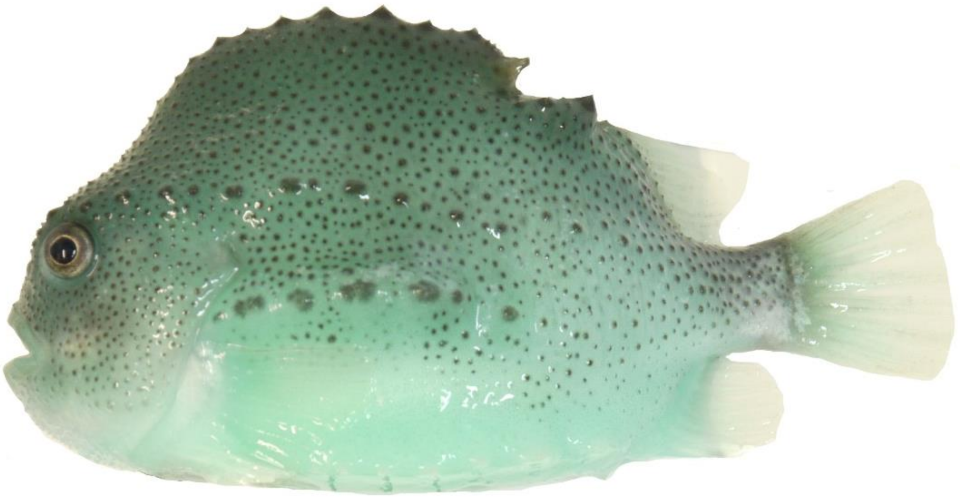
Prifysgol Abertawe
Swansea University

Quantifying phenotypic and genetic variation of the lumpfish (*Cyclopterus lumpus*): application towards the sustainable aquaculture of cleaner fish.



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Submitted to Swansea University in fulfilment of the requirements
for the Degree of Doctor of Philosophy (July, 2019).

Summary

Ectoparasitic lice threaten sustainable Atlantic salmon (*Salmo salar*) aquaculture, as high farm stocking densities facilitate louse infestation thereby increasing mortality rates. Lumpfish (*Cyclopterus lumpus*) have recently been demonstrated to remove lice and are increasingly deployed onto farms as cleaner fish. Commercial stocks of lumpfish are now produced in hatcheries to meet growing demand from farms across the Atlantic, however, concern grows regarding the long-term viability of this practise due to insufficient knowledge on lumpfish biology. Challenges include understanding how patterns of genetic and phenotypic variation influence performance, whether desirable traits can inform captive breeding, potential impacts on wild populations and whether intraspecific variation in cleaning behaviour can be optimized. Therefore, this thesis examined genetic and phenotypic variation in lumpfish across multiple biological scales, ranging from populations down to family stocks and individuals, investigating factors influencing aquaculture performance.

Chapter 1 found genetic structure in populations across the Atlantic, with limited gene flow separating regions of lumpfish aquaculture. Chapter 2 identified phenotypic differences in the body morphology of stocks, including variation in scutes and body depth of northern and southern phenotypes. Sexual dimorphism was identified in the mouth, flag, hump and caudal peduncle. Chapter 3 showed further phenotypic differences in fitness-related traits, with faster growth, more active swimming and lower survival associated with northern stocks. Chapter 4 developed protocols to quantify lumpfish personality and found that bolder individuals interacted more with salmon, whereas, a syndrome for social-aggression was linked to antagonistic behaviour.

Patterns of genetic and phenotypic variation imply population differentiation is reflected in stocks commonly used in aquaculture. Common-garden trials suggested translocated lumpfish show reduced fitness, therefore establishing regionally-specific stocks may benefit performance and minimise detriment to wild populations via introgression through farm escapes. Personality constituted a major source of variation in cleaning behaviour, selecting bold lumpfish could optimise efficiency.

Declaration and Statements

I, Benjamin Alexander Whittaker, declare that this work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed: [redacted]

Date: 10/02/2020

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

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I hereby give consent for my thesis, if accepted, to be available for photocopying and inter-library loan, and for the title and summary to be made available to outside organisations.

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Authorship Declaration

The following people contributed to the publication of work undertaken as part of this thesis.

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Paper:

Whittaker B.A., Consuegra S., and Garcia de Leaniz C. (2018). Genetic and phenotypic differentiation of lumpfish (*Cyclopterus lumpus*) across the North Atlantic: implications for conservation and aquaculture. *PeerJ* **6**: e5974

Authorship Contribution:

The experiment was conceived and designed by SC and CGL, who both wrote the grant and secured funding. Laboratory work was conducted by BW, with SC and CGL contributing reagents, materials and equipment. BW, SC and CGL analysed data. BW, SC and CGL prepared tables and figures. BW, SC and CGL authored and reviewed the manuscript, and all authors approved the final draft.

We, the undersigned, agree with the above Authorship Contribution stated above and agree to the inclusion of this work within the current thesis.

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Disclaimer

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Ethics: Information and protocols for animal research were approved by Swansea University College of Science Ethics Review Committee. Project reference numbers are provided in appropriate chapters.

Chapter 1: Benjamin Whittaker (BW) carried out lab work. Prof. Sofia Consuegra (SC) and BW analysed genotype data, and Prof. Carlos Garcia de Leaniz (CGL) and BW analysed phenotype data. The manuscript, figure and tables were prepared by BW, with supervision and contribution from CGL and SC.

Whittaker B.A., Consuegra S., and Garcia de Leaniz C. (2018). Genetic and phenotypic differentiation of lumpfish (*Cyclopterus lumpus*) across the North Atlantic: implications for conservation and aquaculture. *PeerJ* **6**: e5974

Chapter 2: BW carried out lab work. BW and CGL analysed morphology data. The manuscript, figure and tables were prepared by BW, with supervision and contribution from CGL and SC.

Chapter 3: BW carried out lab work. BW and CGL analysed data. The manuscript, figure and tables were prepared by BW, with supervision and contribution from CGL and SC.

Chapter 4: BW carried out lab work. BW and CGL analysed behavioural data. The manuscript, figure and tables were prepared by BW, with supervision and contribution from CGL and SC.

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“For the Lumpfishes”

by Dr Stephanie Januchowski-Hartley

Dumpling, balloon, or simply lumpy
these fishes are packed with surprises!
There are more than two dozen species,
some travel great distance to humpy.

Members of Scorpaneiformes, scorpions
with fins, some fused for extra suck power.
Their bottom dwelling cousins often cower
below sand. Lumpies are rocky guardians.

Like their order suggests, they bear spines
but who knows if venomous like velvet-
fish or lions. Often hidden by a skin helmet,
they appear absent, fooling the fish who dines.

Heralded as cute, in diverse shapes and sizes.
Their eggs replace caviar as sturgeons have gone,
we rear them for farms, cleaners, salmon’s pawn,
plucking them from the wild, believing they are prizes.

And finally, Lumpfishes...
they are round.
Marvel at their diversity.
Protect their magic.

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Chapter Overview

Overview of Aquaculture

Aquaculture is the production of aquatic organisms within a controlled environment and includes the breeding, rearing and harvesting of commercial stocks (Pillay & Kutty 2005; Stickney 1994). Production occurs in both marine and freshwater systems, across a range of tropical, temperate and arctic environments (Cardia & Lovatelli 2007; Halwart et al. 2007; Jamu & Ayinla 2003; Jobling et al. 1993). Aquaculture is the fastest growing food-production sector in the world, with global production rates increasing by an average of 6.7% per year from 1970 to 2006 (Fisheries Department 2000). Cultured species represent diverse taxa including algae, molluscs, crustaceans and fish (Britton & Orsi 2012; Chopin et al. 2001; Guo et al. 1999; Páez-Osuna 2001). Freshwater finfish aquaculture is largely driven by the production of carp and tilapia, and the production of Atlantic salmon (*Salmo salar*) dominates marine finfish aquaculture (Bostock et al. 2010). Although the primary role of finfish aquaculture is to produce food, the industry also generates stocks for ornamental trade, research facilities and service providers such as cleaner fish (Jonassen et al. 2018b; Tlusty 2002; Varga 2011).

Given the fully or over exploited status of many wild fish populations (Jackson et al. 2001), seafood production through aquaculture is necessary to reduce harvest pressure and conserve wild populations (Diana 2009). The proportion of seafood provided through aquaculture relative to wild capture has increased since the 1980s, with aquaculture producing nearly half of the seafood consumed in 2016 (Fisheries Department 2000). This seafood provides a reliable source of protein amid concerns for global food security as human populations continue to rise (Subasinghe et al. 2009), however, the extent to which aquaculture, particularly open-net cage farming, truly benefits natural biodiversity and ecosystems is a matter of debate (Subasinghe 2003; Valenti et al. 2011).

Sustainable Finfish Aquaculture

The global aquaculture industry has received criticism for violating environmental standards (Bush et al. 2013) and is accused of misrepresenting information on the sustainability of commercial practises in order to prioritise production (Mair et al. 2016). Critics argue that truly sustainable aquaculture should not only seek to maximise productivity but must also aim to reduce its impact on natural systems (Frankic & Hershner 2003). Improved sustainability can be achieved through developing more efficient use of resources, responsible stock management and advancing welfare standards (Huntingford & Kadri 2009; Klinger & Naylor 2012).

Although finfish aquaculture reduces harvest pressure for species otherwise caught for human consumption, farming carnivorous fish is highly dependent on the use of fishmeal and fish oil derived from wild forage fish to manufacture aquafeeds (Tacon 2004). This constitutes substantial resource use, as production of aquafeeds accounts for 68% of the global consumption of fishmeal and 88% of the global consumption of fish oil (Naylor et al. 2009). Uneaten food and detritus produced by farmed fish pollutes water with ammonia, phosphorus and other toxic compounds, which then enters ecosystems surrounding the farm and reduce water quality (Turcios & Papenbrock 2014). Possible waste management solutions include developing plant-based aquafeeds, using land-based facilities (Naylor & Burke 2005) and adopting principles of integrated aquaculture in which multiple species, such as seaweeds and bivalves, are cultured simultaneously alongside carnivorous finfish to reduce pollution (Edwards 1998; Neori et al. 2004). Reducing the level of stress experienced by fish in farm environments could also improve consumption rates, both improving welfare and decreasing food wastage (Gabriel & Akinrotimi 2011).

The production of many farmed species still depends on the use of wild-caught adult fish as broodstock (Bentsen & Olesen 2002). This removes reproductively mature adults from the wild, thereby heightening vulnerability to over-exploitation (Hoenig & Hewitt 2005) and reducing the abundance of wild populations (Halvorsen et al. 2017). Gametes harvested from broodstock are used in artificial fertilisation to produce hatchery stocks (Urbányi et al. 2009), which can bypass processes of sexual selection through the removal of mate choice (Fessehaye et al. 2006; Perry et al. 2019). Genetic analysis is used to inform artificial reproduction to minimise the risk of inbreeding and maintain genetic diversity in stocks (Taniguchi 2003), however, the tools necessary to genetically screen farmed fish are not available for every species and inbreeding depression is often detected in commercial stocks (Saura et al. 2017; Yoshida et al. 2017). Inbreeding depression is associated with compromised stock fitness, including lower resistance to disease (Doyle et al. 2019) and stunted growth (Lyu et al. 2018). Once hatchery stocks have grown to an adequate size they are transported from natal hatchery facilities to reach harvest size on farms, which can involve relocating distinct stocks of fish across natural genetic boundaries of native populations (Stockwell & Leberg 2002). Translocating regionally-specific, potentially inbred, stocks for farm deployment across a wide geographic scale could carry significant risks to native populations should the translocated fish escape from farms (Cross 2000; Gonzalez & de Boer 2017).

Nearly 9 million fish were documented escaping from open-net farms over a 3-year period (Naylor et al. 2005), thus allowing a substantial quantity of farmed fish to enter natural ecosystems. This high number of escapes creates high propagule pressure and increases the risk of escapees interbreeding with wild fish (Karlsson et al. 2016). Hybridisation between escaped farm fish and wild fish has been documented in numerous species from both marine and freshwater habitats (Chang & Connell 2017; Faust et al. 2018; Hashimoto et al. 2016; Wringe et al. 2018). The severity of threat escapees pose to wild populations depends on (1) whether escapees survive in sufficient numbers to colonise wild habitats (Jensen et al. 2013; Skilbrei 2013), (2) the reproductive success of escapees (Clifford et al. 1998; Volpe et al. 2000), (3) whether escaped stocks were deployed outside their native range and thus represent an alien species (De Silva et al. 2006; Naylor et al. 2001), (4) whether stocks were translocated across different genetic populations and carry non-native traits that could prove maladaptive if hybridised with local fish (Cross 2000; Hindar et al. 1991), (5) the extent of inbreeding in commercial stocks that could negatively impact fitness via introgression (Bourret et al. 2011; Consuegra et al. 2011). The consequences of genetic introgression between farmed fish and wild populations are best documented in Atlantic salmon (*Salmo salar*), and are shown to include lower survival rates (McGinnity et al. 2003), reduced predator avoidance behaviour (Houde et al. 2010), and compromised tolerance to high temperature (McGinnity et al. 2009) and low pH (Fraser et al. 2008). Evaluating the degree of genetic and phenotypic differentiation between commercial stocks and wild fish, alongside identifying regionally specific local adaptations by native populations, could help inform appropriate translocation routes in aquaculture by minimising differentiation between populations (Cross 2000; Stockwell & Leberg 2002).

The degree to which aquaculture practises are perceived as being unsustainable is in part linked to the debate surrounding whether fish are capable of feeling pain, fear and stress (Chandroo et al. 2004; Rose et al. 2014). Some argue that fish lack the psychology (Derbyshire 2016) and physiology (Key 2016a; Key 2016b) to experience negative sensation, which implies welfare is not an important component to consider in sustainable aquaculture. Whereas, others show empirical evidence of aversion by fish to negative stimuli that implies the contrary (Braithwaite 2010; Sneddon 2003; Sneddon et al. 2018) and have demonstrated sufficient physiology for pain reception (Sneddon 2009), claiming anthropomorphic bias clouds the viewpoint that fish cannot feel pain (Seth 2016; Sneddon & Leach 2016). Whilst academics have escalated the debate to whether or not fish exhibit self-awareness (de Waal 2019; Kohda et al. 2019), public perception has shifted towards accepting fish as sentient beings capable of experiencing negative emotion and should therefore be legally protected (Browman et al. 2018; Rucinque et al. 2017). This holds significant implications for aquaculture, which is criticised for compromised fish welfare through adverse farm conditions and substandard slaughter methods (Bergqvist & Gunnarsson 2013; Håstein 2004). Unsustainable practise that is deemed to inflict suffering on farm or wild fish has been cited by consumers as reasons against purchasing farmed fish over wild fish (Katrin Schlag & Ystgaard 2013; Osmundsen et al. 2017). Customer surveys have shown consumers are willing to pay higher prices to guarantee better fish welfare conditions (Stubbe Solgaard & Yang 2011), although this preference varies between societies (Kupsala et al. 2013). Nonetheless, concerns over fish welfare have moved to forefront of the debate surrounding sustainability in aquaculture (Olesen et al. 2011; Schlag 2010), with mounting media pressure driving farmers to minimise potential suffering experienced by fish (Amberg & Hall 2008).

Costs and Benefits of Salmon Farming

Intensive farming of Salmonidae has grown dramatically over the over the past 40 years (Asche et al. 2013), and global production increased by one million metric tons from the 1990s to 2000s (Naylor et al. 2003). Atlantic salmon (*Salmo salar*) are typically stocked by age class in open-net cages during the grow-out phase of production (Bron et al. 1993), ranging in stocking density from 9.7 to 34 kg/m^{-3} (Turnbull et al. 2005). The duration of time salmon are stocked on farms depends on numerous bioeconomic factors including market value and fish growth rates (Bjørndal

1988; Forsberg & Guttormsen 2006). Atlantic salmon farming predominantly takes place in Norway, Chile, Scotland, Canada and the Faroe Islands (Asche et al. 2013), with lower levels of farming in Iceland, Ireland, New Zealand and Tasmania (Cheshuk et al. 2003; Clifford et al. 1998; Fløysand et al. 2016; Karbowski et al. 2019).

Approximately 60% of the salmon consumed worldwide in 2004 was derived from aquaculture as, relative to salmon fisheries, farms provide greater quantities of fish at cheaper sale prices and farm produce is available year-round (Eagle et al. 2004). Given that salmon farms are often located in remote coastal areas, the industry is credited as reducing poverty levels by providing sources of income in regions that are otherwise score low for employment prospects (Ceballos et al. 2018). Concern has been raised over potential health risks of consuming farmed salmon (Hites et al. 2004), though subsequent analysis found positive health benefits associated with the proteins, vitamin D and omega-3 fatty acids contained in farmed salmon (Foran et al. 2005; Stokstad 2004; Tuomisto et al. 2004).

Farming Atlantic salmon is perhaps the most controversial form of aquaculture, with intensive farming accused of wasting resources, poor stock management and severely compromising fish welfare in various widespread media campaigns (Olsen & Osmundsen 2017). The World Wildlife Fund (WWF) has established international standards for sustainability in salmon farming which list the degradation of benthic habitats, pollution with organic waste and nutrients, fish escapes, use of chemicals, disease outbreaks and unsustainable feeding practises as being major problems (Burridge et al. 2010). Whether farms meet these international standards is a matter of ongoing contention, though independent assessment models have found salmon farms pose moderate to high risk levels for surrounding ecosystems (Taranger et al. 2014). Proceedings from academic conferences have claimed that there is no ecosystem in which stable populations of wild salmonids and intensive farming can coexist (Morton 2018). Increasing public awareness on the environmental costs of salmon farming is reported to have reduced sales of farmed salmon in Scotland (Whitmarsh & Palmieri 2011; Whitmarsh & Wattage 2006), though telephone surveys conducted in Norway and Ireland suggest that while the general public recognises the economic benefits salmon farming provides in coastal regions opinion is divided over the trade-off inflicted by salmon farms on marine ecosystems (Hynes et al. 2018).

Debate surrounding perceived costs and benefits of salmon farming and the accountability of the industry has proven an emotive topic (Cullen-Knox et al. 2019; Georgakopoulos & Thomson 2005). However, one issue on which salmon farmers, activists, government bodies and academics consistently agree is that parasitic sea-lice pose the biggest threat to the sustainability of salmon aquaculture (Costello 2009a; Forseth et al. 2017; Jansen et al. 2012; Luthman et al. 2019; Staniford 2012; Treasurer 2018; Treasurer 2002).

Threats of Sea-lice

The term sea-lice typically refers to ectoparasitic marine copepods including various species of the genus *Caligus* and *Lepeophtheirus*, the most prevalent being *L. salmonis* which is a specialist of salmonid hosts. The life-cycle begins when planktonic nauplii attach onto hosts during the infective copepodid stage, before developing through two chalimus stages and growing to become mobile adult lice (Hamre et al. 2013). Development from egg to maturity takes approx. 40 days depending on water temperature and salinity (Piasecki & MacKinnon 1995). Mature female lice develop a pair of egg filaments that release 150-300 eggs per filament, with a single female capable of producing 11 filament pairs throughout the summer breeding season (Heuch et al. 2000). High stocking densities found in salmon farms provide ideal conditions for lice to thrive (Torrissen et al. 2013) and the combined fast generation time with high fecundity facilitates rapid population growth, causing farm infestation on a dramatic scale (Asplin et al. 2014). Although sea-lice are typically studied as parasites of farmed Atlantic salmon, they naturally occur alongside wild fish and are capable of infecting numerous species, such as sea trout (*Salmo trutta*), Arctic charr (*Salvelinus alpinus*), Pacific pink salmon (*Oncorhynchus gorbuscha*), Pacific chum salmon (*Oncorhynchus keta*), Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic cod (*Gadus morhua*), and three-spine stickleback (*Gasterosteus aculeatus*) (Brooks 2009; Costello 2009a; Jones et al. 2006a; Jones et al. 2006b).

Adult lice graze on the mucus, skin and epidermal tissue of their host, tending to aggregate in areas with ample blood supply such as the head, behind fins and the gills (Whelan 2010). Extended grazing causes lesions, osmoregulatory failure, secondary infection and stress response in the host, with an infection level of 30 adult lice proving fatal for post-smolt salmon (Grimnes & Jakobsen 1996; Mustafa et al. 2000). Sea-lice also act as vectors of disease and are associated with the emergence and spread of several diseases including Infectious Salmon Anaemia virus (Oelckers et al. 2014), Infectious Haematopoietic Necrosis virus (Jakob et al. 2011), and *Piscine orthoreovius* leading to Heart and Skeletal Muscle Inflammation (Garseth et al. 2013). Sea-lice also induce lethargy in the host, reducing swimming speed and activity levels (Wagner et al. 2003).

The direct economic cost of lice to the global salmon farming industry is estimated at £260 million per year (Costello 2009b), with pesticide treatment and reduced salmon growth rates considered the greatest financial burdens (Sinnott 1998). However, this value does not account for losses incurred by secondary disease transmitted through lice, which may increases the overall loss by a further 7% (Mustafa et al. 2001). Norway is the largest farming region of salmon by a substantial margin, accounting for 51% of global production in 2010 relative to 28% by Chile as second largest producer that same year (Asche et al. 2013). Analysis of data collected from every Norwegian farm over a three year period estimated that sea-lice reduced salmon growth by 17% which, when combined with treatment costs, incurred a total cost of £330 million per year and represented a total 9% loss in farm revenue (Abolofia et al. 2017). The annual loss attributed to sea-lice is greater than the estimated total cost incurred by all parasite species in Brazilian freshwater finfish aquaculture (£75 million) (Tavares-Dias & Martins 2017), and is comparable to economic loss reported for parasites in large scale agriculture systems including gastrointestinal nematodes (£338 million) and horn fly (£231 million) in the Mexican cattle industry (Rodríguez-Vivas et al. 2017). However, inconsistencies in the methods used for calculating revenue loss in agriculture and aquaculture systems makes comparison difficult (Rashid et al. 2019), and standardised meta-analysis would be required to reveal the proportional impact different parasites cost food production sectors.

Reports from the 1970s observed that sea-lice, particularly *Lepeophtheirus*, were found in low numbers on wild fish outside the louse breeding season from August to September (Boxshall 1974; Rikardsen 2004). However, since the late 1980s reports of heavily infested wild fish have become increasingly common throughout the year (Birkeland 1996; Bjørn & Finstad 2002), which coincides with the advent and upscaling of intensive salmon farming (Coull 1988). Numerous lab and field studies have concluded that planktonic nauplii disperse through the open-net cages of salmon farms (Amundrud & Murray 2009; Krkošek et al. 2005; Tully & Whelan 1993), thereby increasing nauplii abundance in natural ecosystems surrounding salmon farms (Costelloe et al. 1998; McKibben & Hay 2004) and raising infection rates of wild fish (Costello 2009a; Frazer 2009; Krkošek et al. 2006). Critics argue that slow nauplii development times, low dispersal potential and low survival rates reduce the overall impact of farm-origin lice on wild fish populations (Brooks 2005; Brooks & Stucchi 2006). Furthermore, it is argued that as the salmonid-specialist, *Lepeophtheirus*, and host-generalist, *Caligus*, have indistinguishable nauplii stages there is inherent uncertainty in identifying the origin of planktonic stage lice (Schram 2004). Despite this criticism, more recent studies continue linking nauplii dispersal from salmon farms to the infestation of wild fish populations (Costello 2009a; Karbowski et al. 2019; Peacock et al. 2016). Comparison of infection rates by adult lice on wild fish in regions with and without salmon farms has demonstrated spikes in *Lepeophtheirus* abundance associated with increased farming activity (Boxaspen 1997; Butler 2002; Thorstad & Finstad 2018). Infection pressure in the immediate areas surrounding farms is documented exceeding 70 times that of an ambient level, and elevated infection pressure is detected up to 30 km from farms (Krkošek et al. 2005). Salmon farms attract many species of wild fish, such as cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and saithe (*Pollachius virens*), in numbers up to three orders of magnitude above ambient abundance (Dempster et al. 2004; Dempster et al. 2009). These wild fish are frequently observed moving between farms, thus creating a highly connected network through which sea-lice can disperse across farms (Uglem et al. 2009). Additionally, farms are often located along the migratory routes of wild salmon and sea trout populations and therefore contact between wild and farmed salmonids becomes common over periods of migration (Krkošek et al. 2009). The high infection pressure surrounding farms significantly impacts wild salmonids to the extent that louse-induced mortality of wild salmon exceeds 80%, and is attributed to the localised extinction of natural populations (Krkošek et al. 2007).

Farmers utilise numerous control methods to reduce louse populations, with the use of chemical therapeutants being the most widespread practise (Treasurer 2002). Treatments include feed additives such as avermectin and baths comprised of organophosphates, pyrethroids and hydrogen peroxide (Haya et al. 2005). The long-term viability of chemotherapeutants is openly criticised, primarily as lice have repeatedly developed resistance to the numerous pesticides used on farms (Aaen et al. 2015; Denholm et al. 2002; Fallang et al. 2004; Treasurer et al. 2000; Tully & McFadden 2000) and bath treatments are shown to negatively impact surrounding ecosystems (Burridge et al. 2010). Pesticide baths can disperse through net-pens and are detected up to 3000 m away from farm sites, with some compounds proving toxic for up to 5 hours after release (Ernst et al. 2001). The active compounds used by the industry target invertebrates and, in excessive dosage, are demonstrated to cause mortality in naturally occurring amphipods (e.g. *Eohaustorius estuaries*), shrimp (e.g. *Crangon septemspinosa, Mysis stenolepsis, Praunus flexuosus*), lobsters (e.g. *Homarus americanus, H .gammarus*), crabs (*Carcinus pagurus, C. maenas*) and mussels (*Mytilus edulis*) (Burridge et al. 2014; Egidius & Møster 1987; Ernst et al. 2014). The safety margin of dose tolerance between lice and salmon is narrow, and active compounds carry significant health risks both for farm fish and staff personnel (Costello 1993; Dunier & Siwicki 1993; Høy et al. 1991).

Alternative control methods include fallowing, a practise of leaving cages unstocked for extended periods between production cycles to remove potential host fish and break the louse lifecycle (Bron et al. 1993). Fallowing is particularly effective when coordinated across the whole farming site (Butler 2002), but inherently leads to reduced farm productivity. Artificial light treatments have been trailed to exploit the positive phototaxis of infective-stage nauplii by separating planktonic lice from salmon at night, however, these trials resulted in increased infection rates due to greater exposure during daylight hours (Hevrøy et al. 2003). Thermolicers are used to mechanically induce the detachment of lice through a natural stress response to heat by passing salmon through pressurised jets of water heated above 30 ^{o}C (Borchel et al. 2018). Although thermolicing appears very effective at reducing louse numbers, this method raises serious concerns over salmon welfare given that lice and salmon share a similar upper thermal limit (Overton et al. 2018). The deployment of cleaner fish in salmon farms is currently accepted as the most viable alternative to chemotherapeutants, and the importance of cleaner fish in pest management programs is predicted to grow over the coming decades (Blanco 2019; Treasurer 2018).

Cleaner fish in Aquaculture

The cleaning mutualism is a well-documented interaction, in which an individual, referred to as the cleaner, removes and consumes parasites from an individual, referred to as the client (Bshary & Côté 2008). Though also observed in birds and crustaceans, the mutualism is best described in tropical species of fish, particularly the bluestreak cleaner wrasse (*Labroides dimidatus*) (Grutter 2010). Cleaner fish can be divided into two categories, dedicated or facultative cleaners, with dedicated species relying on cleaning to meet dietary needs to a greater extent than facultative species (Vaughan et al. 2017). Research on cleaner-client interactions has revealed sophisticated strategies utilised by cleaners to manipulate client behaviour (Bshary 2011). Examples of this includes tactile stimulation in which bluestreak cleaner wrasse maintain body contact with clients using pelvic and pectoral fins, thereby extending the length of cleaning interaction length, reduces the likelihood of clients fleeing and lowers levels of aggression in predatory clients (Bshary & Würth 2001). Bluestreak cleaner wrasse also build positive reputations by performing altruistic behaviour more frequently while observed by bystanders to increase the likelihood of subsequent interaction with those bystanders (Binning et al. 2017; Bshary & Grutter 2006). This level of Machiavellian intelligence allows cleaners to manipulate and deceive clients by advertising parasite removal but then cheating through consuming client skin and mucus (Bshary & Grutter 2002), which blurs the boundary between mutualism and parasitism (Cheney & Côté 2005). Cleaning behaviour is reported among many taxonomic groups of fish (Côté 2000), though is seemingly more common in the Labridae (Baliga & Law 2016). By comparison, research on facultative cleaning behaviour is lacking and factors influencing facultative cleaner fish interaction with clients are largely unknown (Morado et al. 2019; Narvaez et al. 2015). As all the species of cleaner fish used to remove salmon-lice are facultative species (Treasurer 2018) the dynamics of cleaner-client interaction in salmon farms are uncertain, and the abilities of these cleaner fish to facilitate efficient louse removal is yet to be studied.

The use of temperate wrasse to control lice on salmon farms was first investigated in the 1980s (Bjordal 1988). Numerous species of wrasse are now used within the industry, including ballan (*Labrus bergylta*), corkwing (*Symphodus melops*), cuckoo (*Labrus mixtus*), cunner (*Tautogolabrus adspersus*), goldsinny (*Ctenolabrus* *rupestris*) and rock cook (*Centrolabrus exoletus*) (Treasurer 2018). However, cultured wrasse take over one year to reach deployment size (Lekva & Grøtan 2018) and become dormant in colder temperatures which reduces cleaning rates through the winter (Nytrø et al. 2014). In 2014, the delousing of infected salmon by lumpfish (*Cyclopterus lumpus*) was first reported on Norwegian salmon farms (Imsland et al. 2014a). This presented a new opportunity for the industry as lumpfish grow to deployment size in as little as four months and remain active at cold temperatures (Nytrø et al. 2014; Powell et al. 2018b). Aquaculture production of lumpfish in hatcheries began in 2015 and grew exponentially, producing nearly 30 million juveniles to stock salmon farms in 2016, which constituted 64% of all cleaner fish deployed that year (Powell et al. 2018b).

Lumpfish Biology

The lumpfish (*Cyclopterus lumpus*) has many common names including the common lumpfish, Atlantic lumpfish, lumpsucker, stone biter, sea owl, henfish and paddle-cock (Davenport 1985b). The species is the only member of the genus *Cyclopterus* which is one of the eight genera comprising the Cyclopteridae family of the Scorpaeniformes, named for the Greek kyklos (circle) pteryx (fin) in reference to the pelvic suction disc (Lorance et al. 2015).

Lumpfish are classed as globiform with deep bodies compressed both anteriorly and posteriorly (Davenport 1985b). The skin is comprised of bony dermal ossicles called tubercles and spine-like scutes, which are arranged along the dorsal hump, lateral and dorsolateral ridges (Märss et al. 2010). The head is compact with moderately sized eyes, a blunt snout, upturned mouth and slit-like opercula (Powell et al. 2018a). The pelvic fins have fused together forming the characteristic ventral suction disc which lumpfish use to attach onto a variety of substrates with great force (Budney & Hall 2010). Males become sexually mature after one year, typically reach 30 cm in total length (Davenport 1985b) and display red or orange colouration during the spawning season (Goulet et al. 1986). Females are thought to take longer to reach sexual maturity, achieving an average total length of 40 cm and generally displaying green, blue or grey colouration (Powell et al. 2018a). Lumpfish are distributed and spawn across most of the North Atlantic (Figure GI.1), with three genetically distinct populations identified in the West (USA and Canada), East (Iceland and Norway) and Baltic Sea (Poland) (Pampoulie et al. 2014). The extent of phenotypic differentiation between these populations has not been studied in recent years, though dwarfism and reduced skin tubercles have been reported in isolated populations living in areas of the Baltic Sea and Hudson Bay (Cox & Anderson 1922).

Lumpfish spawn in fjords and coastal regions from early spring into summer, with males defending rocky outcrops to attract females onto nesting sites to spawn (Goulet et al. 1986; Mitamura et al. 2012). Males protect and ventilate the egg mass until the larvae hatch and attach onto substrate using the pelvic sucker (Davenport 1983; Powell et al. 2018a), and feed on small copepods, amphipods and crustaceans (Daborn & Gregory 1983). The juveniles stay close to the coast (Moring & Moring 1991) and begin foraging on larger, faster prey items throughout ontogeny (Brown 1986; Ingólfsson & Kristjánsson 2002), sheltering within algae until they reach sufficient size to disperse out into open water (Moring 2001). Juvenile lumpfish will reduce activity when threatened to minimise the risk of detection (Williams & Brown 1991) and can change body colouration to camouflage against substrata within 3 minutes (Davenport & Bradshaw 1995; Davenport & Thorsteinsson 1989). Adult Icelandic lumpfish are recorded to migrate over large distances of open water to reach foraging grounds (Kennedy et al. 2014), though populations in Greenland are shown to exhibit a higher degree of site fidelity (Hedeholm et al. 2017), suggesting different life histories exist between populations. Adult lumpfish are described as showing highly opportunistic foraging behaviour and are reported to prey on small fish, crustaceans, plankton and Cnidaria (Powell et al. 2018a). Known predators of adult lumpfish are predominantly marine mammals such as cetaceans and seals (Hammill et al. 2007; Jourdain et al. 2019; Martin & Clarke 1986), though predation by sharks is also reported (Joyce et al. 2002).

Commercial use of Lumpfish

Prior to 2014 lumpfish were deemed of little commercial value other than for their roe, which is used as an alternative to caviar and harvested predominantly in Iceland, Canada, Norway, Denmark and Greenland (Johannesson 2006). Spawning lumpfish are caught from early spring to late summer, using gillnets with a wide mesh size to selectively harvest ripe females rather than the smaller males (Davenport 1985b). Each female produces up to 4 kg of eggs and global harvest is estimated at being between 3000 and 4000 tonnes (Johannesson 2006). The bias harvest of sexually mature females is thought to be responsible for skewed sex-ratios detected in wild populations of Canadian lumpfish, which has raised concern over the conservation status of populations in regions where roe harvest takes place (Hoenig & Hewitt 2005). Despite the potential threat of overexploitation many lumpfish fisheries remain unregulated, with notable exceptions being licenced fisheries in Iceland and Greenland (Powell et al. 2018b). Numerous uses for the waste carcass have been trialled with limited success, including marketing the flesh for human consumption and using the skin to manufacture oil and glue (Davenport 1985b; Paradis et al. 1975; Powell et al. 2018b).

The cleaner fish industry first began generating commercial stocks of lumpfish in 2015 and production has since grown rapidly, with 30 million lumpfish deployed the following year (Powell et al. 2018b). Iceland, Norway and the UK are the current market leaders in lumpfish aquaculture (Treasurer 2018), utilising a production cycle which takes place across wild spawning sites, hatchery facilities and salmon farms (Figure GI.2). Stock production is currently reliant on collecting milt and eggs from wild broodstock to undertake artificial fertilisation at hatchery facilities (Wittwer & Treasurer 2018). Removing spawning lumpfish from wild populations in this manner may decrease overall abundance of the species, similar to declines recorded in goldsinny wrasse (*Ctenolabrus rupestris*) and corkwing wrasse (*Symphodus melops*) at sites being harvested for cleaner fish aquaculture (Halvorsen et al. 2017). Lumpfish are classified as Near Threatened on the IUCN Red List (Lorance et al. 2015), however, the conservation status of regional populations is yet to be assessed. Milt collection involves sacrificing the male for gonad extraction (Jonassen et al. 2018b). It is not currently possible to assess male spawning condition prior to sampling, which results in the needless sacrifice of sexually immature fish (Norðberg et al. 2015). Although wild females have been transported to hatcheries in hope of establishing a reusable source of eggs, low survival rates induced through disease and females becoming egg-bound has thus far prevented the domestication process (Wittwer & Treasurer 2018). Assessing indicators of spawning status may assist in developing more sustainable methods of broodstock harvest, preventing the removal of immature fish or too many mature adults from the wild.

Juvenile stocks produced from broodstock are grown in hatchery facilities until reaching the deployment size of 20g (Jonassen et al. 2018b). Although juvenile lumpfish naturally show rapid growth, achieving specific growth rates of 1.5 - 3.5% per day (Nytrø et al. 2014), the industry desires faster growth rates to meet greater production targets. Faster growth has been achieved through optimised feeding schedules and methods of delivering food (Imsland et al. 2019a; Imsland et al. 2019b), with evidence for a genetic effect influencing growth rates informing attempts at selective breeding (Sae-Lim et al. 2020). However, increased growth has been demonstrated to incur trade-offs in other fitness-traits in different species of fish, including the mortality rate of brook trout (*Salvelinus fontinalis*) and fecundity of swordtail fish (*Xiphophorus multilineatus*) (Matte et al. 2019; Weinstein et al. 2019). Whether increasing the growth rate of lumpfish incurs similar fitness trade-offs is unknown. Mortality rates for lumpfish in hatcheries are documented reaching 48% (Brooker et al. 2018), and better understanding trade-offs in the performance of different genetic stocks could help determine sustainable approaches to production management and breeding programs.

Once hatchery stocks reach deployment size they are transported internationally for salmon farms deployment (Jonassen et al. 2018a). Though the escape of lumpfish from farms is yet to be confirmed it seems inevitable given the well-documented escape of salmon and other species of cleaner fish from open-net cages (Faust et al. 2018; Jansson et al. 2017; Wringe et al. 2018). The threat severity escaped lumpfish pose to wild populations would depend on the degree of genetic and phenotypic differentiation between groups (Powell et al. 2018b). For example, populations isolated from gene flow or displaying locally adapted phenotypes would be highly vulnerable, whereas, genetically homogenous and phenotypically indistinct populations would have low vulnerability (Cross 2000; Stockwell & Leberg 2002). Comparing genetic and phenotypic differentiation between regional stocks sourced from areas commonly translocating cleaner fish would enable assessment of whether population differences influences vulnerability of native lumpfish to genetic introgression through translocated fish escaping farms.

Lumpfish are a species of facultative cleaner fish and therefore do not rely entirely on cleaning salmon to meet dietary requirements. Stomach content analysis has found food items consumed in farms includes sea-lice, salmon pellets, zooplankton and invertebrates (Eliasen et al. 2018). Studies suggest that lumpfish preferentially graze on sexually mature female lice and, in optimal conditions, can reduce the proportion of adult female lice in cages by 97% relative to cages not stocked with lumpfish (Imsland et al. 2014a). However, only 15-30% of deployed lumpfish consume sea-lice (Imsland et al. 2014a; Imsland et al. 2018b) and factors determining this behavioural variation are poorly understood. To date the majority of studies assessing cleaning efficacy on farms rely on stomach content analysis and the behavioural interaction between lumpfish and salmon is scarcely documented. The only study recording cleaner-client interaction between lumpfish and salmon found interaction accounted for only 0.2% of observations conducted by scuba divers and underwater cameras over a 56 day period (Imsland et al. 2014b), though the nature of interaction observed during these rare interactions were not recorded. Investigating variation in cleaner-client interaction between individual lumpfish and salmon under standardised conditions is essential to identify which factors influence variation in cleaning (Blanco 2019). Research conducted on variation observed in cleaner-client interaction for dedicated cleaner fish has shown different personality traits influenced cleaning efficacy, with bolder individuals exhibiting greater interaction rates (Dunkley et al. 2019; Wilson et al. 2014). Given the lack of information available for facultative cleaners, behavioural research on dedicated cleaner fish may offer valuable insight into the variation documented in delousing rates for lumpfish in salmon farms.

The proportion of lumpfish surviving the salmon production cycle is currently unknown as the figure is not routinely provided by the industry. Government reports issued by the Fish Health Inspectorate recorded lumpfish mortality exceeded 75% during one particular mass mortality event at a farming site in Scotland (Fish Health Inspectorate 2019). Whether this accurately reflects typical mortality rates is unknown, though the report is cause for concern regarding cleaner fish health and welfare. Research on lumpfish welfare requirements is scarce and limited to shelter design (Imsland et al. 2015; Johannesen et al. 2018a), but advancing knowledge on factors behind post-deployment mortality is critical for improving fish welfare standards on farms (Powell et al. 2018b).

Establishing a selective breeding program for captive reared lumpfish has been a key goal since the inception of lumpfish aquaculture (Treasurer 2018). Though the species' life-cycle is yet to be fully closed in aquaculture (Powell et al. 2018b), research has started to quantifying desirable traits to be incorporated in breeding programs once developed (Sae-Lim et al. 2020). Arguably the most important trait to consider is increased cleaning efficacy, which has previously been show to vary amongst different genetic lineages on farms (Imsland et al. 2016a). Current methods of detecting favourable cleaner-client interaction rely on sacrificing cleaner fish to undertake stomach content analysis (Eliasen et al. 2018), which is not a suitable methodology for identifying potential broodstock. Developing protocols to identify the predisposition of individuals to greater cleaning interaction using hatchery reared stocks without killing lumpfish is the next logical step in quantifying this key trait for breeding programs.

Aims and Objectives

Given the growth of salmon farming and the threats represented by sea-lice infestation, the ability to produce lumpfish through aquaculture is of paramount importance. However, for lumpfish deployment to truly improve the sustainability of salmon farming, lumpfish production must be efficient, robust and mitigate any detriment incurred to natural ecosystems. These goals are not achievable without first developing a better understanding of the species biology, including factors effecting the performance of lumpfish in aquaculture. The broad aim of the thesis was to assess patterns of intraspecific genetic and phenotypic variation in lumpfish, on a population, stock and individual scale, while investigating potential factors that influence the species' performance within aquaculture.

