2022	CLCSAR180722-01	69	140	154	52	172		0	2	0	0
26	A7	2	18.07.2022	CLCSAR180722-02	66	146	165	44	152		0	1	0	0
27	A7	1	18.07.2022	CLCSAR180722-03	65	131	145	47	134		0	1	0	0
28	A7	2	18.07.2022	CLCSAR180722-04	61	128	148	44	119		0	1	0	0
29	A7	1	19.07.2022	CLCSAR190722-01	55	119	140	39	93		0	1	0	0
30	A7	2	19.07.2022	CLCSAR190722-02	57	121	140	41	97		0	0	0	0

Excel Calculator			Online Calculator						
Expected Weight	BMI (%)		Expected Weight (g)	Expected Height (mm)	BMI (%)	Class		LOWSI	Welfare Class
146.7582474	96.7578		146.758	81.46	96.76	Normal		10	A - Good
81.46820724	90.4647		81.468	67.6	90.46	Normal		9	A - Good
122.9796582	91.1533		122.98	77	91.15	Normal		9	A - Good
122.9796582	74.8091		122.98	76.9	74.81	Emaciated		8	A - Good
156.4320258	89.4318		156.432	83.07	89.43	Underweight		8	A - Good
131.5600213	101.703		131.56	78.73	101.7	Normal		9	A - Good
195.6443278	80.9632		195.644	89.09	80.96	Underweight		9	A - Good
173.5119327	83.8559		173.512	85.8	83.86	Underweight		9	A - Good
156.4320258	79.2677		156.432	83	79.27	Underweight		9	A - Good
122.9796582	90.828		122.98	77	90.83	Normal		10	A - Good
195.6443278	74.6763		195.644	89.05	74.68	Emaciated		7	B - Compromised
215.5068578	77.8165		215.507	91.84	77.82	Underweight		8	A - Good
223.8244919	101.687		223.824	93.1	101.69	Normal		9	A - Good
163.1185835	91.1607		163.119	84.19	91.16	Normal		10	A - Good
188.0639982	81.3553		188.064	87.99	81.36	Underweight		9	A - Good
131.5600213	97.9021		131.56	78.7	97.9	Normal		9	A - Good
140.5420986	92.0009		140.542	80.32	92	Normal		9	A - Good
122.9796582	86.4371		122.98	76.97	86.44	Underweight		8	A - Good
117.4776834	94.6563		117.478	75.92	94.66	Normal		8	A - Good
137.5028232	77.3075		137.503	79.68	77.31	Underweight		9	A - Good
180.6875352	89.8789		180.688	86.94	89.88	Underweight		9	A - Good
125.7957583	91.0206		125.796	77.55	91.02	Normal		9	A - Good
97.15246041	84.6093		97.152	71.43	84.61	Underweight		8	A - Good
143.6271166	82.8534		143.627	80.82	82.85	Underweight		9	A - Good
166.5341915	102.982		166.534	84.82	102.98	Normal		8	A - Good
207.403665	73.4317		207.404	90.7	73.43	Emaciated		7	B - Compromised
137.5028232	97.0889		137.503	79.8	97.09	Normal		9	A - Good
146.7582474	80.8132		146.758	81.36	80.81	Underweight		8	A - Good
122.9796582	75.9475		122.98	76.9	75.95	Underweight		8	A - Good
122.9796582	79.1188		122.98	76.92	79.12	Underweight		9	A - Good

	BALLAN WRASSE												
Number	Home Tank	Filming Tank	Date	Fish ID	Height_mm	St_length_mm	Total_length_mm	Width_mm	Weight_g		Skin Damage	Caudal Fin Damage	Eye Darkening
1	A6	1	05.07.2022	LBCSAR050722-01	34	108	121	17	27.1		0	0	0
2	A6	2	05.07.2022	LBCSAR050722-02	32	99	110	16	21.2		0	0	1
3	A6	1	05.07.2022	LBCSAR050722-03	36	109	125	18	30.9		0	0	1
4	A6	2	05.07.2022	LBCSAR050722-04	38	115	127	18	36.6		0	0	1
5	A6	1	05.07.2022	LBCSAR050722-05	27	89	101	14	15.7		0	0	0
6	A6	2	05.07.2022	LBCSAR050722-06	34	103	118	17	26.5		0	0	2
7	A6	1	06.07.2022	LBCSAR060722-01	36	113	126	19	35.2		0	0	1
8	A6	2	06.07.2022	LBCSAR060722-02	29	97	107	14	18.7		0	0	1
9	A6	1	08.07.2022	LBCSAR080722-01	28	93	102	14	16.1		0	0	0
10	A6	2	08.07.2022	LBCSAR080722-02	34	95	109	15	23.3		0	0	1
11	A6	1	08.07.2022	LBCSAR080722-03	30	90	104	15	18.1		0	0	1
12	A6	2	08.07.2022	LBCSAR080722-04	33	103	115	17	22.3		0	0	1
13	A6	1	11.07.2022	LBCSAR110722-01	39	117	132	20	38.3		0	0	1
14	A6	2	11.07.2022	LBCSAR110722-02	39	111	127	19	36		0	0	0
15	A6	1	11.07.2022	LBCSAR110722-03	33	92	106	18	22.4		1	1	0
16	A6	2	11.07.2022	LBCSAR110722-04	25	84	95	13	12.2		0	0	0
17	A6	1	13.07.2022	LBCSAR130722-01	44	119	135	19	44.4		0	0	2
18	A6	2	13.07.2022	LBCSAR130722-02	34	103	117	17	24.4		1	0	1
19	A6	1	13.07.2022	LBCSAR130722-03	28	96	109	15	18.8		0	0	1
20	A6	2	13.07.2022	LBCSAR130722-04									
21	A6	1	14.07.2022	LBCSAR140722-01	33	104	116	17	25.3		0	0	0
22	A6	2	14.07.2022	LBCSAR140722-02	38	109	124	19	32		0	0	0
23	A6	1	14.07.2022	LBCSAR140722-03	27	86	99	15	15.1		0	0	0
24	A6	2	14.07.2022	LBCSAR140722-04	34	96	110	17	21.2		0	0	0
25	A6	1	18.07.2022	LBCSAR180722-01	34	104	117	15	26		0	0	0
26	A6	2	18.07.2022	LBCSAR180722-02	31	97	106	15	18		0	0	0
27	A6	1	18.07.2022	LBCSAR180722-03	33	104	117	16	24.5		0	0	0
28	A6	2	18.07.2022	LBCSAR180722-04	39	104	116	19	27		0	0	1
29	A6	1	19.07.2022	LBCSAR190722-01	33	99	113	16	22.7		0	0	0
30	A6	2	19.07.2022	LBCSAR190722-02	34	103	113	17	26.1		0	0	1

	LUMPFISH													
Number	Home Tank	Filming Tank	Date	Fish ID	Height_mm	St_length_mm	Total_length_mm	Width_mm	Weight_g		Skin Damage	Caudal Fin Damage	Eye Condition	Suction Disc
1			05.10.2022	CLMW031022-01	43	89	104	28	38		0	0	0	0
2			05.10.2022	CLMW031022-02	41	87	100	27	32		0	0	0	0
3			05.10.2022	CLMW031022-03	43	81	94	29	31		0	0	0	0
4			05.10.2022	CLMW031022-04	44	78	91	29	30		0	1	0	0
5			05.10.2022	CLMW031022-05	39	81	95	26	26		0	0	0	0
6			05.10.2022	CLMW031022-06	40	86	92	25	28		0	2	0	0
7			05.10.2022	CLMW031022-07	44	84	97	29	32		0	0	0	0
8			05.10.2022	CLMW031022-08	44	80	94	26	31		0	0	0	0
9			05.10.2022	CLMW031022-09	47	89	101	34	42		0	0	0	0
10			05.10.2022	CLMW031022-10	37	80	91	26	24		0	0	0	0
11			05.10.2022	CLMW031022-11	44	90	100	29	34		0	0	0	0
12			05.10.2022	CLMW031022-12	40	79	93	26	27		0	0	0	0
13			05.10.2022	CLMW031022-13	37	73	89	28	21		0	1	0	0
14			05.10.2022	CLMW031022-14	38	79	91	25	24		0	0	0	0
15			05.10.2022	CLMW031022-15	39	81	97	27	28		0	0	0	0
16			05.10.2022	CLMW031022-16	41	83	96	28	31		0	0	0	0
17			05.10.2022	CLMW031022-17	38	78	82	27	26		0	0	0	0
18			05.10.2022	CLMW031022-18	38	80	92	27	28		0	0	0	0
19			05.10.2022	CLMW031022-19	36	74	82	25	22		0	1	0	0
20			05.10.2022	CLMW031022-20	38	79	93	27	24		0	0	0	0
21			05.10.2022	CLMW031022-21	34	76	90	26	22		0	0	0	0
22			05.10.2022	CLMW031022-22	38	76	89	27	26		0	1	0	0
23			05.10.2022	CLMW031022-23	34	74	87	25	22		0	0	0	0
24			05.10.2022	CLMW031022-24	36	76	89	26	23		0	1	0	0
25			05.10.2022	CLMW031022-25	36	81	95	26	24		0	0	0	0
26			05.10.2022	CLMW031022-26	34	68	74	24	16		0	2	0	0
27			05.10.2022	CLMW031022-27	41	85	98	28	30		0	0	0	0
28			05.10.2022	CLMW031022-28	45	85	100	33	38		0	0	0	0
29			05.10.2022	CLMW031022-29	42	90	106	30	34		0	0	0	0
30			05.10.2022	CLMW031022-30	35	80	94	25	23		0	0	0	0
31			05.10.2022	CLMW031022-31	39	79	93	28	27		0	0	0	0
32			05.10.2022	CLMW031022-32	38	82	98	30	29		0	0	0	0

Excel Calculator			Online Calculator		
Expected Weight	BMI (%)		Expected Weight (g)	BMI (%)	Class
47.77305916	79.54273951		47.773	79.54	Underweight
42.16965034	75.88395858		42.17	75.88	Underweight
34.6353597	89.50390661		34.635	89.5	Underweight
31.23997438	96.0308086		31.24	96.03	Normal
35.8210893	72.58294068		35.821	72.58	Emaciated
32.34514324	86.56631937		32.345	86.57	Underweight
38.27550124	83.60439176		38.276	83.6	Underweight
34.6353597	89.50390661		34.635	89.5	Underweight
43.52575268	96.49459783		43.526	96.49	Normal
31.23997438	76.82464688		31.24	76.82	Underweight
42.16965034	80.62670599		42.17	80.63	Underweight
33.47682479	80.65281032		33.477	80.65	Underweight
29.10781534	72.14557243		29.108	72.15	Emaciated
31.23997438	76.82464688		31.24	76.82	Underweight
38.27550124	73.15384279		38.276	73.15	Emaciated
37.0343556	83.70606022		37.034	83.71	Underweight
22.43065029	115.9128231		22.431	115.91	Above Normal
32.34514324	86.56631937		32.345	86.57	Underweight
22.43065029	98.08008112		22.431	98.08	Normal
33.47682479	71.69138695		33.477	71.69	Emaciated
30.16097819	72.94193134		30.161	72.94	Emaciated
29.10781534	89.32308967		29.108	89.32	Underweight
27.0776358	81.24786138		27.078	81.25	Underweight
29.10781534	79.01657932		29.108	79.02	Underweight
35.8210893	66.99963755		35.821	67	Emaciated
16.18177285	98.8766815		16.182	98.88	Normal
39.54486951	75.86319129		39.545	75.86	Underweight
42.16965034	90.11220082		42.17	90.11	Normal
50.75723518	66.98552409		50.757	66.99	Emaciated
34.6353597	66.40612426		34.635	66.41	Emaciated
33.47682479	80.65281032		33.477	80.65	Underweight
39.54486951	73.33441825		39.545	73.33	Emaciated