Chapter 1 aimed to determine the population genetics of wild lumpfish distributed across the species' range, including areas of intensive salmon farming. Through modelling population structure, connectivity and providing estimates of genetic diversity, the conservation status and vulnerability of natural populations to commercial practises can be evaluated for the first time. Chapter 2 aimed to identify sexually dimorphic features and investigate the potential existence of regionally specific phenotypes in stocks representing genetically distinct populations. Conducting geometric analysis on lumpfish reared within a common environment may establish morphological differences between stocks that imply localised adaptation of source populations to different geographic regions, holding implications for stock performance and consequences of genetic introgression via farm escape. Additionally, the description of sexually dimorphic anatomy could offer indicator traits to be further investigated as markers of spawning condition and develop sustainable broodstock harvest. Chapter 3 aimed to compare fitness related traits for growth, foraging activity and survival in regional stocks reared within a common-garden experiment. Quantifying differences in the fitness traits of commercial stocks could reveal patterns in the expression of desirable traits to inform selective breeding programs and offer insight on factors influencing the high mortality rates reported on farms. Chapter 4 aimed to investigate variation in the cleaner-client interaction of lumpfish from different regional stocks. Behavioural protocols to detect cleaning efficacy in hatchery reared stocks were developed through profiling the personality of individual lumpfish, providing tools to inform selective breeding and increase the proportion of cleaning lumpfish deployed in farms.

Chapter Overview

Chapter 1. Genetic and Phenotypic Differentiation of lumpfish (Cyclopterus lumpus) across the North Atlantic: Implications for Conservation and Aquaculture.

Population structure across most of the lumpfish range remains unknown, including areas of intensive salmon aquaculture (Pampoulie et al. 2014). Evidence of natal philopatry (Kennedy et al. 2014) and limited larval dispersal (Moring & Moring 1991) led to the hypothesis that lumpfish showed highly structured populations, with limited gene flow between regions. Extensive harvest for roe across parts of the range could heighten vulnerability to over-exploitation by creating skewed sex-ratios due to sex-bias harvest of female lumpfish (Hoenig & Hewitt 2005), leading to the hypothesis that lumpfish populations vary in genetic diversity across geographic regions. This chapter assessed the population genetics of lumpfish and determined population structure and phenotypic differences at spatial scales relevant to aquaculture.

Chapter 2. Morphological variation in lumpfish (Cyclopterus lumpus): assessing sexual dimorphism and population differentiation to shape sustainable aquaculture.

Historical reports suggest morphological variation is present across the species' range (Cox & Anderson 1922), however, an investigation on the morphological phenotypes of lumpfish from different genetic groups is yet to be made. Marked sexual dimorphism in the size and colour of adult lumpfish reflect different selective pressures between the sexes (Powell et al. 2018a), therefore, I hypothesised that sexual dimorphism may also exist in body anatomy. This chapter investigated differences in geometric morphology and size morphometrics of male and female lumpfish originating from different genetic populations. Results identified new sexually dimorphic traits in the dorsal hump, dorsal flag and mouth which align with established life-history differences between sexes, and distinct regional phenotypes that may imply local adaptation by populations in different regions.

Chapter 3. Clean fast die young: fitness trade-offs challenge sustainable lumpfish aquaculture

Lumpfish have a wide geographic range which spans from the Arctic to the Mediterranean and encompasses contrasting environments (Powell et al. 2018a). Variation is reported in reproductive traits of wild populations across Greenland (Hedeholm et al. 2017), suggesting lumpfish may exhibit different life history strategies. I hypothesised that distinct genetic stocks reared in a common environment may show variation for fitness traits that impact their performance in aquaculture. In this chapter I investigated variation in growth, activity and survival for stocks sourced from the populations in the north and south of the range. Results indicated different paced life history strategies, and a potential trade-off between growth rate and survival probability.

Chapter 4. Personality influences facultative cleaner-client interactions between lumpfish (Cyclopterus lumpus) and Atlantic Salmon (Salmo salar)

Cleaning efficacy of lumpfish varies on farms (Eliasen et al. 2018), with approx. 28% of lumpfish consuming lice (Imsland et al. 2014a). I hypothesised that lumpfish personality may influence cleaner-client interactions, as documented in other species of cleaner fish (Dunkley et al. 2019; Wilson et al. 2014). In this chapter, behavioural differences were quantified using the five-factor model of non-human animal personality (Gosling & John 1999), and behavioural interactions between lumpfish and salmon were recorded to better understand variation in cleaning efficacy. This could help identify desirable traits to inform cleaner fish selection in the industry.

General Discussion:

Findings from the previous chapters are considered relative to the different stages of lumpfish aquaculture, highlighting applications to hatchery practise, farm procedure and interaction with wild populations. The genetic and phenotypic variation identified on a population, stock and individual levels is assessed in terms of the species' biology and implications for facultative cleaning behaviour. The discussion concludes by evaluating the state of current practise, directions for future development, and considers the justification of lumpfish aquaculture.

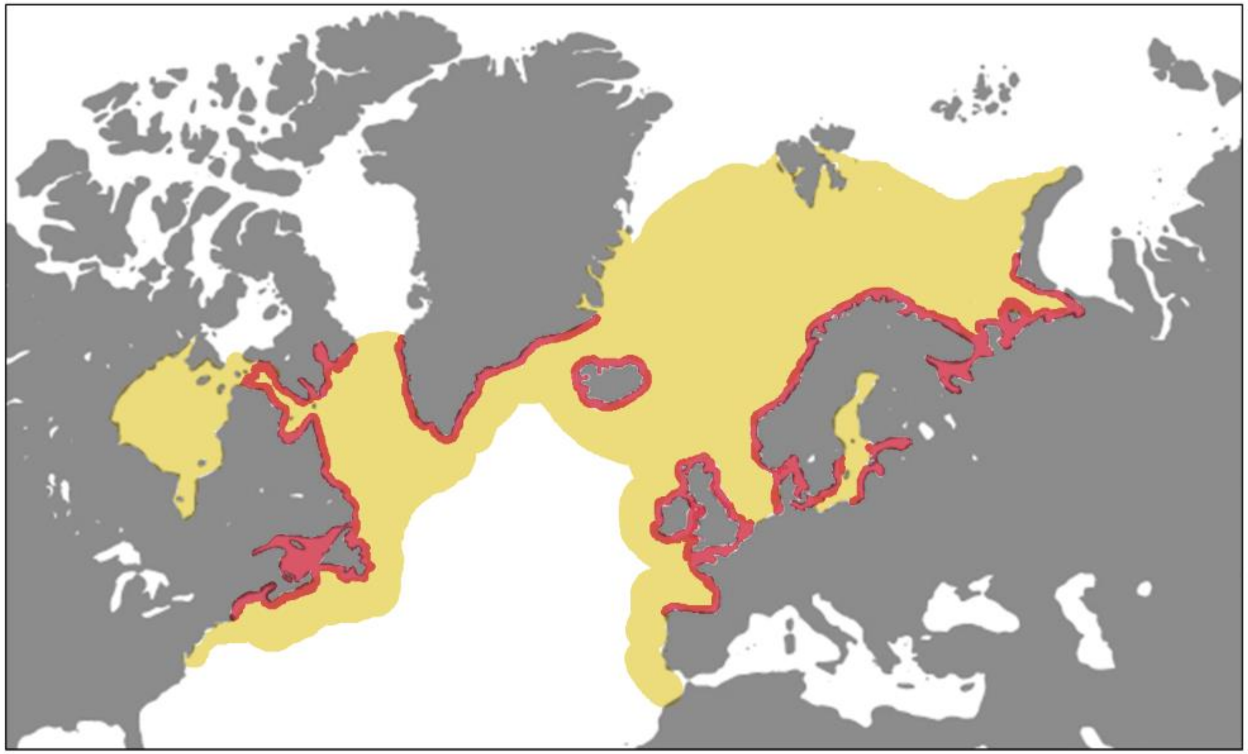

Figure GI.1. Geographic distribution (yellow) and known spawning locations (red) of the lumpfish (*Cyclopterus lumpus*), (Davenport 1985b; Powell et al. 2018a).

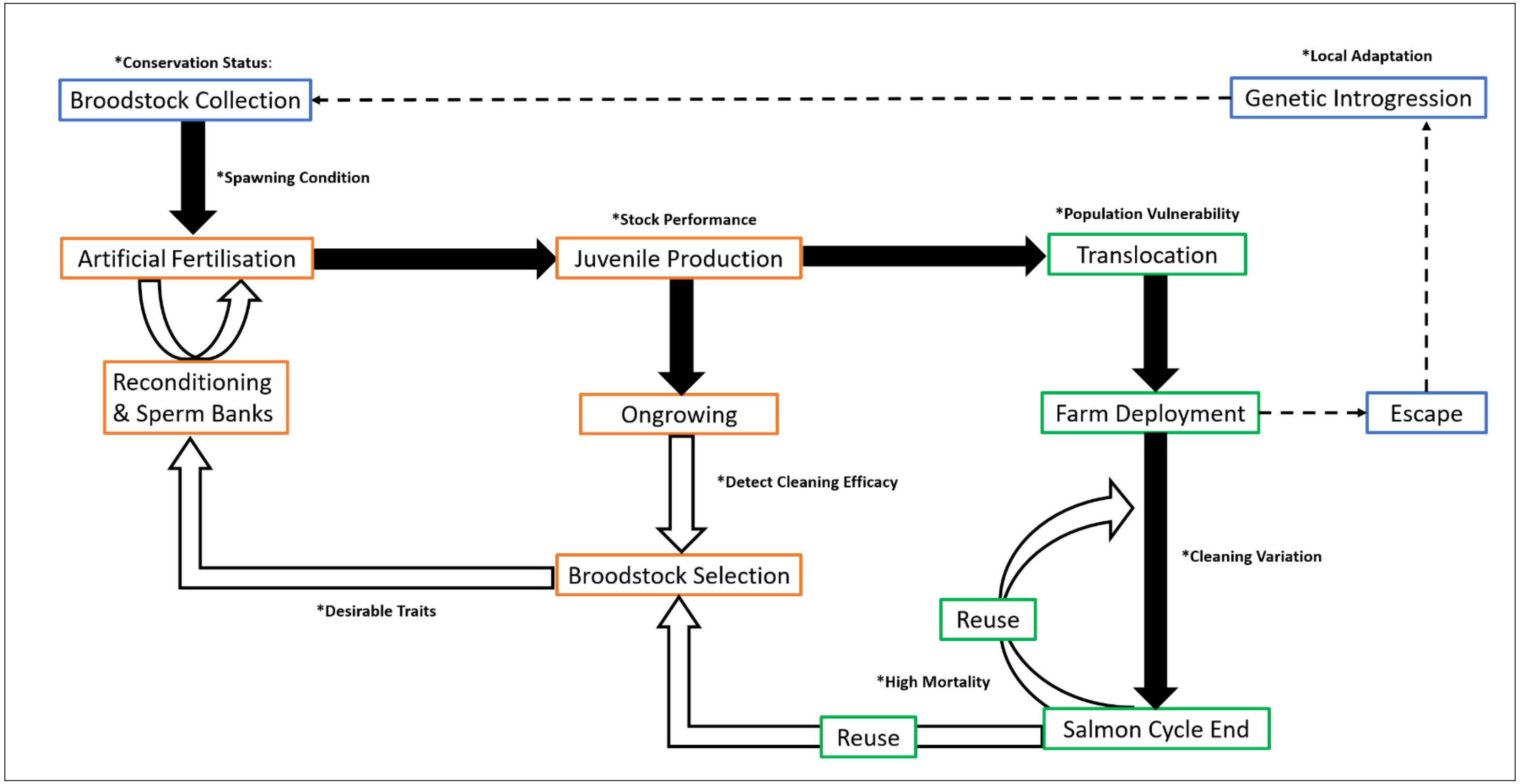

Figure GI.2. Overview of lumpfish aquaculture showing established stages of production (filled arrow), stages under development (empty arrow) and potential consequences (dashed arrow). Box colours indicates the utilisation of wild fish (blue), hatchery stocks (orange) and cleaner fish on salmon farm (green), * details sustainability problems addressed in the thesis.

Chapter 1

Genetic and Phenotypic differentiation of lumpfish (*Cyclopterus lumpus*) across the North Atlantic: Implications for Conservation and Aquaculture.

Abstract

Demand for lumpfish (*Cyclopterus lumpus*) has grown exponentially over the last decade, both for its roe, which is widely used as a caviar substitute, and increasingly as a cleaner fish to control parasitic sea lice in salmon farms. Classed as Near Threatened by the IUCN, there is growing concern that sex-bias harvesting and the escape of translocated hatchery-bred lumpfish from salmon farms endangers the genetic integrity of wild populations. However, information on the structure, size and status of populations across much of the species range is lacking, hindering impact assessment of fishery and aquaculture activity. In this study, 410 lumpfish were sampled across 15 sites and sequenced using ten microsatellite loci. Analyses identified five genetically distinct groups located in the West, Mid and East Atlantic, the English Channel and the Baltic Sea, with little gene exchange detected between groups. Phenotypic differences in body size were also found between smaller lumpfish in the Baltic Sea and larger lumpfish in the Atlantic Ocean. It is therefore recommended that regional population differences are accounted for when deploying cleaner fish on salmon farms, with efforts made to match the genetic background of hatchery-sourced lumpfish to wild populations native in the farm area. Estimates of effective population size suggested low genetic diversity was present in population sampled across the North East Atlantic (Iceland, Faroe Islands and Norway), which is an area characterised by intense roe fishery. The potential vulnerability of these populations should be addressed by closer monitoring of roe fisheries and further evaluating the status of wild stock in the region.

Introduction

The control of parasitic sea-lice (*Lepeophtheirus salmonis*) is commonly cited as the largest problem faced by salmon farmers (Torrissen et al. 2013; Treasurer 2002), particularly as sea-lice have developed resistance to numerous chemotherapeutants (Aaen et al. 2015; Lees et al. 2008) which limits long-term viability of chemical treatment on farms. Alternate control methods includes the deployment of cleaner fish to farms and several species of temperate wrasse, including ballan (*Labrus bergylta*), corkwing (*Symphodus melops*), cuckoo (*Labrus mixtus*), cunner (*Tautogolabrus adspersus*), goldsinny (*Ctenolabrus rupestris*) and rockcook (*Centrolabrus exoletus*), have demonstrated effective parasite removal from farmed salmon (Deady et al. 1995; Tully et al. 1996). However, lumpfish (*Cyclopterus lumpus*) are the most commercially popular cleaner fish in salmon farms as, although they show similar levels of cleaning efficacy to temperate wrasse (Powell et al. 2018b; Treasurer 2018), lumpfish continue feeding on lice at temperatures below 4 ^{o}C (Nytrø et al. 2014) and are relatively faster to rear in hatcheries (Jonassen et al. 2018b; Powell et al. 2018b). Demand for this species has therefore increased exponentially since 2012 and an expected 50 million juveniles will be required for farm deployment in 2020 (Sae-Lim et al. 2020).

Lumpfish have been classified as Near Threatened by the IUCN Red List since 2015 (Lorance et al. 2015), though information on the conservation status of different populations is sparse and it is possible that lumpfish in some regions are already overexploited (Myers et al. 1995). Historical reports suggest wild female lumpfish are reproductively active from 4-10 years of age and mature individuals may spawn multiple times within a single season (Davenport 1985b). Mature females are targeted by fisheries for their roe (Johannesson 2006) which created a sex-bias harvest attributed to decreasing sex ratios (female : male) detected in Newfoundland fisheries, falling from 2.24:1 in 1985 to 1.09:1 in 1994 (Hoenig & Hewitt 2005). Data from Danish fishery authorities report long-term declines in catch per unit effort (CPUE) of lumpfish roe harvest, resulting in a 69% reduction in CPUE across fisheries in Denmark over the past 25 years (Lorance et al. 2015). The expanding cleaner fish market creates additional harvest pressure via the removal of mature adults from spawning sites to act as commercial broodstock and supply juveniles for the salmon industry (Powell et al. 2018b; Wittwer & Treasurer 2018). Harvesting cleaner fish broodstock in this manner has decreased the abundance of commercially exploited wrasse (Halvorsen et al. 2017) and it is feared will also affect wild lumpfish (Blanco 2019; Jónsdóttir et al. 2018b), particularly as the species' life-cycle is not closed in captivity and so production relies entirely on wild broodstock (Jonassen et al. 2018b; Wittwer & Treasurer 2018). Despite concerns over skewed sex-ratios, reduced catch rates and declining abundance, the vast majority of lumpfish fisheries remain unregulated (Kousoulaki et al. 2018).

Widescale translocation of cleaner fish occurs across vast areas of the North Atlantic with little consideration for the genetic background of different stocks (Jonassen et al. 2018a; Jonassen et al. 2018b). For example, over 85% of lumpfish deployed on Scottish farms in 2017 originated from eggs imported from Iceland and Norway (Treasurer et al. 2018). In Ireland, 70% of lumpfish deployed during 2015-2016 were derived from eggs imported from Iceland and Norway (Bolton-Warberg 2018), whilst in the Faroe Islands 100% of lumpfish used from 2014 to 2016 were Icelandic in origin (Johannesen et al. 2018b). Translocated fish can escape open-net cages and may interbreed with local fish, thereby eroding native population structure and result in the genetic introgression of maladaptive traits (Consuegra et al. 2011; Diserud et al. 2019; Naylor et al. 2005). The escape of salmon from open-net farms is well documented in the industry (Karlsson et al. 2016) and the resulting hybridisation of farm and wild fish is associated with reduced fitness in wild salmonid populations, including greater size for maturation and desynchronised spawning (Jonsson & Jonsson 2017; McGinnity et al. 2009; McGinnity et al. 2003). The process of farm escape and hybridisation is also reported for two species of cleaner wrasse deployed in salmon aquaculture (Faust et al. 2018; Jansson et al. 2017), making the escape of lumpfish from open-net cages seem largely inevitable. How escapes impact native lumpfish populations would depend on the number of escapees, their reproductive success and the extent of differentiation between local and introduced fish, however, none of these parameters are currently known. Given that the translocation of lumpfish across international boundaries is predicted to intensify in the near future (Powell et al. 2018b), better understanding genetic and phenotypic differences between populations is a necessity to conserve the integrity of native populations.

Lumpfish are distributed across a vast marine area, extending longitudinally from the Arctic down to the Mediterranean and latitudinally from the eastern coast of Canada across to the Baltic Sea (Powell et al. 2018a), thus representing scope for substantial differentiation across diverse habitats. Lumpfish spawn in fjords and intertidal zones, where males compete for nesting sites to attract females (Wittwer & Treasurer 2018). Once hatched the juveniles attach onto rock and algal substrates using specialized pelvic fins (Moring & Moring 1991), remaining near the nest until they reach sufficient size to disperse out to sea (Moring 2001). Tagging studies suggest adults exhibit a degree of pelagic activity and in some cases swim up to 49 km/day, although most individuals remain within an 80 km home range after +250 days at liberty (Kennedy et al. 2014). Females are shown to exhibit natal philopatry and return to the same spawning site each season (Kennedy et al. 2015). Spawning onset can vary by up to seven months between locations, starting as early as January in the English Channel (Powell et al. 2018a) and as late as August near the Arctic circle (Hedeholm et al. 2017). The extent of genetic differentiation between populations is uncertain, although significant genetic differences have been found on broad geographic scales using microsatellite markers (Pampoulie et al. 2014). Lumpfish sampled along the Norwegian coast appear to be largely undifferentiated (Jónsdóttir et al. 2018b), however, two genetically distinct groups have been found in the north and south of Greenland (Hedeholm et al. 2017).

Given evidence for limited larval dispersal from nest sites and natal philopatry of spawning adults, I hypothesised that lumpfish displayed genetic isolation by distance and that limited gene flow between populations resulted in sites within closer proximity being more genetically similar than those further apart (Rousset 1997). To test this hypothesis, I followed three aims to quantify pan-Atlantic population dynamics and performed a Mantel test to establish genetic isolation by distance. First, I aimed to quantify genetic variation and model population structure through sampling and comparing lumpfish from different sites located across the range. Secondly, I aimed to determine patterns of gene flow between modelled populations and assessed connectivity through estimates of genetic migration (N_m). Finally, I aimed to estimated effective population size (N_e) to quantify the degree of genetic diversity present across populations. By better understanding the structure, connectivity and diversity of natural populations I hoped to evaluate whether lumpfish exhibited genetic isolation by distance and assess the potential impact of commercial practises, such as roe harvest and cleaner fish translocation, on wild populations

Material and Methods

Sample Collection

Tissue was obtained from 410 wild lumpfish originating from 15 sites across the species' range (Table 1.1) and were stored in 96% ethanol at -20 ^{o}C until analysis. Samples were provided by international contributors including academic institutes, government departments and industrial organisations, with each partner collecting tissue from the upper tip of the caudal fin to standardise collection technique. Data for capture method, sex, total length (mm) and weight (g) was requested, though was only available for eight of the 15 sites (Table 1.1), thereby limiting further analysis of samples. However, as lumpfish are seldom caught outside harvest during the spawning season it seems reasonable to assume samples were predominantly mature females caught by gill nets during roe harvest (Johannesson 2006). Information on the geographic origin and capture date was provided for each individual fish, and sites located within an 80 km radius (the estimated maximum range of dispersal, Kennedy et al., 2015) were pooled together to minimise the risk of spatial pseudo-replication. Samples were pooled from the Faroe Islands (Klasvík and Kollafjørður, approx. 20 km), Denmark (Køge Bay and Mosede Havn, approx.13 km) and Sweden (survey hauls from Bornhölm to Öland, and from Gotland to Gotska Sandön). Pooled groups were named after the site contributing the largest number of samples and thereafter considered population units throughout all analyses conducted.

DNA Extraction and Amplification

DNA was extracted using the Nexttec Isolation kit (Nexttec, UK) following the manufacturer's protocol. The concentration of extracted DNA was quantified using a Nanodrop 2000 (Thermo Fisher Scientific Inc., USA) and diluted with DNA free water to 50 ng/µl where necessary. A 2 µl of sample DNA was used for amplification using a QIAGEN Multiplex PCR kit (QIAGEN, UK) in a total reaction volume of 9 µl. Ten microsatellite loci designed for specific use with lumpfish, *Clu*29, *Clu*34, *Clu*36, *Clu*45 and *Clu*12, *Clu*26, *Clu*33, *Clu*37, *Clu*40, *Clu*44 (Skirnisdottir et al. 2013), were genotyped in two separate multiplex reactions (Appendix T1.1). Amplification consisted of a single initial activation step at 95 ^{o}C for 15 minutes, followed by eight cycles of touchdown PCR denaturation at 94 ^{o}C for 30 seconds, annealing from 64 ^{o}C or 60 ^{o}C to 56 ^{o}C in descending two-cycle steps of 2 ^{o}C and an extension at 72 ^{o}C for 90 seconds, 24 additional cycles with an annealing temperature of 56 ^{o}C and a single final extension at 60 ^{o}C for 30 minutes. An Applied Biosystems ABI3130xl Genetic Analyser (Applied Biosystems, UK) was used to resolve the fragments using GeneScan 500-LIZ(-250) as a size standard. Fragment length was established using GeneMapper v5.0 (Applied Biosystems, UK). Genotyping consistency was validated by repeating PCR, fragment analysis and scoring for 10% of samples.

Ethical approval for working with tissue samples was granted by Swansea University College of Science Ethics Review Committee (STUBIOL909201810181328451).

Estimates of Genetic Diversity

I used Microchecker v2.2.3 (Van Oosterhout et al. 2004) to identify null alleles, allele dropout and stutter peaks, and Bayescan v2.1 (Foll 2012) to test for loci neutrality. GENEPOP v4.2 (Rousset 2008) was used to test for linkage disequilibrium, deviations from Hardy-Weinberg equilibrium, and to calculate allelic frequencies across populations. GeneAlEx v6.502 (Peakall and Smouse, 2006) was used to assess the number of alleles (N_{A}), effective alleles (N_E), private alleles (N_{PA}), expected heterozygosity (H_{E}), observed heterozygosity (H_{O}) and to carry out a Mantel test of genetic isolation by geographic distance.

Genetic Structure and Migration

I conducted an Analysis of Molecular Variance (AMOVA) to partition genetic variation at three hierarchical levels (among populations, within populations, and among individuals), and calculated pairwise F_{ST} values between populations using Arlequin v3.5.2.2. (Excoffier et al. 2005). Bayesian cluster analysis was conducted in STRUCTURE v2.3.4 (Falush et al. 2007; Pritchard et al. 2000) to estimate the most likely number of genetic clusters (K) informed by individual genotypes. Admixture models with K values ranging from 2 to 15 were considered using twenty iterations, a burn-in length of 10,000 and 50,000 Markov Chain Monte Carlo repeat simulations to quantify the likelihood of each modelled K value. Results were then fed into STRUCTURESELECTOR (Li & Liu 2018) to identify the most likely number of clusters present based on the median of means (MedMeaK), maximum of means (MaxMeaK), median of medians (MedMedK) and maximum of medians (MaxMedK) criteria (Puechmaille 2016). Bayesian cluster analysis informed by spatial data was conducted using TESS v2.3.1 (Chen et al. 2007) to better understand the extent of spatial genetic structure. Software randomly generated coordinates and assigned to each individual prior to running models, with values falling within one standard deviation of the origin site's coordinates. Admixture models were run with 50,000 total sweeps, 10,000 burn-in sweeps, and 200 runs per $K_{\max}$ ranging from 2 to 15. The average Deviance Information Criterion (DIC) of the lowest 10 DIC values was calculated for each $K_{\max}$ to assess the most likely number of clusters. Runs within 10% of the lowest (DIC) for a given $K_{\max}$ were used for analysis. CLUMMP v1.1.2 (Jakobsson & Rosenberg 2007) was used to average variation between repeated iterations for the most likely K values, and the resulting output was visualised using DISTRUCT v1.1.1 (Rosenberg 2004). A neighbour joining tree assessing the genetic distance of populations was constructed with Populations v1.2.32 (Langella 2002) using Nei's standard genetic distance with 1,000 bootstraps per locus and the resulting tree was visualised using TreeView (Page 2003). Patterns of gene flow were estimated using div-Migrate software, which compares rates of change in allele frequencies between each population and hypothetical migrant pools connecting populations within the model (Sundqvist et al. 2016). The effective number of migrants (N_{m}) was selected for the measure of genetic differentiation, as this uses elements of both G_{ST} and D to give a more robust (Alcala et al. 2014). Simulations were also run to assess the symmetry of gene flow under varying rates of flow (low = 0.0025, mid = 0.005, high = 0.05), each with 5,000 bootstrap iterations.

Population Size and Genetic Bottlenecks

Estimates of effective population size (N_{e}) for sites containing at least 19 individuals were calculated using the Linkage Disequilibrium Model (LDM) with a critical value of 0.02 in NeEstimator v2.1 (Do et al. 2014). Evidence of population expansion was assessed through the *k* intralocus and *g* interlocus tests (Reich et al. 1999), using the application provided in (Bilgin 2007) to evaluate patterns of allele-length distributions. Evidence of genetic bottlenecks was evaluated in Bottleneck v2.1 (Piry et al. 1999) using 1,000 replicates and both the Two-Phase (TPM) and the Stepwise Mutation Models (SMM) to assess heterozygosity deficiency.

Length-Weight Variation

Variation in the length-weight relationship between regions (West Atlantic, n = 30; East Atlantic, n = 65; English Channel, n = 60; Baltic Sea, n = 40), was examined by regression analysis on log-transformed data. Relative weight (Wr) was calculated as the ratio of the observed weight divided by the predicted weight (from the regression obtained above) to obtain an index of body condition that is more appropriate for fish with an unusual body shape (Nahdi et al. 2016). The most plausible number of age classes represented in the samples, and the mean size at age (Macdonald & Pitcher 1979) were calculated through mixture analysis of length-frequency data using PAST v3.17 (Hammer et al. 2001). The Von Bertalanffy growth equation (Kirkwood, 1983) was fitted to estimate growth parameters in each region.

Results

Estimates of Genetic Diversity

All microsatellites exhibited polymorphism. The mean number of alleles (N_A) ranged from 4.5 (Ro) to 6.8 (Kl, VB), mean expected heterozygosity (H_E) ranged from 0.592 (Öl) to 0.700 (Kl), and mean F_{IS} varied from -0.056 (Ro) to 0.110 (Öl) across all loci (Table 1). Initial analysis suggested that null alleles might be present at multiple loci (*Clu34, Clu36, Clu12, Clu33, Clu37* and *Clu40,* Appendix T1.2). However, repeatedly removing each locus in turn showed little variation in F_{ST} values (Appendix T1.3 – T1.8), and therefore all markers were retained for further analyses. No evidence of departure from neutrality or linkage disequilibrium was found after Bonferroni corrections for multiple tests (Rice 1989). Deviations from Hardy-Weinberg equilibrium (HWE) were detected at 5 of the 15 sites (Table 1.1), but these involved only 12% of loci after Bonferroni correction (Appendix T1.9). The mean number of private alleles (N_{PA}) was relatively low, ranging from 0.00 to 0.40, with sites in the West Atlantic (FB = 0.30, WB = 0.40) and Baltic Sea (GS = 0.30) showing the highest values.

Genetic Structure and Migration

Global F_{ST} was 0.095 ($P < 0.001$) indicating a moderate degree of genetic differentiation. Results of AMOVA indicated that 83.5% of molecular variation was due to variation within individuals, 7% amongst individuals within populations, and 9.5% amongst populations. Pairwise F_{ST} showed a significant level of genetic differentiation across most populations (Table 1.2), though populations closer together geographically were genetically similar after Bonferroni correction. On the basis of F_{ST} values, the strongest differentiation was found between West Atlantic and Baltic Sea populations. Results of a Mantel test support the existence of a significant, albeit weak, isolation by distance ($R^2 = 0.123$, $P = 0.01$). The most likely number of genetically distinct groups (K) ranged from $K = 5$ (MedMedK, MedMeaK) to $K = 6$ (MaxMedK, MaxMeaK) using STRUCTURESELECTOR (Appendix F1.1). Spatial cluster analysis using TESS suggested a $K_{max} = 10$ (Appendix F1.2), though only six of these genetic groups showed substantial representation and the remaining four groups contributed only 3.3% to the genetic background. Distinct clusters were detected in the West Atlantic and Baltic Sea by both STRUCTURE and TESS, with a greater level of admixture across the East Atlantic (Figure 1.1). Results were consistent in attributing a genetically unique pattern to the Mid Atlantic, English Channel clusters and a Norwegian site at Averøy. A neighbour joining tree (Figure 1.2) showed similar patterns to that of the structuring analyses, highlighting the separation between the West Atlantic and Baltic Sea populations, and the higher degree of admixture within the East Atlantic group. The effective number of migrants (N_{m}) ranged from 1.00 between sites in the English Channel to 0.03 between sites in the West Atlantic and Baltic Sea. The exchange of migrants was much higher within genetic clusters than among clusters (Appendix T1.10), with the highest levels of gene flow found within the East Atlantic and within the English Channel (Figure 1.3). The only evidence of moderate asymmetric gene flow was from Norway towards the Faroe

Islands (N_m = 0.507), but this was only detected when modelled with a high rate of gene flow.

Population Size and Genetic Bottlenecks

Estimates of effective population size (N_e) based on a Linkage Disequilibrium Model (LDM) varied from 19 (Norway) to 70,148 (Denmark; Appendix T1.11). Sites with low N_e values (<75) were found across Iceland, Faroe Islands and Norway (Figure 1.3). No evidence of recent population expansion was found according to the intra-locus *k* and inter-locus *g* tests (Appendix T1.12). A significant deficiency of heterozygotes was identified in Ireland and Scotland using the Single Mutation Model (SMM) in BOTTLENECK (Wilcoxon signed-rank test, P = 0.0033 after Bonferroni correction), suggesting that these populations could have undergone a recent genetic bottleneck (Appendix T1.13), but this was not detected by the Two-Phase Model of Mutation (TPM).

Length-Weight Variation

The relationship between length and weight differed significantly between regions ($F_{4,192}$ = 917.2, P < 0.001; Figure 1.4). Lumpfish in the Baltic Sea were heavier relative to their size than lumpfish in the East Atlantic and the English Channel (pairwise comparisons: Baltic - East Atlantic, estimate = -0.090 ± 0.036, t = -2.530, P = 0.012; Baltic - English Channel, estimate = -0.145 ± 0.046, t = -3.171, P = 0.002), but were similar to those in the West Atlantic (pairwise comparison Baltic - West Atlantic, estimate = -0.094 ± 0.050, t = -1.891, P = 0.060). The relative weight of lumpfish differed between regions ($F_{3,191}$ = 2.841, P = 0.039) and was highest in the Baltic Sea and the West Atlantic, and lowest in the East Atlantic and the English Channel.

Mixture analysis identified multiple plausible age classes present amongst lumpfish sampled in the Baltic Sea (7 age classes), East Atlantic (4 age classes) and English Channel (3 age classes), but only a single plausible age class in the West Atlantic. Based on the parameters of the Von Bertalanffy Growth equation, the maximum age was estimated to be 6.0 years for Baltic populations, 5.7 years for populations in the East Atlantic and 7.5 years for southern populations spawning in the English Channel.

Fitted growth equations differed significantly between regions (Table 1.3), with lumpfish in the Baltic Sea showing the slowest growth and those in the English Channel showing the fastest.

Discussion

This study reveals a moderate degree of genetic structuring in lumpfish populations which should inform the translocation of lumpfish across the North Atlantic to stock salmon farms. Genetically distinct groups were found in the West Atlantic (USA, Canada), Mid Atlantic (Iceland), East Atlantic (Faroe Islands, Ireland, Scotland, Norway, Denmark), English Channel (England), Averøy (Norway) and Baltic Sea (Sweden). Whilst significant gene flow (N_m) was detected within each of these groups little exchange of migrants was found between groups, with the Mantel test confirming evidence of genetic isolation by geographic distance. Present analyses are limited to the small number of microsatellite markers designed for lumpfish (Skirnisdottir et al. 2013), which narrows the range of detectable polymorphism and therefore reduces the resolution at which trends can be established across more similar populations (Mukesh et al. 2019). However, microsatellites have proven useful in identifying widescale patterns of variation (Jónsdóttir et al. 2018a) and allow comparison with previous studies using the same markers (Jónsdóttir et al. 2018b; Pampoulie et al. 2014), until the more sophisticated tools such as Single Nucleotide Polymorphisms (SNPs) are developed for the species (Jónsdóttir et al. 2018a).

Lumpfish from the Baltic Sea were not only genetically distinct, but were also found to be smaller, grow at a slower rate and weigh more relative to their size compared to lumpfish from other genetic groups in the English Channel, West Atlantic and East Atlantic. Although growth estimates were based on length frequency data that does not distinguish between males and females, they are in line with estimates based on mark and recapture studies in Norway and Iceland ($L\infty = 527 \pm 64$ mm, $K = 0{\cdot}26 \pm 0{\cdot}14$ year^{-1}; (Kasper et al. 2014), which suggests that Baltic lumpfish may grow more slowly and mature at a smaller size (approx. 150 g) than lumpfish from the North Atlantic (2.0-3.0 Kg). However, many environmental factors known to influence growth rate, such as water temperature, salinity and food abundance (Davenport 1985b; Hedeholm et al. 2017), were not accounted for in this study and therefore further investigation is required to establish firmer conclusions on phenotypic variation between wild populations.

Pampoulie et al. (2014) first suggested that lumpfish in the West and East Atlantic were separated by cold southward polar currents, and that populations in the Baltic Sea may have been isolated during the Last Glacial Maximum. Though present analysis supports this broad division, it also indicates further population structure, revealing that lumpfish in the Mid Atlantic and English Channel are genetically distinct from other populations. The conclusion from genetic analyses is consistent with recent tagging studies in Norway and Iceland showing that whilst lumpfish can move offshore to feed on gelatinous zooplankton, they return to spawn in their home waters (Kennedy et al. 2014; Kennedy et al. 2015) and do not migrate between Iceland and Norway (Kasper et al. 2014). There is little information on southern lumpfish populations, though lumpfish in the English Channel appear to spawn earlier in the season than populations further north (Powell et al. 2018a), probably due the warmer temperatures and better feeding opportunities, which are known to influence maturation and spawning onset of lumpfish in Greenland (Hedeholm et al. 2017). It is therefore possible that warmer temperatures found at the species' southern range may favour earlier spawning and lead to a degree of reproductive isolation, hence limiting gene flow along a latitudinal gradient. With the exception of the Averøy population, the remaining sites in the East Atlantic appear to be genetically uniform, as reported along the Norwegian coast (Jónsdóttir et al. 2018b).

Reduced genetic diversity within populations is associated with compromised fitness and has been linked to reduced tolerance of environmental extremes, higher parasite loads (Vrijenhoek 1994) and resulted in localised extinction of fish populations (Faulks et al. 2017). Effective population size (N_e) quantifies the magnitude of genetic drift and inbreeding present within a population to estimate the genetic population size, thereby indicating the relative diversity present between sampled organisms (Wang et al. 2016a). The provided estimates are the first for the species which prevents assessment of temporal patterns, however, estimates were relatively low across the North East Atlantic (Iceland, $N_e = 43$; Faroe Islands, $N_e = 30$; Norway, mean $N_e = 51$) and evidence of genetic bottleneck events were detected at sites in Ireland and Scotland. This region of the North East Atlantic supports some of the largest global roe fisheries surpassing 4,000 tonnes of roe harvested per year (Johannesson 2006) which, given an approximate yield of 4 kg roe/female (Johannesson, 2006), likely exceeds one million spawning females caught each year. Sex and size bias harvesting for roe increases the vulnerability of populations to over-exploitation (Hoenig & Hewitt 2005; Ratner & Lande 2001) and could potentially explain the low estimates of genetic diversity found across this area. Restricting the harvest of mature adults to regulated time periods has been shown to reduce unbalanced population structure in other species of fish (Birkeland & Dayton 2005), and the introduction of similar regulations may help protect vulnerable populations in the North East Atlantic (Kennedy & Ólafsson 2019). Additionally, rearing lumpfish to spawning condition in captivity may present a sustainable source of roe for the caviar industry and juveniles for the cleaner fish market (Jonassen et al. 2018b), therefore closing the life-cycle of the species within captivity should be a research priority.

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Table 1.1. Details of 15 study sites (N: sample size for genetic analysis; *Nb*: sample size for biometric analysis; N_A = mean number of alleles (± SE); N_E = mean number of effective alleles; N_{PA} = number of private alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; F_{IS} = fixation index; * denotes deviation from HWE due to heterozygote deficiency after Bonferroni correction, $P < 0.0033$).

Year	Country	Site	Lat.	Long.	N	*Nb*		N_A	N_E	N_{PA}	H_O	H_E	F_{IS}
2016	USA	Frenchman Bay (FB)	44.33	-68.15	30	-	mean	6.000	3.100	0.300	0.566	0.613	0.100
							± SE	0.775	0.548	0.213	0.058	0.044	0.050
2016	USA	Cobscook Bay (CB)	44.90	-67.05	30	-	mean	6.100	3.452	0.100	0.640	0.668	0.078
							± SE	0.862	0.456	0.100	0.063	0.038	0.055
2016	Canada	Witless Bay (WB)*	47.21	-52.69	30	30	mean	6.700	3.459	0.400	0.630	0.673	0.080
							± SE	0.870	0.425	0.163	0.049	0.036	0.050
2016	Iceland	Hafnir (Ha)	63.93	-22.69	30	-	mean	5.500	2.971	0.000	0.637	0.643	0.019
							± SE	0.500	0.222	0.000	0.041	0.031	0.050
2016	Faroe Is.	Klasvík (Kl)*	62.23	-6.58	30	-	mean	6.800	3.713	0.200	0.668	0.700	0.065
							± SE	0.359	0.453	0.133	0.049	0.030	0.047
2015	Ireland	Ventry Bay (VB)	52.20	-10.12	30	26	mean	6.800	3.255	0.100	0.647	0.658	0.032
							± SE	0.389	0.346	0.100	0.056	0.038	0.050
2017	Scotland	Outer Hebrides (OH)	58.16	-6.38	30	18	mean	6.500	3.247	0.000	0.623	0.644	0.060
							± SE	0.453	0.448	0.000	0.055	0.041	0.036
2015	England	Weymouth (We)	50.61	-2.46	30	30	mean	5.800	2.979	0.000	0.607	0.597	-0.012
							± SE	0.593	0.423	0.000	0.062	0.059	0.053

Table 1.1. (continued)

Year	Country	Site	Lat.	Long.	N	Nb		N_A	N_E	N_{PA}	H_O	H_E	F_{IS}
2015	England	Guernsey (Gu)	49.47	-2.59	30	30	mean	5.600	3.068	0.000	0.618	0.608	0.032
							± SE	0.476	0.431	0.000	0.083	0.055	0.084
2017	Norway	Rogaland (Ro)	59.15	6.01	21	21	mean	6.300	3.080	0.100	0.576	0.614	0.105
							± SE	0.539	0.470	0.100	0.076	0.050	0.078
2016	Norway	Averøy (Av)	63.05	7.48	30	-	mean	5.700	3.077	0.000	0.677	0.638	-0.038
							± SE	0.496	0.336	0.000	0.051	0.038	0.030
2016	Norway	Namsen (Na)	64.45	11.41	19	-	mean	4.500	2.625	0.000	0.600	0.594	-0.056
							± SE	0.342	0.291	0.000	0.071	0.026	0.046
2016	Denmark	Køge Bay (KB)	55.46	12.18	30	-	mean	5.700	3.168	0.000	0.626	0.660	0.067
							± SE	0.423	0.273	0.000	0.036	0.033	0.029
2017	Sweden	Öland (Öl)	55.72	16.39	16	16	mean	4.700	2.838	0.100	0.548	0.592	0.110
							± SE	0.423	0.320	0.100	0.077	0.061	0.073
2017	Sweden	Gotska Sandön (GS)*	57.95	18.97	24	24	mean	5.300	3.192	0.300	0.481	0.611	0.241
							± SE	0.448	0.449	0.213	0.073	0.064	0.081

Table 1.2. Pairwise F_{ST} values (lower) and associated P values (upper) between 15 populations of lumpfish distributed across the natural range of the species, using of 10 microsatellite loci. P values adjusted by Bonferroni correction, $P < 0.00022$.