BALLAN WRASSE

Number	Home Tank	Filming Tank	Date	Fish ID	Height_mm	St_length_mm	Total_length_mm	Width_mm	Weight_g		Skin Damage	Caudal Fin Damage	Eye Darkening	Pectoral Fin Damage	Mouth Deformity
1			04.10.2022	LBOM051022-01	37	104	124	19	30		0	0	1	Y	N
2			04.10.2022	LBOM051022-02	36	107	126	23	32		0	0	1	Y	Y
3			04.10.2022	LBOM051022-03	33	95	111	19	22		1	0	0	Y	N
4			04.10.2022	LBOM051022-04	36	105	123	21	33		0	0	0	Y	N
5			04.10.2022	LBOM051022-05	26	88	99	16	14		1	1	1	Y	Y
6			04.10.2022	LBOM051022-06	37	97	115	21	26		0	0	1	Y	N
7			04.10.2022	LBOM051022-07	41	12	136	22	42		0	0	1	Y	N
8			04.10.2022	LBOM051022-08	35	108	126	20	33		1	0	1	Y	N
9			04.10.2022	LBOM051022-09	36	99	117	19	27		1	0	1	Y	N
10			04.10.2022	LBOM051022-10	26	83	93	17	14		0	0	1	Y	N
11			04.10.2022	LBOM051022-11	45	116	134	24	48		0	0	0	Y	N
12			04.10.2022	LBOM051022-12	38	108	124	18	32		0	0	0	Y	N
13			04.10.2022	LBOM051022-13	35	103	121	19	26		0	0	0	Y	N
14			04.10.2022	LBOM051022-14	31	98	117	18	22		1	0	0	Y	Y
15			04.10.2022	LBOM051022-15	41	108	126	22	36		1	0	2	Y	N
16			04.10.2022	LBOM051022-16	38	109	129	19	32		0	0	1	Y	N
17			04.10.2022	LBOM051022-17	32	100	119	21	24		1	0	1	Y	Y
18			04.10.2022	LBOM051022-18	36	103	116	21	22		1	0	1	Y	Y
19			04.10.2022	LBOM051022-19	30	89	102	16	16		0	0	0	Y	N
20			04.10.2022	LBOM051022-20								0			
21			04.10.2022	LBOM051022-21	32	103	119	19	24		1	0	1	Y	Y
22			04.10.2022	LBOM051022-22	36	102	120	21	27		0	0	1	Y	N
23			04.10.2022	LBOM051022-23	38	111	122	20	31		0	0	1	Y	N
24			04.10.2022	LBOM051022-24	37	112	125	20	32		0	0	0	Y	Y
25			04.10.2022	LBOM051022-25	39	111	130	23	41		1	0	1	Y	N
26			04.10.2022	LBOM051022-26	41	115	134	23	39		0	0	1	Y	N
27			04.10.2022	LBOM051022-27	33	102	112	20	24		1	0	2	Y	Y
28			04.10.2022	LBOM051022-28	33	96	112	19	22		0	0	1	Y	Y
29			04.10.2022	LBOM051022-29	41	111	130	23	39		0	0	1	Y	N
30			04.10.2022	LBOM051022-30	38	109	128	20	32		1	0	1	Y	Y
31			04.10.2022	LBOM051022-31	36	103	115	19	24		0	0	0	Y	N
32			04.10.2022	LBOM051022-32	29	107	125	20	30		0	0	2	Y	Y
33			04.10.2022	LBOM051022-33	29	92	109	17	18		1	0	0	Y	N
34			04.10.2022	LBOM051022-34	26	90	101	18	15		0	0	2	Y	Y

						ImageJ Bottom Measurements				ImageJ Side Measurements				ImageJ Top Measurements		
Fish ID	Height_mm	Sd_length_mm	Total_length_mm	Width_mm		Total Length_mm	Standard Length_mm	Width_mm		Total Length_mm	Standard Length_mm	Height_mm		Total Length_mm	Standard Length_mm	Width_mm
CLCSAR010622-01	48	106	119			125.4	109.1	39.8		118.5	110.7	53.5		123.3	110.3	38.2
CLCSAR010622-02	51	120	136			140.7	119.7	45.6		135.1	119.2	56.1		141.3	123.8	43.3
CLCSAR050722-01	65	135	148	45		157.7	139	51.4		152.6	136	69.3		156.6	139.6	48.7
CLCSAR050722-02	51	108	123	39		126	110	41.9		124.1	109.5	53.9		143.4	127.2	48.5
CLCSAR050722-03	61	125	140	46		148.2	131.5	51.2		146.3	128.3	65.2		148.7	132.9	50
CLCSAR050722-04	56	122	140	38		147.2	127.3	43.4		144.7	123.9	58.2		146.9	125.6	40.7
CLCSAR050722-05	64	132	151	46		161.2	140.2	52.1		154	136.5	69.3		158.5	140.1	49.9
CLCSAR050722-06	58	125	143	64		157.4	133.3	52.4		153	133.1	69.1		153.6	134.9	50.6
CLCSAR060722-01	67	142	162	47		173.8	150.4	56		170.5	147.3	72.5		174.4	157.1	53.4
CLCSAR060722-02	67	136	156	48		164.2	144.2	56		160.1	139.1	71.9		162.9	146.6	54.4
CLCSAR070822-01	63	131	151	42		160.6	139.4	50		154.7	136.2	65.7		156.5	134.9	46.3
CLCSAR070822-02	60	124	140	44		152.6	131.4	50.1		146.5	123	61.8		145.2	125.8	48.3
CLCSAR070822-03	65	143	162	44		174.5	155.3	50.5		169.7	143.4	69.4		170.4	147.9	47.3
CLCSAR070822-04	72	150	167	47		150.1	134.2	46.1		169.2	147	74.6		176.6	153.1	52.8
CLCSAR110722-01	77	151	169	57		180.2	153.3	66.7		170.4	145.2	84.7		174.3	156.7	63.5
CLCSAR110722-02	68	134	153	47		159.9	139.6	53.2		155	131.7	72.9		163.8	141.5	52.1
CLCSAR110722-03	67	139	160	49		171.5	148.7	58.7		159.7	139.3	69.4		170.2	149	53.7
CLCSAR110722-04	63	128	143	47		147.2	129.7	52		149.6	132.2	69.2		149.6	134.8	50.7
CLCSAR130722-01	61	129	146	46		150.7	130.5	50.8		149.3	132.2	66.7		149.1	131.3	47.13
CLCSAR130722-02	59	124	140	44		146	127.6	47.5		144.2	126.5	61.9		145	127.6	44.8
CLCSAR130722-03	60	126	138	42		145.2	129.1	47.6		142.7	125.5	63.6		145.8	129.1	44.8
CLCSAR130722-04	58	126	145	43		149.6	130.4	48.1		141.1	124.9	60.9		147	128.9	46.8
CLCSAR140722-01	69	139	158	54		159.8	137.5	57.9		154.6	133.9	73.6		160.7	138.2	56.4
CLCSAR140722-02	62	126	141	45		145.5	124	50.4		142.7	123.6	65		145.7	128.3	47.6
CLCSAR140722-03	57	116	130	39		135.6	120.3	43		134.6	119.4	57.4		132.8	117.6	41.9
CLCSAR140722-04	63	127	147	43		153	136	48.9		153.4	133	69.1		155	133.8	51.1
CLCSAR180722-01	69	140	154	52		163.9	147.1	58.4		158.8	144.5	76.4		161.2	142.4	56.5
CLCSAR180722-02	66	146	165	44		177.9	1159.4	48.5		171.2	151.1	72.5		176	158	51.5
CLCSAR180722-03	65	131	145	47		156	140.8	55.9		153.1	132.3	72.8		154.8	136.4	54.8
CLCSAR180722-04	61	128	148	44		158.9	140	51.5		156.7	134.5	66.3		157.9	135	49.6
CLCSAR190722-01	55	119	140	39		148.4	131	43.7		147.5	125.2	58.4		149.7	129.7	41.8
CLCSAR190722-02	57	121	140	41		148.7	127.7	46.3		146.8	127.1	60.2		149.5	131.8	44.7
CLMW031022-01	43	89	104	28		104.8	90.4	30.6		102	91.3	42.3		106	90.9	30.1
CLMW031022-02	41	87	100	27		102.9	90	29.2		100.4	84.7	42.3		100.2	88.2	27.4

CLMW031022-03	43	81	94	29		94.1	84.2	30.4		93.5	81.7	42		92.8	82.5	29.5
CLMW031022-04	44	78	91	29		90.9	80.5	30.9		88.4	75.3	42.5		91.3	81.4	29.6
CLMW031022-05	39	81	95	26		94.5	81.7	27.1		94.7	81.4	38.6		90.1	78.6	27.6
CLMW031022-06	40	86	92	25		94.6	86.2	27.3		94.1	86	39.2		91.6	86.1	23.8
CLMW031022-07	44	84	97	29		95.2	83.3	31.9		95.9	83.3	43.4		95.1	89.2	29.8
CLMW031022-08	44	80	94	26		98.3	87.2	29.3		95.4	83.5	42.2		96.9	85.5	28.1
CLMW031022-09	47	89	101	34		101.9	88.4	32.6		104.9	88.8	45.5		100	86.4	32.1
CLMW031022-10	37	80	91	26		90.9	78	26.2		90.4	77.9	37.4		91.4	80.5	26.1
CLMW031022-11	44	90	100	29		104.6	89.7	31.9		105.5	91	41.6		103.9	91.8	29
CLMW031022-12	40	79	93	26		94.8	82.1	29.2		90.7	79.8	39.8		92.4	79.4	28.2
CLMW031022-13	37	73	89	28		84.8	71	27.8		82	69.1	36		84.4	70	26.5
CLMW031022-14	38	79	91	25		89.7	77.2	29.1		90.3	78	37.6		89.2	76.3	26.3
CLMW031022-15	39	81	97	27		97.3	79.9	29.1		99.3	82.1	41.5		98.8	82.2	29.1
CLMW031022-16	41	83	96	28		94.6	80.7	28.4		95.7	79.7	39.4		96.4	81.6	28.8
CLMW031022-17	38	78	82	27		100.5	85.3	30.6		100.3	84.7	42.3		101.1	85.5	31.2
CLMW031022-18	38	80	92	27		98	83.7	28.7		96.6	81.3	38		96.7	81.5	28.7
CLMW031022-19	36	74	82	25		86.6	76.2	27.8		85.3	76.1	37.7		85.6	74.4	27
CLMW031022-20	38	79	93	27		92.4	77.8	27.8		90	79.5	36.7		92.4	80.1	29
CLMW031022-21	34	76	90	26		88.1	76	26.3		89.3	75.5	32.2		89.9	79.8	26.8
CLMW031022-22	38	76	89	27		90	77.9	29.9		88.9	77	39.9		88.7	76.7	29
CLMW031022-23	34	74	87	25		90.2	77.5	26.6		86.1	74.8	74.8		88.9	75.6	27.1
CLMW031022-24	36	76	89	26		93.7	81.1	27.2		91.8	79.2	36.7		93.3	80.3	27
CLMW031022-25	36	81	95	26		92.8	79.3	28.3		93.7	81.5	36.2		92.8	79.9	27.5
CLMW031022-26	34	68	74	24		74	66.6	25.3		73.1	67.3	31.7		72.5	65.5	22.9
CLMW031022-27	41	85	98	28		96.2	83.7	30.1		98.4	88.7	40.2		98.5	85.2	28.3
CLMW031022-28	45	85	100	33		98.3	85.4	34		101.6	85.5	46.5		105.1	90.2	33.6
CLMW031022-29	42	90	106	30		104.7	88.5	30.5		106.8	90.5	42.9		106.2	89.5	30.4
CLMW031022-30	35	80	94	25		97.2	84.1	25.1		94.3	79	32.4		94.8	80.7	24.4
LBCSAR010622-02	32	100	119	18		118.4	104.1	18.8		120.3	107.3	33.4		119.9	103	17.9
LBCSAR050722-01	34	108	121	17		117.4	101.7	18.3		120.3	106	33.8		119.5	103.2	17.4
LBCSAR050722-02	32	99	110	16		106.7	93.7	16.3		110.4	97.3	32.1		108.4	94.8	14.3
LBCSAR050722-03	36	109	125	18		127.1	113.3	19.6		128.1	116.6	37.8		125.9	108.9	20
LBCSAR050722-04	38	115	127	18		123.8	108.2	22.2		128.9	112.6	40.4		123.9	107.9	21.7
LBCSAR050722-05	27	89	101	14		97.2	83	15.9		102.59	88.6	28.9		98.2	82.9	14.7
LBCSAR050722-06	34	103	118	17		118.2	98	18.9		120.1	104.5	35.5		119.8	103.5	19.5
LBCSAR060722-02	29	97	107	14		100.7	89.4	15.3		102.5	89.9	29.7		101.7	92.7	14.3

LBCSAR080722-01	28	93	102	14		105.9	91.6	192.2		108.8	94.6	34.4		107.6	90.3	18.5
LBCSAR080722-02	34	95	109	15		103.4	90.5	17.1		107.3	93.8	30.6		102.8	90.6	17.2
LBCSAR080722-03	30	90	104	15		113.3	98.8	18.3		115	101	31.7		114.7	102.1	18.3
LBCSAR080722-04	33	103	115	17		128.9	111.5	22.7		131.2	114.3	39.2		133.3	116.1	23.3
LBCSAR110722-01	39	117	132	20		127.8	110.7	22.4		128.5	111.3	39.5		129.1	113.9	21.7
LBCSAR110722-02	39	111	127	19		106.3	92.8	18.9		108.6	96.6	33.8		104.9	94	17.7
LBCSAR110722-03	33	92	106	18		94.7	79.1	13.4		96.3	84.2	26.1		93.9	80.9	13.7
LBCSAR110722-04	25	84	95	13		136.6	119.2	24.5		136.2	119	43.6		136.1	119.3	23.3
LBCSAR130722-01	44	119	135	19		112.9	101.5	17.8		115.4	98.3	32.8		113.9	98.9	18.4
LBCSAR130722-02	34	103	117	17		110.5	95.1	15.6		109.5	96.3	30.9		110.5	98.3	15.9
LBCSAR130722-03	28	96	109	15		122.7	106.5	20.2		121	104.5	36.8		125.5	108.6	19.8
LBCSAR140722-01	33	104	116	17		123.2	108.6	21.5		128	113.1	38.3		127.7	114.9	20.6
LBCSAR140722-02	38	109	124	19		98.6	84.8	16.2		97.7	84.7	28.4		100.2	89.4	15.1
LBCSAR140722-03	27	86	99	15		109.5	95.8	19.1		109.7	96	32.4		111.1	95.6	19.4
LBCSAR180722-01	34	104	117	15		119.3	105.5	19		120.1	105.1	36		119.2	102.9	19.2
LBCSAR180722-02	31	97	106	15		109	95.1	16.9		109.1	93.2	30.5		109.3	94.2	15
LBCSAR180722-03	33	104	117	16		117.8	104.2	17.8		116.7	103.3	33.9		118.5	104.9	17.6
LBCSAR180722-04	39	104	116	19		116.9	101.8	19.9		117.3	103.8	34.3		117.7	103.8	21.9
LBCSAR190722-01	33	99	113	16		115.8	100.2	17.3		116	101.4	32.9		113.9	99.1	16.9
LBCSAR190722-02	34	103	113	17		115.2	99	20.8		115.3	103.5	35.1		115.3	98.4	19.9
LBCSAR281022-01	52	154	185	27		186	158	26.7		181.3	156.6	52.3		187.2	162.3	26.3
LBCSAR281022-02	51	156	185	28		184.5	168.6	29.8		180.7	158.2	50.4		185.2	162.1	28.7
LBCSAR281022-03	43	140	161	27		162.3	134.6	28.4		159.3	139	45.1		162.3	142.5	26.5
LBCSAR281022-04	47	148	177	29		176.8	148.1	30.6		175.1	144.9	48.1		176.3	147.3	29.8
LBCSAR281022-05	43	149	173	28		179.2	155.2	28.2		175.6	157.7	46.7		190.4	163.2	28.5
LBCSAR281022-06	34	117	135	21		135.7	119.4	20.9		137.1	120.9	35.3		137.2	122.8	21.1
LBCSAR281022-07	34	121	139	21		144.6	124.2	21.8		140.4	122.3	34.9		143.1	122.1	22.1
LBCSAR281022-08	55	160	187	31		189.2	164	28.3		185.8	161.7	55.9		187.4	159.5	26.9
LBCSAR281022-09	41	131	152	22		158.8	138.4	20.2		154.8	134.9	41.5		155.6	129.3	23.8
LBCSAR281022-10	39	120	139	20		140.5	122.8	19.6		141.7	122.4	40		141	123.1	19.2
LBCSAR281022-11	49	159	183	27		180.6	162.8	29.9		183.1	160.7	50.1		184.8	162.9	28.1
LBCSAR281022-12	50	150	171	29		175.6	157.4	29.8		171.5	150.7	51		180	158.4	29.6
LBCSAR281022-13	64	187	218	28		183.1	158.6	31.7		179.8	156.8	51.8		187.6	162.5	28.7
LBCSAR281022-14	56	164	186	34		190	163.4	33.2		186.5	164.1	55.8		190.4	174.6	32.3
LBCSAR281022-15	51	153	183	31		188.6	165.4	29.3		183.6	162.3	50.6		193	174.6	29.4
LBCSAR281022-16	57	178	208	34		212.8	195.2	33.4		208.4	182	56.6		215	189.7	34.1

LBCSAR281022-17	63	185	211	37		218.4	182.4	37.5		211.4	184.2	60.2		212.6	183.9	37.5
LBCSAR281022-18	182	182	211	30		176.9	155.3	29.9		180.1	158.5	47.7		181.7	160.5	29.5
LBCSAR281022-19	52	177	202	31		204.5	175.7	32.7		204.4	175	53		203.4	180	30.3
LBCSAR281022-20	60	189	218	36		222.1	195.3	34.9		217.5	188.2	61.2		225.1	200.3	34.6
LBCSAR281022-21	59	188	215	35		217.9	189.6	34.7		213.9	187.7	60.8		218.8	189	33.5
LBCSAR281022-22	43	134	152	25		157.2	141	24.6		152.7	135.5	43.1		154.3	135.3	23.9
LBCSAR281022-23	56	171	196	33		202.4	178	32.8		193.8	166.9	55.1		197.1	173.6	29.5
LBCSAR281022-24	50	151	176	31		182.6	158.1	30.4		176.6	157.2	50.7		183.7	157.8	30.6
LBCSAR281022-25	54	178	195	33		201.2	178	31.5		194.8	172.5	54.3		199	171.6	30.6
LBCSAR281022-26	49	154	176	26		181.6	161.7	26.8		175.6	153	48.9		180.1	154.2	26.2
LBCSAR281022-27	51	162	185	30		188.7	162.4	28.7		184.9	162.6	50.7		187.6	162.6	29.4
LBCSAR281022-28	64	210	237	38		238	215.1	35.9		233.8	207.6	60.5		239	209.8	35.1
LBCSAR281022-29	60	176	201	33		202.6	180.7	31.1		200.8	169.3	57.9		207.6	177.5	32
LBCSAR281022-30	55	167	191	32		197	171.3	29.1		192	168.3	52.4		198.1	174.1	30.1
LBCSAR281022-31	59	170	189	32		193.1	165.6	34.4		189.9	165.7	58.4		194.8	170	32.1
LBCSAR281022-32	50	160	178	30		182.8	156.2	27.4		181.2	157.6	50.1		184	159.5	48.8
LBCSAR281022-33	57	184	211	34		214.7	192.9	33.8		211.1	183.8	52.6		216.1	197.6	32.8
LBCSAR281022-34	46	140	161	26		162.9	137.3	26.5		162.5	141.1	45.3		164.8	142.5	25.7

Visifish AI Results					
Fish_ID	stl_w_KM	ttl_w_KM	stl_w_AI	ttl_w_AI	BMI_KM
CLCSAR050722-01	3	3.29	2.74	3.03	96.76
CLCSAR050722-02	2.77	3.15	2.89	3.21	90.46
CLCSAR050722-03	2.72	3.04	2.91	3.24	91.15
CLCSAR070822-02	2.82	3.18	2.72	3.02	88.04
CLCSAR110722-01	2.65	2.97	2.69	2.98	100.76
CLCSAR110722-02	2.85	3.26	2.76	3.06	90.14
CLCSAR110722-04	2.72	3.04	2.6	2.93	94.41
CLCSAR130722-01	2.8	3.17	2.99	3.33	88.07
CLCSAR130722-03	3	3.29	2.83	3.1	85.9
CLCSAR140722-02	2.8	3.13	2.63	3.01	92.28
CLCSAR180722-03	2.79	3.09	2.73	2.94	94.38
CLMW031022-26	2.83	3.08	2.79	3.13	96.49
CLMW031022-28	2.58	3.03	2.82	3.05	96.52
CLCSAR050722-05	2.87	3.28	3.42	3.75	83.8
CLCSAR060722-02	2.83	3.25	2.76	3.08	88.04
CLCSAR130722-04	2.93	3.37	2.84	3.17	79.59
CLMW031022-24	2.92	3.42	3.07	3.39	77.46
CLMW031022-31	2.82	3.32	2.96	3.31	80.65
CLMW031022-13	2.61	3.18	3.16	3.54	85.93
CLMW031022-30	3.2	3.76	2.65	3	66.87
CLMW031022-32	2.73	3.27	2.99	3.33	73.33