	FB	CB	WB	Ha	Kl	VB	OH	We	Gu	Na	Av	Ro	KB	Öl	GS
FB		0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CB	0.013		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
WB	0.030	0.030		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ha	0.130	0.112	0.117		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Kl	0.120	0.101	0.098	0.050		0.081	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
VB	0.117	0.093	0.102	0.042	0.011		0.000	0.000	0.000	0.018	0.000	0.000	0.243	0.000	0.000
OH	0.152	0.111	0.124	0.049	0.034	0.021		0.000	0.009	0.252	0.000	0.000	0.000	0.000	0.000
We	0.177	0.154	0.146	0.065	0.056	0.042	0.024		0.324	0.000	0.000	0.000	0.000	0.000	0.000
Gu	0.188	0.160	0.152	0.083	0.060	0.057	0.014	0.003		0.009	0.000	0.000	0.000	0.000	0.000
Na	0.157	0.121	0.128	0.080	0.035	0.020	0.004	0.029	0.029		0.036	0.000	0.018	0.000	0.000
Av	0.153	0.122	0.108	0.102	0.027	0.021	0.042	0.061	0.061	0.018		0.000	0.000	0.000	0.000
Ro	0.142	0.132	0.138	0.057	0.039	0.041	0.043	0.065	0.061	0.048	0.075		0.000	0.000	0.000
KB	0.113	0.085	0.095	0.034	0.021	0.004	0.028	0.048	0.067	0.022	0.037	0.046		0.000	0.000
Öl	0.194	0.139	0.176	0.087	0.129	0.115	0.097	0.105	0.113	0.126	0.152	0.149	0.088		0.216
GS	0.187	0.154	0.181	0.097	0.140	0.132	0.134	0.136	0.152	0.159	0.175	0.152	0.110	0.011	

Table 1.3. Von Bertalanffy growth parameters (L_∞ = asymptotic length, t_0 = initial condition parameter, and K = Brody growth rate or curvature parameter) and estimated mean weight at first maturity (± 95 CI) for lumpfish from different genetically distinct regions.

Region	Von Bertalanffy Growth parameters			Weight
	L_∞ (mm)	t_0	K (yr^{-1})	at 1st maturity (g)
Baltic Sea	200 ± 6	0.14 ± 0.02	0.51 ± 0.02	150 ± 12.5
East Atlantic	461 ± 14	0.36 ± 0.23	0.56 ± 0.09	2,019 ± 265.5
English Channel	571 ± 22	-1.08 ± 0.59	0.35 ± 0.20	3,007 ± 519.5

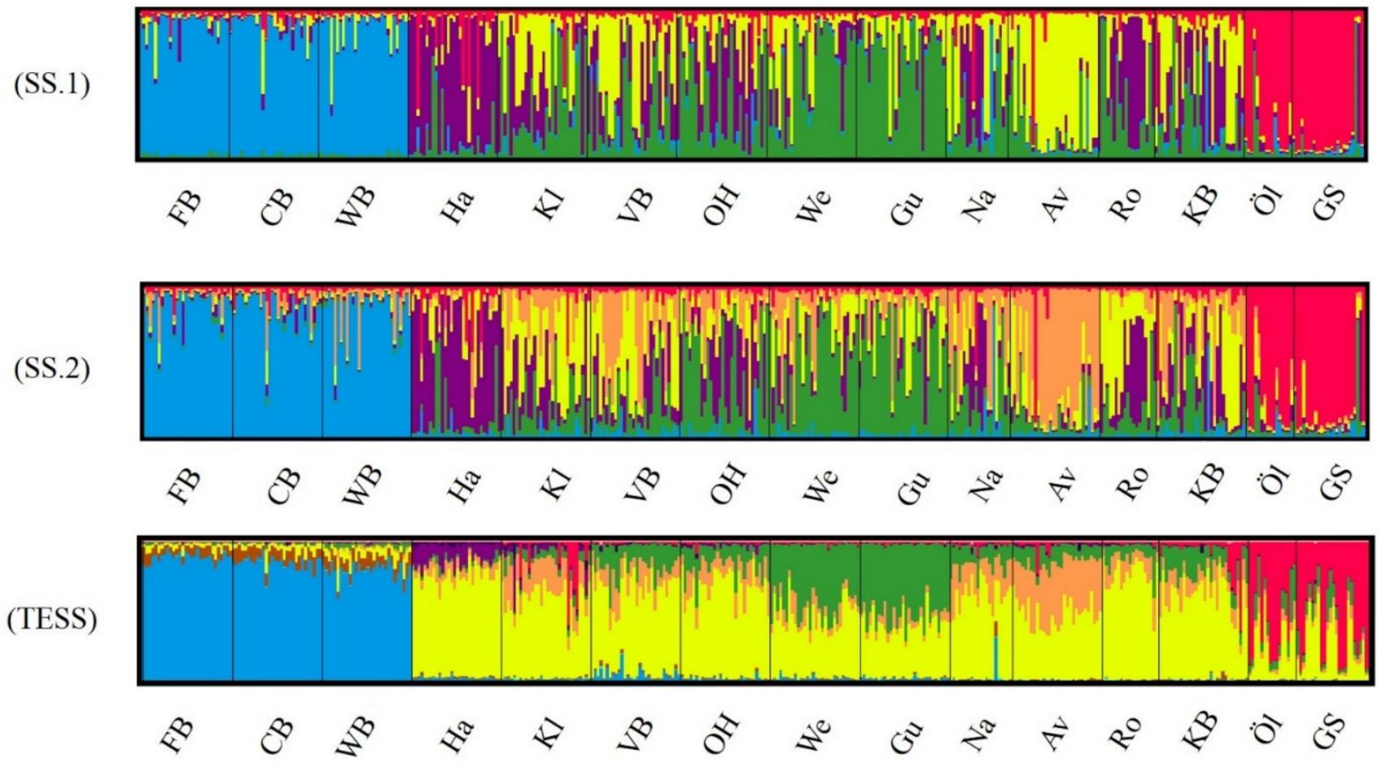

Figure 1.1. Lumpfish genetic structuring according to STRUCTURESELECTOR with (SS.1) MedMedK and MedMeanK of $K = 5$, (SS.2) MaxMedK and MaxMeanK of $K = 6$, and in (TESS) TESS with $K_{\max} = 10$ based on lowest mean DIC value. Each bar represents one individual with colours indicating probability of belonging to different genetically distinct clusters.

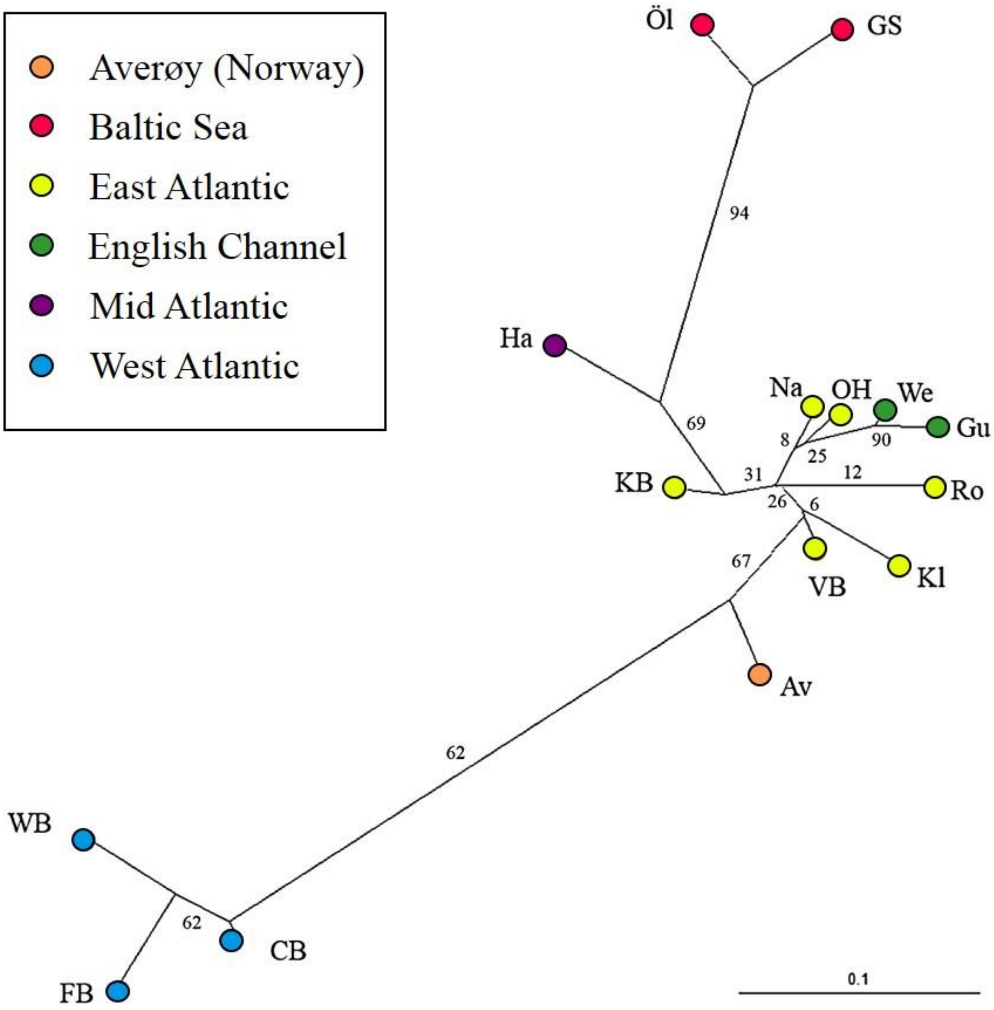

Figure 1.2. Neighbour joining tree based on Nei's Standard Genetic Distance calculated in Populations software with 1000 bootstrap iterations, for 15 lumpfish populations genotyped using 10 microsatellite loci.

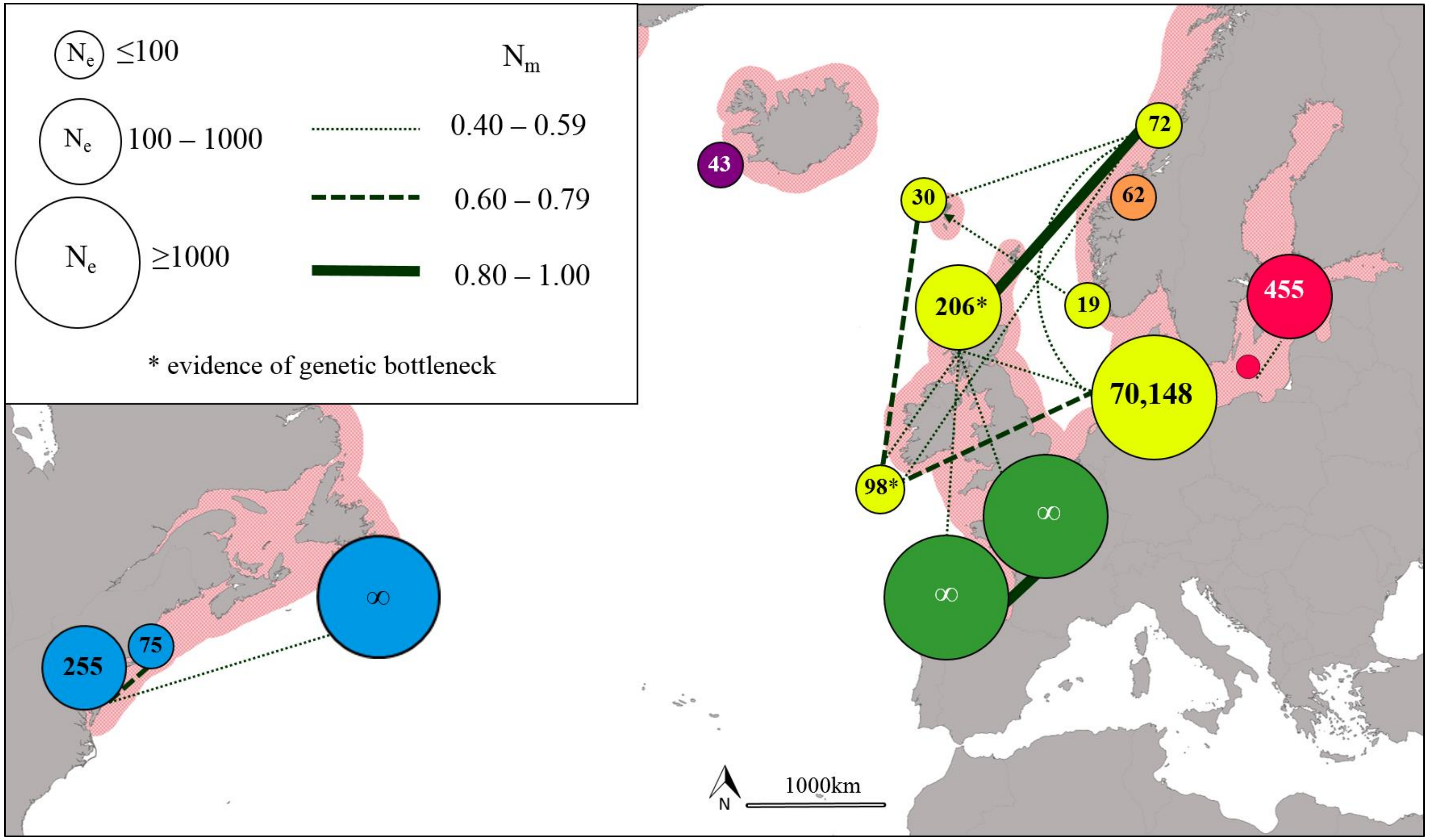

Figure 1.3. Patterns of gene flow among lumpfish populations with colours indicating genetic groups, symbol size proportional to effective population size, line thickness proportional to effective number of migrants, and shaded area representing spawning distribution.

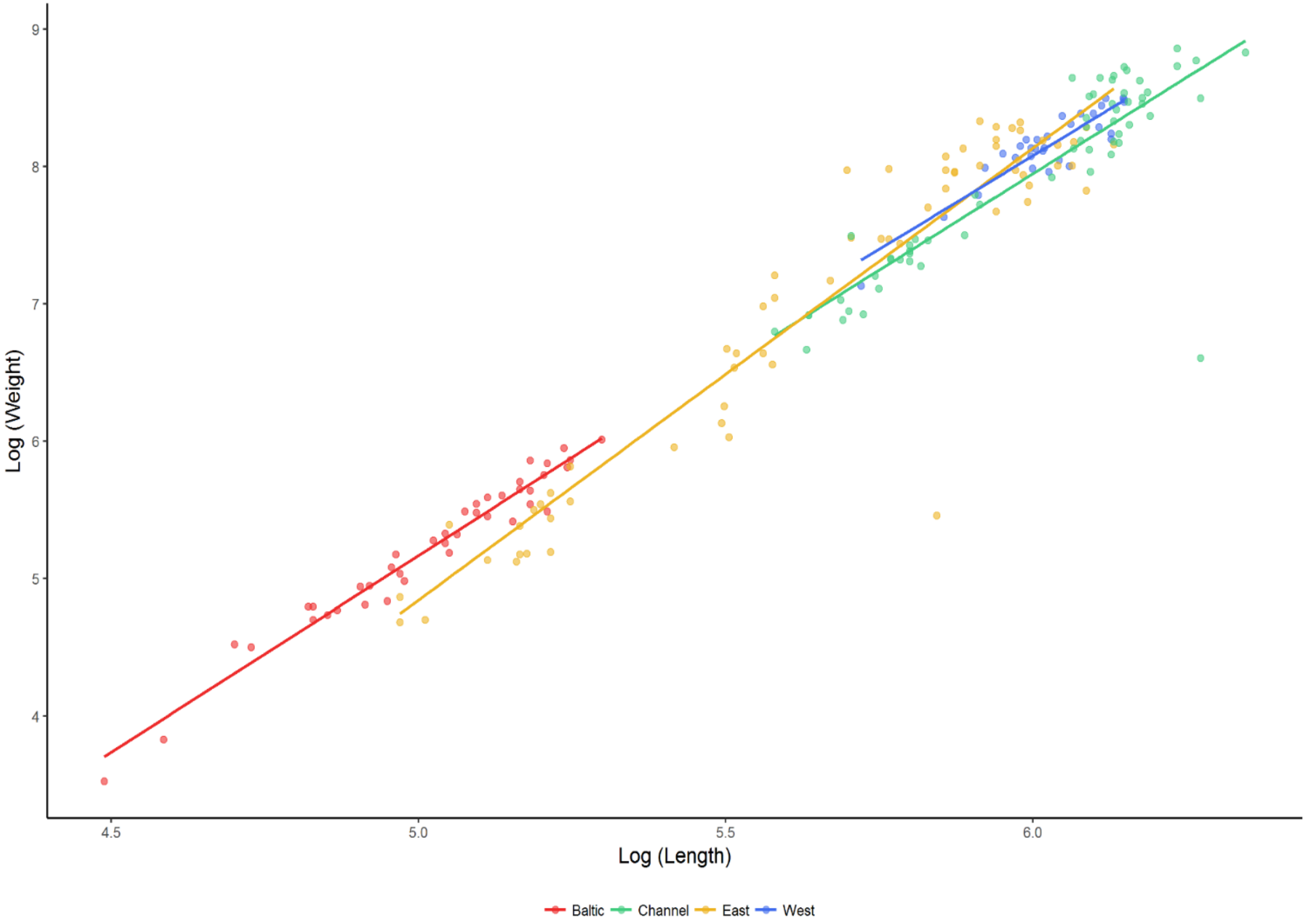

Figure 1.4. Length-weight relationships ($\log_{10}$ scale) for lumpfish sampled in the Baltic Sea (red), English Channel (green), East Atlantic (yellow) and West Atlantic (blue)

Chapter 2

Morphological variation in lumpfish (*Cyclopterus lumpus*): assessing sexual dimorphism and population differentiation to shape sustainable aquaculture practise.

Abstract

Lumpfish (*Cyclopterus lumpus*) are increasingly used as cleaner fish in salmon farms to control parasitic lice, however, gaps in knowledge of the species' biology hinders the development of sustainable practise in hatcheries supplying lumpfish to salmon farms. The inability to determine spawning condition in both sexes prevents the selective harvest of mature adults, thereby decreasing hatchery productivity and resulting in the needless sacrifice of immature fish. Furthermore, genetically distinct populations have been identified across the species' range though the extent of phenotypic variation is largely unknown, causing uncertainty regarding stock differentiation and appropriate management of regional stocks. This study assessed morphological variation between genetically distinct stocks originating from northern and southern lumpfish populations, reared within a common-garden experiment, and evaluated dimorphism between the sexes and between populations. Analysis found that male lumpfish had a longer mouth, longer dorsal flag and smaller dorsal hump than females, which represent new candidate traits to assess spawning condition. Results also indicated northern lumpfish exhibited a more streamlined body shape and that scute arrangement varied between the two stocks, implying different phenotypes exists in commercial stocks commonly used in aquaculture. As the economic significance of lumpfish grows, closing the breeding cycle in captivity and accounting for regional variation in stock management becomes essential for developing sustainable practise.

Introduction

Parasitic copepod lice infect salmon reared in open-net aquaculture, resulting in lowered growth, increased mortality and the spread of disease in fish farms (Costello 2006; Torrissen et al. 2013). Lice infestation incurs significant financial cost to the industry, estimated as exceeding $435 million per annum (Abolofia et al. 2017), whilst also damaging wild salmonid populations in areas of intensive farming (Peacock et al. 2016; Thorstad et al. 2015). Numerous methods of louse control are utilised by farmers and the deployment of cleaner fish is regarded as being among the most efficient and environmentally friendly solutions (Treasurer 2002). The lumpfish (*Cyclopterus lumpus*) is a commonly deployed cleaner fish and, despite being relatively new to the aquaculture industry, has quickly grown in economic importance with tens of millions of lumpfish now required to supply farms each year (Sae-Lim et al. 2020). However, gaps in the fundamental knowledge of lumpfish biology challenges efficient stock management and establishing a breeding program has thus far eluded the industry (Powell et al. 2018b). Research into the basic biology of this species is urgently needed to facilitate the production and management of stocks in a more sustainable manner (Brooker et al. 2018).

Commercial production currently relies on harvesting the gametes of wild adult broodstock (Jonassen et al. 2018b) and typically involves sacrificing males to collect milt through destructive methods (Wittwer & Treasurer 2018). Indicators of male maturity and fecundity are poorly understood, though neither body size nor colouration appear to correlate with milt production or reproductive success (Goulet & Green 1988; Norðberg et al. 2015). Wild-caught females are hand-stripped of eggs and retained at hatcheries in hope of being brought into spawning condition, however, high mortality rates have prevented successful reconditioning (Jonassen et al. 2018b). Egg production incurs significant energetic cost and female lumpfish may only achieve spawning condition every other year in the wild (Kennedy & Ólafsson 2019), with the quality and quantity of eggs noted to vary widely between individuals (Wittwer & Treasurer 2018). Identifying traits that indicate spawning condition would allow increased commercial output (Jonassen et al. 2018b) and prevent the needless sacrifice of non-reproductive fish. Given that established sexually dimorphic traits for colour and body size do not appear to correlate with spawning status or fecundity (Goulet & Green 1988; Norðberg et al. 2015), hatcheries currently lack potential markers of reproductive fitness to inform breeding programs.

Milt and eggs are typically harvested from broodstock native to the hatchery site, where artificial fertilisation is conducted and juveniles are then raised until they reach sufficient size for international translocation onto salmon farms (Jonassen et al. 2018a). Lumpfish are widely distribution across the North Atlantic, ranging longitudinally from Eastern Canada across to the Baltic Sea and latitudinally from the Arctic down to the Mediterranean (Davenport 1985b; Powell et al. 2018a). Distinct genetic groups of wild lumpfish have been identified across different regions of farming (Whittaker et al. 2018) and historical reports suggest extensive morphological variation, including dwarfism and reduced tuberculation, exist between populations (Cox & Anderson 1922). This could imply local adaptation of lumpfish populations to the diverse environmental conditions present across the range (Davenport 1985b). If present, regional differences may constitute an important factor in stock management, however, the extent of phenotypic differentiation between populations across the range is unknown, raising concern over whether regional variation should be considered during stock translocation (Blanco 2019; Jónsdóttir et al. 2018b). Therefore, a comparison of population traits under controlled conditions is needed to ascertain whether any regional phenotypes exist.

Assessing phenotypic diversity through the morphology of fish has proven an effective approach for identifying traits to inform the development of aquaculture practise. For example, morphological predictors of carcass yield in European sea bass (*Dicentrarchus labrax*) (Vandeputte et al. 2017) and Chinese sea bass (*Lateolabrax maculatus*) (Wang et al. 2016b) have been used to establish selective breeding programs to maximise output. Morphological ornamentation, such as the hook-like kype of Atlantic salmon (*Salmo salar*), have acted as indicators of reproductive fitness during the study of sexual selection in farmed fish (Perry et al. 2019). Identified morphological variation between natural, farmed and hybrid populations of tilapia has also aided conservation efforts to protect river systems from invasion via farm escapes (Gu et al. 2016). Although very little is known regarding morphological variation in *Cyclopterus lumpus*, analysis on the body structure of the related spotted lumpsucker (*Eumicrotremus pacificus*) has revealed differences between the sexes that elude to spawning condition (Voskoboinikova & Balanov 2019), suggesting morphological assessment of *C.lumpus* could benefit cleaner fish aquaculture.

Lumpfish are classed as a globiform and have a body that is compressed both anteriorly and posteriorly, with spiny epidermal ossicles known as scutes arranged in parallel lines along body ridges and the dorsal hump (Powell et al. 2018a). They possess a cartilaginous skeleton and lack a swim bladder (Davenport & Kjørsvik 1986), which led to an initial misclassification of the species as an elasmobranch (Gill 1908). Lumpfish have thick, scaleless skin containing melanophores that enable the fish to change colouration (Davenport & Bradshaw 1995). Perhaps the most distinguishing feature of the species is the ventral suction disc, which is formed from fused pelvic fins (Davenport 1985b). This highly specialised organ exerts the strongest suction force of any known fish (Budney & Hall 2010), taking nearly 40 kg of force to dislodge a mature adult (Nachtigall 1974). Adult lumpfish show sexual dimorphism in body size and colour (Goulet et al. 1986). Males are substantially smaller and exhibit bright colours during the spawning season, typically displaying vivid reds and oranges (Davenport & Thorsteinsson 1989). They attract females to spawn in nests on rocky outcrops and then care for the eggs until hatching (Powell et al. 2018a). Mature females develop large ovary sacs that account for a third of the total body weight (Thorsteinsson 1981) and are targeted by roe fisheries (Monfort 2002) when migrating between offshore feeding grounds and coastal spawning sites (Kennedy et al. 2014).

This study investigated morphological variation in the body shape and ventral suction disc of lumpfish reared within a common garden experiment, thereby controlling for environmental covariates. Morphological differences between male and female lumpfish were evaluated to assess whether sexually dimorphic features were present, providing new candidate traits to act to inform future breeding attempts. The morphology of genetically distinct stocks from the north and south of the range was studied to determine whether regional phenotypes were present under common garden conditions, thereby indicating whether population differentiation should be accounted for in stock management.

Material and Methods

Experimental Fish

The lumpfish used in this study were the progeny of wild adults originating from Iceland and the English Channel, which represent two genetically distinct populations in the North Atlantic (Whittaker et al. 2018). Eggs and milt were collected from an unknown number of Icelandic fish by StofnFiskur and from English broodstock (male n =4; female n=4) by the Cleaner Fish Company ltd. Fertilised eggs from both commercial providers were shipped to the Centre for Sustainable Aquatic Research (CSAR) Swansea University (UK), where the eggs hatched from March to May 2016 and the juveniles were reared as commercial stock under common aquaculture conditions (Treasurer et al. 2018) until approximately 1 year of age.

In March 2017, lumpfish stocks were visually screened for external signs of health, including fin, eye and skin damage and malnutrition. Healthy individuals from each stock population were randomly selected by first ensuring all lumpfish were swimming within the water column and then sweeping a hand-net from the tank floor to the water surface. Captured fish were randomly housed in experimental tanks (Iceland, n =4; UK, n =4) using the random number generator at *random.org* (Eddelbuettel 2006) to assign individuals to the four available tanks, until each tank reached a density of 33 fish (total n =264). The row of eight tanks was ordered to alternate between Icelandic and English stocks, and all tanks were connected via a recirculating aquaculture system to ensure uniform water parameters. Each tank held approx.1500 litres (1.4 m diameter, 0.9 m depth). Water was maintained at 11-13 ^{o}C and 28-32 ppt salinity with a room photoperiod of 12D:12L. Fish were fed at 2% of tank biomass over three feeding events per day, using a commercial pellet diet of Amber Neptune (Skretting, UK). The fish were reared under experimental conditions for a further six months (until 18 months old) at which age captive lumpfish have typically developed gonads mature enough to allow definitive determination of sex (Wittwer & Treasurer 2018).

Data Collection

In August 2017, a total of 89 lumpfish from the northern (Icelandic, n =44) and southern (UK, n =45) stocks were humanely euthanised using an overdose of anaesthesia (2-Phenoxyethanol) and exsanguination, by trained staff in accordance with the Animals Scientific Procedures Act (ASPA). The study was undertaken with ethical approval from Swansea University, College of Science Ethics Review Committee (IP1516-6.2).

Each fish was photographed using a Canon EOS 400D digital camera with a SP 90mm F/2.8 Di Macro Lens, against a standard white background. The camera was secured to a tripod which was fixed to a work bench using tape in order to standardise conditions between photographs, and each photograph included a measuring tape for scale reference. All fish were photographed in a left facing, lateral position to assess body morphology and a ventral position to assess suction cup morphology. The sex of individual fish was subsequently determined by the presence of testes (male, n =24) or ovary sacs (female, n =37), with fish of undetermined sex (n =27) excluded from analysis.

Geometric Morphometric Analysis

Photographs of all the lumpfish were digitised using tpsDigs, version 2.26 (Rohlf 2001). Anatomic features were recorded by placing 27 landmarks on lateral body images and 10 landmarks on ventral sucker images (Figure 2.1) by a single observer who was blind to the population and sex of each fish. Landmark analysis was undertaken in MorphoJ, version 1.06 (Klingenberg 2011). Procrustes superimposition was used to align landmarks, which computes average body shape and controls for variation associated with differences in body size and orientation (Vehanen & Huusko 2011).

Canonical Variable Analysis (CVA) was undertaken to examine variation in body and sucker shape for individuals grouped by sex and origin (northern-female, n =18; northern-male, n =10; southern-female, n =19; southern-male, n =14). Pairwise differences in the average shape of these four groups were analysed through permutation tests of Mahalanobis distance with 100,000 permutations (Kelley et al.

2017). Wireframe graphs were used to illustrate morphological differences attributed to each canonical variable. Variation in body and sucker shape was also analysed through Discriminant Function Analysis (DFA), which separately compared the influence of sex (n =61) and population (n =89) using two T^2 Hotelling tests each with 100,000 permutations.

Size Measurement Analysis

The caudal peduncle area (mm^2), dorsal flag length (mm), dorsal hump area (mm^2), mouth length (mm), suction disc area (mm^2) and total length (mm) of each fish (Figure 2.1) was measured from photographs using ImageJ, version 1.52 (Abràmoff et al. 2004).

Linear mixed models (LMM) were carried out using the *lme4* package (Bates et al. 2007) in R, version 3.5 (Core Team 2013). Five LMM were fit using the caudal, flag, hump, mouth and sucker metrics as a dependent variable in each model. The full models contained fixed effects for the sex, population and size (total length) of fish, and included interaction terms between fixed effects. The tank in which lumpfish were housed (n =8) was used as a random factor. The effect of the random factor was assessed using likelihood-ratio tests (LRT) between the full with and without the random factor. Model residuals were plotted to check model assumptions for normality, linearity, heteroscedasticity and the presence of influential data points. The most plausible model was selected using the dredge function applied using the *MuMIn* package (Barton 2009), marginal and conditional coefficients of determination were found using the r.squaredGLMM function, and the ΔAICc of models was used to determine the level of support behind each model (Appendix T2.1). Likelihood-ratio tests (LRT) were carried out between the reduced, most plausible, model a null model that lack explanatory variables to assess whether model reduction improved goodness-of-fit (Appendix T2.2).

Results

Geometric Morphometric Analysis

Permutation tests of Mahalanobis distance found significant variation (P <0.001) for each pairwise comparison, indicating that body shape varied between fish from different population origins and between the sexes. However, permutation tests of Mahalanobis distance found no significant variation in pairwise comparisons for suction disc shape (Table 2.1), which was therefore omitted from further geometric analysis.

Canonical variable 1 accounted for 71.52% of variation in overall body shape and demonstrated differences in fish originating from northern and southern populations. Discriminant function analysis further comparing northern and southern lumpfish found significant difference in the overall body shape between individuals from the two regions ($T^2 = 2567.479$, $P < 0.001$). Wiregraphs showed that northern lumpfish had a more streamlined body shape with scute lines positioned lower on the body relative to southern lumpfish (Figure 2.2).

Canonical variable 2 accounted for 18.12% of body shape variation and showed differences between the sexes. Male lumpfish exhibited a more pronounced mouth and caudal area, with a longer dorsal flag, whereas female lumpfish displayed a more prominent dorsal hump (Figure 2.2). However, discriminant function analysis did not detect a significant difference in the overall body shape of male and female lumpfish ($T^2 = 342.871$, $P = 0.425$).

Size Measurement Analysis

Body size was included as a covariate in each linear mixed model (Table 2.2), and was found to positively correlate with caudal peduncle size (2.413 ±0.307, t =7.853, *P* <0.001), suction disc area (5.018 ±1.052, t =4.770, *P* <0.001), dorsal hump size (7.251 ±0.490, t =14.787, *P* <0.001) and mouth length (0.051 ±0.007, t =6.842, *P* <0.001).

Male lumpfish had a longer dorsal flag (4.082 ±0.702, t =5.819, *P* <0.001), longer mouth (1.209 ±0.350, t =3.454, *P* = 0.001) and smaller dorsal hump (-71.635 ±22.801, t =14.787, *P* <0.001) than female lumpfish (Figure 3.2). Females had a smaller caudal peduncle than males (-434.778 ±102.031, t =-4.261, *P* <0.001), with the sexual dimorphism growing as size increased (3.520 ±0.763, t =4.614, *P* <0.001), and was most dimorphic in the largest, northern lumpfish (-3.102 ±0.0890, t =-3.484, *P* <0.001).

Results from likelihood-ratio tests indicated that the tank in which lumpfish were housed did not account for variation in size measurements, and that model selection significantly improved goodness-of-fit (Appendix T2.2).

Discussion

This is the first study to compare morphology of male and female *C.lumpus* from different genetic stocks and found that, despite identifying anatomic differences in body shape, the morphology of the ventral suction disc was highly conserved among all studied fish. Lumpfish use this sucker to cling onto substrates in order to conserve energy (Killen et al. 2007), remain motionless to avoid predators and ambush prey (Williams & Brown 1991), maintain locomotion in challenging water currents (Davenport & Kjørsvik 1986) and is integral to the development of foraging behaviours throughout larval and juvenile life-stages (Brown 1986; Ingólfsson & Kristjánsson 2002). The conserved sucker morphology found in the present study therefore implies little difference in its function between sexes or populations, and serves to highlight the importance of this organ in lumpfish biology.

The majority of variation detected in lumpfish body shape (72%) was found between the two stocks used within the study, representing populations from the north and south of the species distribution (Whittaker et al. 2018). Differences included the positioning of lateral and dorsolateral scute lines, which are pronounced ridges of dermal ossicles tipped with one or more spiny points (Märss et al. 2010). Whether these spine-like structures hold any adaptive significance is unknown, though abnormal scute patterns have been reported in lumpfish inhabiting areas of low salinity (Davenport 1985a) and the number of scutes is thought to reduce with the age (Ojaveer et al. 2003). Given the spine-like nature of scutes it is possible they confer a degree of physical defence, similar in manner to the scutes of pallid sturgeon (*Scaphirhynchus albus*) which offer protection from larger predatory fish (French et al. 2014). Further variation between the two stocks was associated with body depth, with the northern phenotype exhibiting a shallower, relatively more streamlined body compared to the southern phenotype. Similar intraspecific variation in body depth has been attributed to phenotypes of fish inhabiting areas with contrasting environmental factors. For example, cichlid populations living in lotic habitats have more streamlined bodies, better suited to fast flowing water currents, relative to populations in slow flowing lentic habitats (Berbel-Filho et al. 2016). Body depth could also reflect adaptation of phenotypes to different life-history strategies. This is demonstrated by migrant roach (*Rutilus rutilus*) having a more slender body profile than resident populations, which is assumed to reduce energy expenditure during migration (Chapman et al. 2015). Whether the differences described in lumpfish morphology show localised adaptation by the origin populations to their respective native environments cannot be firmly established without comparison of multiple families, of known genetic pedigree, under varying environmental conditions. However, the notion that the northern phenotype has a shallower body which benefits a more mobile life-history does align with studies indicating that whilst Icelandic fish travel great distances (Kennedy et al. 2014). Furthermore, lumpfish spawning in northern regions experience high levels of seasonal predation from orca (*Orcinus orca*) (Jourdain et al. 2019) that is not yet assessed documented in the range. Whether differences in migration and predation pressure exist across wild lumpfish populations remains uncertain, but developing knowledge on regional phenotypes could aid the development of hatchery stocks to supply farms across North Atlantic (Powell et al. 2018b).

Sexual dimorphism accounted for variation (18%) the body shapes of male and female lumpfish, which could reflect established differences in the life-histories of sexes. The mouth length of males was found to be significantly longer than females which could aid males in providing parental care, as they are observed to expel water from their mouth and circulate flow around eggs (Goulet et al. 1986) preventing a build-up of waste ammonia (Davenport et al. 1983). Males have also been witnessed spouting water from tidal pools onto eggs exposed by low tides (Davenport 1985a), though it is unknown how frequently this behaviour occurs. The fleshy protrusion commonly known as the dorsal flag was identified as being the most divergent trait between sexes and, as this protrusion serves no obvious function, could be speculated as a form of sexual ornamentation. Highly ornamented individuals may exhibit reproductive traits that are beneficial to selective breeding in aquaculture, for example whitefish (*Coregonus lavaretus*) displaying greater ornamental tubercles during spawning produce offspring with increased survival rates (Kekäläinen et al. 2010). The lumpfish analysed in the present study were not yet sexually mature and may not accurately reflect the morphology of spawning adults, therefore collecting biometric data for dorsal flag length alongside milt volume and viability is necessary in order to evaluate an association of this trait with spawning condition. Contrariwise, female lumpfish were found to have a larger dorsal hump, which contains buoyant gelatinous tissue (Davenport & Kjørsvik 1986) that may aid the heavier, gravid females in migration between feeding grounds and coastal spawning sites (Mitamura et al. 2012). It has been theorised that older females have taller dorsal humps (Davenport 1985a), though as the present study only analysed a single age class this cannot be further substantiated. Nonetheless, collecting biometric data on hump size along with egg quality could help determine whether this trait does indicate spawning condition and enable the selective breeding and harvest of more productive broodstock to increase commercial productivity (Jonassen et al. 2018b; Powell et al. 2018b) and prevent the over-exploitation of mature females (Hoenig & Hewitt 2005).

Given the rapid expansion of the cleaner fish industry and intensifying demand for lumpfish in salmon farms, establishing a breeding program is now vital for the long-term sustainability of lumpfish aquaculture (Imsland et al. 2018b; Powell et al. 2018b). The current inability to supply domesticated stocks necessitates the translocation of available lumpfish from natal hatchery sites to salmon farms on an international scale (Jonassen et al. 2018a; Jonassen et al. 2018b) often resulting in farm deployment outside the native range of different genetic groups (Bolton-Warberg 2018; Johannesen et al. 2018b; Treasurer et al. 2018). Translocating fish across sites of open-net aquaculture in this manner could pose a risk to native populations around the farm, as fish escaping open-net pens may hybridise with wild fish (Diserud et al. 2019; Faust et al. 2018). Introducing non-native genes into wild fish could prove maladaptive, particularly if the source population shows considerable phenotypic differentiation from fish native to the deployment region (Cross 2000; Stockwell & Leberg 2002). Whilst the present study does not confirm the existence of local adaptation between lumpfish populations, morphological differences in body depth and scute arrangement were identified in fish collected from different genetic groups in different geographic regions, despite being reared in a common environment. This implies that phenotypic differentiation is present between genetic groups across the range, though further study is required to evaluate the potential consequences of translocated lumpfish escaping farms. Evaluating regional differences to develop more efficient stocks has benefitted breeding programs of African catfish (*Clarias gariepinus*), with identified variation in growth and mortality between genetic lines facilitating broodstock selection (Opiyo et al. 2017), though this would first require the ability to identify reproductively mature lumpfish. In light of uncertainty for body size and colouration to act as indicators of spawning condition (Goulet & Green 1988; Norðberg et al. 2015), the present study proposes sexually dimorphic traits identified in mouth length and dorsal flag length for males and dorsal hump size for females should now be evaluated alongside data collected on milt and egg quality to determine the validity of these as indicators of reproductive success. Further quantifying desirable traits in broodstock, such as robust growth (Sae-Lim et al. 2020) and predisposition to cleaning behaviours (Imsland et al. 2016a), should also be undertaken to inform the domestication process of more efficient hatchery stocks. Failure to close the breeding cycle in captivity leaves the cleaner fish industry reliant on wild-caught lumpfish (Wittwer & Treasurer 2018), which if harvested unsustainably to meet growing commercial demand could reduce abundance and heighten the vulnerability of populations to overexploitation (Halvorsen et al. 2017; Hoenig & Hewitt 2005).

Acknowledgments

I wish to thank Mia Berwick and Niall Coates for their assistance in photographing lumpfish and conducting necropsy.

Table 2.1. Malahanobis distances (lower) and permutation test *P* values (upper) of body (a) and suction disc (b) geometric morphology of female and male lumpfish originating from northern and southern populations.

(a)

Body		Northern		Southern	
		Female	Male	Female	Male
Northern	Female		<0.001	<0.001	<0.001
	Male	7.520		<0.001	<0.001
Southern	Female	10.321	13.244		<0.001
	Male	8.842	11.014	6.021	

(b)

Suction Disc		Northern		Southern	
		Female	Male	Female	Male
Northern	Female		0.407	0.719	0.256
	Male	2.472		0.500	0.807
Southern	Female	2.269	2.501		0.432
	Male	2.920	2.390	2.774	

Table 2.2. Most plausible models for caudal area (mm^2), sucker area (mm^2), dorsal hump area (mm2), mouth length (mm) and dorsal flag length (mm). Values include marginal (R2m) and conditional (R2c) coefficients of models and significant P values shown in bold.