Chapter 3

Fish	Date	Time	Species	Experime	Weight	Temperat	Left_shelt		Latency_h	Lego_appi	Lego_touc	Mirror_ap	Mirror_toi	Leaving_h	Entering_	Model_ap	Status_mc	Latency_r	Approach	Approach	Approach	Contacts_	Contacts_	Contacts_	Time_0lice	Time_1lice	Time_6lice
F1	24/11/2022	11:00	Labrus bei	2	29	13	Yes	1	140	4	0	2	1	9	9	Yes	1	90	26	19	25	0	1	288	463	528
F2	24/11/2022	12:45	Labrus bei	3	38	13	No	0	1200	0	0	0	0	0	0	Yes	1	122								
F3	25/11/2022	11:00	Labrus bei	2	39	12	Yes	1	34	1	0	5	4	30	30	Yes	1	383	46	72	65	1	0	3	278	738	624
F4	25/11/2022	12:45	Labrus bei	3	27	12	Yes	1	383	0	0	2	8	2	2	Yes	1	791									
F5	29/11/2022	12:00	Labrus bei	2	39	12	No	0	1200	0	0	0	0	0	0	Yes	1	3330	0	1	2	0	0	0	0	7	24
F6	30/11/2022	13:00	Labrus bei	3	28	12	Yes	1	63	3	0	3	85	4	4	Yes	1	979									
F7	01/12/2022	11:00	Labrus bei	2	28	12	Yes	1	88	0	0	1	0	15	15	Yes	1	85	23	21	30	0	0	0	223	216	312
F8	01/12/2022	12:45	Labrus bei	3	37	12.5	No	0	1200	0	0	0	0	0	0	Yes	1	1808									
F9	02/12/2022	10:00	Labrus bei	2	26	12.5	Yes	1	55	6	0	5	8	14	14	Yes	1	30	8	5	8	0	0	0	264	216	343
F10	08/12/2022	12:00	Labrus bei	3	29	12	Yes	1	748	0	0	0	0	2	2	Yes	1	748									
F11	09/12/2022	10:00	Labrus bei	2	54	12.5	Yes	1	147	2	0	0	0	6	7	Yes	1	1860	0	7	0	0	0	0	0	104	0
F12	09/12/2022	13:30	Labrus bei	3	35	12.5	No	0	1200	0	0	0	0	0	0	Yes	1	190									
F13	12/12/2022	11:00	Labrus bei	2	40	12.5	Yes	1	0	0	0	3	35	13	14	Yes	1	258	22	18	5	0	0	0	120	67	24
F14	19/12/2022	10:00	Labrus bei	3	39	12.1	Yes	1	27	1	0	3	4	9	9	Yes	1	624									
F15	19/12/2022	13:30	Labrus bei	2	37	12	Yes	1	43	3	0	0	0	19	19	Yes	1	269	36	10	55	0	0	0	152	40	216
F16	20/12/2022	10:00	Labrus bei	3	40	12.4	Yes	1	0	6	0	4	8	15	15	Yes	1	373									
F17	20/12/2022	13:30	Labrus bei	2	28	12.3	Yes	1	814	0	0	0	0	0	0	Yes	1	2882	4	0	1	0	0	0	48	0	32
F18	21/12/2022	10:00	Labrus bei	3	33	12.4	Yes	1	575	0	0	0	0	1	1	No	0	3600									
F19	21/12/2022	13:30	Labrus bei	2	44	12.5	Yes	1	268	1	0	3	11	7	7	Yes	1	2414	0	4	1	0	0	0	1	0	0
F20	22/12/2022	10:00	Labrus bei	3	30	12.2	No	0	1200	0	0	0	0	0	0	Yes	1	6									
F21	05/12/2022	11:00	Cyclopteri	2	43	12.2	Yes	1	0	17	0	26	123	16	16	Yes	1	16	60	78	90	0	0	1	344	408	512
F22	05/12/2022	12:45	Cyclopteri	3	61	12.4	No	0	1200	0	0	0	0	0	0	Yes	1	1680									
F23	06/12/2022	11:00	Cyclopteri	2	38	12.3	Yes	1	0	12	0	2	0	14	14	Yes	1	971	23	23	125	0	0	1	200	152	600
F24	06/12/2022	12:45	Cyclopteri	3	37	12.1	Yes	1	0	52	0	17	71	11	11	Yes	1	389									
F25	07/12/2022	10:00	Cyclopteri	2	44	12	Yes	1	95	11	0	28	26	32	32	Yes	1	129	35	91	146	0	0	0	168	208	560
F26	07/12/2022	11:45	Cyclopteri	3	35	12	No	0	1200	0	0	0	0	0	0	Yes	1	329									
F27	07/12/2022	13:30	Cyclopteri	2	41	12.3	No	0	1200	0	0	0	0	0	0	Yes	1	1093	35	34	36	0	0	0	264	256	272
F28	08/12/2022	13:45	Cyclopteri	3	40	12.2	No	0	1200	0	0	0	0	0	0	Yes	1	692									
F29	09/12/2022	11:45	Cyclopteri	2	47	12.5	No	0	1200	0	0	0	0	0	0	Yes	1	1466	17	15	18	0	0	0	128	104	128
F30	09/12/2022	15:15	Cyclopteri	3	44	12.3	Yes	1	10	10	0	2	63	20	20	Yes	1	64									
F31	12/12/2022	12:45	Cyclopteri	2	47	12	No	0	1200	0	0	0	0	0	0	Yes	1	1272	21	33	30	0	0	0	176	344	296
F32	15/12/2022	11:00	Cyclopteri	3	54	12.5	No	0	1200	0	0	0	0	0	0	Yes	1	1869									
F33	19/12/2022	11:45	Cyclopteri	2	35	12.1	Yes	1	60	12	0	17	8	35	35	Yes	1	506	13	8	6	0	0	0	152	104	56
F34	19/12/2022	15:15	Cyclopteri	3	55	12.4	Yes	1	0	8	0	2	3	35	35	Yes	1	321									
F35	20/12/2022	11:45	Cyclopteri	2	57	12	Yes	1	0	35	0	14	17	24	24	Yes	1	129	45	24	22	2	3	1	536	232	200

F36	20/12/2022	15:15	Cyclopteri	3	65	12	No	0	1200	0	0	0	0	0	0	Yes	1	258									
F37	21/12/2022	11:45	Cyclopteri	2	64	12.5	Yes	1	1110	0	0	0	0	1	1	Yes	1	244	51	42	50	0	0	0	448	280	432
F38	21/12/2022	15:15	Cyclopteri	3	55	12.6	Yes	1	0	3	0	2	0	40	40	Yes	1	249									
F39	22/12/2022	11:45	Cyclopteri	2	54	12.2	No	0	1200	0	0	0	0	0	0	Yes	1	664	6	5	10	0	0	0	64	32	184
F40	22/12/2022	13:30	Cyclopteri	3	66	12.3	No	0	1200	0	0	0	0	0	0	No	0	3600									
F41	12/01/2023		Labrus ber	2	33	12.2	Yes	1	16	1	0	0	0	3	4	Yes	1	270	8	16	16	0	1	0	72	130	84
F42	13/01/2023		Labrus ber	2	65	12.2	Yes	1	74	5	0	3	3	12	13	No	0	0	0	0	0	0	0	0	0	0	0
F43	13/01/2023		Labrus ber	2	40	12.2	Yes	1	56	5	0	0	0	6	6	Yes	1	3120	1	3	7	0	1	0	5	36	28
F44	16/01/2023		Labrus ber	2	26	12.4	No	0	1200	0	0	0	0	0	0	No	0	0	0	0	0	0	0	0	0	0	0
F45	18/01/2023		Labrus ber	2	42	12.2	Yes	1	267	0	0	0	0	2	2	Yes	1	3180	3	1	0	0	0	0	15	9	0
F46	19/01/2023		Labrus ber	3	32	12.5	Yes	1	280	0	0	1	2	2	3	Yes	1	115									
F47	19/01/2023		Labrus ber	3	40	12.5	No	0	1200	0	0	0	0	0	0	No	0	0									
F48	20/01/2023		Labrus ber	3	39	12.5	No	0	1200	0	0	0	0	0	0	Yes	1	890									
F49	12/01/2023		Cyclopteri	3	69	12.2	Yes	1	990	1	1	60	1	5	6	Yes	1	30									
F50	13/01/2023		Cyclopteri	3	71	12.2	No	0	1200	0	0	0	0	0	0	Yes	1	800									
F51	13/01/2023		Cyclopteri	3	78	12.2	No	0	1200	0	0	0	0	0	0	Yes	1	199									
F52	16/01/2023		Cyclopteri	3	113	12.4	No	0	1200	0	0	0	0	0	0	Yes	1	780									
F53	18/01/2023		Cyclopteri	3	92	12.2	No	0	1200	0	0	0	0	0	0	Yes	1	390									
F54	19/01/2023		Cyclopteri	2	100	12.5	Yes	1	15	4	1	0	0	1	1	No	0	0	0	0	0	0	0	0	0	0	0
F55	19/01/2023		Cyclopteri	2	88	12.5	Yes	1	77	10	0	2	0	3	4	Yes	1	1020	8	22	51	0	0	6	88	204	617
F56	20/01/2023		Cyclopteri	2	160	12.5	No	0	1200	0	0	0	0	0	0	Yes	1	2820	16	5	10	2	0	2	107	30	6
F57	23/01/2023		Cyclopteri	2	119	12.2	Yes	1	3	5	0	11	18	38	39	Yes	1	590	51	36	120	3	0	14	153	106	490
F58	23/01/2023		Labrus ber	3	41	12.2	No	0	1200	0	0	0	0	0	0	No	0	0									
F59	24/01/2023		Cyclopteri	2	112	12.3	No	0	1200	0	0	0	0	0	0	Yes	1	510	47	92	80	2	2	3	141	273	202
F60	24/01/2023		Labrus ber	3	26	12.3	Yes	1	40	0	0	3	10	6	7	Yes	1	362									
F61	26/01/2023		Cyclopteri	2	82	12.4	Yes	1	7	13	0	7	4	2	2	Yes	1	20	54	66	158	4	2	0	87	166	213
F62	26/01/2023		Labrus ber	3	44	12.4	No	0	1200	0	0	0	0	0	0	No	0	0									
F63	27/01/2023		Cyclopteri	2	105	12.4	Yes	1	2	2	0	2	0	1	0	Yes	1	448	74	55	53	3	1	0	171	122	95
F64	27/01/2023		Labrus ber	3	34	12.4	No	0	0	0	0	0	0	0	0	Yes	1	2100									
F65	30/01/2023		Cyclopteri	3	112	12.4	Yes	1	0	19	0	38	35	18	17	Yes	1	135									
F66	30/01/2023		Labrus ber	2	51	12.4	No	0	1200	0	0	0	0	0	0	No	0	3600	0	0	0	0	0	0	0	0	0
F67	02/06/2023	09:30	Cyclopteri	2	175	12.4	No	0	1200	0	0	0	0	0	0	Yes	1	446	32	47	18	5	0	0	70	60	79
F68	02/06/2023	09:30	Labrus ber	3	38	12.4	No	0	1200	0	0	0	0	0	0	Yes	1	225									
F69	02/06/2023	14:00	Cyclopteri	3	187	12.4	Yes	1	0	2	0	32	83	32	31	Yes	1	0									
F70	02/06/2023	14:00	Labrus ber	2	35	12.4	No	0	1200	0	0	0	0	0	0	Yes	1	2935	1	6	4	0	0	0	55	231	230
F71	02/07/2023	09:30	Cyclopteri	2	149	12.4	No	0	1200	0	0	0	0	0	0	Yes	1	2292	26	11	9	0	0	0	285	130	65