Trait	Factor	Estimate ±SE	R2m	R2c	t	*P*
Caudal Area	Size	2.413 ±0.307	0.863	0.863	7.853	**<0.001**
	Sex	-434.778 ±102.031			-4.261	**<0.001**
	Origin	-34.940 ±52.152			-0.670	0.506
	Size : Sex	3.520 ±0.763			4.614	**<0.001**
	Size : Origin	0.317 ±0.406			0.780	0.439
	Sex : Origin	388.100 ±117.009			3.317	**0.002**
	Size : Sex : Origin	-3.102 ±0.890			-3.484	**<0.001**
Sucker Area	Size	5.018 ±1.052	0.272	0.272	4.770	**<0.001**
Hump Area	Size	7.251 ±0.490	0.794	0.794	-3.142	**0.003**
	Sex	-71.635 ±22.801			14.787	**<0.001**
Mouth Length	Size	1.209 ±0.350	0.489	0.497	3.454	**0.001**
	Sex	0.051 ±0.008			6.842	**<0.001**
Flag Length	Sex	4.082 ±0.702	0.357	0.357	5.819	**<0.001**

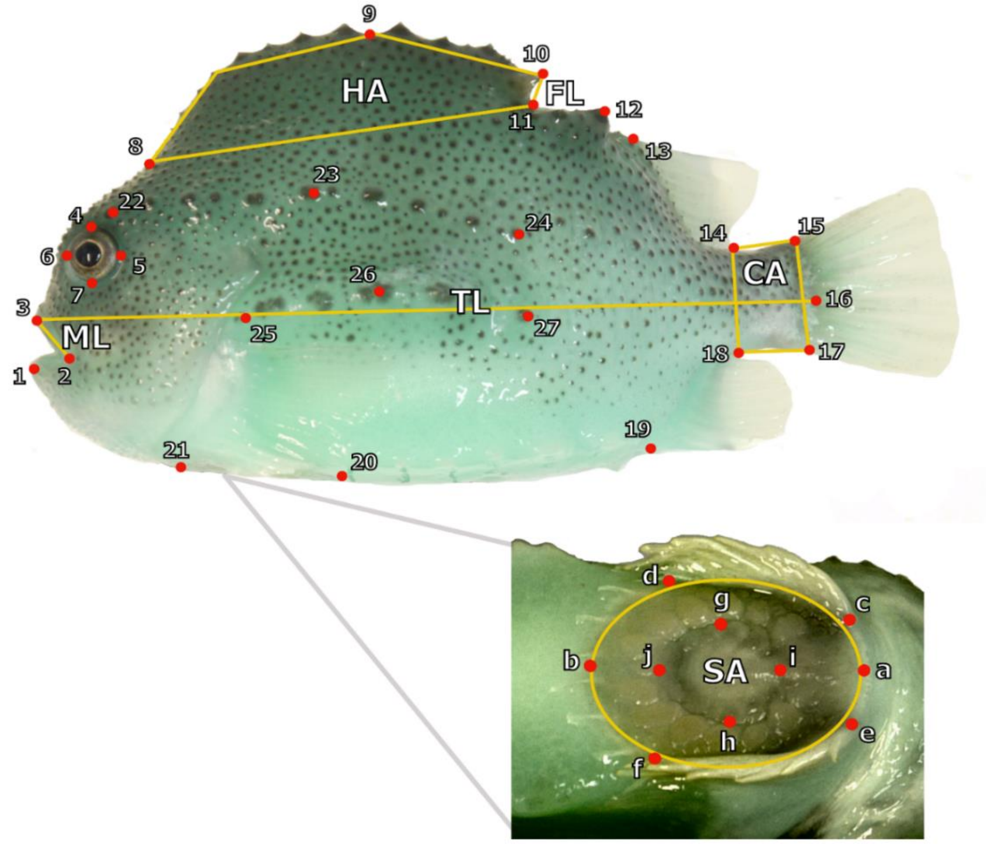

Figure 2.1. Landmarks (red) used for morphometric analyses. The 27 body landmarks: (1) distal lower jaw, (2) mandible (3) distal upper jaw, (4) upper eye, (5) rightmost of eye, (6) leftmost of eye, (7) lower eye, (8) start of hump incline, (9) tallest point of hump (excluding tubercles), (10) flag tip, (11) flag recess, (12) dorsal ridge, (13) anterior dorsal fin base, (14) posterior dorsal fin base, (15) upper caudal peduncle, (16) midpoint of caudal peduncle, (17) lower caudal peduncle, (18) posterior anal fin base, (19) anterior anal fin base, (20) posterior pelvic fin base, (21) anterior pelvic fin base, (22) anterior dorsolateral scute line, (23) largest scute of dorsolateral line, (24) posterior dorsolateral scute line, (25) anterior lateral scute line, (26) largest scute of lateral line, (27) posterior ventral lateral line. The 10 sucker landmarks include: (a) anterior start of suction disc (b) posterior end of suction disc (c) anterior left pectoral fin, (d) posterior left pectoral fin, (e) anterior right pectoral fin, (f) posterior right pectoral fin, (g) leftmost point of suction cup, (h) rightmost point of suction cup, (i) anterior start of suction cup, (j) posterior end of suction cup. Size measurements recorded for size analysis (yellow) include: total length (TL), mouth length (ML), caudal peduncle area (CA), hump area (HA), flag length (FL) and sucker area (SA).

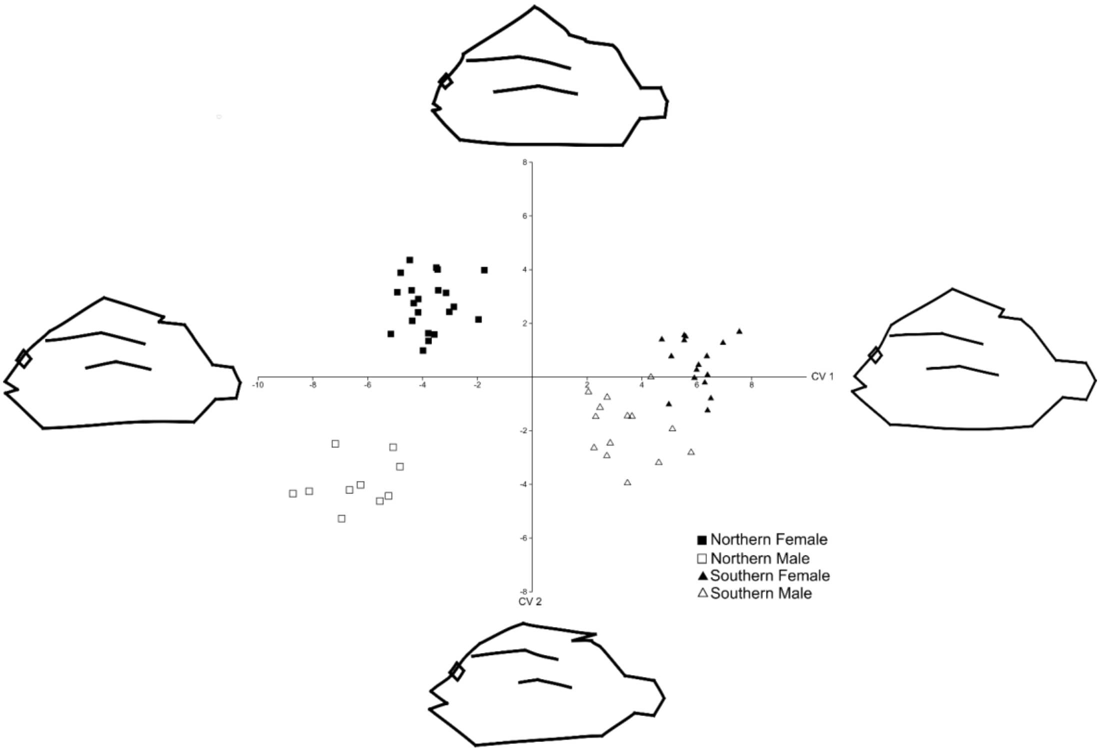

Figure 2.2. Canonical Variable Analysis of body morphology for northern female (■), northern male (□), southern female (▲) and southern male (△) lumpfish. Wireframe graphs show extremes in variation of negative and positive values for canonical variable 1 (origin) along the x axis and canonical variable 2 (sex) along the y axis.

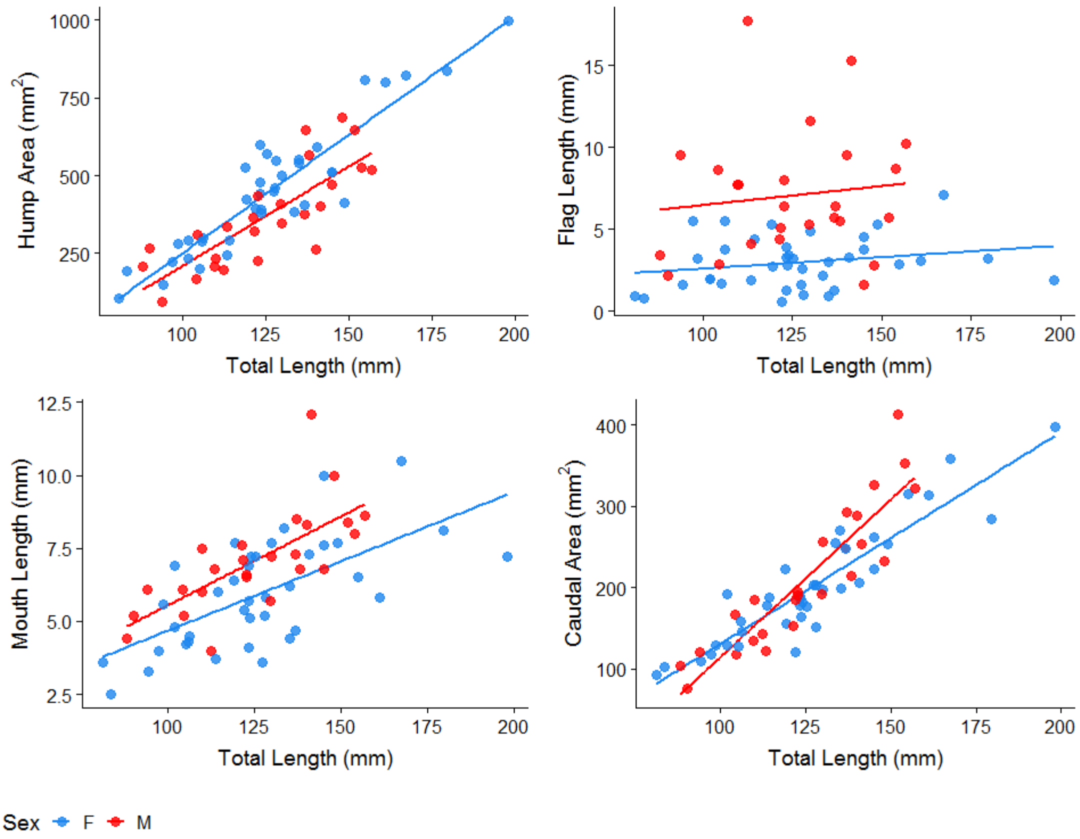

Figure 2.3. Sexually dimorphic relationships between the dorsal hump area (mm^2), dorsal flag length (mm), mouth length (mm) and caudal area (mm^2) relative to lumpfish body size (total length - mm).

Clean fast die young: fitness trade-offs challenge sustainable lumpfish aquaculture

Abstract

Lumpfish (Cyclopterus lumpus) are increasingly used as a cleaner fish in salmon aquaculture to control parasitic sea-lice in farms. Commercial lumpfish stocks are produced using wild broodstock, with juveniles reared in hatcheries and then transported into salmon farms. The species is widely distributed across the North Atlantic Ocean and, although distinct genetic groups are documented in salmon farming regions, the extent of phenotypic differentiation between populations is largely unknown. This study evaluated variation in fitness traits for growth rate, survival probability and feeding activity in two commercially available stocks obtained from populations in the north and south of the range. Despite a common environment, the northern stock showed faster growth and higher feeding activity while the southern stock showed greater survival probability. These findings suggest differences in life history strategy between stocks, potentially reflecting fitness trade-offs in origin populations. Lumpfish aquaculture should balance fast growth with high survival rate in order to improve sustainability, while further investigating variation along latitudinal gradients to inform translocation practise.

Introduction

The Pace of Life Syndrome hypothesis (POLS) predicts differences in the life history strategies for a species or population, in which physiological and behavioural traits correlate to prioritise reproduction (a fast life history) or survival (a slow life history) (Ricklefs & Wikelski 2002; Royauté et al. 2018). Trade-offs between different life history paces are incurred when an organism invests more energy and nutrient resources into one strategy, such as faster growth rate, thereby reducing resource allocation for other fitness traits, such as lower immune function (Soler et al. 2003; van der Most et al. 2011) or reduced longevity (Chen et al. 2019; Hou 2013). Fitness trade-offs in life history strategies are well documented across natural populations, with the associated cost-benefit of different strategies shown to vary both spatially and temporally (Christie et al. 2018; Mangel & Stamps 2001). For example, faster growth rates may benefit fitness by enhancing the competitive ability to claim territory (Moginie & Shima 2018) but the need to forage frequently in order to sustain growth could incur lower survival in areas of high predation risk or during times of environmental stress (de la Mata et al. 2017; Mangel & Munch 2005; Meister et al. 2017). Successful life histories are inherited by offspring, which therefore possess a genetic advantage when competing under the same conditions as their predecessors (Boots et al. 2018; Santostefano et al. 2017). Therefore populations exhibit different adaptive life histories to particular geographic regions or temporal patterns (Hereford 2009; Montiglio et al. 2018), and show suites of correlated life history traits to form an optimised POLS for a specific context (Dammhahn et al. 2018). Faster paced life histories may be beneficial in warm, productive ecosystems with ample resources, whereas, slower life histories may be more advantageous in cold, barren ecosystems, thereby creating climatic gradient in life history strategies (Segev et al. 2017). However, artificially manipulating selection processes for a particular fitness trait or modifying the context in which a particular strategy is adapted could hold implications for wider life history trade-offs (Kotrschal et al. 2016; Matsumura & Miyatake).

As human populations grow and the abundance of wild fish decreases, aquaculture takes a leading role in securing a reliable supply of seafood for future generations (Alleway et al. 2018; Naylor et al. 2000; Troell et al. 2014). Selective breeding programs are used to maximise production output by selecting for desired traits in parental broodstock to create genetically enhanced commercial stock (Duarte et al.

2007; Gjedrem et al. 2012). This process has successfully increased the harvest weight of Nile tilapia (*Oreochromis niloticus*) over successive generations (Bentsen et al. 2017) and delayed time to maturation of rainbow trout (*Oncorhynchus mykiss*) to improve meat quality (Kause et al. 2003). Perhaps the most common trait integrated in selective breeding for finfish aquaculture is increased growth rate, reducing the time required to reach harvest weight, and approximately 80% of European finfish aquaculture is driven by stocks selectively bred for increased growth (Janssen et al. 2017). Maximal growth rates are rarely observed in wild fish, as life history traits increasing growth often incur trade-offs which lower survival (Biro et al. 2006). Faster growing swordtail (*Xiphophorus multilineatus*) exhibit a shorter life-span in a manner consistent with the growth-maturity optimisation hypothesis, which predicts growth rate should not incur the cost of mortality before maturity (Weinstein et al. 2019). Balancing potential fitness trade-offs is an important process in sustainable aquaculture and reducing genetic selection for high growth in favour of lower mortality is considered a more sustainable business model (Omasaki et al. 2017). Spatially significant patterns influencing fitness trade-offs should also be considered, particularly as stocks of fish are commonly translocated internationally from hatchery sites to grow-out on farms (Cross 2000; Vlachos & Malindretos 2017). Contrasting fitness traits described between genetic stocks originating from different geographic regions could imply locally adapted life history strategies. For example, varying survival, fecundity and growth rates identified among regional strains of African catfish (*Clarias gariepinus*) raised in a common environment is consistent with a different paced life histories of origin populations (Opiyo et al. 2017). Understanding fitness trade-offs in a species' biology and factors driving regional variation between populations could help develop more sustainable approaches to selective breeding programs and stock translocation.

Lumpfish (*Cyclopterus lumpus*) are widely used as a species of cleaner fish to control ectoparasitic copepods (*Lepeophtheirus salmonis*) on Atlantic salmon (*Salmo salar*) farms (Imsland et al. 2018b). Commercial hatcheries have recently been established to produce stocks of juvenile lumpfish using adult broodstock collected from different wild populations (Jonassen et al. 2018b; Wittwer & Treasurer 2018). Estimates from the last five years of production growth predict 50 million juveniles may be needed to supply salmon farms in 2020 (Powell et al. 2018b). Therefore hatcheries aim to maximise production output through increased growth rate of juvenile lumpfish, and have focused on optimising husbandry parameters including water temperature (Nytrø et al. 2014), lighting schedule (Imsland et al. 2018a), feeding frequency and food delivery mechanisms such as grazing blocks (Imsland et al. 2019a; Imsland et al. 2019b). The industry is also in the process of establishing a selective breeding program for the species (Blanco 2019; Imsland et al. 2016a), with a genetic component identified for growth to be incorporated in future breeding attempts (Sae-Lim et al. 2020). Low survival rates are cited as a major problem for sustainability in both the hatchery and farm phases of lumpfish aquaculture, with mortality rates reportedly reaching 48% in some cases (Brooker et al. 2018). Sustainability is also challenged by the variation in lumpfish cleaning behaviour in salmon farms, as stomach content analysis has found as little as 14% of lumpfish forage on sea-lice while 30% had empty stomachs (Eliasen et al. 2018). Neither high mortality rates nor low cleaning rates are beneficial for lumpfish aquaculture, reducing waste and improving the proportion of healthy, actively cleaning lumpfish on salmon farms is a top priority for the industry (Blanco 2019; Powell et al. 2018b).

Lumpfish are distributed latitudinally from the Artic down to the Mediterranean and longitudinally from Canada to the Baltic Sea (Powell et al. 2018a), representing diverse habitats to which local populations may show regional adaptation (Davenport 1985b). Juveniles grow rapidly throughout early life history (Moring 2001), achieving a specific growth rate of 1.5-3.5% per day under optimal conditions (Nytrø et al. 2014). Lumpfish behave as energy satisfiers rather than maximising net energy gain and conserve energy through adopting less-active feeding strategies when prey is plentiful, thereby allocating more resources towards sustaining growth (Killen et al. 2007). The species is also sexually dimorphic and the larger females achieve faster growth rates than the males (Powell et al. 2018a). Commercial stocks of lumpfish are routinely translocated from natal hatchery sites for deployment in salmon farms across the North Atlantic (Jonassen et al. 2018a), crossing natural genetic boundaries between wild populations (Whittaker et al. 2018). Information detailing whether differences in fitness or life history strategies exist between genetic populations is lacking, although female fecundity is documented to vary along a latitudinal gradient in Greenland (Hedeholm et al. 2017).

This study aimed to quantify fitness traits in a production cohort of lumpfish, comprised of two genetically distinct populations originating from the north (Iceland) and south (England) of the range, reared within a common environment. Individual specific growth rate, survival probability and tank-level foraging activity were quantified for hatchery fish. I hypothesised that stock-level variation in fitness traits, maintained despite a common environment, may indicate a genetic basis of fitness trade-offs and therefore reflect life history differences in origin populations. Investigating patterns of life history variation could give insight on how aquaculture practises, such as selective breeding or stock translocation, could be developed to enhance sustainability in terms of reduced mortality and increased feeding activity.

Material and Methods

Experimental Fish

The lumpfish used in this project represent two populations from the north (Iceland) and south (England) of the species' range. Commercial stocks were sourced from StofnFiskur (Hafnir) bred from an unknown number of Icelandic broodstock, and the Cleaner Fish Company ltd. (Weymouth), bred from eight English broodstock (male n =4; female n =4) (Figure 3.1). Fertilised eggs were shipped from both suppliers to the Centre for Sustainable Aquatic Research (CSAR) Swansea University (Wales), where the eggs then hatched as a single age cohort in March 2016, and then reared under common hatchery conditions for one year (Treasurer et al. 2018).

Experimental Setup

Experimental lumpfish (total n =264; northern, n =132; southern, n=132) were selected from each commercial stock in March 2017. The selection process was randomised by first ensuring all lumpfish were swimming within the water column and then sweeping a hand-net from the tank floor up to the surface to capture lumpfish at random. Captured fish were first visually inspected for external signs of good health, including absence of fin, eye or skin damage and malnutrition. To allow individual identification each lumpfish was PIT tagged (7 x 1.35 mm, Loligo) in accordance with the manufacturer's protocols, using a standardised position at the base of the dorsal hump. Tagging studies have found that using PIT tags to individually identify lumpfish in this manner does not influence growth or survival rates (D'Arcy et al. 2020). Tagged lumpfish were then assigned to one of eight tanks (north, n =4; south, n =4), using a random number generator (Eddelbuettel 2006) until each tank reached a stocking density of 33 individuals. The row of tanks was labelled numerically and ordered to alternate between those housing northern and southern lumpfish. All tanks were connected via a recirculating aquaculture system to ensure uniform water parameters, with temperature maintained between 11-13 ^{o}C and salinity between 28-32 ppt. The room photoperiod was kept at 12D:12L throughout the experiment. Lumpfish were fed at 2% tank biomass over three feeding events per day, using a pellet diet of Amber Neptune (Skretting, UK). Experimental lumpfish were reared under these standardised conditions for five months until August 2017, during which time growth rate, mortality and feeding activity were recorded. The study was undertaken by trained personnel in accordance with the Animals Scientific Procedures Act (ASPA) and received ethical approval from Swansea University, College of Science Ethics Review Committee (IP1516-6.2).

Growth Rate

All lumpfish (n =264) were individually weighed during PIT tagging in March 2017 to give the first time-point for growth analysis. All surviving individuals were weighed again five months later in August 2017 (n =201), giving the second time-point. Specific Growth Rate (SGR) was calculated for each individual and expressed as percentage weight change over time (%), using the formula:

$$\mathrm{SGR} = \mathrm{Ln}\ (W_2 - W_1) / (T_2 - T_1)$$

Where W_2 and W_1 are the weights (g) of an individual and T_2 and T_1 are the time points at which an individual was weighed. Sex was determined through the dissection of gonads at the end of the study (north male, n= 15; north female, n=35; south male, n=37; south female =28), and only sexed individuals were considered in growth analysis to account for dimorphic differences in growth rate.

Statistical analysis was conducted in R (Core Team 2013). A linear model was used to compare the initial weight and growth rate of fish across all tanks and populations.

The full model included population, sex, tank and the interaction between population and sex as predictors. Model residuals were inspected to check that assumptions for linearity, normality and homoscedasticity were met and influential data points were assessed using Cook's Distance. Multi-model inference was used to select the most plausible model via the dredge function in the *MuMIn* package (Barton 2009), with both marginal and conditional coefficients of determination found using the r.squaredGLMM function. The difference in Akaike Information Criterion (ΔAICc) was used to identify models for ΔAICc <2 (Appendix T3.1), model parsimony and weight were used to inform model selection. The final model was refitted using Restricted Maximum Likelihood (REML).

Survival Probability

Each tank was checked twice daily for mortalities to record survival data over the course of the experiment. Frailty models are analogous to regression models performed on individual survival data and provide a hazard function (HF) and variance (θ) term, while shared frailty models also account for random effects among groups commonly referred to as grouping factors (Gutierrez 2002). A shared frailty model was used to assess the effect of population and individual starting weight on the survival probability of individual lumpfish (n =264), fitted using the *frailtypack* package (Rondeau et al. 2012) and including tank (n =8) as a grouping factor. The number of knots used in the model was selected by increasing knots until plots of the hazard function became unstable. Cross validation was used to estimate the best smoothing parameters, and the maximum number of iterations was set at 350 (Rondeau et al. 2012). Variance in the frailty term was used to assess heterogeneity across tanks.

Feeding Activity

Behavioural observations were undertaken at feeding events in order to quantify tank-level foraging behaviour. Observations were conducted for each of the eight tanks twice per day, on two days per week, over a four-week period midway through the experiment in May 2017 (daily, n =16; weekly, n =32; total, n =128). The order in which the numerically labelled tanks were observed was alternated by randomly selecting a different starting tank each day using a number generator (Eddelbuettel 2006) then working sequentially along the tanks, returning to the Tank 1 at the end of the row, until each tank had been fed. As there were eight observation days in total this ensured each tank was observed in every sequential position once during the total observation period. Observations were conducted at the first morning feed (10:00) and last afternoon feed (16:00), during which time the number of lumpfish actively swimming and consuming food pellets was recorded at 30 second intervals for five minutes. This was converted into the average proportion (%) of individuals actively foraging during five minutes, in order to account for different stocking densities as a result of mortality.

A generalised linear mixed model (GLMM), using average proportion of fish swimming as a dependent variable, was fit with a binomial distribution in the *lme4* package (Bates et al. 2007). The full model included fixed terms for population origin (north, south), observation session (morning, afternoon) and the interaction between origin and observation session. All models included a random factor for observation (observation number) and time (day) to account for cumulative observations and a repeated measures design. The full model contained a random factor for the tank in which fish were housed, however, this was removed if a likelihood ratio test (LRT) between the full model with and without the random factor was not significant. Model residuals were checked to verify model assumptions for linearity, normality, homoscedasticity and influential data points were assessed using Cook's Distance. The most plausible model was selected through the dredge function using the *MuMIn* package (Barton 2009), marginal and conditional coefficients of determination were found using the r.squaredGLMM function. Model parsimony and weight were used to inform selection from models within ΔAICc <2 (Appendix T3.2) and the final model was refitted using Restricted Maximum Likelihood (REML).

Results

The initial weight of lumpfish did not vary significantly between tank replicates ($F_{7,178}$ =1.471, P = 0.180). Northern lumpfish showed a significantly faster Specific Growth Rate (0.330 ±0.080, t=4.143, P <0.001), though sex did not account for variation in growth rate (-0.149 ±0.079, t=-1.874, P = 0.064). The average weight of northern lumpfish (n =89, 285.9 ±116.4g) was higher than the average weight of southern lumpfish (n =98, 215.0 ±103.7g) by the end of the five-month period (Figure 3.2).

Analysis of survival data found that northern lumpfish had a significantly lower survival probability (hazard ratio: 0.44, 95% CI: 0.26 – 0.75, P = 0.003) than lumpfish from the south (Figure 3.3). The starting weight of individual fish did not influence survival probability of individuals (Table 3.1). Variance in the frailty term was not significant ($\theta > 0.001$, $P = 0.500$), indicating that survival probability was homogenous across tanks and therefore the tank in which fish were reared had no effect on individual survival (Appendix F3.1).

The average proportion of lumpfish observed actively foraging in tanks was significantly higher in tanks housing northern lumpfish (-0.344 ±0.092, t =-3.758, P <0.001) (Table 3.2) than those containing fish from the south (Figure 3.4). Tank identity, and therefore observation order, had no influence on fish activity (χ^2 =272.990, df =1, P =0.998).

Discussion

The lumpfish reared in the present study were the progeny of two genetically distinct wild populations, unconnected by gene flow, that originated from different geographic regions of the species’ range (Whittaker et al. 2018). When raised in a common environment the hatchery stocks demonstrated different average growth rates, survival probability and feeding activity, suggesting a genetic component influenced variation in the measured fitness traits. The northern lumpfish grew faster and exhibited more active feeding behaviour, whereas, the southern fish showed a higher survival rate. These patterns are consistent with the hypothesis that different populations of lumpfish show different life history strategies as described by the Pace of Life Syndrome hypothesis (POLS) (Dammhahn et al. 2018; Montiglio et al. 2018), as northern lumpfish showed a faster paced life history relative to the southern lumpfish. Investigating additional physiological traits including standard and maximum metabolic rates alongside behavioural factors such as bold-shyness (Auer et al. 2018; Binder et al. 2016), would give greater confidence in describing POLS of lumpfish populations. Stock-level differences in POLS might reflect adaptation by origin populations to different regional pressures (Hereford 2009), and evaluating trade-offs between these fitness traits could inform development of aquaculture practises such as stock translocation and selective breeding programs.

Fast growth provides multiple fitness advantages for fish, as larger body size is often correlated with greater competitive ability to secure territory and mates (Jennions et al. 2001). Given that fecundity is size-dependent in many teleost, attaining a larger body size by the time of maturation improves fitness through enhanced quantity and quality of gametes, thereby benefitting offspring survival rate (Karjalainen et al. 2016; Tsoukali et al. 2016; Yokouchi et al. 2018). However, maintained fast growth may incur fitness costs that lower survival probability, including increased exposure to predators and prey-borne pathogens while foraging (Henriksen et al. 2019; Mangel & Stamps 2001). More energy invested towards fast growth reduces resource availability for allocation towards other physiological process like immune function (Causey 2018; van der Most et al. 2011) and depletes energy reserves that would aid future survival (Ejsmond et al. 2019; Post & Parkinson 2001). The benefits associated with fast growth are ultimately lost if the individual does not survive to reproduce, therefore, the Growth-Maturity Optimisation hypothesis (GMO) predicts that maximum growth rate should not exceed the threshold for mortality before an individual reaches maturity (Morris et al. 2016). The stock differences recorded for growth and survival in the present study suggest that northern lumpfish exceeded the GMO threshold to a greater extent than their southern counterparts, in spite of higher feeding activity. This is disadvantageous for the cleaner fish industry which desires fast growing juveniles, low mortality rates and successful maturation of captive broodstock to facilitate breeding programs (Powell et al. 2018b). Although GMO is effected by factors influencing spatial and temporal food availability, like population density and prey abundance (Vrtílek et al. 2019), these conditions were standardised during the present study and do not represent likely candidates driving stock variation. Different growth rates are found between genetically distinct populations of anchovy (*Engraulis encrasicolus*) across Europe (Huret et al. 2019). Wider patterns of life history variation in which include growth rate, maturation and longevity are reported in fish populations along latitudinal gradients, however, this trend usually favours faster life history strategies by populations at lower latitudes (Denit & Sponaugle

2004; Hughes et al. 2017; L. Trip et al. 2014; Vinagre et al. 2009) unlike the current findings for lumpfish. Ectotherms may favour faster life history strategies at higher latitudes through a process of counter gradient variation (Conover & Schultz 1995), presumably in response to seasonal fluctuation in temperature and food availability (Conover & Present 1990), though the physiological mechanisms underlying this process are yet to be fully determined (Lenard & Gifford 2019; Martin & Targett 2019; Rödin-Mörch et al. 2019). Comparative analysis of growth and survival rates are lacking in wild populations of lumpfish (Kasper et al. 2014) though variation in age at maturity is documented along a latitudinal gradient in Greenland (Hedeholm et al. 2017), implying geographic trends are associated with population-level life history variation in the species. Transplant studies comparing the performance of northern stocks reared in southern hatcheries and southern stocks reared in northern hatcheries could help elucidate this unexpected trend in growth rate.

Multiple factors influence latitudinal clines in life history, including population density and ecosystem productivity, though temperature gradients across latitudes are considered a key component (Hedeholm et al. 2017; Riesch et al. 2018). The thermal-tolerance of marine fish is shown to determine a species' geographic distribution, as ectothermic populations contract from regions in which temperature regime adversely affects physiological function (Sunday et al. 2012). This process can be illustrated through aerobic scope, which is defined as the difference between standard metabolic rate and maximum metabolic rate (Fry 1971) and represents the capacity to undertake physiological processes including growth, activity and reproduction (Nati et al. 2016). When exposed to warm conditions cold-water fish, including lumpfish, show restricted cardiorespiratory function which decreases oxygen transportation throughout the body and reduces aerobic scope thereby increasing mortality risk (Ern et al. 2016; Jensen et al. 2017; Killen et al. 2006). As lumpfish naturally operate within a narrow aerobic scope and therefore possess a limited ability to satisfy physiological processes (Killen et al. 2007), sensitivity to warm water is hypothesised to determine geographic distribution by creating thermal-niche boundaries (Hvas et al. 2018). However, the geographic distribution of the species stretches along a considerable temperature gradient, spanning from the Arctic Ocean down to the Mediterranean Sea (Powell et al. 2018a), which seems at odds with the thermal-niche boundary hypothesis. Most studies concerning lumpfish physiology focus on a single genetic population (Ern et al. 2016; Hvas et al. 2018; Killen et al. 2007) therefore it is unknown whether different regional populations show variation in thermal tolerance, though intraspecific variation in thermal-niche is observed across populations of other widespread fish species (Dabruzzi et al. 2019; Feiner et al. 2017; Matte et al. 2019; Raby et al. 2016; Santiago et al. 2016). Population differences in ability to occupy thermal-niche would account for the wide latitudinal distribution of this aerobically restricted species, and could explain the contrasting performance of stocks in the present study. Higher feeding activity indicated northern lumpfish struggled to satisfy energy requirements under the experimental conditions relative to southern fish (Killen et al. 2007), perhaps owing to a faster growth rate, and therefore showed increased mortality. Indeed, latitudinal variation in the onset and duration of lumpfish spawning season could reduce latitudinal gene flow across European populations (Whittaker et al. 2018), representing a potential mechanism for genetic isolation by thermal-niche. In order to test this new hypothesis further common-garden experiments utilising three or more populations obtained from high, mid and low latitudes are needed, alongside respirometry data to compare the aerobic scope of each population under a range of temperatures.

Stomach content analysis of lumpfish on salmon farms shows variation in foraging behaviour, with a higher proportion of lumpfish found to have empty stomachs than containing sea-lice (Eliasen et al. 2018; Imsland et al. 2014a). Different families of lumpfish show variation in the type and quantity of prey consumed, which suggests a genetic component influences foraging behaviour in farms (Imsland et al. 2016a). The present study also found differences in feeding activity between stocks, with tanks containing northern lumpfish exhibiting higher activity during feeding events. This is consistent with a difference in POLS between the stocks, as fish following a faster life history pace demonstrate rapid growth, high activity levels and forage frequently (Auer et al. 2018; Binder et al. 2016; White et al. 2016). As faster growing lumpfish showed greater feeding activity it is logical to assume these individuals are more likely to consume prey items on salmon farms, including sea-lice, in order to maintain rapid growth. However, for logistical reasons the present study only provided a pellet diet and did not assess sea-lice consumption, therefore variables such as individual variation in food preference and ability to interact with salmon need further investigation in order to examine the relationship between pellet consumption and delousing.

The interaction between growth, survival and feeding activity observed in the two regional stocks presents a conundrum for the development of sustainable lumpfish aquaculture. Hatcheries desire fast growth in order to reduce the time taken for juvenile lumpfish to reach farm deployment size (Imsland et al. 2019b; Powell et al. 2018b) and the onset of breeding programs is set to favour selection for improved growth rate (Sae-Lim et al. 2020). As faster growth rate is associated with higher feeding activity, this could potentially benefit delousing rates by lumpfish in salmon farms. However, the fitness trade-off identified in the present study implies selection for faster growth could decrease survival and therefore fewer lumpfish will survive in farms. Contrariwise, selection for enhanced survival may slow stock production and possibly reduce feeding activity. In order to achieve sustainable breeding programs, the industry needs to find compromise between rapid hatchery production and the longevity of cleaner fish on farms. It cannot be assumed that commercial stocks will respond in a homogenous manner under aquaculture conditions and better understanding stock-specific husbandry requirements should aid in tailoring hatchery conditions, for example by providing different diets, feeding regimes or environmental enrichment (Imsland et al. 2015; Johannesen et al. 2018a). A stock-specific approach to husbandry should extend throughout the aquaculture process, with hatchery data on genetic origin and phenotype informing suitable farm deployment locations. Stocks are currently transported against a latitudinal gradient, primarily from Iceland to the Faroe Islands (Johannesen et al. 2018b), Ireland (Bolton-Warberg 2018) and Scotland (Treasurer et al. 2018) and latitudinally along the Norwegian coast (Jónsdóttir et al. 2018b). Although further research is needed to establish the presence of local adaptation and factors driving differentiation between wild populations, this study demonstrated life history variation in regional stocks commonly translocated on an international scale and highlights the need to develop greater awareness of how the species performs in aquaculture.

Acknowledgments

I wish to thank Mia Berwick, Niall Coates, Ben Jennings, Jessica Minett, Andre Pires, Maria Scolamacchia and Rob Smith for assistance in collecting weight data and recording tank mortalities.

Table 3.1. Shared frailty model for the survival probability of lumpfish, accounting for stock origin and initial weight of individuals at the start of the experiment. Output includes the coefficient, standard error (SE), hazard ratio (HR), Wald statistic (Z) and significant probability (*P*) shown in bold.

Factor	Coef.	±SE	HR	Z	*P*
Stock	-0.815	0.271	0.443	-3.011	**0.003**
Initial Weight	0.005	0.003	1.005	1.877	0.061

Table 3.2. Most plausible models for Specific Growth Rate (SGR) and foraging activity. Values include marginal (R2m) and conditional (R2c) coefficients of models and significant *P* values shown in bold.

Trait	Factor	Estimate ±SE	R2m	R2c	t	*P*
SGR	Origin	-0.330 ±0.080	0.193	0.272	4.143	**<0.001**
	Sex	-0.149 ±0.079			-1.874	0.064
Activity	Origin	-0.344 ±0.092	0.190	0.192	-3.758	**<0.001**

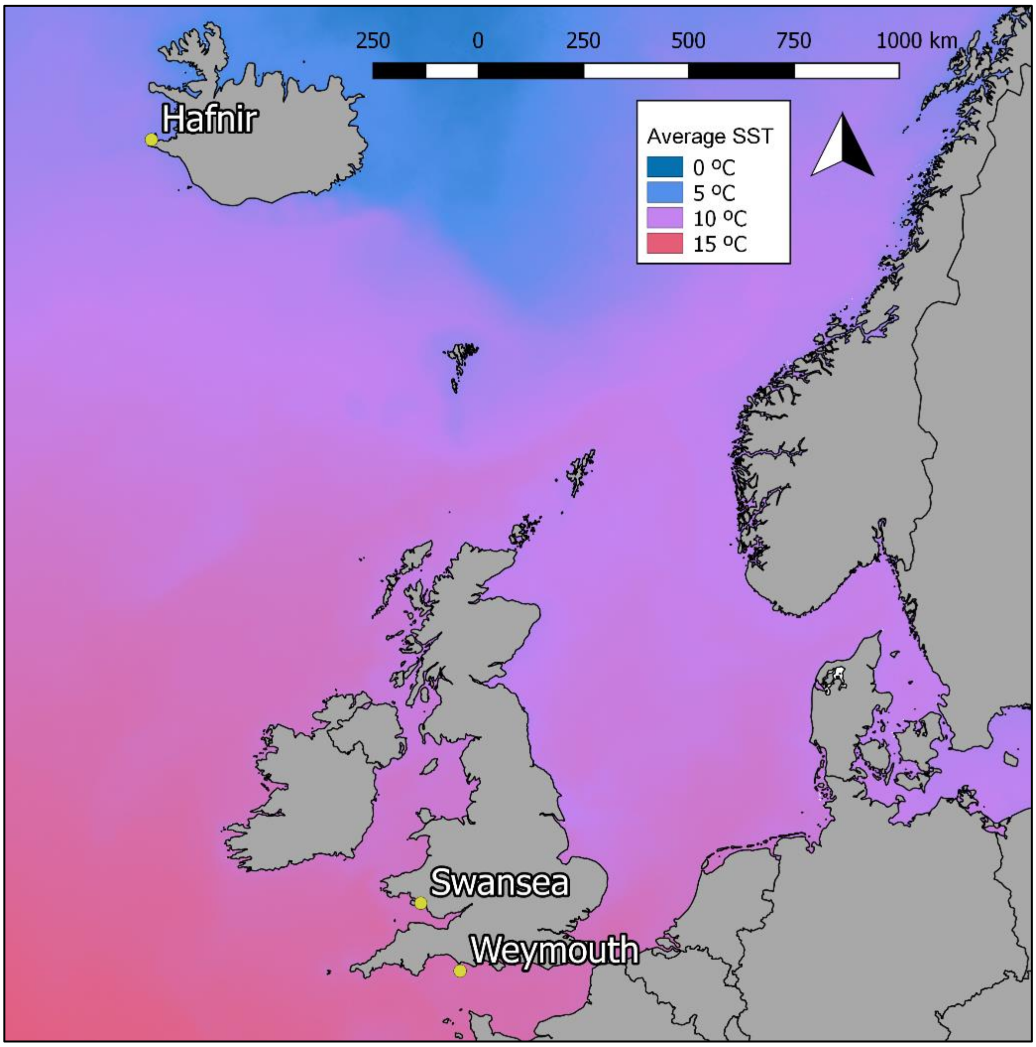

Figure 3.1. Mean sea surface temperature (SST) at the locations of origin of the experimental stocks, Hafnir (Iceland) and Weymouth (England) as well as the common rearing location (Swansea, UK), obtained from marine climate archive (Sbrocco & Barber 2013).

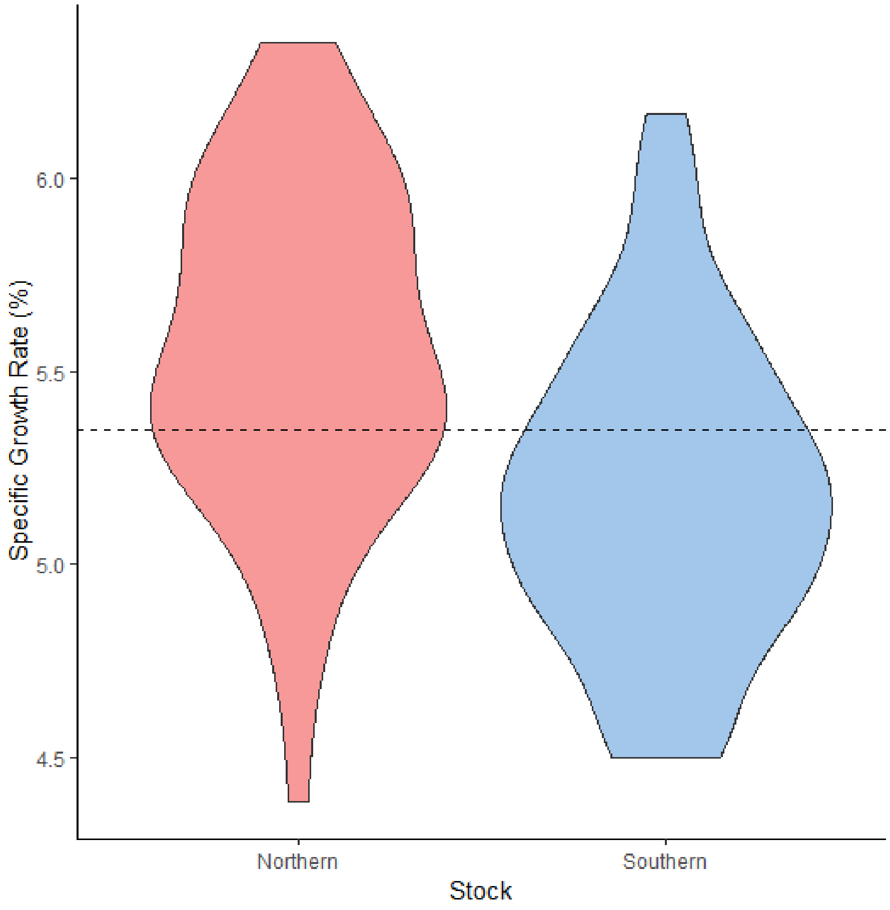

Figure 3.2. Spread and distribution of Specific Growth Rates (SGR) for lumpfish originating from northern and southern populations reared in a common-garden experiment from March to August 2017. Dotted line shows median SGR across all individuals.

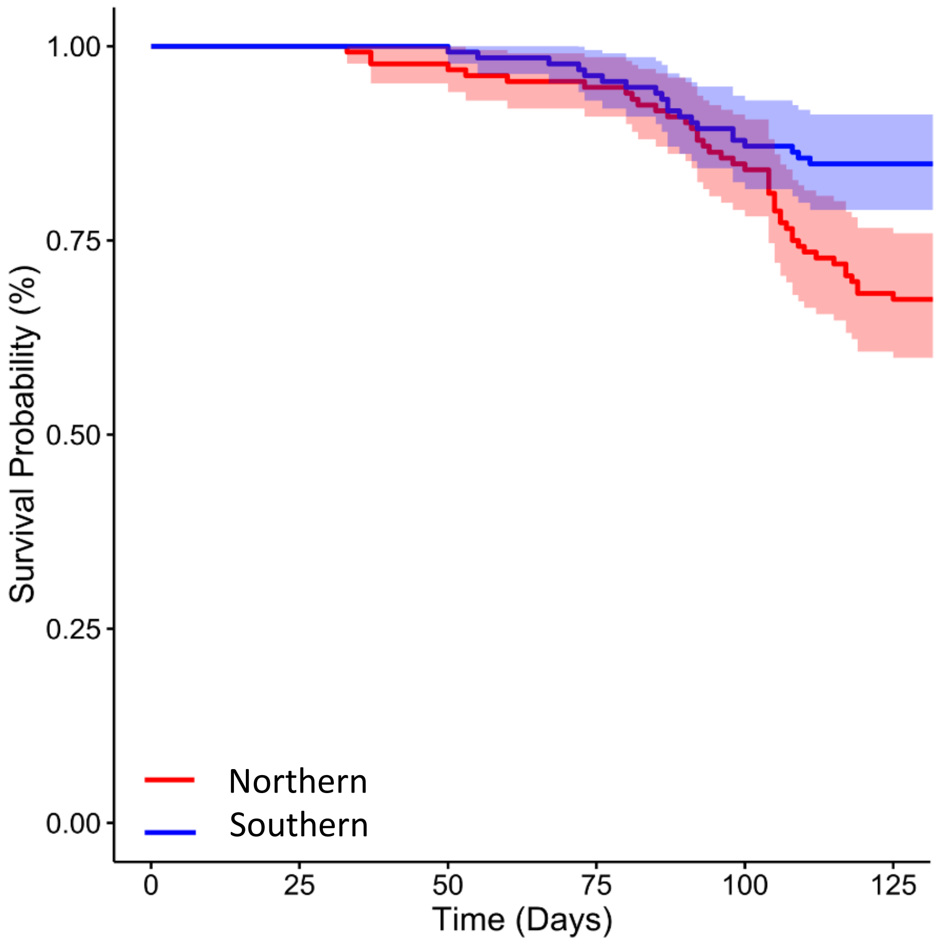

Figure 3.3. Survival probability of northern and southern lumpfish stocks reared in a common-garden experiment from March to August 2017.

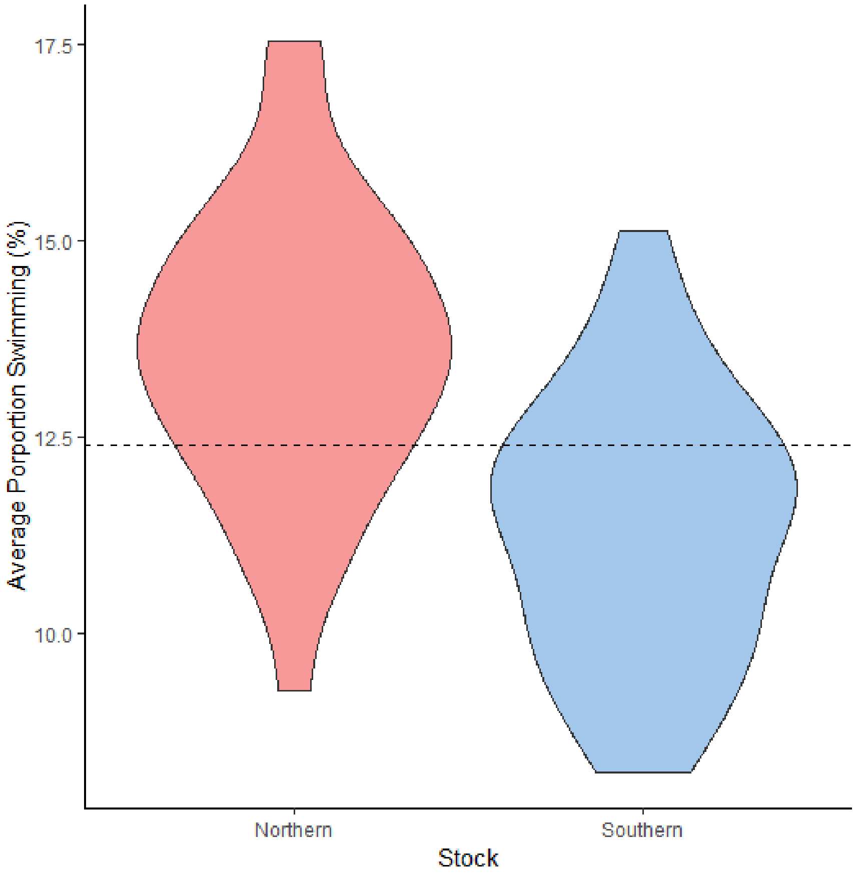

Figure 3.4. Spread and distribution of average proportion of lumpfish swimming (%) during observations of northern and southern stocks reared in a common-garden experiment from March to August 2017. Dotted line shows median proportion of activity across all observations.

Chapter 4

Personality influences facultative cleaner-client interactions between lumpfish (*Cyclopterus lumpus*) and Atlantic salmon (*Salmo salar*).

Abstract

Little is known of the interaction between facultative cleaner fish and their clientele, though individual variation in cleaning efficacy is reported among species deployed on salmon farms and show some individuals remove parasitic sea-lice whilst others compete for salmon pellets. Research has largely focused on tropical, dedicated species of cleaner fish and illustrate differences in personality traits influence client engagement. This study followed assumptions used in personality profiling by screening for traits associated with successful dedicated cleaners to determine whether cold-water, facultative cleaners sharing similar traits are also predisposed to client interaction. Repeatable variation was quantified for five traits in lumpfish (*Cyclopterus lumpus*), the most commonly used cleaner species on salmon farms, and variation in the interaction with Atlantic salmon (*Salmo salar*) was then observed. Bolder individuals were found to pursue salmon for longer, whereas, lumpfish exhibiting a syndrome for social-aggression were observed to incur more frequent jolt rates, which may imply antagonism. These findings reflect those established for dedicated cleaner fish, suggesting variation in personality is also an important aspect of facultative cleaning. The screening protocols developed in this study could inform more efficient use of facultative cleaner fish in aquaculture.

Introduction

Personality profiling is used by psychologists to identify common personality traits amongst individuals exhibiting a focal behaviour, on the premise that others sharing similar traits are also predisposed to the focal behaviour and can therefore be detected within a wider group via behavioural screening (McCann 1992). The methodology was pioneered in the 1970s by the FBI Behavioural Science Unit to aid police through generating the profiles of criminals responsible for violent acts (Pinizzotto 1984), but has since been developed to identify those susceptible to mental health conditions (Oyeleke et al. 2017; Papava et al. 2015) and inform candidate selection within business programs (Wahab et al. 2017; Wilsher 2015). Screening often incorporates the five-factor model of personality, quantifying openness to risk, conscientiousness, extraversion, agreeableness and neuroticism (Neal et al. 2012; Van Dijk et al. 2017), which is well established as a robust model for investigating multiple dimensions surrounding a focal trait (Costa Jr & McCrae 1990). The study of personality in non-human animals, also referred to as temperament, coping style or consistent individual differences, investigates the adaptive function of behavioural variation between individuals both temporally and across different contexts (Dingemanse et al. 2010; Piersma & Drent 2003). Correlated personality traits form behavioural syndromes which, through limiting behavioural plasticity, carry ecologically significant trade-offs for behaviours such as cognitive style, migration and response to anthropogenic factors (Found & Clair 2016; Sih et al. 2012; Sih & Del Giudice 2012). Numerous studies have demonstrated the repeatability of personality traits in a variety of taxa, including fish (Castanheira et al. 2017; Elias et al. 2018; Vargas et al. 2018), therefore, screening for repeatable personality traits may allow the identification of non-human individuals predisposed to focal behaviours.

The cleaning mutualism is a behavioural interaction in which an individual referred to as the cleaner removes and consumes ectoparasites from an individual referred to as the client (Bshary & Côté 2008). Species of cleaner fish can be classed as either dedicated cleaners, which heavily rely on cleaning to meet dietary needs, or facultative cleaners, which show greater variation in the extent of cleaning behaviour (Vaughan et al. 2017). Research has focused on iconic species of tropical, dedicated cleaner fish such as the bluestreak cleaner wrasse (*Labroides dimidatus*) (Grutter 2010). Dedicated cleaners display sophisticated behavioural interactions with clients in order to better facilitate the mutualism, including tactile stimulation with fins to ease client aggression (Bshary & Würth 2001; Grutter 2004; Soares et al. 2011), posing to advertise receptiveness (Côté 2000), and social eavesdropping to maintain cooperative reputations in the presence of bystanders (Bshary & Grutter 2006; Pinto et al. 2011). However, dedicated cleaners may also cheat the mutualism and parasitize clients by consuming skin or mucus (Cheney & Côté 2005), often followed by aversive jolting behaviour from the client (Bshary & Grutter 2002; Soares et al. 2008a). The personality of tropical cleaners is found to influence interaction with client fish, for example more active sharknose goby (*Elacatinus evelynae*) have reduced rates of cleaning while bolder individuals pose to clients more frequently (Dunkley et al. 2019). Bluestreak cleaner wrasse with artificially raised cortisol levels are shown to favour larger clients (Soares et al. 2018), indicating that anxiety-level influences client preference. Social communication between cleaners and clients forms a key component of the mutualism (Vaughan et al. 2017), whereas, parasitic interactions may be associated with aggression-levels of cleaners (Cardoso et al. 2015), implying individual differences in sociality and aggression are also important aspects of cleaner-client interaction. Although facultatively cleaning species are more numerous and widespread than the dedicated cleaners of the tropics (Baliga & Mehta 2018), studies investigating facultative cleaner-client interactions are relatively uncommon (Narvaez et al. 2015) and therefore little is known regarding the dynamics of this behaviour. However, individual variation is reported in the expression of facultative cleaning, with some individuals attending clients whilst others do not show any cleaning behaviour (Morado et al. 2019). Understanding this variation in facultative cleaning is of particular importance to the salmon farming industry, which deploys cold-water, facultative cleaner fish into farms to remove parasitic lice from salmon (Bjordal 1988; Kousoulaki et al. 2018; Treasurer 2002). Studies suggest only a minority of facultative cleaners (approx. 15 - 30%) consume lice on farms, whilst the majority compete with salmon for pellet food or graze on plankton (Deady et al. 1995; Eliasen et al. 2018; Imsland et al. 2014a; Powell et al. 2018b). The ability to screen an individual's predisposition to cleaning prior to deployment would enable the industry to identify elite cleaner fish for stocking farms, and select individuals showing desirable personality traits to establish breeding programs (Imsland et al. 2016a; Jónsdóttir et al. 2018a).

This study aimed to use personality profiling as a method to identify facultative cleaner fish predisposed to client interaction, thereby allowing more efficient use of cleaner fish on salmon farms. Given the lack of available information on facultative cleaning, I hypothesised that personality profiles associated with successful dedicated cleaners, such as high boldness and low activity, were similar to the personality traits of successful facultative cleaners. I screened the personality of lumpfish (*Cyclopterus lumpus*), which is the most commonly deployed species of cleaner used by the salmon farming industry (Powell et al. 2018b), according to the five-factor model for non-human animal personality (Gosling & John 1999). Though lumpfish have been shown to effectively remove and consume salmon lice (Eliasen et al. 2018; Imsland et al. 2018b), as with other facultatively cleaning species (Morado et al. 2019), the dynamics of lumpfish-salmon interaction are largely unknown. I therefore observed the interaction of screened lumpfish with uninfected Atlantic salmon (*Salmo salar*) to assess whether personality influenced interaction in the predicted manner, whilst developing baseline knowledge on lumpfish-salmon interaction. Whilst the absence of lice may have reduced overall interaction rates, it should allow preliminary detection of mutualistic or antagonistic interaction without confounding variation attributed to the parasite.

Materials and Methods

Experimental Lumpfish

Lumpfish eggs were collected from wild adults caught in Iceland and the English Channel, representing an unknown number of families from two genetically distinct populations (Whittaker et al. 2018). In March 2016, the eggs were hatched at the Centre for Sustainable Aquatic Research (CSAR), Swansea University (UK), and the juveniles were then reared under standard aquaculture conditions for one year (Treasurer et al. 2018). In March 2017, experimental fish (114.8 ±9.7 g) were PIT tagged (7 x 1.35 mm, Loligo) to allow individual identification, and housed in eight 1500 litre tanks (1.4 m diameter, 0.9 m depth) at a stocking density of 33 fish per tank (initial biomass = 75.37 ±30.99 g/m^3). Tanks were connected through a recirculating aquaculture system, ensuring uniform water parameters of 11-13^{o}C and 28-32ppt salinity. Lighting was maintained at a 12D:12L photoperiod. Lumpfish were fed a pellet diet of Amber Neptune (Skretting, UK) at 2% of tank biomass.

The study was undertaken with ethical approval from Swansea University, College of Science Ethics Review Committee (IP1617-27).

Experimental Setup

Behavioural screening took place at CSAR from March to July 2017, when the lumpfish were approx. 1 year old. Lumpfish (Iceland, n = 16; England, n = 22) were analysed twice, with a four-week interval between trials in order to assess the repeatability of behaviour over time. Behavioural screening was undertaken in white, rectangular tanks (55 cm x 120 cm x 25 cm) fitted with air stones midway along the tank to maintain oxygen levels at 100% saturation (Figure 4.1). Water in the screening tanks was replaced between each fish. A CCTV camera system with 1080p cameras (Sannce, Hong Kong) was installed above each tank to continuously record behaviour. The filming area was curtained with tarpaulin sheets in order to minimise the effect of background stimuli on fish behaviour.

Screening Lumpfish Personality

Fish personality was quantified using the five-factor model for nonhuman animal personality (Gosling & John 1999), with behaviours measured to score activity, aggression, anxiety (neuroticism/stress coping style), boldness (openness/willingness to take risk) and sociality (extraversion/agreeableness). Behavioural screening was conducted over four consecutive phases, each lasting for 10 minutes. Starting in the Neutral Phase, fish were left undisturbed to acclimatise without the addition of any stimulus in the tank. During the Shelter Phase a black, acrylic panel was introduced to one end of the tank providing a refuge, as lumpfish preferentially attach onto smooth, dark substrates (Imsland et al. 2015). In the Mirror Phase an acrylic mirror was added at the opposite end of the tank to the shelter. Finally, in the Novel Phase, a novel object was placed in the centre of the tank (Figure 4.1). To prevent habituation to a novel stimulus, the object was a yellow golf ball in the first repeat and a green Lego brick in the second repeat. Phases were conducted in the same order for every fish. To determine whether individual growth rates influenced measured behaviours, the weight of fish was recorded prior to each repeat of screening and Specific Growth Rate (%) was calculated as:

$$\mathrm{SGR} = \mathrm{Ln}\ (W_2 - W_1) / (T_2 - T_1)$$

Where W_2 and W_1 are the weights (g) of an individual and T_2 and T_1 are the time points at which the individual was weighed.

Behavioural tests were conducted for each personality trait across different phases of screening in order to determine whether individual differences influenced cleaner-client interaction. Activity was recorded by the total time an individual spent swimming throughout the tank across all four screening phases to give the total *swimming duration*. During video analysis of the Neutral Phase three equal sections (40 cm) were superimposed over the tank and the time taken for fish to cross the central section was recorded to calculate *crossing speed* (cm per second), alongside the number of times fish travelled between the sections to give *crossing frequency* as two further measures of individual activity. A mirror test was conducted during the Mirror Phase by counting the *bite frequency* of individuals making mouth contact with their own reflection to score aggression (Scherer et al. 2016). The number of times an individual approached the mirror but did not make body contact (*mirror frequency*) and the length of time individuals spent in close proximity (<10 cm) with the mirror (*mirror duration*) were both recorded in the Mirror Phase to quantify individual sociality (Cattelan et al. 2017). An open-field test was conducted during the Neutral Phase, recording the total time an individual spent within a 10cm perimeter of the tank walls (*perimeter duration*), whilst a shelter-seeking test was carried out during the Shelter Phase by recording the total time an individual spent in close proximity (<10 cm) to the black panel (*shelter duration*). Open-field and shelter-seeking tests are both established methods of assessing behavioural anxiety in fish (Cianca et al. 2013; Godwin et al. 2012). In the Novel Phase the number of times an individual closely approached the novel object (*novel frequency*) and the time latency to first approach the object (*novel latency*) were recorded to quantify individual boldness (Thomson et al. 2016).

Quantifying Cleaner-Client Interactions

Atlantic salmon (*Salmo salar*) smolts (n = 20, 175.6 ±37.6g) were obtained from the Marine Harvest Scotland production site at Loch Ness, Inverness in July 2017. The salmon were quarantined at CSAR for one month in two 1500 litre recirculating aquaculture tanks (1.4m diameter, 0.9m depth), at a density of 10 salmon per tank (initial biomass = 117.04 ±25.06 g/m^3). In August 2017, lumpfish (n = 38) which had previously been screened for personality traits were individually introduced into the salmon tanks and recorded via CCTV for 20 minutes. An ethogram was constructed using literature for dedicated cleaner fish (Bshary & Côté 2008; Horton 2011) in order to evaluate facultative interactions between lumpfish and salmon, and whether such behaviours were observed between the two species was noted in the ethogram (Table 4.1). Salmon were not infected with sea-lice during observations which may have reduced overall cleaner-client interaction, particularly once lumpfish had first inspected salmon and detected no parasites. However, the absence of lice allows for baseline observation of lumpfish-salmon interaction and to detect the presence of mutualistic interaction (e.g. inspection) or antagonistic interaction (e.g. jolting) occurring between the two species, whilst controlling for any variation (e.g. parasite load or life stage) that sea-lice may introduce to the interaction. All lumpfish were naive to salmon during their trial and salmon were naive to lumpfish at the beginning of trials, however, the cumulative exposure of salmon to lumpfish over successive trials was addressed in statistical analysis.

Data Analysis

Video recordings were scored using the event-scoring software BORIS, version 6.24 (Friard & Gamba 2016), by a single observer who was blind to trial conditions. Statistical analysis was conducted in R, version 3.5 (Core Team 2013).

Identifying Covariates of Personality

Adjusted repeatability estimates the repeatability (R) of traits by accounting for the influence of significant covariates through their inclusion as fixed or random effects in repeatability models (Nakagawa & Schielzeth 2010). The inclusion of significant covariates in repeatability models was first determined using linear mixed models (LMM) and generalized linear mixed models (GLMM) (Nakagawa & Schielzeth 2010; Schuster et al. 2017) applied using the *lme4* package (Bates et al. 2007). Dependent variables for LMM included *swimming duration*, *crossing speed, mirror duration*, *shelter duration*, *perimeter duration* and *novel latency*, and GLMM fit with Poisson distribution used the dependent variables of *crossing frequency*, *bite frequency*, *mirror frequency* and *novel frequency*. Geographic origin (Iceland, UK), sex, specific growth rate (%) of individuals, the tank in which lumpfish were housed (Iceland, n = 4; England, n = 4) and the time period between screening repeats (days) were used as fixed effects in the full models. Fish ID was included as a random factor in all models to account for repeated measures (Schuster et al. 2017). The most plausible model was selected using the dredge function applied with the *MuMIn* package (Barton 2009), using ∆AICc to assess the weight of support attributed to each model (Appendix. T4.1). The distribution of model residuals was plotted to check model assumptions and influential data points were assessed using Cook's Distance. Data for *crossing speed* and *mirror duration* was log10 transformed, and the *swimming duration* and *shelter duration* was square-root transformed to meet model assumptions. The most plausible model was compared to the null model using a Likelihood-Ratio Test (LRT) to determine whether model fit had improved (Appendix. T4.2), marginal and conditional coefficients of determination were obtained through the r.squaredGLMM function, and the final model was refitted using Restricted Maximum Likelihood (REML).

Repeatability of Personality

Repeatability (R) quantifies the consistency of measurements through assessing within-group and between-group sources of variation, referred to as intra-class correlation (ICC), with estimates ranging from R = 0 (no repeatability) to R = 1 (perfect repeatability) (Nakagawa & Schielzeth 2010). The repeatability of behaviour measured for individuals during screening was analysed using the *rptR* package (Stoffel et al. 2017). Adjusted repeatability for the *swimming duration, crossing speed*, *crossing frequency*, *bite frequency*, *mirror frequency*, *mirror duration*, *perimeter duration*, *shelter duration*, *novel frequency* and *novel latency* was estimated through the inclusion of appropriate covariates as previously identified through LMM and GLMM (Nakagawa & Schielzeth 2010; Schuster et al. 2017). Repeatability (R) and 95% confidence intervals were calculated using 1000 bootstrap runs and 1000 permutations for each dependent variable. Models were fitted using the rptGaussian or rptPoisson function depending on the distribution of each dependent variable. Behaviour was considered repeatable if the lower 95% confidence interval did not fall below or include a value of 0 (Neumann et al. 2013). As with other studies on animal personality, $R < 0.2$ was considered to show low repeatability, $0.2 \leq R \leq 0.4$ showed moderate repeatability and $R > 0.4$ denoted strong repeatability (Bohn et al. 2017). Behaviours with values that were either non-repeatable (lower confidence interval below 0) or had low repeatability ($R > 0.2$) were excluded from further analysis.

Effects of Personality on Cleaner-Client Interactions

Behaviours that showed moderate to high levels of repeatability ($R > 0.2$) were averaged across screening repeats to give each fish a single value per measured behaviour, and average values were then used to generate personality scores for individual fish (Gosling & John 1999; Wilson et al. 2014). The average *bite frequency* and average *novel frequency* were both rescaled from 0 to 1, using the *scales* package (Wickham 2016), to provide comparable scores for aggression and boldness respectively. The average *swimming duration*, average *crossing speed* and average *crossing frequency* were included in principal component analysis (PCA) to give the activity score (PC1 - 61.1%), average *mirror frequency* and the average *mirror duration* were used in PCA to produce the sociality score (PC1 - 58.6%), and the average *shelter duration* and average *perimeter duration* were used in PCA to give the anxiety score (PC1 - 52.5%) (Appendix. T4.3). Scores for activity, sociality and anxiety were also rescaled from 0 to 1 using the *scales* package (Wickham 2016) to aid comparison of scores. A correlation matrix including each of the five personality factors was created using the *Hmisc* package (Harrell Jr & Dupont 2006) in order to assess whether these traits showed correlation with one another to form a behavioural syndrome (Sih et al. 2012).

In order to assess the influence of lumpfish personality on interactions with salmon, the most common behaviours recorded with the cleaner-client ethogram (Table 4.1) were analysed in linear mixed models using the *lme4* package (Bates et al. 2007). Dependent variables included the total time each lumpfish spent inspecting (secs) or pursuing (secs) salmon, and the jolt rate of salmon (jolt per min). Full models included fixed terms for each of the five personality scores (activity, aggression, anxiety, boldness and sociality), the geographic origin of fish (UK or Iceland), sex, specific growth rate of fish and the salmon observation tank. The trial day was included as a random factor to account for the cumulative exposure of salmon to lumpfish and was included in all models. The most plausible model was selected through the dredge function applied using the *MuMIn* package (Barton 2009), with ΔAICc used to assess the weight of support behind each model (Appendix. T4.4). Model assumptions were checked through plotting the model residuals and the influence of data points were assessed using Cook's Distance. Data for time chasing was square-root transformed and data for time inspecting and jolt rate were log10 transformed in order to meet test assumptions. The most plausible model and the null model were compared using a Likelihood-Ratio Test to determine if goodness-of-fit (Appendix T4.2), marginal and conditional coefficients of determination were found using the r.squaredGLMM function, and the final model was refitted using Restricted Maximum Likelihood (REML).

Results

Identifying Covariates of Personality

The number of times individual lumpfish crossed the tank was influenced by sex (0.524 ±0.219, z = 2.400, *P* = 0.016) and specific growth rate (0.635 ±0.200, z = 3.181, *P* = 0.001), as females and faster growing fish crossed the tank more frequently. The genetic origin of lumpfish influenced the amount of times individuals spent around the tank perimeter (0.056 ±0.028, t =2.016, *P* =0.047) and the number of approaches made to novel objects (-1.632 ±0.386, z =-4.171, *P* <0.001), with fish of UK origin spending more time in the centre of tanks and lower levels of interaction with novel objects. Lumpfish also showed lower *mirror frequency* in the second repeat of screening (-0.005 ±0.001, z =-3.783, *P* <0.001). Other covariates included in linear mixed models and generalized linear mixed models did not influence variation in the measured behaviours (Table 4.2). Therefore, sex and specific growth rate were included in adjusted repeatability models for *crossing frequency*, geographic origin was included in models for *perimeter duration* and *novel frequency*, and time between trials was included in models for *mirror frequency*.

Repeatability of Personality

Repeatability estimates showed that *crossing speed* (R =0.451 ±0.125, *P* =0.001), *swimming duration* (R =0.460 ±0.126, *P* =0.001), *bite frequency* (R =0.661 ±0.268, *P* =0.009) and *mirror frequency* (R =0.677 ±0119, *P* <0.001) were strongly repeatable behaviours (R > 0.4) and that *crossing frequency* (R =0.338 ±0.147, *P* =0.018), *mirror duration* (R =0.293 ±0.114, *P* =0.038), *perimeter duration* (R =0.269 ±0.097, *P* =0.001), *shelter duration* (R =0.217 ±0.141, *P* =0.009) and *novel frequency* (R =0.398 ±0.193, *P* =0.033) were moderately repeatable behaviours (0.2 ≤ R ≤ 0.4) (Table 4.3). However, *novel latency* was not repeatable (R = 0.061 ±0.095, *P* = 0.101) and was therefore omitted from further analysis. A correlation matrix found a positive relationship between the aggression score and sociality score for lumpfish (R(37) = 0.360, *P* = 0.028), though no further behavioural syndromes were identified between the other personality factors (Table 4.4).

Cleaner-Client Interactions

The most commonly observed interactions between lumpfish and salmon included lumpfish visually inspecting salmon (37.1% of total observations), lumpfish pursuing salmon (28.5% of total observations) and salmon jolting from lumpfish (34.4% of total observations) (Figure 4.2).

Lumpfish which scored highly for boldness, those that displayed greater *novel frequency*, spent more time pursuing salmon (17.932 ±3.846, t =4.663, *P* <0.001). None of the variables included in the linear mixed model accounted for significant variation in the time lumpfish spent inspecting salmon (Table 4.2). Lumpfish that scored highly for aggression incurred more frequent jolt rates when interacting with salmon (0.523 ±0.225, t= 2.335, *P* =0.026), as did lumpfish that scored highly for sociality (0.925 ±0.313, t =2.955, *P* = 0.006), and had a faster specific growth rate (0.349 ±0.106, t =3.297, *P* = 0.002) (Figure 4.3). Other factors included in models, such as sex, origin and observation tank, were not found to influence cleaner-client interactions.

Discussion

This study found that lumpfish showed individual variation in the extent of client engagement, and the personality profiles of lumpfish which exhibited higher interaction rates were similar to the profiles reported for dedicated cleaner fish also showing greater client engagement (Dunkley et al. 2019; Wilson et al. 2014). Repeatable personality traits demonstrated that bolder individuals pursued clients for longer, whilst a syndrome for social-aggression was associated with lumpfish incurring frequent salmon jolts. These results build on an growing interspecific trend of personality influencing cleaner-client interaction, which could hold significance for the utilisation of other cleaner fish within aquaculture in terms of hatchery management and breeding programs (Imsland et al. 2016a; Powell et al. 2018b).

Bluestreak cleaner wrasse manipulate client behaviour in order to reduce predation risk, using tactile fin stimulation and visual displays to calm larger clients to an extent where the wrasse willingly clean inside the mouths of predatory fish (Cheney & Côté 2005). These Machiavellian behaviours are not observed in all cleaning systems (Soares et al. 2008b) and the degree to which other species, particularly cold-water cleaners, manipulate clients is largely unknown (Narvaez et al. 2015). Lumpfish were not observed to interact with salmon via tactile stimulation or communicate through posing in the present study which implies this species does not manipulate client behaviour in the same manner as Machiavellian cleaners, however, developing a species-specific ethogram for lumpfish may detect refined behaviours absent from the broader ethogram used in the present study. An inability to pacify clients could incur a greater degree of risk during client interaction for non-Machiavellian cleaners. Indeed, mortality rates are higher in populations of goby exhibiting cleaning behaviour than in non-cleaning populations of the same species, which supports the notion that client interaction carries an element of risk (White et al. 2007). This may account for finding that bolder lumpfish pursued salmon for longer, indicating that the individuals most open to taking risk are those more likely to interact with clients. An interspecific trend of bolder individuals exhibiting greater client interaction is reported in the posing behaviour of sharknose goby (Dunkley et al. 2019) and cheating behaviour of bluestreak cleaner wrasse (Wilson et al. 2014), which may hold wider implications for the cleaning mutualism and could be investigated in additional species from different regions.

Client jolts are a well-established indicator of cheating by dedicated cleaners, occurring in response to the consumption of client tissue or mucus rather than ectoparasite removal (Bshary & Grutter 2002). The nature of client jolts during interaction with facultative cleaners is yet to be determined and could indicate either an honest or antagonistic interaction (Morado et al. 2019). Salmon in the present study were not infected with lice, which may have reduced overall interaction rates, yet some lumpfish readily interacted without triggering jolts (implying honesty) whereas others incurred frequent jolt rates (implying antagonism). Although lumpfish are a demonstrated cleaner fish (Eliasen et al. 2018; Imsland et al. 2018b), personality screening found individuals incurring greater jolt rates also exhibited a syndrome for social-aggression, lending to a more antagonistic interpretation of salmon jolting. Cheating behaviour aligns well with the opportunistic foraging behaviour often described for lumpfish in farms (Imsland et al. 2014a; Imsland et al. 2016b) and is more common in artificial environments (Soares et al. 2008a). However, the absence of parasitic lice limits current understanding of this behaviour and requires further trials on infected salmon.

In the present study, 34% of lumpfish interacted with salmon at rates above the group average of 25 interactions per 20 minutes, and the majority of lumpfish had limited engagement with salmon (Figure 4.2). This aligns with the approx. 30% of lumpfish reported to consume lice on farms (Imsland et al. 2018b) and could suggest that variation in cleaner personality is associated with delousing rates. By developing protocols to screen fish personality on a large scale the industry could identify bolder individuals for farm deployment, thereby reducing the proportion of lumpfish released on farms that are unlikely to clean but rather compete with salmon for pellet food. As personality is driven by both biological and social factors (Kandler et al. 2019), optimising the hatchery management of lumpfish stocks could provide further opportunities to increase cleaning efficacy on farms. For example, breeding programs currently seek to establish pedigree lines from a handful of lumpfish families with proven delousing ability (Imsland et al. 2016a), however, identifying desirable traits associated with client engagement, such as greater boldness and faster growth rate, could help inform broodstock selection on an individual basis to further expand these programs. Likewise, the environment in which commercial cleaner fish are raised may also influence behavioural development, particularly as living in socially restricted habitats with few clients is shown to decrease cleaning efficacy in wrasse (Wismer et al. 2014). Whilst the need for structural enrichment is considered important for the welfare of lumpfish (Imsland et al. 2015; Johannesen et al. 2018a), assessing social enrichment prior to farm deployment could also be of benefit, especially in terms of evaluating potentially antagonistic behaviour. Hatcheries face pressure to supply small size classes of lumpfish (approx. 20g) as this is thought to be an optimal size for cleaning behaviour (Imsland et al. 2016b), although recent studies report that body size accounts for little variation in louse consumption and that smaller lumpfish are more likely to have empty stomachs (Eliasen et al. 2018). The finding that faster growth rate is linked to higher salmon interaction supports the notion that larger lumpfish may also engage with salmon, and that deploying different size classes could be a sustainable approach to stocking farms (Powell et al. 2018b).

Acknowledgments

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Table 4.1. Adapted ethogram detailing cleaner-client interactions (Bshary & Côté 2008; Horton 2011).

Behaviour	Description	Observed in study?
Tactile Stimulation	Slow, massaging contact with client's body	No
Posing	Immobile head or tail stand, irregular zig-zag swimming	No
Pursuit	Changing direction to follow client	Yes
Inspection	Close visual assessment of client, often from multiple angles	Yes
Bite	Biting of client resulting in a jolt	No
Peck	Biting of client without resulting in a jolt	No
Jolt	Immediate motion by client away from cleaner	Yes

Table 4.2. Most plausible models for (a) covariates of behaviours measured during personality screening using fish ID as a random factor and (b) observed cleaner-client interactions using observation day as a random factor. Values include marginal (R2m) and conditional (R2c) coefficients of models and significant *P* values shown in bold.

	Behaviour	Variable	Estimate ±SE	R2m	R2c	t value	z value	*P*
a)	Crossing Freq.	Sex	0.524 ± 0.219	0.256	0.979		2.400	**0.016**
		SGR	0.635 ± 0.200				3.181	**0.001**
		Origin	0.344 ± 0.203				1.701	0.089
	Crossing Speed	Sex	-0.173 ± 0.063	0.155	0.645	-2.741		0.097
		SGR	-0.167 ± 0.056			-3.002		0.365
	Swim Duration			0.126	0.444			
	Bite Freq.	Day	-0.010 ± 0.004	0.111	0.931		-2.352	0.119
	Mirror Freq.	Day	-0.005 ± 0.001	0.124	0.978		-3.783	**<0.001**
	Mirror Duration			0.028	0.028			
	Shelter Duration	Day	-2.039 ± 1.320	0.048	0.250	-1.545		0.130
	Perimeter Duration	Origin	-0.012 ± 0.015	0.111	0.111	-0.711		0.650
	Novel Freq.	Origin	-1.612 ± 0.386	0.370	0.727		-4.171	**<0.001**
	Novel Latency			0.030	0.030			

Table 4.2. Continued

Behaviour	Variable	Estimate ±SE	R2m	R2c	t value	z value	*P*
Inspection	Activity	0.721 ± 0.598	0.153	0.153	1.199		0.239
	Boldness	0.837 ± 0.417			2.008		0.053
	Sex	-0.404 ± 0.239			1.206		0.1
Jolt	Aggression	0.523 ± 0.225	0.441	0.441	2.335		**0.026**
	Boldness	0.194 ± 0.194			0.997		0.326
	Origin	0.199 ± 0.112			1.78		0.085
	SGR	0.349 ± 0.106			3.297		**0.002**
	Sociality	0.925 ± 0.313			2.955		**0.006**
	Tank	0.131 ± 0.101			1.301		0.203
Pursuit	Boldness	17.932 ± 3.846	0.312	0.312	4.663		**<0.001**

Table 4.3. Repeatability values (R) with 95% confidence intervals for individual fish across repeat trials. Including the total time spent swimming (*swimming duration*), speed crossing central portion of tank (*crossing speed*) and the number of crossing events (*crossing frequency*), number of bites of the mirror reflection (*bite frequency*), total time spent sitting by mirror reflection (*mirror duration*) and the number of approaches to mirror reflection (*mirror frequency*), performance in an open field test (*perimeter duration*), performance in a shelter-seeking test (*shelter duration*), the number of times the novel object was approached (*novel frequency*) and the time latency from introduction of the novel object to the first approach (*novel latency*).

Personality Factor	Behaviour	R	95% CI	*P*
Activity	Crossing Freq. (no.)	0.338	0.191 - 0.485	0.018
	Crossing Speed (cm/sec)	0.451	0.326 - 0.576	0.001
	Swimming Duration (sec)	0.460	0.334 - 0.586	0.001
Aggression	Bite Freq. (no.)	0.615	0.347 - 0.883	0.009
Sociality	Mirror Freq. (no.)	0.677	0.558 - 0.796	<0.001
	Mirror Duration (secs)	0.293	0.179 - 0.407	0.038
Anxiety	Perimeter Duration (secs)	0.269	0.172 - 0.366	0.001
	Shelter Duration (secs)	0.217	0.076 - 0.358	0.009
Boldness	Novel Freq. (no.)	0.398	0.205 - 0.591	0.033
	Novel Latency (secs)	0.061	0.000 - 0.156	0.101

Table 4.4. Correlation coefficients R (lower) and associated *P* values (upper) of personality scores for 38 lumpfish screened for activity (Act), aggression (Agg), sociality (Soc), boldness (Bld) and anxiety (Anx).