F72	02/07/2023	09:30	Labrus bei	3	44	12.4	Yes	1	30	0	0	2	0	5	5	No	0	3600									
F73	02/07/2023	14:00	Cyclopteri	3	136	12.4	Yes	1	0	3	0	28	15	45	45	Yes	1	30									
F74	02/07/2023	14:00	Labrus bei	2	40	12.4	Yes	1	60	1	0	0	0	3	3	Yes	1	720	13	16	13	0	0	0	105	180	1630
F75	02/08/2023	09:30	Cyclopteri	3	137	12.4	No	0	1200	0	0	0	0	0	0	Yes	1	260									
F76	02/08/2023	09:30	Labrus bei	2	43	12.4	Yes	1	60	0	0	0	0	6	5	Yes	1	2580	0	0	3	0	0	0	0	0	0
F77	02/08/2023	14:00	Cyclopteri	2	14	12	Yes	1	30	6	0	6	152	8	7	Yes	1	430	13	8	49	0	0	0	0	5	30
F78	02/08/2023	14:00	Labrus bei	3	31	12	Yes	1	240	0	0	1	0	2	1	No	0	3600									
F79	02/09/2023	09:30	Cyclopteri	2	18	11.7	No	0	1200	0	0	0	0	0	0	Yes	1	3260	14	9	7	0	0	0	15	15	0
F80	02/09/2023	09:30	Labrus bei	3	40	11.7	Yes	1	20	3	0	9	54	19	18	Yes	1	405									
F81	02/10/2023	09:30	Cyclopteri	3	171	12.2	No	0	1200	0	0	0	0	0	0	Yes	1	216									
F82	02/10/2023	09:30	Labrus bei	2	43	12.2	No	0	1200	0	0	0	0	0	0	Yes	1	2400	1	0	0	0	0	0	0	0	0
F83	13/02/2023	10:00	Cyclopteri	3	10	12.1	Yes	1	35	2	0	0	0	2	2	Yes	1	3210									
F84	13/02/2023	10:00	Labrus bei	2	47	12.1	Yes	1	1020	0	0	0	0	1	0	yes	1	525	2	3	2	0	0	0	420	360	120
F85	17/02/2023	10:00	Cyclopteri	3	19	12.5	No	0	1200	0	0	0	0	0	0	Yes	1	18									
F86	17/02/2023	10:00	Labrus bei	2	42	12.5	Yes	1	0	5	0	8	105	18	18	No	0	3600	0	0	0	0	0	0	0	0	0
F87	20/02/2023	10:30	Cyclopteri	3	23	12.7	No	0	1200	0	0	0	0	0	0	Yes	1	23									
F88	20/02/2023	10:30	Labrus bei	2	31	12.7	No	0	1200	0	0	0	0	0	0	Yes	1	1920	7	11	0	0	0	0	0	0	0
F89	21/02/2023	10:00	Cyclopteri	2	24	13	No	0	1200	0	0	0	0	0	0	No	0	3600	0	0	0	0	0	0	0	0	0
F90	21/02/2023	10:00	Labrus bei	3	33	13	No	0	1200	0	0	0	0	0	0	Yes	1	310									
F91	22/02/2023	13:45	Cyclopteri	2	24	13	Yes	1	10	12	0	12	20	9	10	Yes	1	60	37	63	24	0	1	0	60	15	20
F92	22/02/2023	13:45	Labrus bei	3	36	13	No	0	1200	0	0	0	0	0	0	Yes	1	480									
F93	23/02/2023	10:00	Cyclopteri	2	33	12.9	Yes	1	0	7	0	14	113	12	12	Yes	1	2490	3	10	7	0	0	0	0	5	15
F94	23/02/2023	10:00	Labrus bei	3	53	12.9	No	0	1200	0	0	0	0	0	0	No	0	0									
F95	23/02/2023	13:45	Cyclopteri	3	23	13.1	No	0	1200	0	0	0	0	0	0	Yes	1	240									
F96	23/02/2023	13:45	Labrus bei	2	40	13.1	No	0	1200	0	0	0	0	0	0	No	0	3600	0	0	0	0	0	0	0	0	0
F97	24/02/2023	13:30	Cyclopteri	2	26	13	Yes	1	0	8	0	18	3	10	9	Yes	1	510	29	7	11	0	0	0	`10	0	0
F98	24/02/2023	13:30	Labrus bei	3	42	13	Yes	1	335	2	2	0	0	4	4	Yes	1	2280									
F99	27/02/2023	10:30	Cyclopteri	3	25	13.4	Yes	1	205	2	0	0	0	1	1	Yes	1	250	0	0	0	0	0	0	0	0	0
F100	27/02/2023	10:30	Labrus bei	2	41	13.4	Yes	1	200	4	0	0	0	7	7	Yes	1	1955	6	17	0	0	1	0	10	20	0
F101	27/02/2023	14:45	Cyclopteri	3	26	13.5	Yes	1	25	1	0	3	27	3	2	Yes	1	30									
F102	27/02/2023	14:45	Labrus bei	2	40	13.5	No	0	1200	0	0	0	0	0	0	Yes	1	1010	12	10	11	0	0	0	0	0	30
F103	28/02/2023	11:00	Cyclopteri	3	28	13.7	Yes	1	175	6	0	14	3	4	4	Yes	1	1									
F104	28/02/2023	11:00	Labrus bei	2	52	13.7	No	0	1200	0	0	0	0	0	0	Yes	1	120	0	0	2	0	0	0	0	0	0
F105	28/02/2023	14:20	Cyclopteri	2	28	13.8	No	0	1200	0	0	0	0	0	0	Yes	1	50	40	41	79	0	0	0	15	0	0
F106	28/02/2023	14:20	Labrus bei	3	38	13.8	Yes	1	13	4	0	0	0	5	5	Yes	1	220									
F107	03/01/2023	13:30	Cyclopteri	2	34	14	Yes	1	5	3	0	0	1	5	4	Yes	1	120	59	83	65	0	0	0	300	0	0

F108	03/01/2023	13:30	Labrus bei	3	48	14	No	0	1200	0	0	0	0	0	0	Yes	1	2									
F109	03/02/2023	09:30	Cyclopteri	3	21	14.1	Yes	1	5	16	0	21	5	3	2	Yes	1	3									
F110	03/02/2023	09:30	Labrus bei	2	49	14.1	Yes	1	180	0	0	0	0	2	2	Yes	1	2990	1	1	1	0	0	0	0	0	0
F111	03/02/2023	13:50	Labrus bei	3	41	14.2	Yes	1	145	1	0	2	1	3	3	Yes	1	270									
F112	03/02/2023	13:50	Labrus bei	2	38	14.2	Yes	1	145	1	0	2	5	15	14	Yes	1	2340	1	0	4	0	0	0	0	0	0
F113	03/03/2023	09:30	Cyclopteri	3	30	14	Yes	1	13	4	0	2	7	2	2	Yes	1	180									
F114	03/03/2023	09:30	Labrus bei	2	32	14	No	0	1200	0	0	0	0	0	0	No	0	3600	0	0	0	0	0	0	0	0	0
F115	03/03/2023	13:00	Cyclopteri	2	23	14.1	No	0	1200	0	0	0	0	0	0	Yes	1	210	12	11	19	0	0	0	0	0	5
F116	03/03/2023	13:00	Labrus bei	3	41	14.1	No	0	1200	0	0	0	0	0	0	Yes	1	1350									
F117	03/06/2023	09:30	Cyclopteri	3	29	14.5	Yes	1	1	9	1	11	6	10	9	Yes	1	1									
F118	03/06/2023	09:30	Labrus bei	2	52	14.5	Yes	1	70	3	0	0	0	4	3	Yes	1	930	1	2	0	0	0	0	0	10	0
F119	03/06/2023	13:00	Cyclopteri	2	32	14.6	Yes	1	45	3	0	39	10	14	14	Yes	1	1020	2	1	3	0	0	0	0	0	0
F121	03/07/2023	13:30	Cyclopteri	2	30	14.6	Yes	1	510	0	0	1	0	7	7	Yes	1	30	96	40	60	3	0	0	135	0	0

Chapter 4

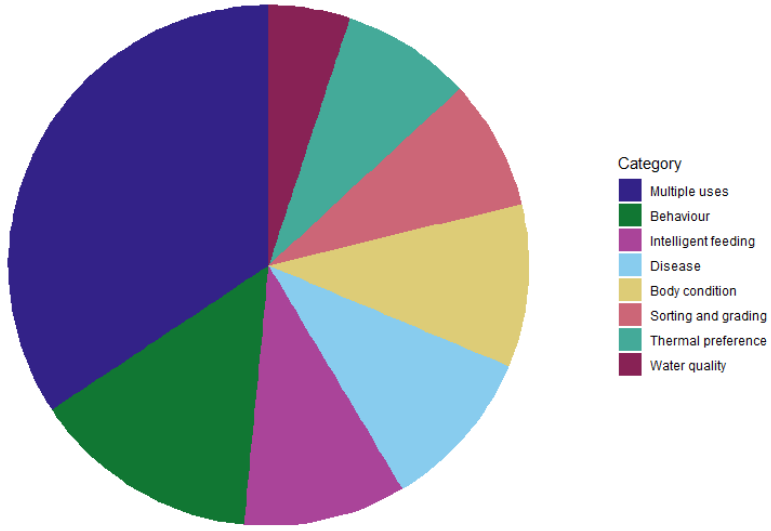
Date	File	Total_distan	Lowzone_	Highzone_	Offzone_c	Lowzone_	Highzone_	Offzone_t	Lowzone_	Highzone_	Offzone_r	Number_r	Preferenc	Lowzone_	Highzone_	Avoidtem	Lowzone_	Highzone_	Lowzone_	Weight
23/11/2022	Wrasse#1	129475.05	46131.91	73405.55	9937.59	41526.78	22422.04	3069.65	61.96	33.46	4.58	931	10.5	10.82	11.25	11.04	9.77	11.86	11.56	13.2	38.7
24/11/2022	Wrasse#2	205421.19	62203.21	128180.8	15037.19	13044.25	42724.94	7538.06	20.6	67.49	11.91	2628	13.74	13.06	13.26	13.16	10.11	12.79	11.86	14.54	32
30/11/2022	Wrasse#3	32374.7	23373.64	8418.84	582.22	80845.72	3349.24	106.71	95.9	3.97	0.13	115	9.98	10.41	11.12	10.77	9.77	12.05	11.58	13.5	33.8
01/12/2022	Wrasse#4	91138.35	16022.69	72883.66	2232	2091.09	42998.12	207.55	4.62	94.93	0.46	297	14.26	13.27	13.51	13.39	10.75	12.55	11.79	14.33	38
05/12/2022	Wrasse#5	526136.48	60716.58	377210.8	88209.08	8757.65	63855.99	13017.19	10.23	74.57	15.2	3941	12.42	12.38	12.5	12.44	10.14	11.99	11.86	13.24	35.6
06/12/2022	Wrasse#6	264411.65	48222.09	193015.3	23174.31	8744.86	72431.98	4933.32	10.16	84.12	5.73	1238	13.21	12.98	13.28	13.13	10.56	12.58	11.89	14.38	22.5
07/12/2022	Wrasse#7	323123.81	60162.8	233593.6	29367.42	12769.74	65607.06	7852.95	14.81	76.08	9.11	2343	12.21	11.65	11.92	11.78	9.82	12.11	11.75	12.65	41.2
08/12/2022	Wrasse#8	173182.9	34656.3	111008.1	27518.54	10588.06	61763.63	11644.12	12.61	73.53	13.86	1143	13.89	12.73	13.03	12.88	10.61	12.42	11.92	14.24	44.3
12/12/2022	Wrasse#9	119793.9	22072.11	87314.63	10407.16	5282.46	78190.35	2812.41	6.12	90.62	3.26	1164	12.39	11.97	12.21	12.09	9.77	12.05	11.78	13.42	19.4
13/12/2022	Wrasse#10	288036.92	55156.11	170322.6	62558.24	13034.64	48368.65	24740.87	15.13	56.15	28.72	2785	11.44	11.19	11.39	11.29	9.72	11.96	11.62	12.51	38.7
14/12/2022	Wrasse#11	265019.76	57666.59	91670.98	115682.2	18976.99	18067.23	48300.15	22.24	21.17	56.59	5571	12.24	11.52	11.6	11.56	9.85	11.99	11.41	13.63	33.5
15/12/2022	Wrasse#12	184482.19	24392.72	142932	17157.43	3590.79	79741.1	2989.72	4.16	92.38	3.46	849	14.47	13.66	13.89	13.77	10.46	12.86	11.84	14.68	25
19/12/2022	Wrasse#13	92072.57	40433.58	40731.85	10907.14	36313.95	27923.27	4812.64	52.59	40.44	6.97	1484	10.68	11.2	11.49	11.34	9.77	11.93	11.39	13.16	35.5
20/12/2022	Wrasse#14	118281.49	21248.96	85990.08	11042.45	4327.81	66418.82	5491.11	5.68	87.12	7.2	930	15.46	13.84	14.04	13.94	10.61	13.75	11.89	15.57	39
21/12/2022	Wrasse#15	89007.3	29525.2	38002.87	21479.23	20376.53	25846.69	23121.77	29.38	37.27	33.34	1608	12.06	11.31	11.56	11.44	9.74	12.14	11.47	13.46	30.8
05/01/2023	Wrasse#16	72381.5	30250.01	38246.71	3884.77	33461.42	50590.19	850.55	39.41	59.59	1	497	14.38	12.44	13.04	12.74	9.81	12.86	11.36	14.67	27.9
09/01/2023	Wrasse#17	150583.44	11148.5	132893.6	6541.3	2330.4	70981.59	4014.84	3.01	91.79	5.19	465	14.73	13.52	13.8	13.66	10.11	13.09	11.74	14.91	38.7
10/01/2023	Wrasse#18	127523.79	31727.53	92706.45	3089.8	5014.97	80515.38	463.5	5.83	93.63	0.54	787	14.25	13.1	13.45	13.28	10.27	12.49	11.94	14.32	32
11/01/2023	Wrasse#19	174241.53	48467.64	110754	15019.85	12619.33	59131.12	9618.13	15.51	72.67	11.82	1761	13.15	12.37	12.66	12.52	9.81	12.26	11.58	14.12	40.5
12/01/2023	Wrasse#20	91687.52	13930.26	76835.5	921.75	2368.78	63839.67	267.33	3.56	96.03	0.4	157	13.85	12.65	13.24	12.95	10.49	12.24	12	14.06	41.8
16/01/2023	Wrasse#21	169871.66	37936.95	124855.6	7079.09	5698.46	77877.37	2277.63	6.64	90.71	2.65	952	12.04	11.64	11.97	11.8	9.42	12.12	11.55	13.7	44
17/01/2023	Wrasse#22	67065.08	15302.87	51506.84	255.37	30460.61	55857.66	117.79	35.24	64.62	0.14	70	13.01	11.3	12.04	11.67	9.77	12.05	11.22	13.12	29.6
18/01/2023	Wrasse #23	32635.48	8846.91	22488.14	1300.43	8390.96	67218.37	486.59	11.03	88.33	0.64	130	13.31	11.76	12.42	12.09	9.56	12.1	11.65	13.49	24.5
19/01/2023	Wrasse #24	98181.72	13704.1	76030.41	8447.21	4506.4	73866.52	6345.03	5.32	87.19	7.49	589	13.65	11.89	12.2	12.05	9.73	12.09	11.54	13.89	46.1
23/01/2023	Wrasse#25	75817.32	21577.92	52537.85	1701.55	6254.26	79509.46	484.1	7.25	92.19	0.56	285	12.05	11.44	12.09	11.76	9.43	12.34	11.5	13.91	41.8
24/01/2023	Wrasse #26	59836.29	25292.76	23490.21	11053.31	21527.05	35874.82	10598.24	31.66	52.76	15.59	669	12.35	11.48	11.91	11.69	9.8	12	11.32	13.43	40.5
30/01/2023	Wrasse#27	77297.62	23444.95	25815.52	28037.15	20481.4	28258.29	29334.24	26.23	36.19	37.57	1591	11.57	11.53	11.83	11.68	9.87	12.48	11.47	13.35	46
31/01/2023	Wrasse#28	68834.4	7385.05	58649.43	2799.92	2197.08	81746.82	1398.03	2.57	95.79	1.64	280	13.78	13.04	13.33	13.18	10.85	12.32	11.91	14.13	34.7
01/02/2023	Wrasse#29	198266.07	91763.94	89001.69	17500.44	43352.86	30033.42	9812.51	52.11	36.1	11.79	1891	10.85	10.69	11.07	10.88	9.71	12.03	11.28	13.19	31.1
02/02/2023	Wrasse#30	75298.82	22474.38	50064.85	2759.59	15882.91	12883.83	933.55	53.48	43.38	3.14	453	11.47	12	12.25	12.12	9.84	12.06	11.32	13.35	42.9