	Act	Agg	Soc	Bld	Anx
Act		0.069	0.233	0.498	0.793
Agg	0.300		0.028	0.451	0.606
Soc	0.200	0.360		0.273	0.056
Bld	-0.110	0.130	-0.180		0.274
Anx	0.040	0.090	-0.310	0.180	

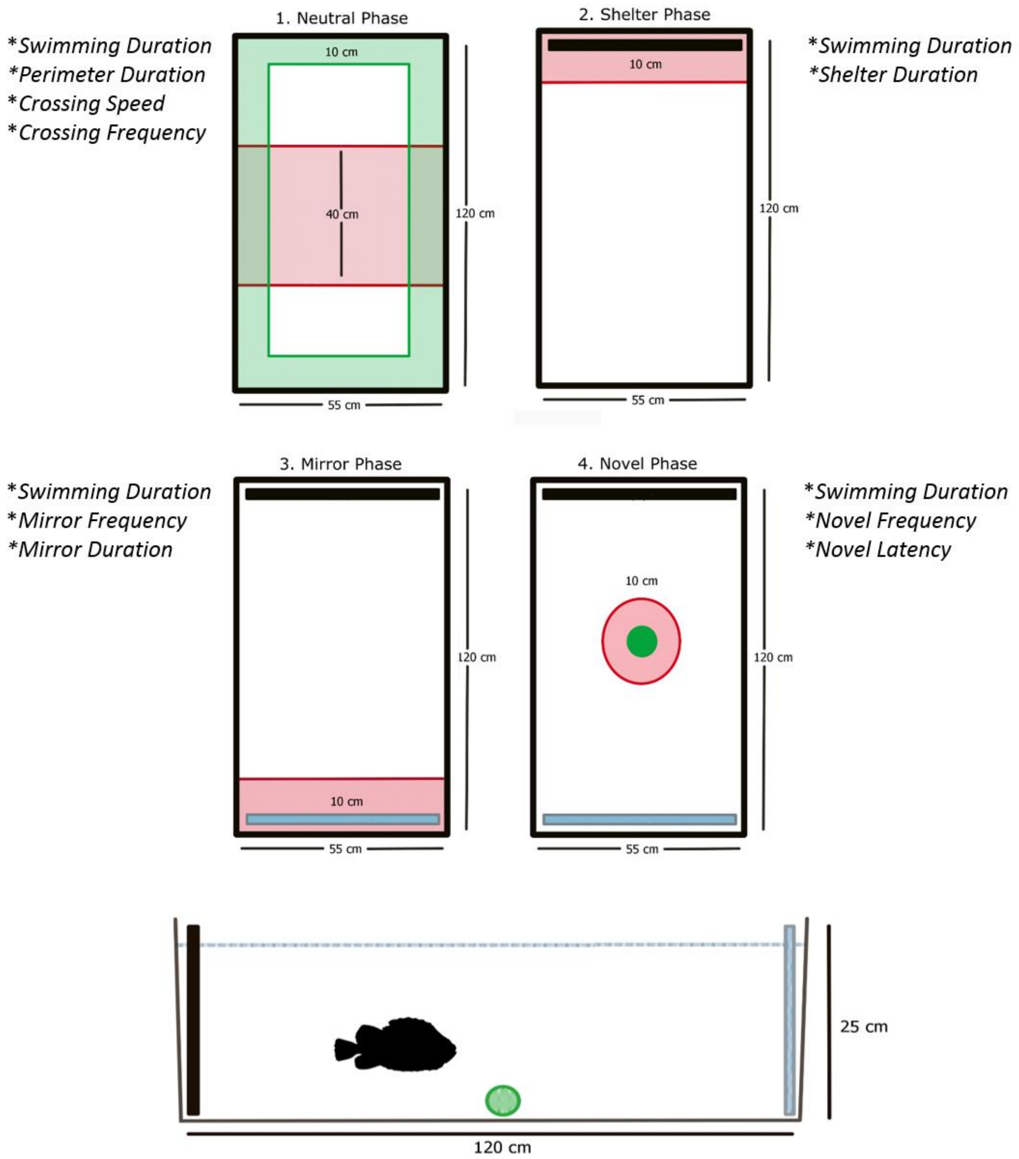

Figure 4.1. Scheme of experimental design. Personality screening of lumpfish took place across four experimental phases, each lasting 10 minutes. Swimming duration was recorded across all four phases. In the *neutral phase* a perimeter (green) was superimposed over video footage to conduct an open field test and a middle section a third of the tank length (red) was superimposed to measure crossing frequency and speed across the central section. In the *shelter phase* the duration of time individuals spent in close proximity (red) the black panel was recorded. In the *mirror phase* the duration of time and the frequency of visits made to close proximity (red) of the mirror was recorded. In the *novel phase* the number of visits and time latency of approach to the area of close proximity (red) was recorded. * denotes behaviour measured in each phase.

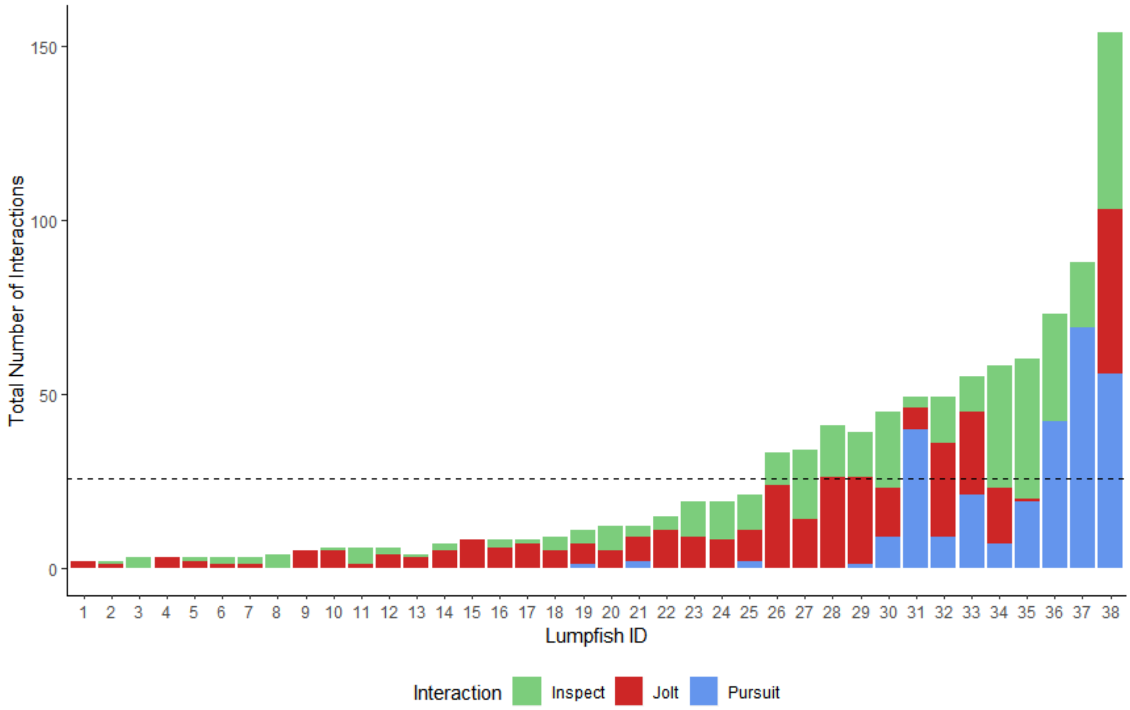

Figure 4.2. Total interactions observed between focal lumpfish (n=38) and salmon smolts during a 20-minute observation session per lumpfish. Dotted line shows mean number of interactions across all observations.

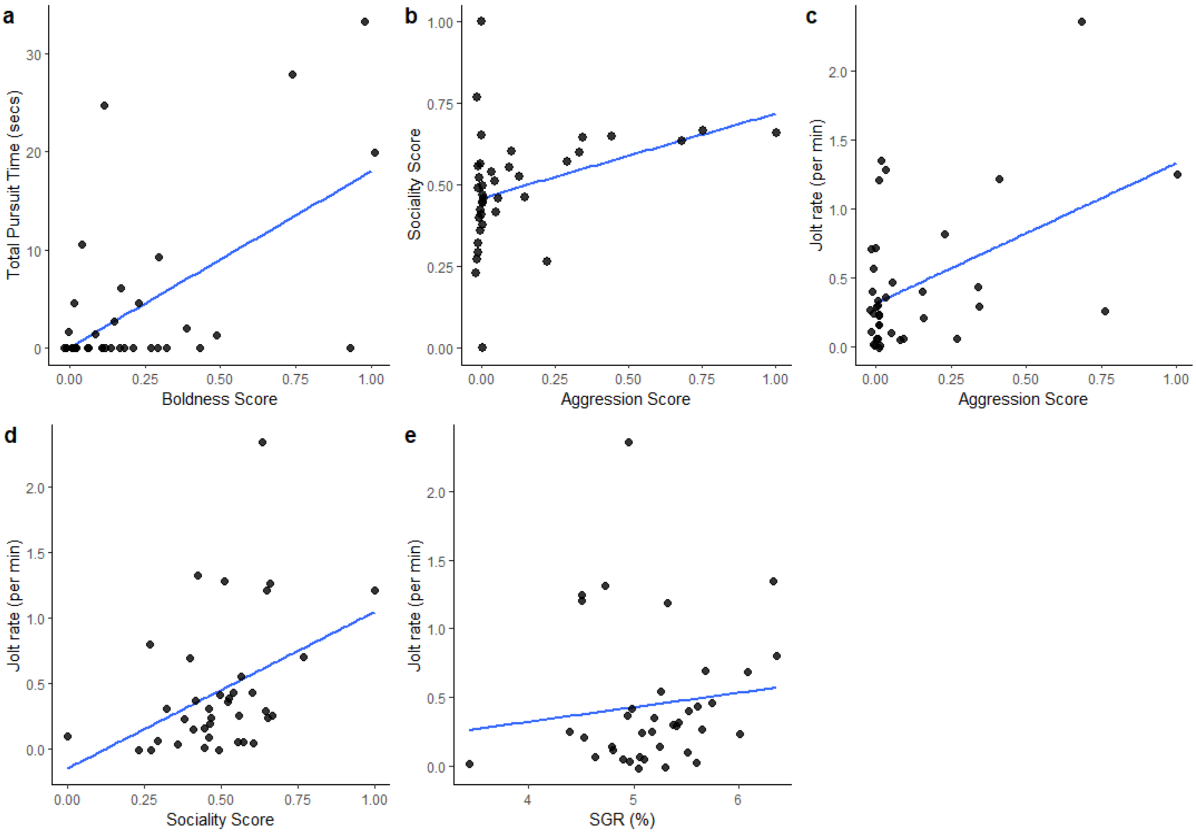

Figure 4.3. Significant relationships between (a) total time pursuing salmon and lumpfish boldness score, (b) social-aggression syndrome identified in lumpfish, salmon jolt rate and lumpfish aggression score (c), sociality score (d) and specific growth rate (e).

General Discussion:

Lumpfish Hatcheries

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Lumpfish in Salmon Farms

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Insights on Facultative Cleaner Fish

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The Future of Lumpfish Aquaculture

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Conclusions

For lumpfish deployment in salmon farms to be considered a truly sustainable method of sea-louse control the production process must be optimised and any impact on natural ecosystems minimised. The process of refinement and risk mitigation has barely begun, yet the utilisation of lumpfish in aquaculture has developed rapidly and will likely continue expanding, both in the volume of stock production and area of farm deployment. Research has struggled to match the pace of industry, and peer-reviewed literature on the application of cleaner fish in aquaculture remains sparse. Key questions regarding fundamental aspects of lumpfish biology remain unanswered, particularly with respect to individual differences in cleaning behaviour, variation in commercial stock performance and patterns of differentiation between wild populations. The overarching aim of this thesis is to provide insight on factors driving variation across these biological scales, assessing how potential factors may impact aquaculture practise, and provide a foundation on which to evaluate sustainability.

Lumpfish Hatcheries

At present, the aquaculture process begins with harvesting adult lumpfish from wild populations to act as broodstock (Figure GD.1) (Jonassen et al. 2018b). This is not a desired source as collecting gametes from wild fish has introduced disease into hatcheries (Wittwer & Treasurer 2018), reduced the abundance of wild populations (Halvorsen et al. 2017) and skewed population dynamics which resulted in heightened vulnerability to overexploitation (Hoenig & Hewitt 2005). The industry currently lacks the ability to determine spawning condition of wild adults as traits including body size and colouration are not correlated with gamete production (Goulet & Green 1988; Norðberg et al. 2015), therefore, surplus adults are sacrificed in order to ensure enough viable milt and eggs is collected to generate commercial stocks (Wittwer & Treasurer 2018). Geometric analysis conducted in Chapter 2 identified new anatomic aspects of sexual dimorphism in the species. Males had a longer dorsal flag and mouth whereas females had a larger dorsal hump. These features align with previously described life history variation between the sexes (Davenport 1985b), as a larger mouth may facilitate males in brooding eggs (Davenport 1983) and a larger hump could aid the buoyancy of gravid females (Davenport & Kjørsvik 1986). Assessing whether these newly identified traits are correlated with spawning condition through collecting biometrics on teste and ovary mass, alongside data on gamete viability, could validate if these sexually dimorphic traits are indicators of maturity. If so, this would enable selective harvest of mature broodstock, preventing the sacrifice of immature fish and the overharvest of spawning adults. The number of sexually mature lumpfish that can be sustainably removed from wild populations requires further assessment (Kennedy & Jónsson 2017). Estimates of effective population size (N_e) provided in Chapter 1 found genetic diversity varied across the range and therefore the number of spawning adults that can be sustainably harvested will vary across regions, and populations in Iceland, Norway and the Faroes Islands showed greatest vulnerability. Recent work in Iceland suggests females spawn twice per season, which could allow broodstock collection to be staggered across the spawning season (Kennedy 2018; Kennedy & Ólafsson 2019). Assessing temporal patterns of genetic diversity across spawning seasons is needed to ascertain the effect of broodstock collection on genetic diversity as the cleaner fish industry grows.

Artificial fertilisation is conducted in hatcheries using broodstock gametes from and the juveniles are then reared until a deployment size of 20g (Jonassen et al. 2018b). The industry aims to reduce the time taken for juveniles to reach deployment size, which would allow faster stock production and more frequent deployment (Powell et al. 2018b). Reports from Norwegian hatcheries have suggested that attempts to close the species life cycle in captivity have neared fruition (Sveier & Olav 2018), therefore, selective breeding programs may soon be established. A genetic component for enhanced growth has been identified and will likely be incorporated into future breeding attempts (Sae-Lim et al. 2020). Chapter 3 conducted a comparison of fitness traits between different commercial stocks reared in a common-garden experiment, and results implied a trade-off occurred between faster growth rate and lower survival probability. Similar growth-mortality trade-offs are documented in numerous species of fish (Feiner et al. 2017; Matte et al. 2019; Weinstein et al. 2019), though the factors which contributed towards this trade-off in lumpfish remain unclear. Data on lumpfish mortality rates in hatcheries and farms are not routinely reported, but are estimated within the region of 48-75% (Brooker et al. 2018; Fish Health Inspectorate 2019). Accelerating production rates without having first addressed the issue of low survival probability does not represent a sustainable business model (Omasaki et al. 2017) nor does it demonstrate sufficient protection of animal welfare (Browman et al. 2018; Huntingford & Kadri 2009). The trade-off outlined in Chapter 3 should caution against selection for increased output without first considering the potential economic and ethical consequences, and further comparison of fitness traits in three of more stocks of known pedigree is needed.

Increasing the low proportion of lumpfish delousing salmon in farms represents a substantial obstacle for the industry (Blanco 2019). Selective breeding programs may aim to enhance delousing efficacy, as louse consumption has previously been found to vary between families of lumpfish and therefore assumed to carry a genetic component (Imsland et al. 2016a). Delousing efficacy is currently recorded through post-mortem stomach content analysis (Eliasen et al. 2018; Imsland et al. 2014a; Imsland et al. 2018b) and therefore does not represent a viable method for identifying potential broodstock. Introducing sea-lice or salmon into lumpfish hatcheries to assess cleaning behaviour would incur a biosecurity risk, as some pathogens transmit horizontally between the two species of fish (Haugland et al. 2017) and *Caligus* lice are known to infect lumpfish (Heuch et al. 2007; Øines et al. 2006). Therefore, developing methodologies to assess variation in cleaning predisposition among individuals within a hatchery environment and without exposure to clients or parasites represents a significant hurdle. Chapter 4 attempted to bridge this gap by demonstrating how personality profiling techniques could be adopted to identify individuals predisposed to a focal behaviour. After screening individuals along 5 axis of animal personality (Gosling & John 1999) results indicated that bolder individuals showed greater client interaction, while lumpfish that expressed a syndrome for social-aggression displayed potentially antagonistic behaviour towards salmon. Further study is needed to validate whether observed behaviours correlate with louse removal and assess heritability of these personality traits. Further developing profiling protocols to simultaneously screen multiple individuals across different contexts could offer vital tools for selective breeding programs.

Lumpfish in Salmon Farms

Once stocks have reached deployment size the fish are transported from hatcheries and introduced into salmon farms (Jonassen et al. 2018a). This movement takes place across most of the North-east Atlantic, with lumpfish exchanged between salmon farming regions in Iceland, Norway, Faroe Islands, Scotland and Ireland (Bolton-

Warberg 2018; Johannesen et al. 2018b; Jónsdóttir et al. 2018b; Treasurer et al. 2018). Although genetic differentiation is reported across broad geographic regions in the West Atlantic (Canada-USA), East Atlantic (Iceland-Norway) and Baltic Sea (Poland) (Pampoulie et al. 2014), the existence of fine scale genetic structure in wild populations across regions where stock translocation takes place is unknown. Given the high likelihood that lumpfish escape open-net farms and hybridise with native fish in the same manner as salmon and cleaner wrasse (Faust et al. 2018; Wringe et al. 2018), stock translocation carries a risk of introducing non-native genotypes into wild populations should genetic differences exist between populations utilised in aquaculture. Cluster analysis conducted in Chapter 1 identified distinct genetic groups of lumpfish in the West Atlantic (Canada-USA), Mid Atlantic (Iceland), East Atlantic (Faroe Islands, Ireland, Scotland, Norway, Denmark), English Chanel (England), Baltic Sea (Sweden) and a site in Norway (Averøy). Furthermore, estimated effective migration (N_m) found gene flow within each of these genetic groups but not between genetic groups. Therefore, it can be confirmed hatchery stocks are translocated across areas with genetically distinct wild populations, which heightens the risk severity posed by hybridisation between escapees and wild fish (Cross 2000; Stockwell & Leberg 2002). The consequence of genetic introgression would also depend upon phenotypic differences between populations.

The common-garden experiment conducted in Chapter 3 found that genetic origin of stocks was associated with differences in aquaculture performance, and that a lower survival probability was attributed to the stock translocated furthest from the natal collection site. The factors which induced this difference in mortality require further assessment, as the data collected in Chapter 3 is insufficient to establish firm conclusions. These results could imply native stocks show greater survival probability on local farms and that the high mortality rates reported on salmon farms (Brooker et al. 2018)reflect the large proportion of translocated lumpfish (Jonassen et al. 2018a). A low survival probability would reduce the risk of genetic introgression via escapes into wild populations but would not benefit farm productivity or lumpfish welfare. Routine reports on the deployment and mortality rates on farms are needed to assess the potential scale of lumpfish escapes.

Differences in growth, feeding activity and mortality identified in Chapter 3 could imply a contrast in the Pace of Life Syndrome (POLS) exhibited by each stock.

Chapter 4 also recorded significantly higher interaction with a novel object by northern lumpfish, and greater boldness would fit the trend of a faster life history pace in this stock (Binder et al. 2016). The consequence of translocated stocks with differing POLS escaping farms and hybridising with native populations would vary depending on the difference in POLS. Escapees with a fast POLS which entered an area where native fish showed a slower POLS would have an advantage, as faster POLS confers greater competitive ability in terms of acquiring resources and faster reproduction (Yang et al. 2019). This would be the case for surviving Icelandic lumpfish that escaped farms and became established in southern regions of the range. Therefore, the genetic and phenotypic differences outlined in Chapters 1 and 3 of this thesis should act as a caution against the continued widescale translocation of lumpfish into salmon farms. Stock movement should be restricted to within the genetic groups identified by Chapter 1, matching the genotype of farm and wild lumpfish to reduce differentiation and therefor consequence of genetic introgression. Further assessment on the temporal and spatial patterns of population dynamics are needed, and tagging studies similar to those conducted around the Icelandic coast (Kennedy et al. 2014; Kennedy et al. 2015) are required elsewhere in the range to evaluate population connectivity. Regionally specific stocks should be developed to supply local farms, and translocation against a latitudinal gradient should be avoided.

Lumpfish, which survive and do not escape from farms, are found to differ in cleaning efficacy and the proportion of individuals shown to have consumed lice ranges from approximately 15% to 30% (Eliasen et al. 2018; Imsland et al. 2018b). Chapter 4 found a similar proportion (34%) of lumpfish exhibited behavioural interaction with salmon, and that traits including personality profile and growth rate were associated with the type and extent of interaction. Unfortunately, restrictions prevented the use of infected salmon during these trials and so the relationship between client interaction and delousing remain unclear. Human personality is described as being formed from a matrix of biogenic (e.g. genetic, brain anatomy, hormonal), sociogenic (e.g. environment, social group, parental care) and idiogenic (e.g. experience, motivation, emotional state) factors (Little 2006; Little 2008). Although the translation to fish personality is unfounded, these three categories might offer a basis on which to further explore how personality influences fish behaviour, with respects to manipulating cooperative and antagonistic interactions and optimising cleaning in salmon farms.

Though caution should be exercised as to not anthropomorphise fish, the bluestreak cleaner wrasse (*Labroides dimidatus*) has demonstrated that traits falling into these three categories of personality genesis influence client interaction. Artificially manipulating the neurochemical arginine vasotocin is shown to regulate wrasse sociality (Soares et al. 2018) and low habitat complexity is found to reduce cleaning activity (Bansemer et al. 2002; Horton 2011). The wrasse has even demonstrated the ability to apply learned behaviour and feed against prey preference when motivated by future reward (Danisman et al. 2010). Through evaluating how biogenic, sociogenic and idiogenic factors influence the 5 axis of animal personality and the translation to cleaning interaction, farms could adapt practises to facilitate improved cleaning behaviour. For example, environmental enrichment is considered important for welfare on farms (Imsland et al. 2015; Johannesen et al. 2018a), however, social enrichment has not been evaluated and lumpfish do not presently have opportunity to learn cleaning skills.

Insights on Facultative Cleaner Fish

Research focused on facultative cleaning is rare in comparison to studies of dedicated cleaner fish, despite that majority of cleaner fishes doing so in a facultative manner (Baliga & Law 2016), therefore knowledge is limited on how, when and why facultative cleaners exhibit cleaning behaviour. All the species utilised in salmon aquaculture are facultative cleaners (Treasurer 2018) and stomach content analysis has found variation in the proportion of individuals consuming lice, ranging from 14% to 28% of lumpfish and approximately 16% of goldsinny wrasse (*Ctenolabrus rupestris*) (Deady et al. 1995; Eliasen et al. 2018; Imsland et al. 2014a; Imsland et al. 2016a). Variation in cleaning efficacy is also noted in ballan wrasse (*Labrus bergylta*), rock cook wrasse (*Labrus ossifagus*) cunner wrasse (*Tautogolabrus adspersus*) and cuckoo wrasse (*Labrus mixtus*), though stomach content analysis data is insufficient to provide a proportion of individuals consuming lice for these species (Bjordal 1988; Costa et al. 2016; Leclercq et al. 2014; MacKinnon 1995; Skiftesvik et al. 2013; Tully et al. 1996). Although Chapter 4 did not assess parasite removal the observations demonstrated that the majority of lumpfish (66%) do not show notable interaction with salmon, implying facultative cleaning is not necessarily a species-wide behaviour and that some individuals show a greater predisposition to cleaning than others.

Individuals that did interact with clients showed repeatable traits for boldness, sociality and aggression. Boldness, increased willingness to take risk, is associated with greater client interaction in other cleaner fish species (Dunkley et al. 2019; White et al. 2007) and this growing interspecific trend could indicate risk taking is an important component of client interaction for both dedicated and facultative cleaners. No antagonistic behaviour has been reported of salmon towards lumpfish (Imsland et al. 2014a; Imsland et al. 2014b), however, given the difference in size at deployment (lumpfish, approx.20g; salmon, approx.3500g) it is possible that salmon represent a predation risk for juvenile lumpfish. The high stocking density associated with salmon farms is not a conducive environment to test this hypothesis through observation, but stomach content analysis of salmon could determine whether farmed salmon prey on lumpfish. Quantifying the cost and benefit of interaction between lumpfish and salmon could elucidate the risk associated with cleaning and therefore the importance of a bolder personality type.

Bluestreak cleaner wrasse are well studied for their sophisticated client interaction, Machiavellian intelligence (Bshary 2011; Bshary & Würth 2001), and the species has recently passed the mirror-mark test which implies self-awareness (Kohda et al. 2019). The highly specialised behaviour of this dedicated cleaner may not be comparable to that of the more generalist facultative species, however, the limited literature on facultative cleaner-client interaction suggests similarities do exist. The rainbow wrasse (*Coris julis*) and ornate wrasse (*Thalassoma pavo*) both show inspection behaviour, in which clients are visually assessed for parasite removal (Narvaez et al. 2015). Wild rock cook wrasse are also reported to incur client jolts (Morado et al. 2019), which are commonly interpreted as an indicator of antagonism when consume client tissue (Soares et al. 2008a). Both inspection and client jolt behaviours were also observed between lumpfish and salmon in Chapter 4. As these interactions are increasingly documented in multiple species of facultative cleaner, this implies client-oriented behaviour is not exclusive to dedicated cleaner fish.

Comparison between bluestreak cleaner wrasse and the facultative yellowtail tubelip wrasse (*Diproctacanthus xanthurus*) found no difference in the number of clients cleaned nor parasites consumed (Grutter & Feeney 2016). Indeed, some populations of this iconic cleaner species have failed to replicate advanced cleaning behaviours described in populations elsewhere in the range (Bansemer et al. 2002; Wismer et al.

2014). Furthermore, phylogenetic analysis does not support an evolutionary division between dedicated and facultative wrasse, and concludes that both categories of cleaner has evolved numerous times among the Labridae (Baliga & Law 2016). These examples blur the boundary between dedicated and facultative cleaner fish, which are categories originally defined by the extent to which a species' diet is sourced through cleaning rather than the expression of any specific behaviour (Côté 2000). Lumpfish do not represent a typical wrasse-like cleaner fish, yet some individuals have been demonstrated to preferentially feed by removing lice from salmon (Eliasen et al. 2018; Imsland et al. 2014a; Imsland et al. 2014b; Imsland et al. 2016a; Imsland et al. 2016b; Imsland et al. 2018b; Johannesen et al. 2018b), and Chapter 4 presents potential mechanisms that facilitate louse removal. While it is unnecessary to describe individual lumpfish with high cleaning preference as a dedicated cleaner, the example serves to highlight that a binary system of classifying cleaner fish as a dedicated species or facultative species masks the diversity of behavioural variation underlying the mutualism.

The Future of Lumpfish Aquaculture

Sea-lice have posed a growing threat to sustainable Salmonid aquaculture since its beginnings in the 1980s (Treasurer 2018), and there is no evidence to suggest this trend will not continue into the future (Bergheim 2012). The industry has developed numerous control strategies (Costello 1993; Treasurer 2002) yet none, including the deployment of cleaner fish, currently represent a sustainable solution. The use of cleaner fish has been described as a "green revolution", although the present system of lumpfish aquaculture lacks optimised production and likely impacts on wild ecosystems (Powell et al. 2018b). This is perhaps unsurprising given the recent inception of lumpfish aquaculture and the short time frame of development. However, the magnitude at which industrial growth out-paces the peer-review process is concerning and, despite numerous reviews outlining challenges facing lumpfish aquaculture (Blanco 2019; Brooker et al. 2018; Gonzalez & de Boer 2017; Powell et al. 2018b), solutions are not developed at a speed the required by industry. The time lag between research innovation and industry application ultimately results in the welfare of lumpfish and wrasse being sacrificed, demonstrated through high mortality rates (Brooker et al. 2018; Fish Health Inspectorate 2019), in an attempt to increase salmon welfare. This poses an ethical dilemma: does the need to improve welfare for one species justify compromising the welfare of another species? Given the growing public awareness of fish welfare (Braithwaite, 2010) it could be expected this question will become more pertinent over time. Despite the numerous concerns outlined throughout this thesis, cleaner fish deployment may represent the only viable long-term form of parasite control in open-net salmon cages (Treasurer 2002) and lumpfish the most promising candidate to fill the need for rapid production (Powell et al. 2018b). Therefore, it is paramount for salmon aquaculture that lumpfish aquaculture not only persists but quickly develops refined protocols for improved application.

Future genetic analysis is needed to examine temporal dynamics of lumpfish populations in farming areas to assess impact on wild fish. The development of new genetic tools will facilitate genome and transcriptome analysis, providing a basis for studying the molecular ecology of the species (Maduna et al. 2020) and the development of SNPs will allow more powerful genetic analysis to allow fine scale population assessment and heritability analysis. Sampling efforts should focus on areas of roe and broodstock harvest in order to monitor genetic diversity and around salmon farms to assess potential hybridisation from escaped fish. Newly identified elements of sexual dimorphism require further evaluation as markers of spawning condition and fecundity to aid broodstock harvest. The significance of these features on mate choice and reproductive success could also provide insight on the likelihood of successful hybridisation between translocated lumpfish from different regions. Morphological variation across different age classes could be undertaken to investigate whether body or sucker shape varies throughout ontogeny, and the implications on different stages of aquaculture. Comparison of life history variation between three or more populations is required to assess latitudinal trends in fitness traits. Data on the aerobic scope of different populations under varying temperature regimes would enable assessment of thermal-niche, thereby providing insight on factors determining population distribution and the implications of translocation. Assessing lumpfish from the West Atlantic and Baltic Sea in a common-garden experiment could determine whether phenotypic variation occurs across a longitudinal gradient. Personality screening can be used to identify individuals predisposed to client interaction, however, the ability to successful delouse salmon requires validation. Assessing the heritability of personality traits through determining breeding coefficients could inform selective breeding for greater delousing rates. Similarly, screening prior to farm deployment could reduce the number of non-cleaning lumpfish deployed on farms that compete with salmon for food pellets. Lumpfish which are not required by the cleaner fish industry could be reared for roe production, further reducing the need for wild harvest. Factors influencing the development of personality, such as environment and experience, could be explored to determine methodologies of manipulating the expression of cleaning behaviours.

Conclusions

- Distinct genetic populations of lumpfish are found across the North Atlantic, including regions where wild lumpfish are harvested for aquaculture. Gene flow between populations is limited. Populations in the North East Atlantic have lower genetic diversity than elsewhere in the range.

- Morphological variation is attributed to different genetic groups, including body depth and scute arrangement. Lumpfish show sexual dimorphism in mouth length, dorsal flag length and dorsal hump area.

- Northern and southern lumpfish show different fitness traits when reared in a common environment. Northern lumpfish grow faster, are more active and have lower survival probability.

- Interaction between lumpfish and salmon varies with the personality of lumpfish. Bold lumpfish show greater client pursuit and individuals exhibiting a syndrome for social-aggression display potentially antagonistic behaviour. Only 34% of individual interacted with salmon.

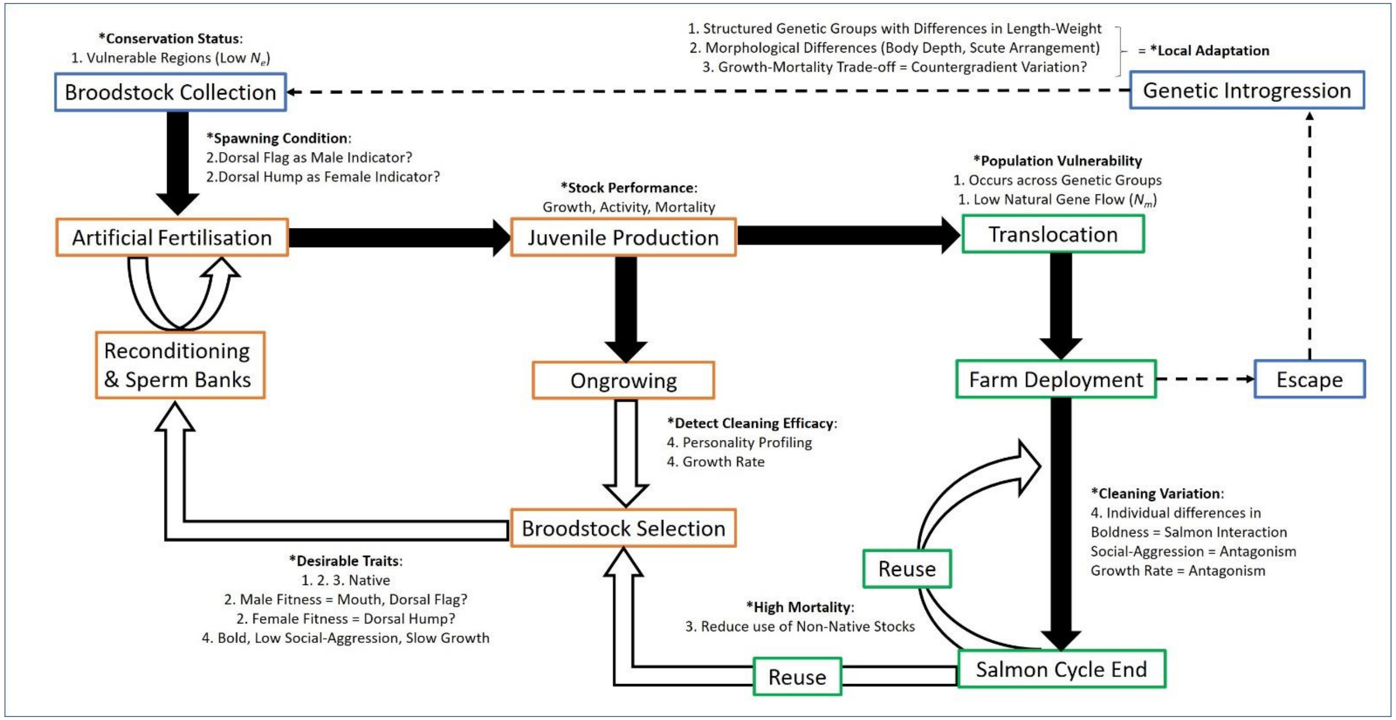

Figure GD.1. Overview of lumpfish aquaculture showing established stages of production (filled arrow), stages under development (empty arrow) and potential consequences (dashed arrow). Box colours indicates the utilisation of wild fish (blue), hatchery stocks (orange) and cleaner fish on salmon farm (green), * details sustainability problems addressed in the thesis. Numbers (1-4) represent thesis Chapter contributing to problem.

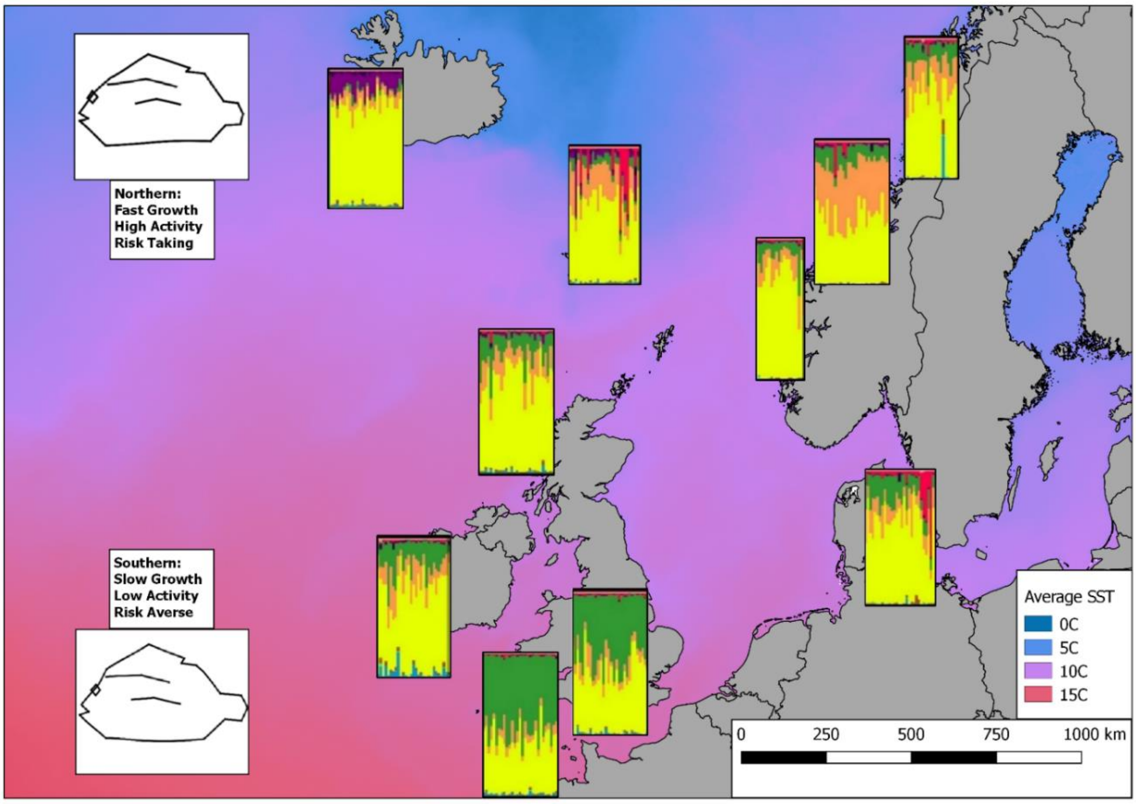

Figure GD.2. Mean sea surface temperature (SST) across the north-west Atlantic, obtained from marine climate archive (Sbrocco & Barber 2013). TESS output, with K_{max} = 10, shows genetic groups across the region, with each bar representing one individual and colours indicating probability of belonging to different genetically distinct clusters. Wireframe graphs show extremes in morphological variation for canonical variable depicting stock variation.

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Appendix

T1.1. Information for 10 microsatellite loci used in analysis (Skirnisdottir et al. 2013), including allele size ranges found across populations analysed in the present study.

Locus	Primer sequence (5' - 3')	Dye	Allele size range (bp)
Multiplex 1			
Clu29	F: CGCGCGGTCAGCTCATCCTTAG R: TCGCGTGACGGACAGGTTTCG	PET	126-142
Clu34	F: TCTGCGATAGTAGCGTCAGGGTTC R: AGGCCGGCTGATCAAGAGCAC	NED	179-219
Clu36	F: CACGGCGAGTCAGACGAGGC R: GCTGCCGCTACTCCGCACAG	6-FAM	183-203
Clu45	F: GCGCAGGAATGCGCCTGAAG R: ACCGCAGCTTGTTGGGCAGG	PET	268-298
Multiplex 2			
Clu12	F: CCACAACCGGTGGGTCCCG R: ACGCTCCTTCTGATCTTCGCCC	6-FAM	188-202
Clu26	F: CGAGAGAGGAGAACGCACGGC R: GGCACAAGTGCATGGGCACG	6-FAM	97–121
Clu33	F: TCATGCAAGCATTTGAGCGCCG R: TGTTGCCTTGTAACTGCGCTTGAG	VIC	173–193
Clu37	F: CTTCACAGGTCGGGCGACGG R: GCACAGCGATGACGCTTGCAG	PET	200-226
Clu40	F: TGGGCATACAGGTCTGAACACGC R: GCCACCTGCTGCAGCCTCTC	NED	246-266
Clu44	F: CCGGCCCAGCCTGCCTTATG R: TGCCTGGAAACAGTGTATGGCAC	6-FAM	269-287

T1.2. Presence of null alleles identified at 10 microsatellite loci across 15 lumpfish populations using Microchecker v.2.2.3 (Van Oosterhout et al. 2004). Values indicate the estimated null allele frequency at each locus using the Oosterhout algorithm, and asterix denotes confidence interval of 95%*, 99%** or 100%***.

Population	*Clu29*	*Clu34*	*Clu36*	*Clu45*	*Clu12*	*Clu26*	*Clu33*	*Clu37*	*Clu40*	*Clu44*
FB	-	-	-	-	0.175*	-	-	-	-	-
CB	-	-	-	-	-	-	0.189**	-	-	-
WB	-	-	-	-	0.140*	-	-	-	0.143***	-
Ha	-	-	-	-	-	0.310***	-	-	0.213***	-
Kl	-	0.193**	-	-	-	-	-	-	-	-
VB	-	-	-	-	-	0.145*	-	-	-	-
OH	-	-	-	-	-	-	-	-	-	-
We	-	-	-	-	-	-	-	-	-	-
Gu	-	0.289***	-	-	-	-	-	-	-	-
Na	-	0.181*	-	-	-	0.168*	-	0.243***	-	-
Av	-	-	-	-	-	-	-	-	-	-
Ro	-	0.387***	-	-	-	-	-	-	-	-
KB	-	-	-	-	-	-	-	-	-	-
Öl	-	-	0.209***	-	-	-	0.242**	-	-	-
GS	-	0.282***	0.197***	-	0.204**	-	0.185*	-	0.121*	-

T1.3. Pairwise F_{ST} values of 9 microsatellite loci (*Clu40* removed) across 15 populations, * denotes significance after Bonferroni correction ($P < 0.00022$).

	FB	CB	WB	Ha	Kl	VB	OH	We	Gu	Na	Av	Ro	KB	Öl	GS
FB	0.000	*NS*	*	*	*	*	*	*	*	*	*	*	*	*	*
CB	0.010	0.000	*	*	*	*	*	*	*	*	*	*	*	*	*
WB	0.032	0.037	0.000	*	*	*	*	*	*	*	*	*	*	*	*
Ha	0.132	0.116	0.122	0.000	*	*	*	*	*	*	*	*	*	*	*
Kl	0.130	0.103	0.103	0.050	0.000	*	*	*	*	*	*	*	*	*	*
VB	0.129	0.100	0.112	0.046	0.013	0.000	*	*	*	*	*	*	*NS*	*	*
OH	0.165	0.124	0.139	0.053	0.035	0.023	0.000	*	*NS*	*NS*	*	*	*	*	*
We	0.195	0.166	0.160	0.058	0.060	0.044	0.021	0.000	*NS*	*	*	*	*	*	*
Gu	0.206	0.173	0.167	0.077	0.058	0.057	0.009	0.003	0.000	*	*	*	*	*	*
Na	0.177	0.133	0.144	0.080	0.041	0.023	0.001	0.033	0.027	0.000	*	*	*	*	*
Av	0.170	0.130	0.118	0.102	0.029	0.020	0.040	0.071	0.065	0.023	0.000	*	*	*	*
Ro	0.152	0.128	0.140	0.044	0.042	0.041	0.031	0.070	0.055	0.052	0.082	0.000	*	*	*
KB	0.125	0.090	0.104	0.034	0.026	0.007	0.031	0.054	0.072	0.026	0.041	0.048	0.000	*	*
Öl	0.198	0.148	0.190	0.077	0.123	0.112	0.105	0.107	0.127	0.125	0.155	0.129	0.083	0.000	*NS*
GS	0.208	0.173	0.205	0.102	0.155	0.151	0.157	0.158	0.178	0.185	0.198	0.163	0.127	0.005	0.000

T1.4. Pairwise F_{ST} values of 9 microsatellite loci (*Clu37* removed) across 15 populations, * denotes significance after Bonferroni correction ($P < 0.00022$).

	FB	CB	WB	Ha	Kl	VB	OH	We	Gu	Na	Av	Ro	KB	Öl	GS
FB	0.000	*NS*	*	*	*	*	*	*	*	*	*	*	*	*	*
CB	0.012	0.000	*	*	*	*	*	*	*	*	*	*	*	*	*
WB	0.025	0.024	0.000	*	*	*	*	*	*	*	*	*	*	*	*
Ha	0.139	0.112	0.134	0.000	*	*	*	*	*	*	*	*	*	*	*
Kl	0.112	0.092	0.109	0.061	0.000	*NS*	*	*	*	*	*	*	*	*	*
VB	0.118	0.092	0.117	0.050	0.014	0.000	*	*	*	*	*	*	*NS*	*	*
OH	0.147	0.105	0.137	0.050	0.036	0.017	0.000	*	*	*NS*	*	*	*	*	*
We	0.148	0.126	0.139	0.065	0.052	0.034	0.021	0.000	*NS*	*	*	*	*	*	*
Gu	0.152	0.128	0.142	0.085	0.055	0.047	0.015	0.001	0.000	*	*	*	*	*	*
Na	0.151	0.114	0.142	0.075	0.035	0.017	0.000	0.023	0.024	0.000	*	*	*	*	*
Av	0.109	0.088	0.094	0.116	0.036	0.029	0.052	0.058	0.054	0.030	0.000	*	*	*	*
Ro	0.148	0.133	0.158	0.060	0.043	0.039	0.035	0.056	0.054	0.039	0.085	0.000	*	*	*
KB	0.115	0.087	0.115	0.040	0.024	0.006	0.025	0.041	0.058	0.017	0.045	0.043	0.000	*	*
Öl	0.224	0.180	0.221	0.145	0.135	0.157	0.147	0.146	0.151	0.164	0.183	0.192	0.125	0.000	*NS*
GS	0.212	0.184	0.217	0.141	0.135	0.159	0.164	0.159	0.170	0.178	0.194	0.179	0.133	0.000	0.000

T1.5. Pairwise F_{ST} values of 9 microsatellite loci (*Clu33* removed) across 15 populations, * denotes significance after Bonferroni correction ($P < 0.00022$).

	FB	CB	WB	Ha	Kl	VB	OH	We	Gu	Na	Av	Ro	KB	Öl	GS
FB	0.000	*NS*	*	*	*	*	*	*	*	*	*	*	*	*	*
CB	0.011	0.000	*	*	*	*	*	*	*	*	*	*	*	*	*
WB	0.017	0.023	0.000	*	*	*	*	*	*	*	*	*	*	*	*
Ha	0.159	0.124	0.129	0.000	*	*	*	*	*	*	*	*	*	*	*
Kl	0.125	0.107	0.115	0.039	0.000	*NS*	*	*	*	*	*	*	*NS*	*	*
VB	0.131	0.104	0.121	0.037	0.005	0.000	*	*	*	*NS*	*NS*	*	*NS*	*	*
OH	0.171	0.128	0.151	0.038	0.033	0.018	0.000	*	*NS*	*NS*	*	*	*	*	*
We	0.181	0.156	0.156	0.058	0.048	0.032	0.023	0.000	*NS*	*	*	*	*	*	*
Gu	0.182	0.158	0.162	0.071	0.054	0.045	0.016	0.000	0.000	*	*	*	*	*	*
Na	0.160	0.128	0.152	0.050	0.026	0.011	0.000	0.013	0.018	0.000	*	*	*	*	*
Av	0.106	0.094	0.098	0.075	0.026	0.014	0.043	0.039	0.046	0.026	0.000	*	*	*	*
Ro	0.173	0.154	0.168	0.056	0.027	0.031	0.033	0.055	0.048	0.027	0.057	0.000	*	*	*
KB	0.132	0.103	0.124	0.028	0.011	0.003	0.022	0.037	0.052	0.014	0.028	0.041	0.000	*	*
Öl	0.234	0.184	0.214	0.142	0.119	0.152	0.141	0.147	0.145	0.148	0.154	0.191	0.116	0.000	*NS*
GS	0.220	0.183	0.202	0.135	0.105	0.141	0.145	0.149	0.153	0.145	0.147	0.170	0.111	0.000	0.000

T1.6. Pairwise F_{ST} values of 9 microsatellite loci (*Clu12* removed) across 15 populations, * denotes significance after Bonferroni correction ($P < 0.00022$).

	FB	CB	WB	Ha	Kl	VB	OH	We	Gu	Na	Av	Ro	KB	Öl	GS
FB	0.000	*NS*	*	*	*	*	*	*	*	*	*	*	*	*	*
CB	0.011	0.000	*	*	*	*	*	*	*	*	*	*	*	*	*
WB	0.027	0.021	0.000	*	*	*	*	*	*	*	*	*	*	*	*
Ha	0.146	0.122	0.129	0.000	*	*	*	*	*	*	*	*	*	*	*
Kl	0.131	0.113	0.115	0.060	0.000	NS	*	*	*	*	*	*	*	*	*
VB	0.126	0.106	0.113	0.046	0.012	0.000	*	*	*	*	*	*	*NS*	*	*
OH	0.154	0.118	0.131	0.047	0.034	0.017	0.000	*	*	*NS*	*	*	*	*	*
We	0.148	0.136	0.124	0.056	0.041	0.030	0.018	0.000	*NS*	*	*	*	*	*	*
Gu	0.163	0.146	0.139	0.081	0.051	0.047	0.016	0.000	0.000	*	*	*	*	*	*
Na	0.150	0.121	0.128	0.069	0.029	0.015	0.000	0.022	0.023	0.000	*	*	*	*	*
Av	0.130	0.113	0.099	0.114	0.035	0.029	0.050	0.053	0.052	0.027	0.000	*	*	*	*
Ro	0.151	0.138	0.150	0.058	0.039	0.028	0.031	0.039	0.045	0.025	0.074	0.000	*	*	*
KB	0.120	0.097	0.107	0.037	0.022	0.007	0.024	0.039	0.059	0.016	0.044	0.034	0.000	*	*
Öl	0.202	0.167	0.193	0.131	0.124	0.150	0.139	0.143	0.149	0.156	0.176	0.172	0.116	0.000	*NS*
GS	0.209	0.186	0.207	0.136	0.134	0.159	0.161	0.157	0.172	0.175	0.195	0.171	0.129	0.000	0.000

T1.7. Pairwise F_{ST} values of 9 microsatellite loci (*Clu36* removed) across 15 populations, * denotes significance after Bonferroni correction ($P < 0.00022$).

	FB	CB	WB	Ha	Kl	VB	OH	We	Gu	Na	Av	Ro	KB	Öl	GS
FB	0.000	*NS*	*	*	*	*	*	*	*	*	*	*	*	*	*
CB	0.012	0.000	*	*	*	*	*	*	*	*	*	*	*	*	*
WB	0.028	0.022	0.000	*	*	*	*	*	*	*	*	*	*	*	*
Ha	0.152	0.122	0.133	0.000	*	*	*	*	*	*	*	*	*	*	*
Kl	0.142	0.116	0.123	0.053	0.000	NS	*	*	*	*	*	*	*	*	*
VB	0.145	0.111	0.128	0.035	0.011	0.000	*	*	*	*	*	*	*NS*	*	*
OH	0.179	0.131	0.151	0.047	0.035	0.016	0.000	*	*	*NS*	*	*	*	*	*
We	0.187	0.156	0.159	0.060	0.054	0.034	0.023	0.000	*NS*	*	*	*	*	*	*
Gu	0.192	0.161	0.164	0.083	0.056	0.047	0.017	0.002	0.000	*	*	*	*	*	*
Na	0.180	0.137	0.154	0.068	0.036	0.015	0.000	0.025	0.025	0.000	*	*	*	*	*
Av	0.143	0.113	0.112	0.104	0.036	0.030	0.051	0.059	0.053	0.029	0.000	*	*	*	*
Ro	0.176	0.154	0.170	0.051	0.044	0.036	0.036	0.060	0.058	0.039	0.084	0.000	*	*	*
KB	0.142	0.107	0.125	0.035	0.023	0.002	0.026	0.044	0.063	0.017	0.041	0.043	0.000	*	*
Öl	0.230	0.184	0.216	0.147	0.134	0.148	0.147	0.149	0.154	0.163	0.176	0.191	0.121	0.000	*NS*
GS	0.218	0.189	0.213	0.142	0.133	0.152	0.163	0.158	0.169	0.177	0.189	0.176	0.126	0.000	0.000

T1.8. Pairwise F_{ST} values of 9 microsatellite loci (*Clu34* removed) across 15 populations, * denotes significance after Bonferroni correction ($P < 0.00022$).

	FB	CB	WB	Ha	Kl	VB	OH	We	Gu	Na	Av	Ro	KB	Öl	GS
FB	0.000	*NS*	*	*	*	*	*	*	*	*	*	*	*	*	*
CB	0.014	0.000	*	*	*	*	*	*	*	*	*	*	*	*	*
WB	0.024	0.018	0.000	*	*	*	*	*	*	*	*	*	*	*	*
Ha	0.161	0.118	0.133	0.000	*	*	*	*	*	*	*	*	*	*	*
Kl	0.139	0.106	0.110	0.062	0.000	*NS*	*	*	*	*	*	*	*	*	*
VB	0.141	0.100	0.113	0.049	0.014	0.000	*	*	*	*NS*	*	*	*NS*	*	*
OH	0.165	0.105	0.122	0.040	0.025	0.012	0.000	*	*	*NS*	*	*	*	*	*
We	0.171	0.132	0.126	0.056	0.043	0.030	0.022	0.000	*NS*	*	*	*	*	*	*
Gu	0.171	0.130	0.125	0.077	0.044	0.042	0.015	0.000	0.000	*	*	*	*	*	*
Na	0.161	0.107	0.118	0.060	0.018	0.006	0.000	0.023	0.022	0.000	*NS*	*	*NS*	*	*
Av	0.141	0.104	0.101	0.117	0.034	0.029	0.049	0.055	0.047	0.022	0.000	*	*	*	*
Ro	0.160	0.129	0.141	0.057	0.041	0.034	0.023	0.045	0.048	0.018	0.076	0.000	*	*	*
KB	0.143	0.098	0.118	0.038	0.025	0.007	0.017	0.036	0.050	0.005	0.047	0.034	0.000	*	*
Öl	0.237	0.180	0.211	0.136	0.135	0.153	0.132	0.136	0.137	0.146	0.180	0.180	0.118	0.000	*NS*
GS	0.231	0.190	0.216	0.138	0.139	0.158	0.149	0.148	0.157	0.160	0.196	0.174	0.129	0.000	0.000

T1.9. Global tests of deviation from Hardy-Weinberg equilibrium by heterozygote excess (H^e) and heterozygote deficiency (H^d) conducted in GenePop, across 15 populations of lumpfish genotyped using 10 microsatellite loci.

Population	H^e		H^d	
	P	S.E.	*P*	S.E.
FB	0.971	0.005	0.042	0.005
CB	0.955	0.005	0.060	0.007
WB	0.998	0.001	0.001*	0.001
Ha	0.676	0.014	0.332	0.016
Kl	1.000	0.001	0.001*	0.001
VB	0.904	0.011	0.099	0.008
OH	0.906	0.011	0.100	0.010
We	0.849	0.011	0.160	0.013
Gu	0.521	0.017	0.493	0.016
Na	0.994	0.002	0.004	0.001
Av	0.306	0.014	0.689	0.014
Ro	0.376	0.011	0.580	0.009
KB	0.996	0.001	0.006*	0.001
Öl	1.000	0.001	0.001*	0.001
GS	1.000	0.001	0.001*	0.001

T1.10. Effective number of migrants (N_m) amongst 15 populations estimated using div-Migrate across 10 microsatellite loci, * denotes significant asymmetric gene flow at 0.05 alpha level. There was no significant asymmetric gene flow detected at lower alpha levels of 0.005 or 0.0025.

	FB	CB	WB	Ha	Kl	VB	OH	We	Gu	Na	Av	Ro	KB	Öl	GS
FB		0.639	0.465	0.066	0.083	0.102	0.068	0.057	0.053	0.076	0.076	0.050	0.096	0.047	0.053
CB	0.470		0.399	0.071	0.078	0.109	0.081	0.061	0.047	0.089	0.077	0.037	0.102	0.054	0.048
WB	0.247	0.325		0.073	0.099	0.099	0.084	0.072	0.059	0.091	0.095	0.043	0.086	0.046	0.054
Ha	0.076	0.089	0.094		0.192	0.246	0.270	0.161	0.144	0.158	0.104	0.141	0.241	0.108	0.134*
Kl	0.078	0.070	0.077	0.181		0.441	0.276	0.190	0.204	0.197	0.227	0.134	0.232	0.070	0.076
VB	0.071	0.088	0.099	0.234	0.611		0.503	0.294	0.227	0.329	0.320	0.213	0.505	0.125	0.115*
OH	0.075	0.119	0.082	0.231	0.376	0.548		0.351	0.497	0.574	0.212	0.238	0.345	0.130	0.119
We	0.069	0.086	0.094	0.226	0.297	0.359	0.514		1.000	0.303	0.194	0.197	0.200	0.132*	0.138*
Gu	0.062	0.080*	0.088	0.149	0.232	0.259	0.509	0.825		0.290	0.205	0.166	0.157	0.113*	0.108*
Na	0.069	0.103	0.082	0.183	0.473	0.546	0.892	0.378	0.365		0.349	0.302	0.479	0.100	0.091
Av	0.082	0.120	0.101	0.119	0.331	0.347	0.242	0.242	0.185	0.323		0.115	0.209	0.073	0.066
Ro	0.071	0.046	0.090*	0.216	0.507*	0.306	0.284	0.178	0.205	0.181	0.143		0.259	0.062	0.078
KB	0.078	0.117	0.106	0.207	0.355	0.636	0.457	0.272	0.189	0.337	0.232	0.218		0.127	0.110
Öl	0.056	0.084	0.046	0.055	0.057	0.058	0.093	0.062	0.056	0.076	0.090	0.053	0.137		0.441
GS	0.057	0.064	0.039	0.053	0.055	0.057	0.085	0.049	0.048	0.081	0.093	0.046	0.141	0.245	

T1.11. Effective population size (N_e) and 95% confidence intervals estimated using the LD method in NeEstimator for populations containing at least 19 individuals, genotyped using 10 microsatellite loci.

	NeEstimator		
	Estimated N_e	CI	JackKnife Method
FB	254.8	64.7	65.0
CB	75.4	38.9	36.0
WB	Infinite	204.9	106.5
Ha	43.0	25.6	23.2
Kl	30.3	21.1	19.9
VB	97.9	48.7	40.3
OH	205.6	66.0	60.9
We	Infinite	Infinite	536.1
Gu	Infinite	113.5	91.1
Na	72.2	32.1	25.9
Av	62.3	33.4	30.9
Ro	19.2	10.7	10.6
KB	70147.8	84.5	79.6
GS	454.6	40.00	26.4

T1.12. *k* intralocus test and *g* interlocus test for recent population expansion across 10 microsatellite loci in 15 populations calculated using Excel Macro application (Bilgin 2007), * denotes significance after Bonferroni correction (P = 0.0033).

	k test	*g* test
FB	0.586	2.803
CB	0.045	0.926
WB	0.341	0.586
Ha	0.045	1.269
Kl	0.341	2.179
VB	0.008	0.804
OH	0.341	0.554
We	0.148	0.246
Gu	0.341	5.488
Na	0.148	0.471
Av	0.341	0.622
Ro	0.148	0.310
KB	0.045	2.731
Öl	0.586	2.526
GS	0.586	2.330

T1.13. Wilcoxon signed-rank tests for heterozygosity excess (H^e) and heterozygosity deficiency (H^d) across 10 microsatellite loci in 15 populations calculated using Bottleneck, under the Two-Phase Model of Mutation (TPM) and the Stepwise Mutation Model (SMM), * denotes significance after Bonferroni correction (*P* = 0.0033).

	TPM		SSM	
	H^e	H^d	H^e	H^d
FB	0.754	0.278	0.991	0.012
CB	0.188	0.839	0.958	0.053
WB	0.500	0.539	0.984	0.042
Ha	0.784	0.688	0.784	0.246
Kl	0.461	0.577	0.991	0.012
VB	0.884	0.138	0.999	0.002*
OH	0.754	0.278	0.998	0.003*
We	0.754	0.278	0.947	0.065
Gu	0.423	0.615	0.984	0.042
Na	0.920	0.910	0.988	0.016
Av	0.500	0.539	0.984	0.042
Ro	0.500	0.539	0.138	0.884
KB	0.754	0.278	0.947	0.065
Öl	0.754	0.278	0.884	0.138
GS	0.500	0.539	0.839	0.188

T2.1. Model reduction for caudal peduncle area (CA), dorsal flag length (FL), dorsal hump area (HA), mouth length (ML), and sucker area (SA), using fixed factors of sex (Se), origin population (O) and size (Si).

Trait	Model	Se	Si	Or	Se:Si	Se:Or	Si:Or	Se:Si:Or	df	logLik	AICc	Δ	weight
CA	CA.128	X	X	X	X	X	X	X	9	-199.85	621.1	0.00	0.811
FL	FL.2	X							3	-151.51	309.4	0.00	0.282
	FL.4	X	X						4	-150.88	310.4	1.01	0.170
	FL.22	X		X		X			5	-150.18	311.4	1.97	0.105
HA	HA.4	X	X						4	-370.28	749.2	0.00	0.394
	HA.12	X	X		X				5	-369.60	750.2	1.00	0.239
ML	ML.4	X	X						4	-107.14	223	0.00	0.295
	ML.40	X	X	X			X		6	-105.2	223.9	0.93	0.185
	ML.8	X	X	X					5	-106.7	224.5	1.49	0.14
	ML.12	X	X		X				5	-106.79	224.6	1.67	0.128
SA	SA.3		X						3	-418.92	844.2	0.00	0.204
	SA.12	X	X			X			5	-416.67	844.4	0.15	0.189
	SA.4	X	X						4	-418.18	845.0	0.81	0.136
	SA.16	X	X	X		X			6	-416.08	845.7	1.42	0.100
	SA.32	X	X	X		X	X		7	-414.82	845.7	1.43	0.100

T2.2. Likelihood-ratio tests (LRT) carried out for the full models of each morphometric trait with and without inclusion of a random factor, assessing morphological variance attributed to differences in the tanks in which fish were reared. LRT were also carried out between the reduced, most plausible, model and a null model that lacked explanatory variables. The null model was rejected if LRT found a significant improvement in goodness-of-fit.

Trait	Random Factor			Null Model		
	χ^2	df	*P*	χ^2	df	*P*
Caudal Area	1.075	1	0.300	49.332	7	<0.001
Flag Length	0.000	1	1.000	33.861	1	<0.001
Hump Area	0.000	1	1.000	115.79	2	<0.001
Mouth Length	0.793	1	0.373	28.671	2	<0.001
Sucker Area	0.000	1	1.000	22.754	1	<0.001

T3.1. Model selection for predictors of lumpfish stock, sex, stock:sex and tank on Specific Growth Rate (SGR). Factors included for models within Δ2 AIC of the best fitting model. Values include the degree of freedom (df), log likelihood (logLik), Akaike information criterion (AIC), delta AIC from best model (Δ) and model weight.

Model	Stock	Sex	Stock:Sex	Tank	df	logLik	AICc	Δ	weight
SGR.4	X	X			4	-58.525	125.4	0.00	0.504
SGR.2	X				3	-60.301	126.8	1.40	0.250
SGR.12	X	X	X		5	-58.240	127.0	1.62	0.225

T3.2. Model selection for predictors of stock, observation session (AM/PM), observation week, stock:time and stock:week on the proportion of tank activity (%). Factors included for models within ∆2 AIC of the best fitting model. Values include the degree of freedom (df), log likelihood (logLik), Akaike information criterion (AIC), delta AIC from best model (∆) and model weight.

Model	Stock	Session	Stock:Session	df	logLik	AICc	∆	weight
Act.3	X			3	-137.493	281.4	0.00	0.477
Act.4	X	X		4	-136.545	281.8	0.38	0.394

T4.1. Model selection for assessing influence of covariates sex, origin, specific growth rate (SGR), home tank and time between repeats (day) on behaviour during screening of lumpfish, including a random factor of fish ID. Factors included for models within ∆2 AIC of the best fitting model. Values include the degree of freedom (df), log likelihood (logLik), Akaike information criterion (AIC), delta AIC from best model (∆) and model weight.

Behaviour	Model	Sex	Origin	SGR	Tank	Day	df	logLik	AICc	∆	weight
Crossing Freq.	CF15	X	X	X			5	-770.85	1552.6	0.00	0.236
	CF13	X		X			4	-772.25	1553.1	0.51	0.183
Crossing Speed	CS13	X		X			5	-213.28	437.4	0.00	0.134
	CS29	X		X	X		6	-212.65	438.5	1.10	0.077
	CS17				X		4	-214.99	438.5	1.11	0.077
	CS1						3	-216.13	438.6	1.18	0.074
	CS9			X			4	-215.20	439.0	1.54	0.062
Swim Duration	SD1						3	-567.75	1141.8	0.00	0.082
	SD2					X	4	-566.69	1141.9	0.09	0.079
	SD13	X		X			5	-565.77	1142.4	0.55	0.063
	SD3		X				4	-566.99	1142.5	0.70	0.058
	SD9			X			4	-566.99	1142.6	0.71	0.058
	SD14	X		X		X	6	-564.74	1142.7	0.86	0.054
	SD10			X		X	5	-566.01	1142.9	1.04	0.049
	SD4		X			X	5	-566.13	1143.1	1.28	0.043
	SD7	X	X				5	-566.29	1143.4	1.59	0.037
	SD15	X	X	X			6	-565.16	1143.5	1.69	0.035
	SD5	X					4	-567.59	1143.8	1.91	0.032
	SD6	X				X	5	-566.49	1143.8	1.99	0.030

T4.1. Continued

Behaviour	Model	Sex	Origin	SGR	Tank	Day	df	logLik	AICc	Δ	weight
Bite Freq.	BF2					X	3	-105.93	218.2	0	0.166
	BF6	X				X	4	-104.86	218.3	0.08	0.159
Mirror Freq.	MF2					X	3	-352.06	710.5	0	0.218
	MF6	X				X	4	-351.67	711.9	1.45	0.106
	MF4		X			X	4	-351.7	712	1.51	0.103
	MF8	X	X			X	5	-350.73	712.3	1.85	0.086
Mirror Duration	MD1						3	-506.46	1019.2	0	0.175
	MD3		X				4	-505.65	1019.9	0.61	0.128
	MD17					X	4	-506.22	1021	1.75	0.073
Shelter Duration	SD2					X	4	-542.46	1093.5	0	0.147
	SD1						3	-543.65	1093.6	0.15	0.137
	SD18				X	X	5	-542.15	1095.2	1.66	0.064
	SD17				X		4	-543.31	1095.2	1.7	0.063
Perimeter Duration	PD3		X				4	-455.11	918.8	0	0.157
	PD7	X	X				5	-454.49	919.8	1.06	0.097
	PD19		X		X		5	-454.7	920.2	1.47	0.075

T4.1. Continued

Behaviour	Model	Sex	Origin	SGR	Tank	Day	df	logLik	AICc	Δ	weight
Novel Freq.	NF2		X				3	-125.83	258	0	0.325
	NF4	X	X				4	-125.52	259.6	1.61	0.145
	NF6		X	X			4	-125.55	259.7	1.67	0.141
	NF10		X		X		4	-125.59	259.7	1.75	0.136
Novel Latency	NL1						3	-451.69	909.7	0	0.174
	NL5	X					4	-451.07	910.7	0.99	0.106
	NL2					X	4	-451.31	911.2	1.47	0.084
	NL9			X			4	-451.57	911.7	1.99	0.064

T4.2. Likelihood ratio tests comparing the most plausible model and a null model for (a) behaviours measured during personality screening, (b) behaviours observed during cleaner-client interaction.

	Behaviour	χ^2	df	*P*
a)	Crossing Freq.	11.157	5	0.048
	Crossing Speed	254.530	2	<0.001
	Swim Duration		0	
	Bite Freq.	5.696	1	0.017
	Mirror Freq.	14.183	1	<0.001
	Mirror Duration	0.000	5	1.000
	Perimeter Duration	2229.100	1	<0.001
	Shelter Duration	346.590	1	<0.001
	Novel Freq.	15.709	1	<0.001
	Novel Latency	0.579	1	0.446
b)	Pursuit	17.166	1	<0.001
	Inspection	0.000	3	1.000
	Jolt	25.248	6	<0.001

T4.3. Principal component loadings of behaviours measured during screening used to generate personality scores for activity, sociality and anxiety.

Personality Factor	Behaviour	PC1 Loadings	Variation Explained
Activity	Crossing Speed	0.598	61.1%
	Crossing Freq.	0.664	
	Swimming Duration	0.448	
Sociality	Mirror Freq.	0.707	58.6%
	Mirror Duration	0.707	
Anxiety	Perimeter Duration	0.709	52.5%
	Shelter Duration	0.709	

T4.4. Model selection for assessing influence of activity score (Act), aggression score (Agg), sociality score (Soc), anxiety score (Anx), boldness score (Bld), sex, specific growth rate (SGR), genetic origin and home tank on behaviour during screening of lumpfish, with a random factor for observation day. Factors included for models within Δ2 AIC of the best fitting model. Values include the degree of freedom (df), log likelihood (logLik), Akaike information criterion (AIC), delta AIC from best model (Δ) and model weight.

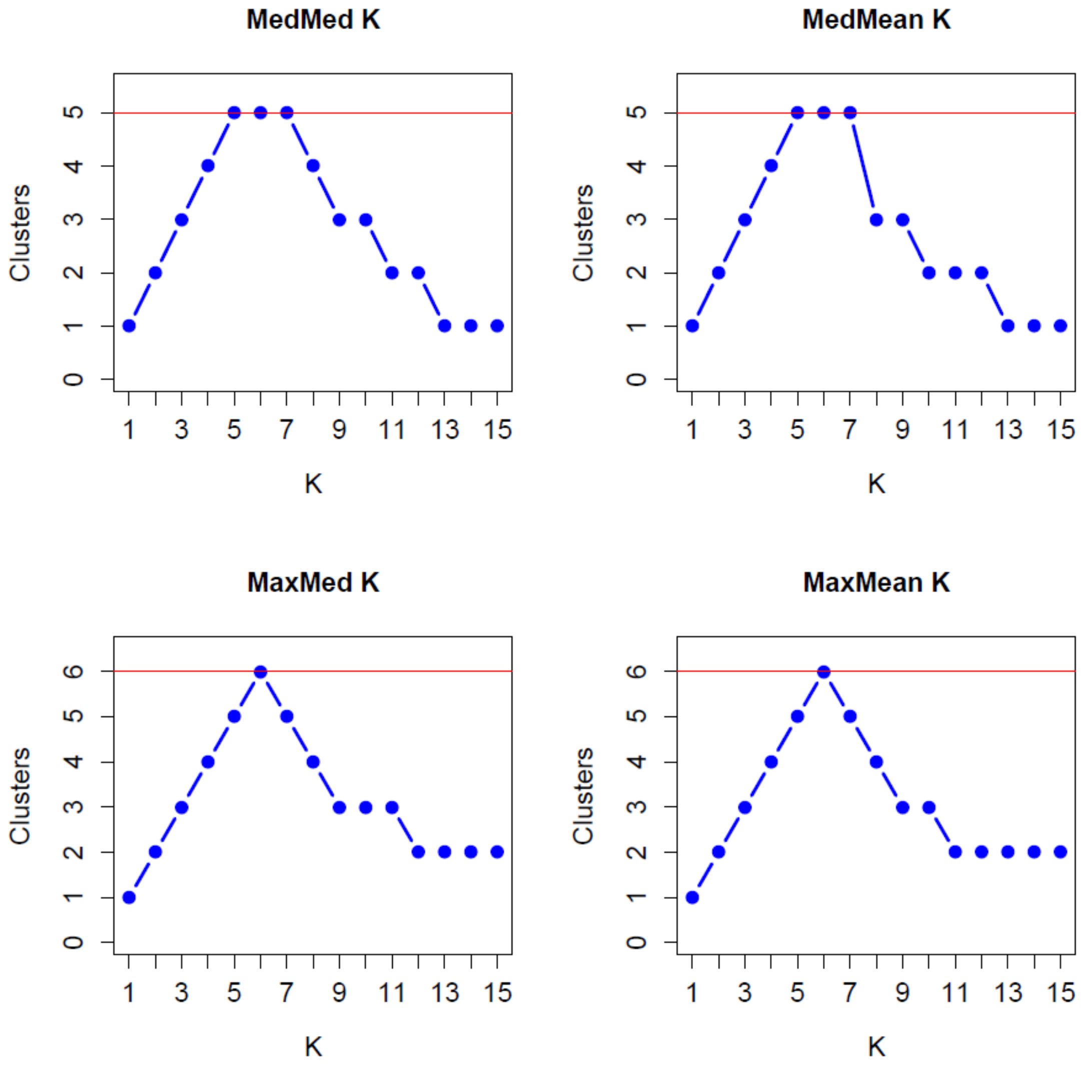
Fish Interaction	Model	Act	Agg	Soc	Anx	Bld	Sex	SGR	Origin	Tank	df	logLik	AICc	Δ	weight
Pursuit	P17					X					4	-125.514	260.2	0.00	0.060
	P22	X		X		X					6	-123.063	260.8	0.60	0.045
	P18	X				X					5	-124.544	261.0	0.72	0.042
	P21			X		X					5	-124.555	261.0	0.74	0.041
	P49					X			X		5	-124.764	261.4	1.16	0.034
	P273					X				X	5	-124.952	261.8	1.54	0.028
	P54	X		X		X			X		7	-122.196	262.1	1.89	0.023
	P50	X				X			X		6	-123.721	262.2	1.91	0.023

T4.4. Continued

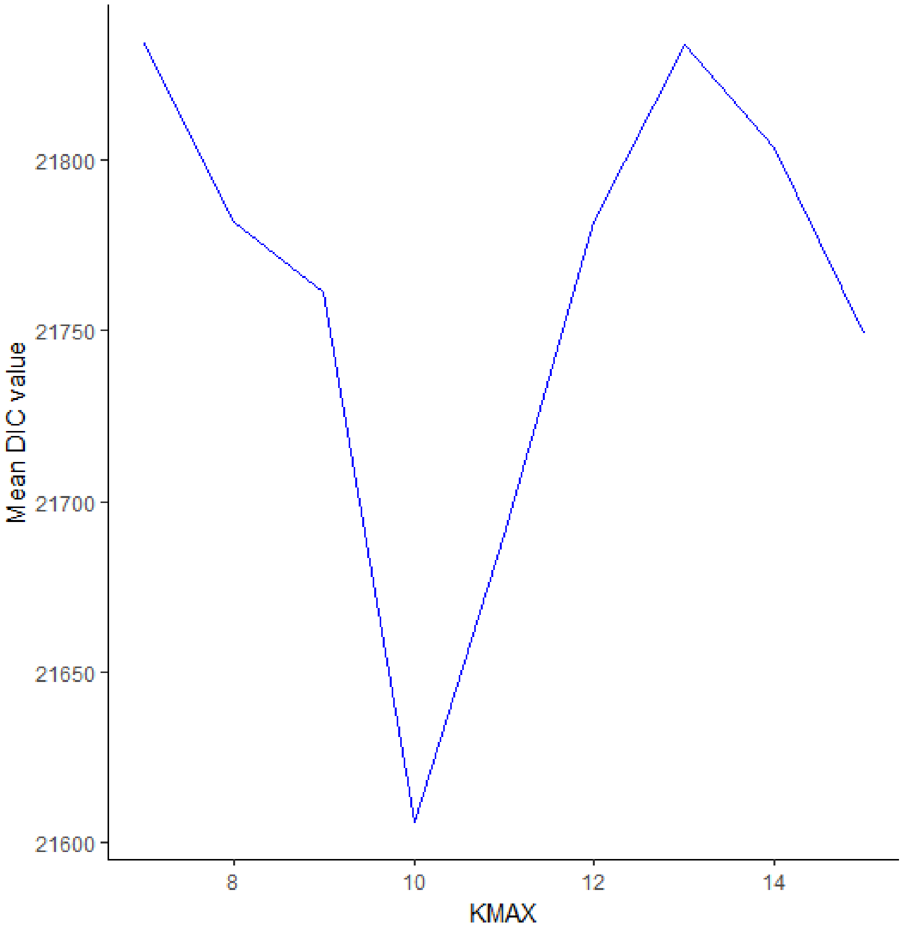
Fish Interaction	Model	Act	Agg	Soc	Anx	Bld	Sex	SGR	Origin	Tank	df	logLik	AICc	Δ	weight
Inspection	I82	X				X	X				6	-153.518	321.7	0	0.037
	I18	X				X					5	-154.982	321.8	0.09	0.035
	I149			X		X		X			6	-153.648	322	0.26	0.032
	I146	X				X		X			6	-153.848	322.4	0.66	0.026
	I147		X			X		X			6	-153.996	322.7	0.96	0.023
	I150	X		X		X		X			7	-152.536	322.8	1.06	0.022
	I145					X		X			5	-155.573	323	1.27	0.019
	I22	X		X		X					6	-154.298	323.3	1.56	0.017
	I86	X		X		X	X				7	-152.789	323.3	1.57	0.017
	I22	X									4	-157.133	323.5	1.73	0.015
	I151		X	X		X		X			7	-152.94	323.6	1.87	0.014

T4.4. Continued

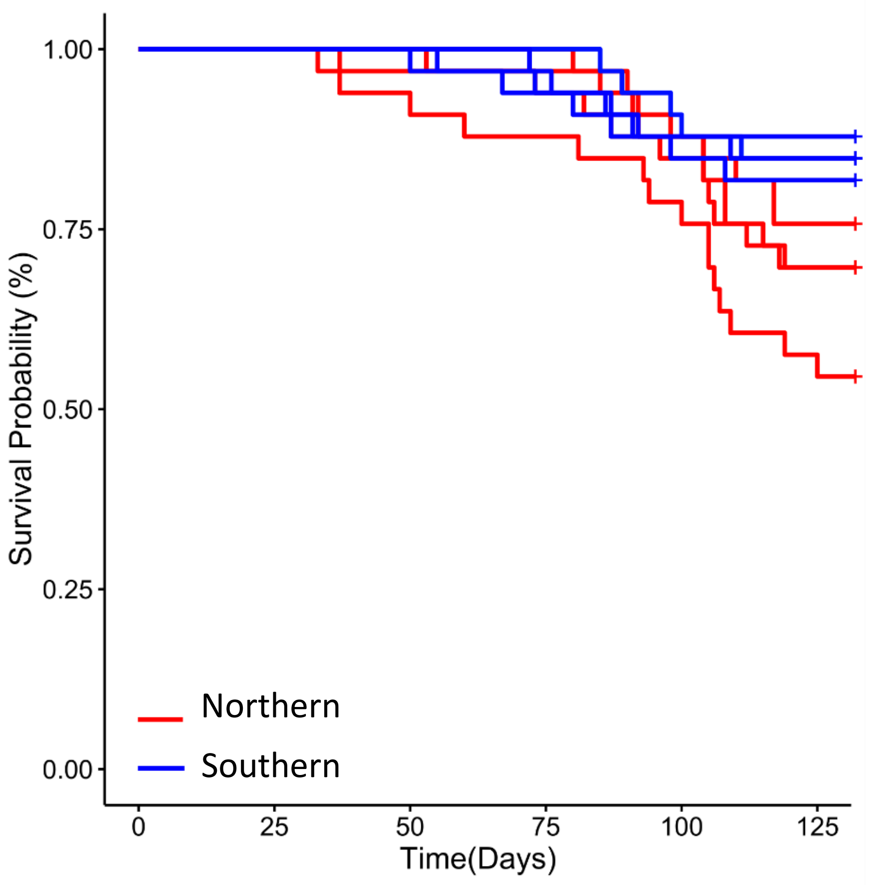
Fish Interaction	Model	Act	Agg	Soc	Anx	Bld	Sex	SGR	Origin	Tank	df	logLik	AICc	Δ	weight
Jolt	J439		X	X		X	X		X	X	9	-14.438	53.3	0	0.032
	J183		X	X		X	X		X		8	-16.406	53.8	0.47	0.025
	J151		X	X		X		X			7	-18.055	53.8	0.54	0.025
	J167		X	X				X	X		7	-18.133	54	0.69	0.023
	J135		X	X				X			6	-29.689	54.1	0.78	0.022
	J8	X	X	X				X			6	-19.842	54.4	1.09	0.019
	J423		X	X				X	X	X	8	-16.718	54.4	1.1	0.019
	J4	X	X								5	-21.268	54.4	1.11	0.018
	J7		X	X							5	-21.35	54.6	1.27	0.017
	J407		X	X		X		X		X	8	-17.011	55	1.68	0.014
	J131		X					X			5	-21.587	55	1.74	0.013
	J3		X								4	-22.953	55.1	1.81	0.013
	J263		X	X						X	6	-20.247	55.2	1.9	0.012



F1.1. Likelihood plots showing number of clusters present in STRUCTURE analysis of 15 populations genotyped using 10 microsatellite loci, informed by median of means (MedMeaK), maximum of means (MaxMeaK), median of medians (MedMeaK) and maximum of medians (MaxMedK) implemented in STRUCTURESELECTOR.



F1.2. Likelihood plot showing mean Deviance Information Criterion (DIC) of the lowest 10 DIC values per K_{MAX} within lowest 10% of DIC, implemented in TESS analysis using 15 populations genotyped with 10 microsatellite loci.



F3.1. Survival probability of northern and southern lumpfish stocks tanks from March to August 2017.

Ethics

HMO Training Certificate

SAB

SCOTTISH ACCREDITATION BOARD

This is to certify that

Benjamin Whittaker

has successfully achieved the learning outcomes of the following modules as required under UK and EU training frameworks.

MODULES: L (EU1 Legislation), E1 (EU2 Ethics)

PILA (EU3.1 Basic Biology (theory), EU4 Animal Care, Health & Management (theory), EU5 Recognition of PSD, EU7 Minimally Invasive Procedures (theory), EU3.2 Basic Biology (practical), EU8 Minimally Invasive Procedures (skills))

K (EU6.1 Humane Killing (theory), EU6.2 (skills))

PILB (EU20 Anaesthesia – Minor Procedures)

PILC (EU21 Advanced Anaesthesia, EU22 Principles of Surgery)

TRAINING IN (list animal/types species of PILA):

Freshwater Fish, Zebrafish, Estuarine Fish, Marine Fish

Training organised by: CHARLES RIVER UK LTD

Certificate Number: BE2771 Date: 4th July 2016

[redacted]

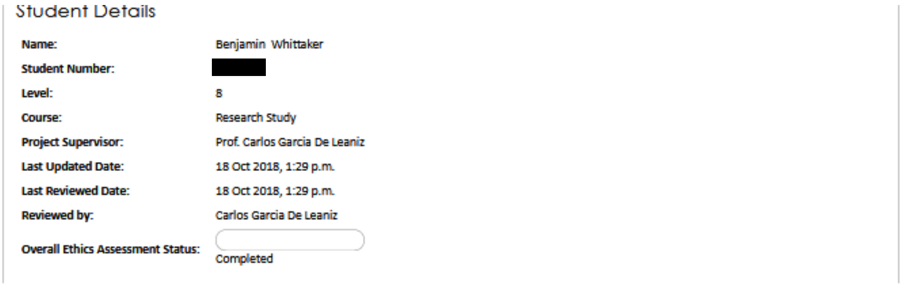
Signature 1
For Course Organiser

[redacted]

Signature 2
For Scottish Accreditation Board

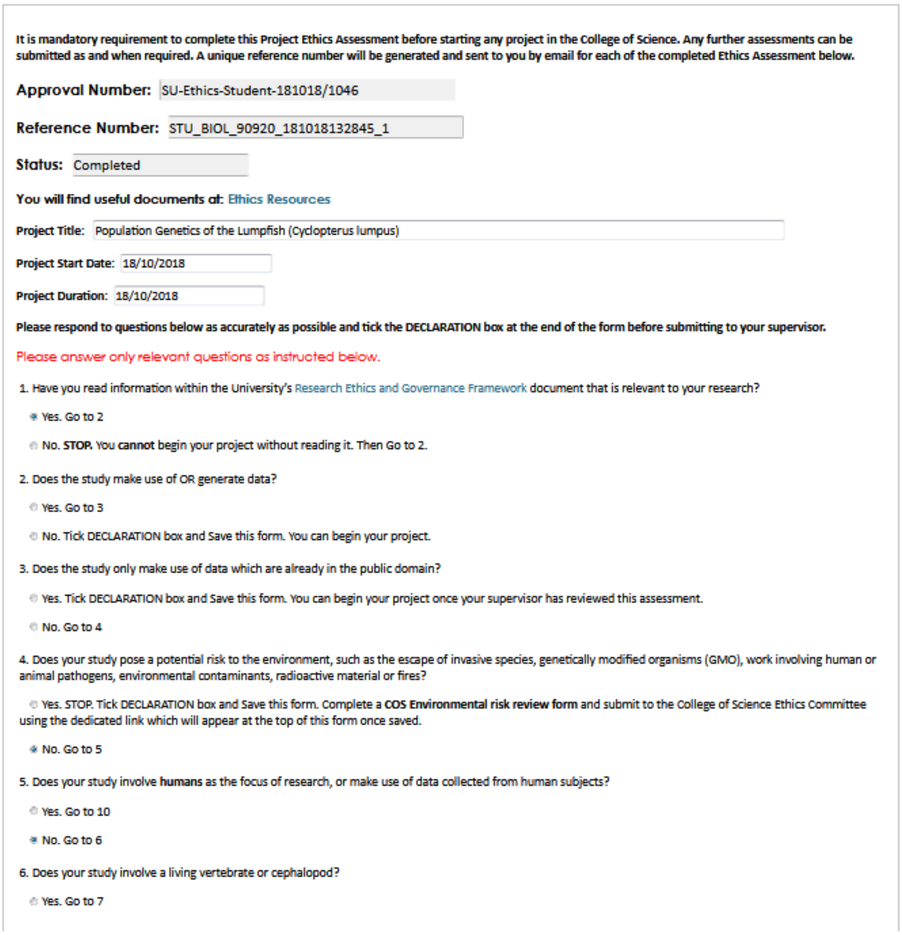
Please note, this Certificate allows you to apply for a Licence, but it is <u>not</u> a licence to perform procedures under the Animals (Scientific Procedures) Act 1986.
References to EU Modules relates to requires arising from Directive 2010/63/EU – further information can be obtained at:
http://ec.europa.eu/environment/chemicals/lab_animals/pdf/Endorsed_E-T.pdf

Chapter 1 – Ethical Approval



Student Details

Name:	Benjamin Whittaker
Student Number:	[redacted]
Level:	8
Course:	Research Study
Project Supervisor:	Prof. Carlos Garcia De Leaniz
Last Updated Date:	18 Oct 2018, 1:29 p.m.
Last Reviewed Date:	18 Oct 2018, 1:29 p.m.
Reviewed by:	Carlos Garcia De Leaniz
Overall Ethics Assessment Status:	Completed



It is mandatory requirement to complete this Project Ethics Assessment before starting any project in the College of Science. Any further assessments can be submitted as and when required. A unique reference number will be generated and sent to you by email for each of the completed Ethics Assessment below.