R Script

R script Chapter 1

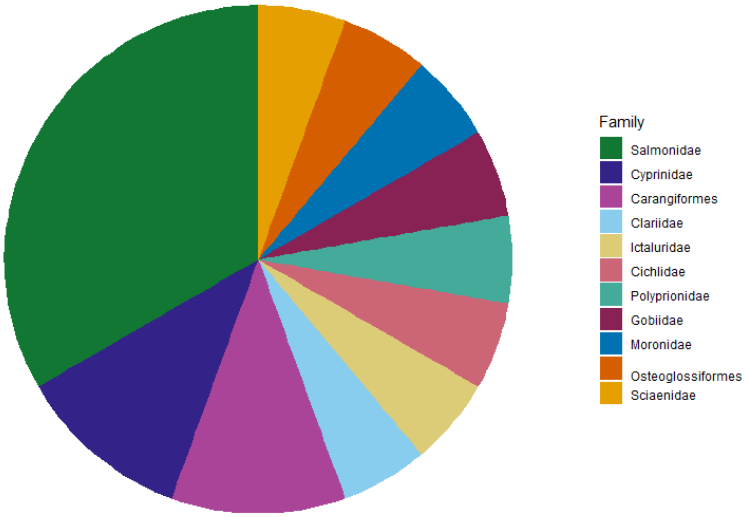
 # all use pie graph

```
dataLit <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RDataLiterature.csv")

install.packages("readxl")

library(readxl)

ggplot(dataLit) + theme_bw() +
  geom_bar(aes(x = "", y = Percent, fill = as.factor(Place)),
           stat = "identity") +
  coord_polar("y", start = 0) +
  theme(plot.title = element_text(hjust = 0.5, size = 20),
        axis.title = element_blank(),
        axis.text = element_blank(),
        axis.ticks = element_blank(),
        panel.grid = element_blank(),
        panel.border = element_blank()) +
  labs(fill = "Category") +
  scale_fill_manual(labels = c("Multiple uses", "Behaviour", "Intelligent feeding", "Disease",
                               "Body condition", "Sorting and grading", "Thermal preference", "Water quality"),
                    values = c("#332288", "#117733", "#AA4499", "#88CCEE", "#DDCC77", "#CC6677", "#44AA99", "#882255"))
```



 # family pie graph

```
dataLit2 <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RDataLiterature2.csv")

ggplot(dataLit2) + theme_bw() +
  geom_bar(aes(x = "", y = No_studies, fill = as.factor(Place)),
           stat = "identity") +
  coord_polar("y", start = 0) +
  theme(plot.title = element_text(hjust = 0.5, size = 20),
        axis.title = element_blank(),
        axis.text = element_blank(),
        axis.ticks = element_blank(),
        panel.grid = element_blank(),
        panel.border = element_blank()) +
  labs(fill = "Family") +
  scale_fill_manual(labels = c("Salmonidae", "Cyprinidae", "Carangiformes", "Clariidae", "Ictaluridae", "Cichlidae", "Polyprionidae", "Gobiidae", "Moronidae", "
Osteoglossiformes", "Sciaenidae"),
                    values = c("#117733","#332288", "#AA4499", "#88CCEE", "#DDCC77", "#CC6677", "#44AA99", "#882255", "#0072B2", "#D55E00", "#E69F00"))
```


Family
Salmonidae
Cyprinidae
Carangiformes
Clariidae
Ictaluridae
Cichlidae
Polyprionidae
Gobiidae
Moronidae
Osteoglossiformes
Sciaenidae

R script Chapter 2

 # T-tests to compare Image J and Visifish AI results with known (true) measurements

 # Visifish

```
VS<- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RawdataVisifish_corr.csv", stringsAsFactors=TRUE)

View(VS)

t.test(VS$w_stl_KM, VS$w_stl_AI, paired = TRUE, alternative = "two.sided")

t.test(VS$w_ttl_KM, VS$w_ttl_AI, paired = TRUE, alternative = "two.sided")
```

#Boxplots to compare Visifish ratios with ratios of true (known) measurements (figures 2.7 and 2.8)

```
dataBox<- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RDataVisifishBox.csv")

data_meansB <- aggregate(dataBox$w_stl,
                         list(dataBox$Type),
                         mean)

boxplot(dataBox$w_stl ~ dataBox$Type, xlab = "Type", ylab = "Width/Standard Length")

points(x = 1:nrow(data_meansB),
       y = data_meansB$x,
       col = "black",
       pch = 16)

text(x = 1:nrow(data_meansB),
     y = data_meansB$x + 10,
     labels = paste("Mean =", round(data_meansB$x, 1)),
     col = "black")


data_meansB2 <- aggregate(dataBox$w_ttl,
                          list(dataBox$Type),
                          mean)

boxplot(dataBox$w_ttl ~ dataBox$Type, xlab = "Type", ylab = "Width/Total Length")

points(x = 1:nrow(data_meansB2),
```

```
      y = data_meansB2$x,
      col = "black",
      pch = 16)
text(x = 1:nrow(data_meansB2),
    y = data_meansB2$x + 10,
    labels = paste("Mean =", round(data_meansB2$x, 1)),
    col = "black")
```

 # ImageJ T-tests

```
data1 <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RDataImageJ.csv")
library(ggplot2)
library(tidyverse)
t.test(data1$height_KM, data1$height_IJ, paired = T, data = data1)
t.test(data1$stl_KM, data1$stl_IJ, paired = T, data = data1)
t.test(data1$ttl_KM, data1$ttl_IJ, paired = T, data = data1)
t.test(data1$wid_KM, data1$wid_IJ, paired = T, data = data1)
```

 # Boxplots to compare known measurements to ImageJ measurements (height, standard length, total length and width) (figures 2.9 to 2.12)

```
data6 <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RDataImageJ2.csv")
View(data6)
```

 # Height

```
boxplot(data2$height ~ data2$type, xlab = "Type", ylab = "Height (mm)")
points(x = 1:nrow(data_means),
      y = data_means$x,
      col = "black",
      pch = 16)
text(x = 1:nrow(data_means),
    y = data_means$x + 45,
```

```
    labels = paste("Mean =", round(data_means$x, 1)),
    col = "black")

# Standard length
data_means2 <- aggregate(data6$stl,
                         list(data6$type),
                         mean)
boxplot(data6$stl ~ data6$type, xlab = "Type", ylab = "Standard Length (mm)")
points(x = 1:nrow(data_means2),
       y = data_means2$x,
       col = "black",
       pch = 16)
text(x = 1:nrow(data_means2),
    y = data_means2$x + 45,
    labels = paste("Mean =", round(data_means2$x, 1)),
    col = "black")

# Total length
data_means3 <- aggregate(data6$ttl,
                         list(data6$type),
                         mean)
boxplot(data6$ttl ~ data6$type, xlab = "Type", ylab = "Total Length (mm)")
points(x = 1:nrow(data_means3),
       y = data_means3$x,
       col = "black",
       pch = 16)
text(x = 1:nrow(data_means3),
    y = data_means3$x + 45,
    labels = paste("Mean =", round(data_means3$x, 1)),
    col = "black")
```

 # Width

```
data_means4 <- aggregate(data6$wid,
                         list(data6$type),
                         mean)
boxplot(data6$wid ~ data6$type, xlab = "Type", ylab = "Width (mm)", ylim = c(0,80))
points(x = 1:nrow(data_means4),
       y = data_means4$x,
       col = "black",
       pch = 16)
text(x = 1:nrow(data_means4),
     y = data_means4$x + 40,
     labels = paste("Mean =", round(data_means4$x, 1)),
     col = "black")
```

 # To predict BMI from body ratios (true/known measurements, ImageJ and Visifish AI)

```
rm(list=ls())
library(ggplot2)
library(performance)
Ratios_final <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RDataRatios_final.csv")
View(Ratios_final)
m1<-lm(BMI_KM~height_ttl_KM+width_ttl_KM,data=Ratios_final)
summary(m1)
```

 # Plot results

```
ggplot(data=Ratios_final,aes(x=BMI_KM, y=height_ttl_KM))+
  geom_point()+
  geom_smooth(method="lm")+
  theme_bw()
ggplot(data=Ratios_final,aes(x=BMI_KM, y=width_ttl_KM))+
  geom_point()+
  geom_smooth(method="lm")+
```

```
  theme_bw()
```

 # There is an obvious outlier in width, check and remove

```
check_outliers(m1)
```

 # remove outlier (case # 6)

```
m2<-lm(BMI_KM~height_ttl_KM+width_ttl_KM,data=na.omit(Ratios_final[-6,]))
summary(m2)      # USE THIS SUMMARY FOR PREDICTED BMI CALCULATION
```

 # It is highly significant > BMI can be predicted from ratios of hight to length + width to length

 # Plot results again (final code for graphs)

```
ggplot(data=na.omit(Ratios_final[-6,]), aes(x=BMI_KM, y=height_ttl_KM))+
  geom_point()+
  ylim(0.35,0.5)+
  xlim(65,120)+
  geom_smooth(method="lm")+
  theme_bw()
ggplot(data=na.omit(Ratios_final[-6,]), aes(x=BMI_KM, y=width_ttl_KM))+
  geom_point()+
  ylim(0.25,0.38)+
  xlim(65,120)+
  geom_smooth(method="lm")+
  theme_bw()
```

 # Trying model with only height/length

```
h1<-lm(BMI_KM~height_ttl_KM,data=na.omit(Ratios_final[-6,]))
summary(h1)
```

 # Trying model only width/length

```
w1<-lm(BMI_KM~width_ttl_KM,data=na.omit(Ratios_final[-6,]))
summary(w1)
```

 # Trying model with both ratios

```
w1<-lm(BMI_KM~width_ttl_KM + height_ttl_KM,data=na.omit(Ratios_final[-6,]))
summary(w1)
```
 # Model fit summary to predict true BMI from true ratios

 # Same models and graphs but with ImageJ ratios

```
i1<-lm(BMI_KM~height_ttl_IJ+width_ttl_IJ,data=Ratios_final)
summary(i1)
```

 # Plot results

```
ggplot(data=Ratios_final,aes(x=BMI_KM, y=height_ttl_IJ))+
  geom_point()+
  geom_smooth(method="lm")+
  theme_bw()
```

 # There is an obvious outlier in height, check and remove

```
check_outliers(i1)
```
 # remove outlier (case # 53)

 # Plot results again (final code for graphs)

```
ggplot(data=Ratios_final,aes(x=BMI_KM, y=width_ttl_IJ))+
  geom_point()+
  geom_smooth(method="lm")+
  theme_bw()
```

 # Trying model with both ratios

```
i2<-lm(BMI_KM~height_ttl_IJ+width_ttl_IJ,data=na.omit(Ratios_final[-53,]))
summary(i2)
```
 # Model fit summary to predict true BMI from true ratios

 # Same models and graphs but with different ratios

```
library(ggplot2)
Ratios_visifish <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RawdataVisifish_corr.csv")
View(Ratios_visifish)
# To check if these ratios work first
checkvs<-lm(BMI_KM~w_stl_KM+w_ttl_KM,data=Ratios_visifish)
summary(checkvs)
# Plot results
ggplot(data=Ratios_visifish,aes(x=BMI_KM, y=w_stl_KM))+
  geom_point()+
  geom_smooth(method="lm")+
  theme_bw()

ggplot(data=Ratios_visifish,aes(x=BMI_KM, y=w_ttl_KM))+
  geom_point()+
  geom_smooth(method="lm")+
  theme_bw()
# To check for outliers
check_outliers(checkvs)
# None

# To check if they work separately or have to be together like the known measurements
# stl/width
s1<-lm(BMI_KM~w_stl_KM,data=Ratios_visifish)
summary(s1)

# ttl/width
t1<-lm(BMI_KM~w_ttl_KM,data=Ratios_visifish)
summary(t1)

# To check if the Visifish AI ratios be used
```

```
vf<-lm(BMI_KM~w_stl_AI+w_ttl_AI,data=Ratios_visifish)
summary(vf)
# Plot results
ggplot(data=Ratios_visifish,aes(x=BMI_KM, y=w_stl_AI))+
  geom_point()+
  geom_smooth(method="lm")+
  theme_bw()
# Check for outliers
check_outliers(vf)
# Plot results again (final code for graph)
ggplot(data=Ratios_visifish,aes(x=BMI_KM, y=w_ttl_AI))+
  geom_point()+
  geom_smooth(method="lm")+
  theme_bw()

# To test correlation between known BMI and predicted BMI
t.test(Ratios_final$BMI_KM, Ratios_final$BMI_pred, paired = T, data = Ratios_final)

# Boxplot to display this
dataP <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R
DATA/RDataPredictedBMI.csv")
View(dataP)
data_meansP <- aggregate(dataP$BMI,
                         list(dataP$Type),
                         mean)
boxplot(dataP$BMI ~ dataP$Type, xlab = "Type", ylab = "BMI")
points(x = 1:nrow(data_meansP),
       y = data_meansP$x,
       col = "black",
       pch = 16)
text(x = 1:nrow(data_meansP),
```

```
y = data_meansP$x + 22,
labels = paste("Mean =", round(data_meansP$x, 1)),
col = "black")
```

R script Chapter 3

3.4.1. Experiment 1

 # Clear environment

```
rm(list=ls())
```

 # Load data and install necessary packages

```
data3 <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RDataBehaviourWrasseLumpfish.csv")

View(data3)

install.packages("pscl")

library(pscl)
```

 # Test to see if Latency to leave the hide if influenced by species

```
t.test(Latency_hide ~ Species, data = data3)
```

 # Test to see if Latency to leave the hide if influenced by weight

 # wrasse

```
datawrasse <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/WrasseBehaviourData.csv")

View(datawrasse)

t.test(datawrasse$Weight, datawrasse$Latency_hide, paired = T, data = datawrasse) # weight vs latency for wrasse only
```

 # lumpfish

```
datalumpfish <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/LumpfishBehaviourData.csv")

View(datalumpfish)


t.test(datalumpfish$Weight, datalumpfish$Latency_hide, paired = T, data = datalumpfish)
```

 # To see if species influenced number of touches to the Lego (novel object)

```
t.test(Lego_touch ~ Species, data = data3)
```

 # To see if weight influenced number of touches to the Lego (novel object)

 # lumpfish

```
t.test(datalumpfish$Weight, datalumpfish$Lego_touch, paired = T, data = datalumpfish)
```

 # wrasse

```
t.test(datawrasse$Weight, datawrasse$Lego_touch, paired = T, data = datawrasse)
```

 # effect of latency and species on approaches Lego (novel object)

```
g6 <- glm(data = data3, Lego_approach ~ Latency_hide * Species)
summary(g6)
```

 # Plot to see strength of model

```
plot(g6)
```

 # Boxplot to display species vs latency to leave hide (figure 3.5)

```
boxplot(data3$Latency_hide ~ data3$Species, xlab = "Species", ylab = "Latency to leave hide (seconds)")
points(x = 1:nrow(data_meansB),
       y = data_meansB$x,
       col = "black",
       pch = 16)
text(x = 1:nrow(data_meansB),
    y = data_meansB$x + 10,                    # Adjust this number to change position of mean value
    labels = paste("Mean =", round(data_meansB$x, 1)),
    col = "black")
```

 # Boxplot to display species vs number of approaches to Lego (novel object) (figure 3.6)

```
boxplot(data3$Lego_approach ~ data3$Species, xlab = "Species", ylab = "No. of approaches to novel object")
points(x = 1:nrow(data_meansA),
       y = data_meansA$x,
       col = "black",
       pch = 16)
text(x = 1:nrow(data_meansA),
    y = data_meansA$x + 10,                    # Adjust this number to change position of mean value
```

```
    labels = paste("Mean =", round(data_meansA$x, 1)),
    col = "black")
```

 # Boxplot to display species vs number of approaches to mirror (figure 3.7)

```
boxplot(data3$Mirror_approach ~ data3$Species, xlab = "Species", ylab = "No. of approaches to mirror
points(x = 1:nrow(data_meansC),
       y = data_meansC$x,
       col = "black",
       pch = 16)
text(x = 1:nrow(data_meansC),
    y = data_meansC$x + 10,                  # Adjust this number to change position of mean value
    labels = paste("Mean =", round(data_meansC$x, 1)),
    col = "black")
```

 # Boxplot to display species vs number of touches to mirror (figure 3.8)

```
boxplot(data3$Mirror_approach ~ data3$Species, xlab = "Species", ylab = "No. of touches to mirror
points(x = 1:nrow(data_meansD),
       y = data_meansD$x,
       col = "black",
       pch = 16)
text(x = 1:nrow(data_meansD),
    y = data_meansD$x + 10,                  # Adjust this number to change position of mean value
    labels = paste("Mean =", round(data_meansD$x, 1)),
    col = "black")
```

 # effect of latency and species on number of touches to novel object

```
g7 <- glm(data = data3, Lego_touch ~ Latency_hide * Species)  # effect of latency and species on no. of touches to novel object
summary(g6)
```

 # Plot to see strength of model

plot(g6)

 # To test for relationship between mirror approaches and touches

ZIP2 <- zeroinfl(Mirror_touch ~ Mirror_approach | Mirror_approach, dist = "poisson", link = "logit", data = data3)

summary(ZIP2)

 # AIC to determine strength of model

AIC(ZIP2)

ZINB2 <- zeroinfl(Mirror_touch ~ Mirror_approach | Mirror_approach, dist = "negbin", link = "logit", data = data3)

summary(ZINP2)

 # AIC to determine strength of model

AIC(ZINB2)

 # Negative binomial distribution model chosen due to lower AIC (better model)

 # effect of latency and species on number of approaches to the mirror

g6 <- glm(data = data3, Mirror_approach ~ Latency_hide * Species)

summary(g6)

 # Plot to see strength of model

plot(g6)

 # effect of latency and species on touches Mirror

gBB <- glm(data = data3, Mirror_touch ~ Latency_hide * Species

summary(gBB)

 # Plot to see strength of model

plot(gBB)

3.4.2 Experiment 2

 # Violin plot to display number of approaches to each salmon model (figure 3.9)

 # Load data

data2 <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RDataBehaviourModels.csv")

View(data2)

a= ggplot(data=data2, mapping = aes(fill=Species, x=Model, y=No_approaches))

summary(a)

a+

geom_violin()

 # Violin plot to display number of approaches to each salmon model (figure 3.10)

b= ggplot(data=data2, mapping = aes(fill=Species, x=Model, y=No_touches))

summary(b)

b+

geom_violin()

 # Boxplot to display the time spent within 10cm of each salmon model (figure 3.11)

data_means3 <- aggregate(data6$time,

list(data6$Model),

mean)

boxplot(data6$time ~ data6$Model, xlab = "Model type", ylab = "Time spend within 10cm each model (secs)", ylim = c(0,800)) # approaches to each model

points(x = 1:nrow(data_means3),

y = data_means3$x,

col = "black",

pch = 16)

text(x = 1:nrow(data_means3),

y = data_means3$x + 35, **# Adjust this number to change position of mean value**

labels = paste("Mean =", round(data_means3$x, 1)),

```
    col = "black")
```

 # Boxplot to display the time taken to first approach a salmon model in experiment 2 (figure 3.12)

```
boxplot(data3$Latency_model ~ data3$Species, xlab = "Species", ylab = "Time to first approach model (seconds)”

points(x = 1:nrow(data_meansE),

      y = data_meansE$x,

      col = "black",

      pch = 16)

text(x = 1:nrow(data_meansE),

    y = data_meansE$x + 10,                    # Adjust this number to change position of mean value

    labels = paste("Mean =", round(data_meansE$x, 1)),

    col = "black")
```

 # Welch’s T-tests to see if species had an effect on the number of approaches and touches to each salmon model

```
t.test(Approach_0lice ~ Species, data = data3)


t.test(Approach_1lice ~ Species, data = data3)


t.test(Approach_6lice ~ Species, data = data3)


t.test(Contacts_0lice ~ Species, data = data3)


t.test(Contacts_1lice ~ Species, data = data3)


t.test(Contacts_6lice ~ Species, data = data3)
```

 # LMs created to test if the time spent within 10cm of each model was influenced by latency to first approach a salmon model, species and weight

```
g15 <- lm(data = data3, Time_1lice ~ Latency_model * Species * Weight)
```

summary(g15)

 # Plot to see strength of model

plot(g15)

g16 <- lm(data = data3, Time_6lice ~ Latency_model * Species * Weight)

summary(g16)

 # Plot to see strength of model

plot(g16)

g17 <- lm(data = data3, Time_0lice ~ Latency_model * Species * Weight)

summary(g17)

 # Plot to see strength of model

plot(g17)

 # T-test to see if the species had an effect on the time taken to first approach a salmon model

t.test(Latency_model ~ Species, data = data3)

 # T-test to see if the weight had an effect on the time taken to first approach a salmon model

t.test(data3$Weight, data3$Latency_model, paired = T, data = data3)

3.4.3. Is there a relationship between the personality components and the fish reactions to the salmon models?

 # Clear environment

rm(list=ls())

 # Load data

data4 <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RDataBehaviourSection3.csv")

View(data4)

 # lm for comparing data with time (seconds)

 # Do species and anxiety have an effect on latency to first approach a salmon model

```
g8 <- lm(data = data4, Latency_model ~ Species + Anxiety)
summary(g8)
plot(g8)
```

 # Violin model to display each species and anxiety and latency to approach the first salmon model (figure 3.13)

```
a= ggplot(data=data4, mapping = aes(fill=Species, x=Anxiety, y=Latency_model))
summary(a)

a+
  geom_violin()
```

 # Do species and boldness have an effect on latency to first approach a salmon model

```
g9 <- lm(data = data4, Latency_model ~ Species + Boldness)
summary(g9)
plot(g9)
```

 # Violin model to display each species and boldness and latency to approach the first salmon model (figure 3.15)

```
b= ggplot(data=data4, mapping = aes(fill=Boldness, x=Species, y=Latency_model))
summary(b)

b+
  geom_violin()
```

 # Do species and sociality have an effect on latency to first approach a salmon model

```
g10 <- lm(data = data4, Latency_model ~ Species + Sociality)
summary(g10)
plot(g10)
```

 # Do species and aggression have an effect on latency to first approach a salmon model

```
g11 <- lm(data = data4, Latency_model ~ Species + Aggression)
```

```
summary(g11)
plot(g11)
```

 # Quasipoisson GLM method for count data

 # To see if species and boldness had an effect on number of approaches to each of the salmon models (0, 1 and 6 sea lice)

```
AModel <- glm(Approach_0lice ~ Species + Boldness, data = data4, family = quasipoisson)
summary(AModel)
plot(AModel)

BModel <- glm(Approach_1lice ~ Species + Boldness, data = data4, family = quasipoisson)
summary(BModel)
plot(BModel)

CModel <- glm(Approach_6lice ~ Species + Boldness, data = data4, family = quasipoisson)
summary(CModel)
plot(CModel)
```

 # Violin plot to display species and aggression on approaches to the model with 6 sea lice (figure 3.18)

```
d= ggplot(data=data4, mapping = aes(fill=Aggression, x=Species, y=Approach_6lice))
summary(d)

d+
  geom_violin()
```

 # To see if species and sociality had an effect on number of approaches to each of the salmon models (0, 1 and 6 sea lice)

```
DModel <- glm(Approach_0lice ~ Species + Sociality, data = data4, family = quasipoisson)
summary(DModel)
plot(DModel)
```

```
EModel <- glm(Approach_1lice ~ Species + Sociality, data = data4, family = quasipoisson)
summary(EModel)
plot(EModel)

FModel <- glm(Approach_6lice ~ Species + Sociality, data = data4, family = quasipoisson)
summary(FModel)
plot(FModel)
```

 # Violin plot to display species and sociality on approaches to the model with 6 sea lice (figure 3.19)

```
e= ggplot(data=data4, mapping = aes(fill=Sociality, x=Species, y=Approach_6lice))
summary(e)

e+
  geom_violin()
```

 # To see if species and aggression had an effect on number of approaches to each of the salmon models (0, 1 and 6 sea lice)

```
GModel <- glm(Approach_0lice ~ Species + Aggression, data = data4, family =
quasipoisson)
summary(GModel)
plot(GModel)

HModel <- glm(Approach_1lice ~ Species + Aggression, data = data4, family =
quasipoisson)
summary(HModel)
plot(HModel)

IModel <- glm(Approach_6lice ~ Species + Aggression, data = data4, family = quasipoisson)
summary(IModel)
plot(IModel)
```

 # To see if species and boldness had an effect on number of contacts to each of the salmon models (0, 1 and 6 sea lice)

```
JModel <- glm(Contacts_0lice ~ Species + Boldness, data = data4, family = quasipoisson)
summary(JModel)
plot(JModel)

KModel <- glm(Contacts_1lice ~ Species + Boldness, data = data4, family = quasipoisson)
summary(KModel)
plot(KModel)

LModel <- glm(Contacts_6lice ~ Species + Boldness, data = data4, family = quasipoisson)
summary(LModel)
plot(LModel)
```

 # To see if species and sociality had an effect on number of contacts to each of the salmon models (0, 1 and 6 sea lice)

```
MModel <- glm(Contacts_0lice ~ Species + Sociality, data = data4, family = quasipoisson)
summary(MModel)
plot(MModel)

NModel <- glm(Contacts_1lice ~ Species + Sociality, data = data4, family = quasipoisson)
summary(NModel)
plot(NModel)

OModel <- glm(Contacts_6lice ~ Species + Sociality, data = data4, family = quasipoisson)
summary(OModel)
plot(OModel)
```

 # To see if species and aggression had an effect on number of contacts to each of the salmon models (0, 1 and 6 sea lice)

```
PModel <- glm(Contacts_0lice ~ Species + Aggression, data = data4, family = quasipoisson)
```

```
summary(PModel)
plot(PModel)

QModel <- glm(Contacts_1lice ~ Species + Aggression, data = data4, family = quasipoisson)
summary(QModel)
plot(QModel)

RModel <- glm(Contacts_6lice ~ Species + Aggression, data = data4, family = quasipoisson)
summary(RModel)
plot(RModel)
```

 # lm to see if species and anxiety had an effect on approaches to each of the salmon models (0, 1 and 6 sea lice)

```
g1 <- lm(data = data4, Approach_0lice ~ Species + Anxiety)
summary(g1)
plot(g1)

g2 <- lm(data = data4, Approach_1lice ~ Species + Anxiety)
summary(g2)
plot(g2)

g3 <- lm(data = data4, Approach_6lice ~ Species + Anxiety)
summary(g3)
plot(g3)
```

 # Violin plot to display species and anxiety on approaches to the model with 6 sea lice (figure 3.14)

```
a= ggplot(data=data4, mapping = aes(fill=Anxiety, x=Species, y=Approach_6lice))
summary(a)

a+
```

```
  geom_violin()
```

 # lm to see if species and anxiety had an effect on contacts to each of the salmon models (0, 1 and 6 sea lice)

```
g4 <- lm(data = data4, Contacts_0lice ~ Species + Anxiety)
summary(g4)
plot(g4)

g5 <- lm(data = data4, Contacts_1lice ~ Species + Anxiety)
summary(g5)
plot(g5)

g6 <- lm(data = data4, Contacts_6lice ~ Species + Anxiety)
summary(g6)
plot(g6)
```

 # lm to see if species and anxiety had an effect on time spent within 10cm of each salmon model (0, 1 and 6 sea lice)

```
g7 <- lm(data = data4, Time_0lice ~ Species + Anxiety)
summary(g7)
plot(g7)

g8 <- lm(data = data4, Time_1lice ~ Species + Anxiety)
summary(g8)
plot(g8)

g9 <- lm(data = data4, Time_6lice ~ Species + Anxiety)
summary(g9)
plot(g9)
```

 # lm to see if species and boldness had an effect on time spent within 10cm of each salmon model (0, 1 and 6 sea lice)

```
g10 <- lm(data = data4, Time_0lice ~ Species + Boldness)
summary(g10)
plot(g10)

g11 <- lm(data = data4, Time_1lice ~ Species + Boldness)
summary(g11)
plot(g11)

g12 <- lm(data = data4, Time_6lice ~ Species + Boldness)
summary(g12)
plot(g12)
```

 # Violin plot to display species and boldness on time spent within 10cm of the salmon model with 6 sea lice (figure 3.16)

```
b= ggplot(data=data4, mapping = aes(fill=Boldness, x=Species, y=Time_6lice))
summary(b)

b+
  geom_violin()
```

 # lm to see if species and sociality had an effect on time spent within 10cm of each salmon model (0, 1 and 6 sea lice)

```
g13 <- lm(data = data4, Time_0lice ~ Species + Sociality)
summary(g13)
plot(g13)

g14 <- lm(data = data4, Time_1lice ~ Species + Sociality)
summary(g14)
plot(g14)
```

```
g15 <- lm(data = data4, Time_6lice ~ Species + Sociality)
summary(g15)
plot(g15)
```

 # lm to see if species and aggression had an effect on time spent within 10cm of each salmon model (0, 1 and 6 sea lice)

```
g16 <- lm(data = data4, Time_0lice ~ Species + Aggression)
summary(g16)
plot(g16)

g17 <- lm(data = data4, Time_1lice ~ Species + Aggression)
summary(g17)
plot(g17)

g18 <- lm(data = data4, Time_6lice ~ Species + Aggression)
summary(g18)
plot(g18)
```

Chapter 4

```
# Clear environment
rm(list=ls())
# Load data and necessary packages
install.packages("ggplot2")
library(ggplot2)
dataThermal <- read.csv("C:/Users/[redacted]/Downloads/RDataThermalPref.csv")

# glm to see if weight had an effect on preference temperature
g1 <- glm(Preference_temperature ~ Weight, data = dataThermal)
summary(g1)
plot(g1)

# Boxplot to display thermal niche of Ballan wrasse
data_means1 <- aggregate(dataThermal$Preference_temperature,
                         list(dataThermal$Species),
                         mean)

boxplot(dataThermal$Preference_temperature ~ dataThermal$Species, xlab = "Ballan
wrasse", ylab = "Temperature (ºC)")
points(x = 1:nrow(data_means1),
       y = data_means1$x,
       col = "black",
       pch = 16)
text(x = 1:nrow(data_means1),
     y = data_means1$x + 10,
     labels = paste("Mean =", round(data_means1$x, 1)),
     col = "black")

# Barplot to display the numbers of fish at each temperature
```

```
dataT <- read.csv("C:/Users/[redacted]/OneDrive - Swansea University/R DATA/RDataThermalPref3.csv")

barplot(height = dataT$No_fish, names = dataT$Temperatures,
        col = rgb(0.2, 0.4, 0.6, 0.6),
        xlab = "Preference Temperatures",
        ylab = "No. of fish",
        ylim = c(0,12),
        cex.lab = 1, cex.names = 0.8)
```

 # T-test to determine if there is significance between distance travelled in each zone.

```
t.test(distance ~ zone, data = data2)
```