Approval Number: SU-Ethics-Student-181018/1046

Reference Number: STU_BIOL_90920_181018132845_1

Status: Completed

You will find useful documents at: Ethics Resources

Project Title: Population Genetics of the Lumpfish (Cyclopterus lumpus)

Project Start Date: 18/10/2018

Project Duration: 18/10/2018

Please respond to questions below as accurately as possible and tick the DECLARATION box at the end of the form before submitting to your supervisor.

Please answer only relevant questions as instructed below.

1. Have you read information within the University's Research Ethics and Governance Framework document that is relevant to your research?

● Yes. Go to 2

○ No. **STOP.** You **cannot** begin your project without reading it. Then Go to 2.

2. Does the study make use of OR generate data?

○ Yes. Go to 3

○ No. Tick DECLARATION box and Save this form. You can begin your project.

3. Does the study only make use of data which are already in the public domain?

○ Yes. Tick DECLARATION box and Save this form. You can begin your project once your supervisor has reviewed this assessment.

○ No. Go to 4

4. Does your study pose a potential risk to the environment, such as the escape of invasive species, genetically modified organisms (GMO), work involving human or animal pathogens, environmental contaminants, radioactive material or fires?

○ Yes. STOP. Tick DECLARATION box and Save this form. Complete a **COS Environmental risk review form** and submit to the College of Science Ethics Committee using the dedicated link which will appear at the top of this form once saved.

● No. Go to 5

5. Does your study involve **humans** as the focus of research, or make use of data collected from human subjects?

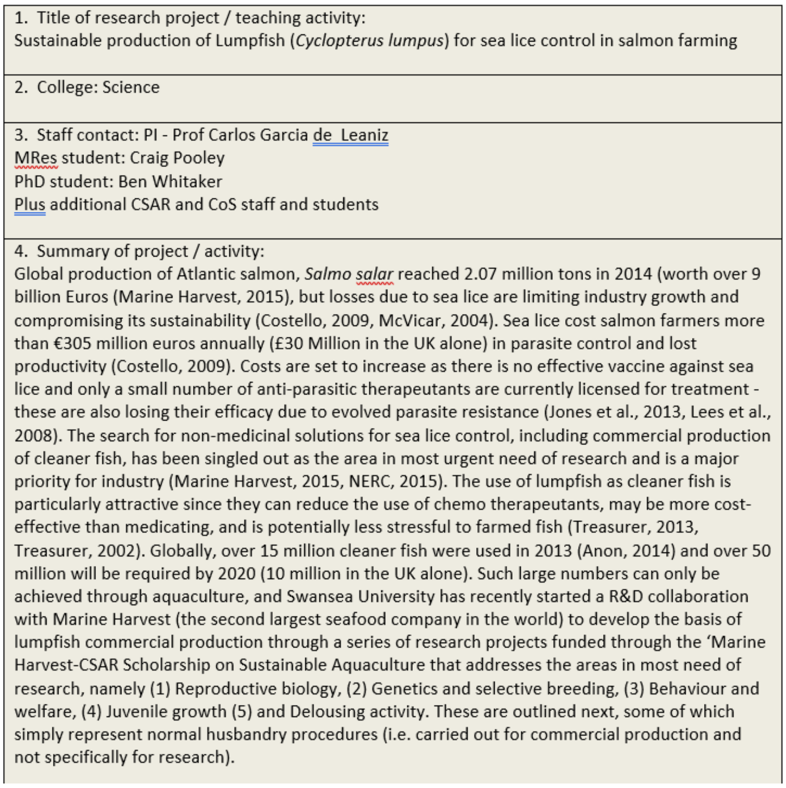
○ Yes. Go to 10

● No. Go to 6

6. Does your study involve a living vertebrate or cephalopod?

○ Yes. Go to 7

Chapters 2 and 3 – Ethical Approval

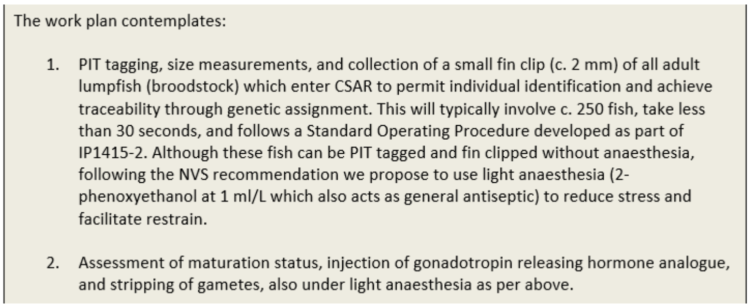


1. Title of research project / teaching activity:
Sustainable production of Lumpfish (*Cyclopterus lumpus*) for sea lice control in salmon farming

2. College: Science

3. Staff contact: PI - Prof Carlos Garcia de Leaniz
MRes student: Craig Pooley
PhD student: Ben Whitaker
Plus additional CSAR and CoS staff and students

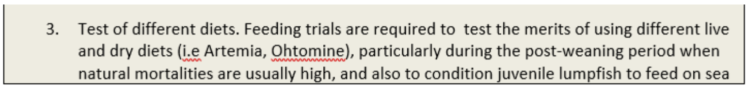
4. Summary of project / activity:
Global production of Atlantic salmon, *Salmo salar* reached 2.07 million tons in 2014 (worth over 9 billion Euros (Marine Harvest, 2015), but losses due to sea lice are limiting industry growth and compromising its sustainability (Costello, 2009, McVicar, 2004). Sea lice cost salmon farmers more than €305 million euros annually (£30 Million in the UK alone) in parasite control and lost productivity (Costello, 2009). Costs are set to increase as there is no effective vaccine against sea lice and only a small number of anti-parasitic therapeutants are currently licensed for treatment - these are also losing their efficacy due to evolved parasite resistance (Jones et al., 2013, Lees et al., 2008). The search for non-medicinal solutions for sea lice control, including commercial production of cleaner fish, has been singled out as the area in most urgent need of research and is a major priority for industry (Marine Harvest, 2015, NERC, 2015). The use of lumpfish as cleaner fish is particularly attractive since they can reduce the use of chemo therapeutants, may be more cost-effective than medicating, and is potentially less stressful to farmed fish (Treasurer, 2013, Treasurer, 2002). Globally, over 15 million cleaner fish were used in 2013 (Anon, 2014) and over 50 million will be required by 2020 (10 million in the UK alone). Such large numbers can only be achieved through aquaculture, and Swansea University has recently started a R&D collaboration with Marine Harvest (the second largest seafood company in the world) to develop the basis of lumpfish commercial production through a series of research projects funded through the 'Marine Harvest-CSAR Scholarship on Sustainable Aquaculture that addresses the areas in most need of research, namely (1) Reproductive biology, (2) Genetics and selective breeding, (3) Behaviour and welfare, (4) Juvenile growth (5) and Delousing activity. These are outlined next, some of which simply represent normal husbandry procedures (i.e. carried out for commercial production and not specifically for research).



The work plan contemplates:

1. PIT tagging, size measurements, and collection of a small fin clip (c. 2 mm) of all adult lumpfish (broodstock) which enter CSAR to permit individual identification and achieve traceability through genetic assignment. This will typically involve c. 250 fish, take less than 30 seconds, and follows a Standard Operating Procedure developed as part of IP1415-2. Although these fish can be PIT tagged and fin clipped without anaesthesia, following the NVS recommendation we propose to use light anaesthesia (2-phenoxyethanol at 1 ml/L which also acts as general antiseptic) to reduce stress and facilitate restrain.

2. Assessment of maturation status, injection of gonadotropin releasing hormone analogue, and stripping of gametes, also under light anaesthesia as per above.



3. Test of different diets. Feeding trials are required to test the merits of using different live and dry diets (i.e Artemia, Ohtomine), particularly during the post-weaning period when natural mortalities are usually high, and also to condition juvenile lumpfish to feed on sea

4. Habitat preferences. Behavioural preference tests are required to infer the optimal habitat of lumpfish in captivity. These include preference for different substrate textures, colours, depths, water velocities, densities, or other essential ecological niche features. In practice this will involve counting the number of fish that settled in different habitats in tanks offering a choice, obtained through video filming and/or direct visual observation.

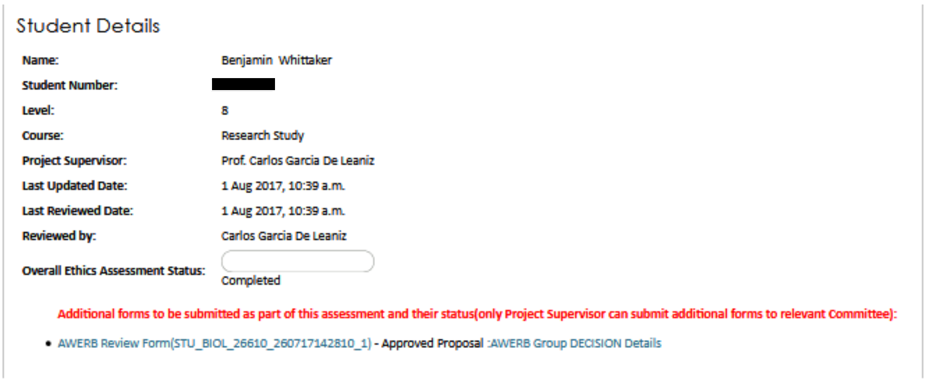
5. Welfare, environmental and social enrichment. Virtually nothing is known about how to measure the welfare of lumpfish in captivity or how this can be improved. We propose to assess welfare by considering growth, size differences, stereotyped swimming behaviours, body colouration (from photographs or video filming), fin erosion, and spatial distribution of fish in tanks that differ in enrichment structures (e.g. pipes, buoys, artificial kelp, cover, lighting) as well as presence of other species as a form of social enrichment. In the latter case, we propose to use a small number of sea bass to act as 'prefects' in duo-culture (estimated ratio 1:20), as these are available at CSAR and may well benefit from cleaner fish in the future. We will ensure (by measuring gape size) that 'prefects' cannot prey on lumpfish and will monitor their behaviour to make sure they are not overly aggressive, terminating the study if this is the case. Duo-culture can greatly reduce intra-specific competition and result in better welfare in other species (e.g. Latremouille et al 2003), so we wish to explore whether this approach will also benefit lumpfish.

Replacement: As the project is about developing the commercial production of lumpfish no replacement is possible. The use of live fish is essential.

Reduction: Being a new species to aquaculture, little or no information is available on this species so little or no pilot data are available to inform power analysis in most cases. However, we will employ mixed modelling (i.e. hierarchical analysis that combines both fixed and random factors such as tank identity; Crawley, 2007) to increase statistical power and keep the number of individuals required to a minimum. To further increase power we also propose three additional measures: (1) to use at least three replicates per treatment whenever possible (this is limited by tank numbers), (2) to use at least 20 experimental fish per tank, and (3) to consider the inclusion of covariates (such as water temperature or differences in body size) to increase precision.

Refinement: Any experimental conditions likely to cause stress (such as handling or netting) will be kept within limits typical of commercial fish farms; their severity can in all cases be considered mild.

Chapter 4 – Ethical Approval

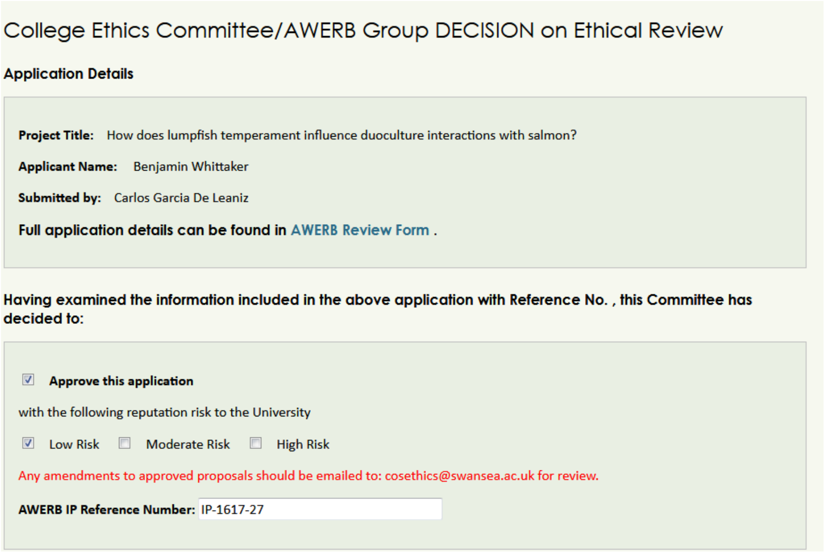


Student Details

Name: Benjamin Whittaker
Student Number: [redacted]
Level: 8
Course: Research Study
Project Supervisor: Prof. Carlos Garcia De Leaniz
Last Updated Date: 1 Aug 2017, 10:39 a.m.
Last Reviewed Date: 1 Aug 2017, 10:39 a.m.
Reviewed by: Carlos Garcia De Leaniz
Overall Ethics Assessment Status: Completed

Additional forms to be submitted as part of this assessment and their status(only Project Supervisor can submit additional forms to relevant Committee):

- AWERB Review Form(STU_BIOL_26610_260717142810_1) - **Approved Proposal** :AWERB Group DECISION Details



College Ethics Committee/AWERB Group DECISION on Ethical Review

Application Details

Project Title: How does lumpfish temperament influence duoculture interactions with salmon?
Applicant Name: Benjamin Whittaker
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. , this Committee has decided to:

☑ **Approve this application**

with the following reputation risk to the University

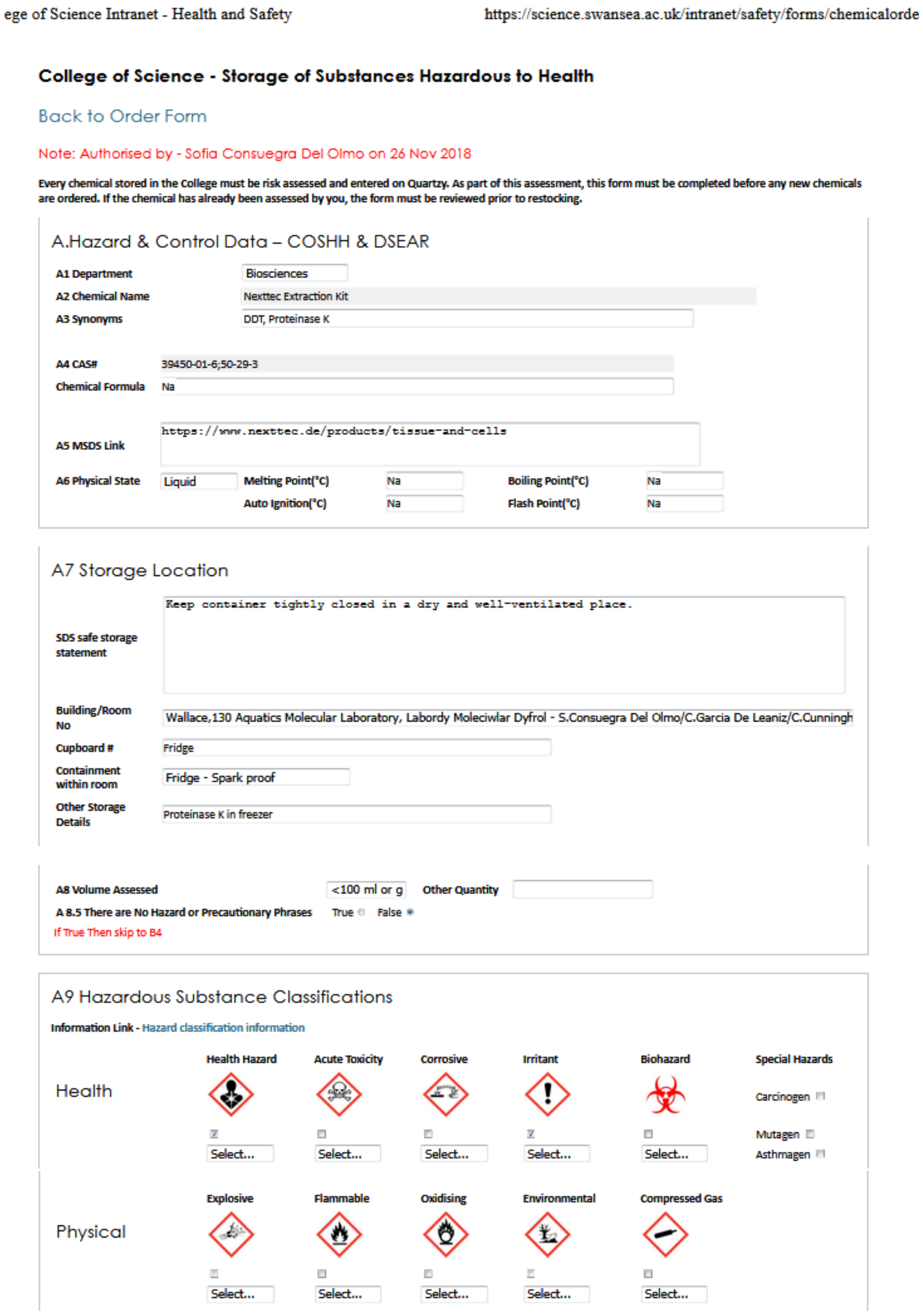
☑ Low Risk ☐ Moderate Risk ☐ High Risk

Any amendments to approved proposals should be emailed to: cosethics@swansea.ac.uk for review.

AWERB IP Reference Number: IP-1617-27

Health and Safety

Nexttec Extraction Kit



ege of Science Intranet - Health and Safety https://science.swansea.ac.uk/intranet/safety/forms/chemicalorde

College of Science - Storage of Substances Hazardous to Health

Back to Order Form

Note: Authorised by - Sofia Consuegra Del Olmo on 26 Nov 2018

Every chemical stored in the College must be risk assessed and entered on Quartzy. As part of this assessment, this form must be completed before any new chemicals are ordered. If the chemical has already been assessed by you, the form must be reviewed prior to restocking.

A.Hazard & Control Data – COSHH & DSEAR

A1 Department	Biosciences				
A2 Chemical Name	Nexttec Extraction Kit				
A3 Synonyms	DDT, Proteinase K				
A4 CAS#	39450-01-6;50-29-3				
Chemical Formula	Na				
A5 MSDS Link	`https://www.nexttec.de/products/tissue-and-cells`				
A6 Physical State	Liquid	**Melting Point(°C)**	Na	**Boiling Point(°C)**	Na
		Auto Ignition(°C)	Na	**Flash Point(°C)**	Na

A7 Storage Location

SDS safe storage statement	`Keep container tightly closed in a dry and well-ventilated place.`
Building/Room No	Wallace,130 Aquatics Molecular Laboratory, Labordy Moleciwlar Dyfrol - S.Consuegra Del Olmo/C.Garcia De Leaniz/C.Cunningh
Cupboard #	Fridge
Containment within room	Fridge - Spark proof
Other Storage Details	Proteinase K in freezer

A8 Volume Assessed <100 ml or g **Other Quantity**

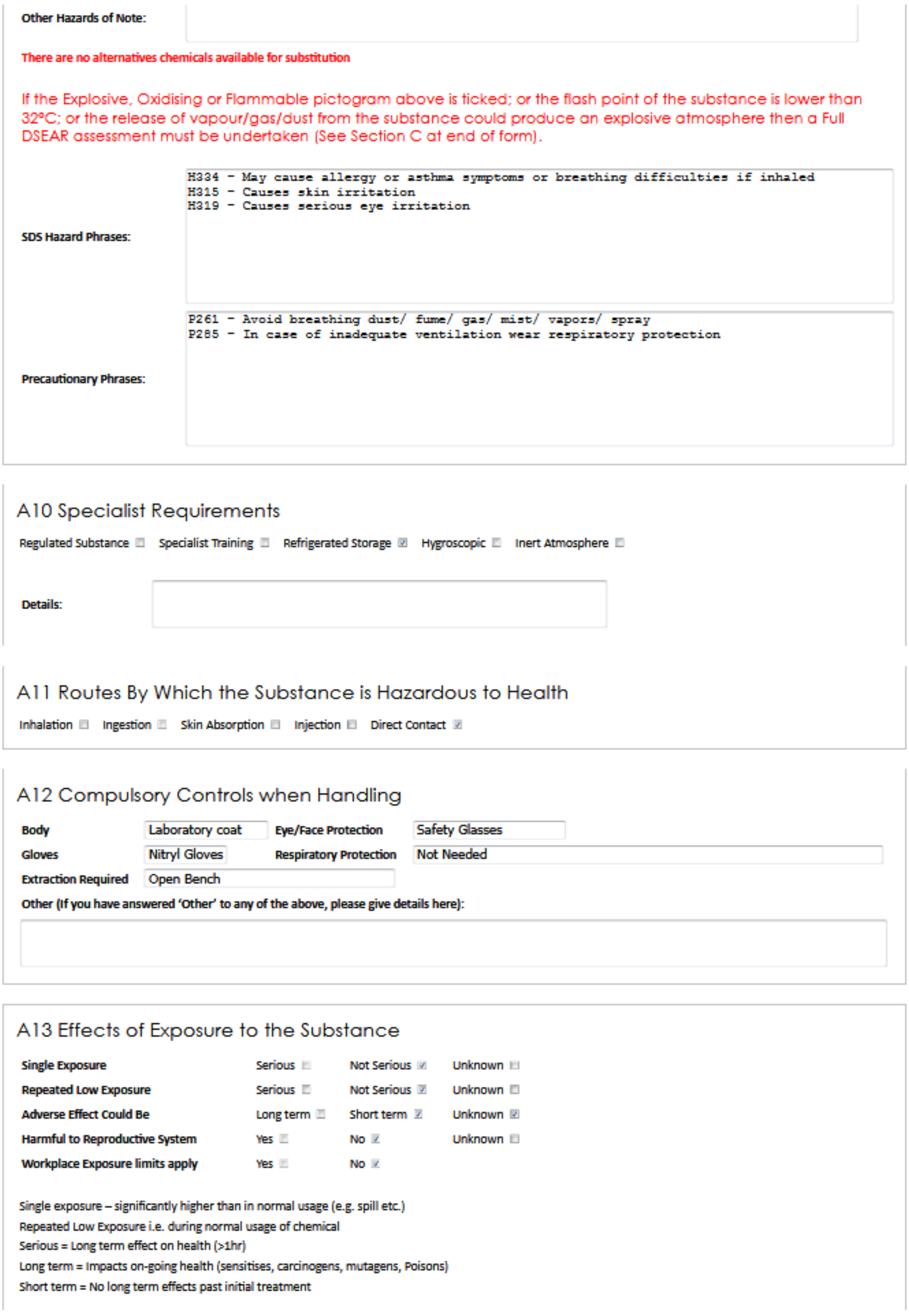
A 8.5 There are No Hazard or Precautionary Phrases True ○ False ●

If True Then skip to B4

A9 Hazardous Substance Classifications

Information Link - Hazard classification information

Health	**Health Hazard** ☑ Select...	**Acute Toxicity** ☐ Select...	**Corrosive** ☐ Select...	**Irritant** ☑ Select...	**Biohazard** ☐ Select...	**Special Hazards** Carcinogen ☐ Mutagen ☐ Asthmagen ☐
Physical	**Explosive** ☐ Select...	**Flammable** ☐ Select...	**Oxidising** ☐ Select...	**Environmental** ☐ Select...	**Compressed Gas** ☐ Select...	



Other Hazards of Note:

There are no alternatives chemicals available for substitution

If the Explosive, Oxidising or Flammable pictogram above is ticked; or the flash point of the substance is lower than 32°C; or the release of vapour/gas/dust from the substance could produce an explosive atmosphere then a Full DSEAR assessment must be undertaken (See Section C at end of form).

SDS Hazard Phrases:

H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled
H315 - Causes skin irritation
H319 - Causes serious eye irritation

Precautionary Phrases:

P261 - Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray
P285 - In case of inadequate ventilation wear respiratory protection

A10 Specialist Requirements

Regulated Substance ☐ Specialist Training ☐ Refrigerated Storage ☑ Hygroscopic ☐ Inert Atmosphere ☐

Details:

A11 Routes By Which the Substance is Hazardous to Health

Inhalation ☐ Ingestion ☐ Skin Absorption ☐ Injection ☐ Direct Contact ☑

A12 Compulsory Controls when Handling

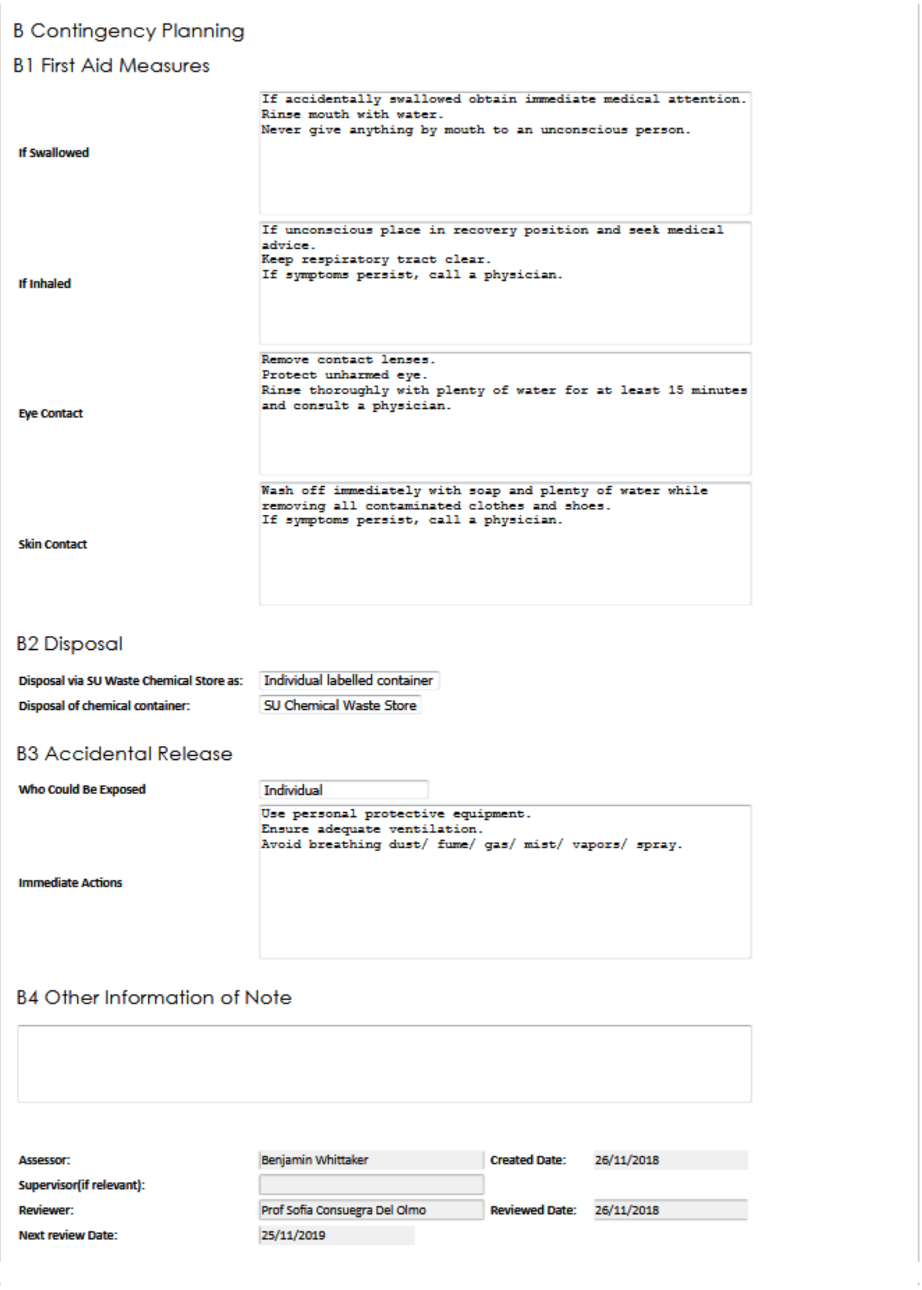
Body	Laboratory coat	**Eye/Face Protection**	Safety Glasses
Gloves	Nitryl Gloves	**Respiratory Protection**	Not Needed
Extraction Required	Open Bench		

Other (If you have answered 'Other' to any of the above, please give details here):

A13 Effects of Exposure to the Substance

Single Exposure	Serious ☐	Not Serious ☑	Unknown ☐
Repeated Low Exposure	Serious ☐	Not Serious ☑	Unknown ☐
Adverse Effect Could Be	Long term ☐	Short term ☑	Unknown ☑
Harmful to Reproductive System	Yes ☐	No ☑	Unknown ☐
Workplace Exposure limits apply	Yes ☐	No ☑	

Single exposure – significantly higher than in normal usage (e.g. spill etc.)
Repeated Low Exposure i.e. during normal usage of chemical
Serious = Long term effect on health (>1hr)
Long term = Impacts on-going health (sensitises, carcinogens, mutagens, Poisons)
Short term = No long term effects past initial treatment



B Contingency Planning

B1 First Aid Measures

If Swallowed	If accidentally swallowed obtain immediate medical attention. Rinse mouth with water. Never give anything by mouth to an unconscious person.
If Inhaled	If unconscious place in recovery position and seek medical advice. Keep respiratory tract clear. If symptoms persist, call a physician.
Eye Contact	Remove contact lenses. Protect unharmed eye. Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
Skin Contact	Wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes. If symptoms persist, call a physician.

B2 Disposal

Disposal via SU Waste Chemical Store as:	Individual labelled container
Disposal of chemical container:	SU Chemical Waste Store

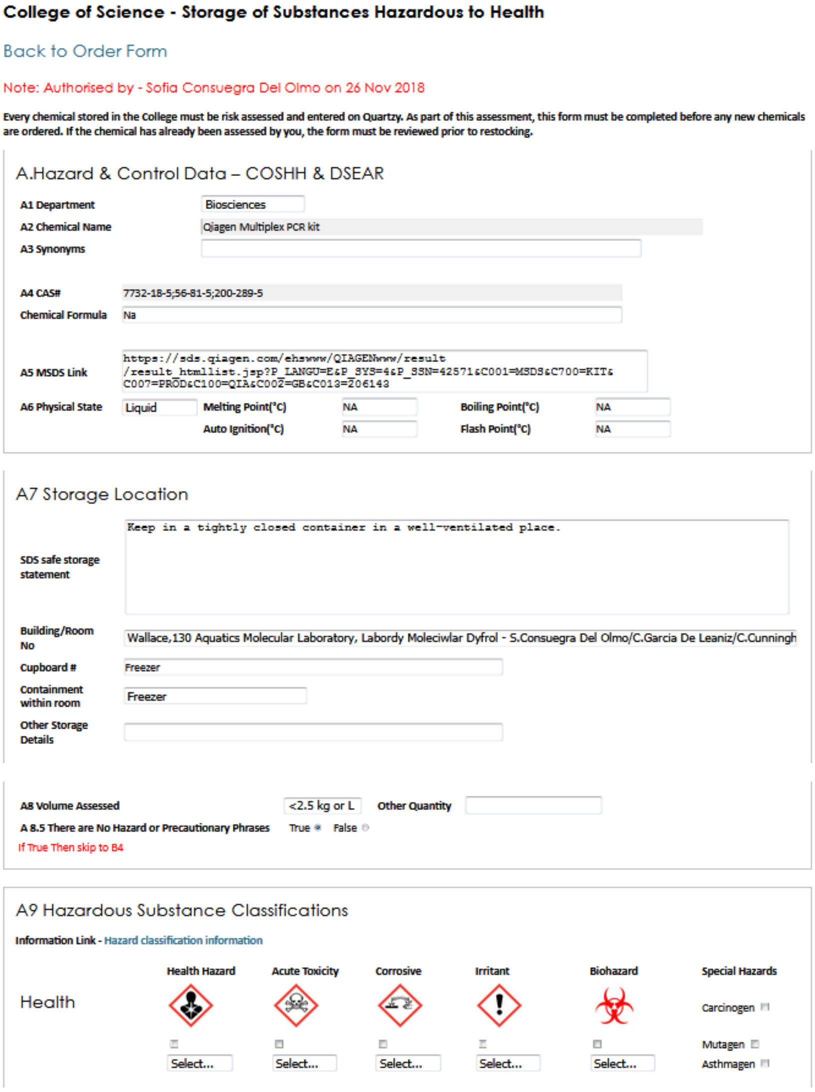
B3 Accidental Release

Who Could Be Exposed	Individual
Immediate Actions	Use personal protective equipment. Ensure adequate ventilation. Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.

B4 Other Information of Note

Assessor:	Benjamin Whittaker	**Created Date:**	26/11/2018
Supervisor(if relevant):			
Reviewer:	Prof Sofia Consuegra Del Olmo	**Reviewed Date:**	26/11/2018
Next review Date:	25/11/2019		

Qiagen Multiplex PCR Kit



College of Science - Storage of Substances Hazardous to Health

Back to Order Form

Note: Authorised by - Sofia Consuegra Del Olmo on 26 Nov 2018

Every chemical stored in the College must be risk assessed and entered on Quartzy. As part of this assessment, this form must be completed before any new chemicals are ordered. If the chemical has already been assessed by you, the form must be reviewed prior to restocking.

A.Hazard & Control Data – COSHH & DSEAR

A1 Department Biosciences
A2 Chemical Name Qiagen Multiplex PCR kit
A3 Synonyms

A4 CAS# 7732-18-5;56-81-5;200-289-5
Chemical Formula Na

A5 MSDS Link

```
https://sds.qiagen.com/ehswww/QIAGENwww/result
/result_htmllist.jsp?P_LANGU=E&P_SYS=4&P_SSN=42571&C001=MSDS&C700=KIT&
C007=PROD&C100=QIA&C002=GB&C013=206143
```

A6 Physical State	Liquid	**Melting Point(°C)**	NA	**Boiling Point(°C)**	NA
		Auto Ignition(°C)	NA	**Flash Point(°C)**	NA

A7 Storage Location

SDS safe storage statement

```
Keep in a tightly closed container in a well-ventilated place.
```

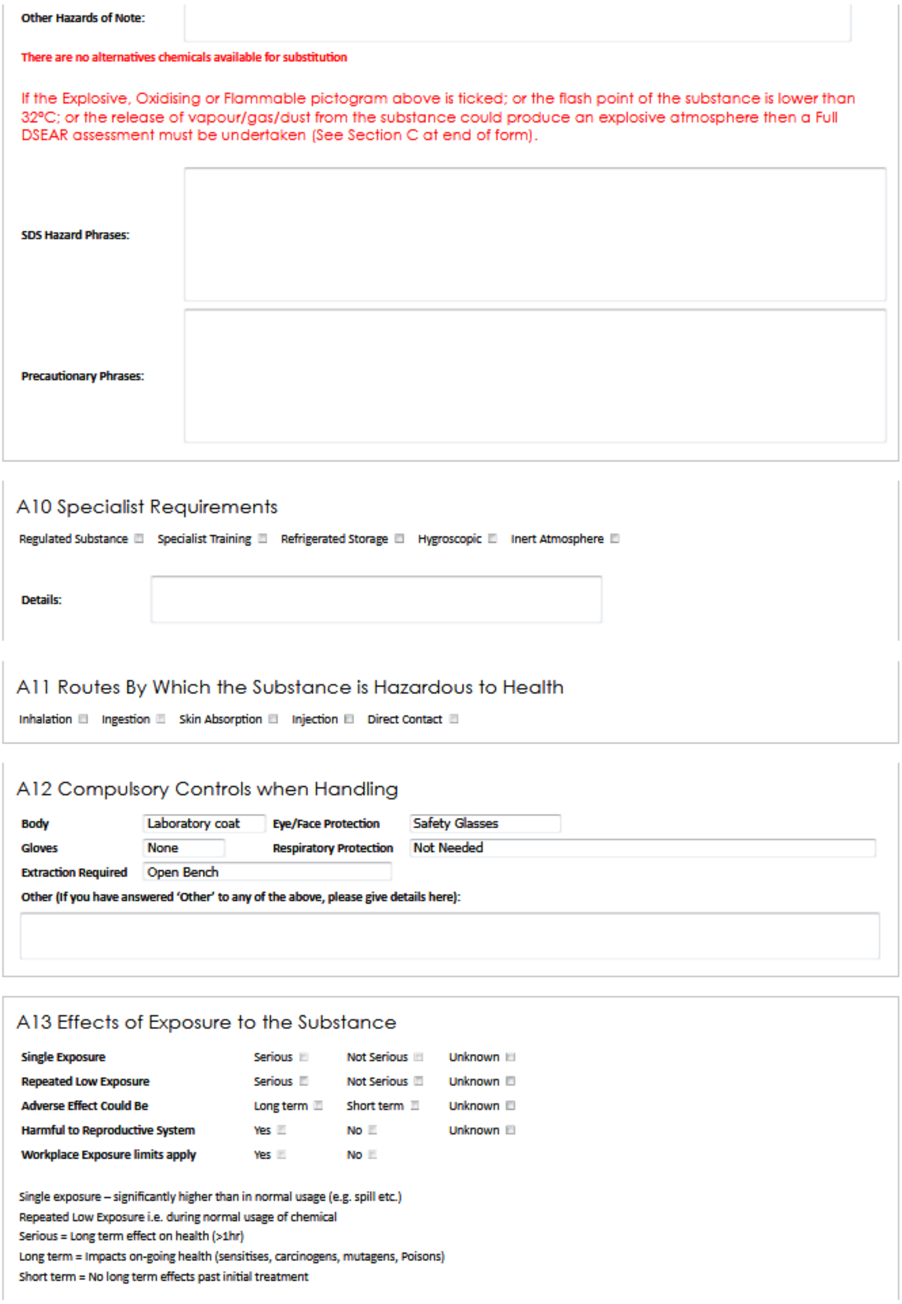
Building/Room No Wallace,130 Aquatics Molecular Laboratory, Labordy Moleciwlar Dyfrol - S.Consuegra Del Olmo/C.Garcia De Leaniz/C.Cunningh
Cupboard # Freezer
Containment within room Freezer
Other Storage Details

A8 Volume Assessed <2.5 kg or L **Other Quantity**
A 8.5 There are No Hazard or Precautionary Phrases True ● False ○
If True Then skip to B4

A9 Hazardous Substance Classifications

Information Link - Hazard classification information

	Health Hazard	Acute Toxicity	Corrosive	Irritant	Biohazard	Special Hazards
Health	☐ Select...	☐ Select...	☐ Select...	☐ Select...	☐ Select...	Carcinogen ☐ Mutagen ☐ Asthmagen ☐



Other Hazards of Note:

There are no alternatives chemicals available for substitution

If the Explosive, Oxidising or Flammable pictogram above is ticked; or the flash point of the substance is lower than 32°C; or the release of vapour/gas/dust from the substance could produce an explosive atmosphere then a Full DSEAR assessment must be undertaken (See Section C at end of form).

SDS Hazard Phrases:

Precautionary Phrases:

A10 Specialist Requirements

Regulated Substance ☐ Specialist Training ☐ Refrigerated Storage ☐ Hygroscopic ☐ Inert Atmosphere ☐

Details:

A11 Routes By Which the Substance is Hazardous to Health

Inhalation ☐ Ingestion ☐ Skin Absorption ☐ Injection ☐ Direct Contact ☐

A12 Compulsory Controls when Handling

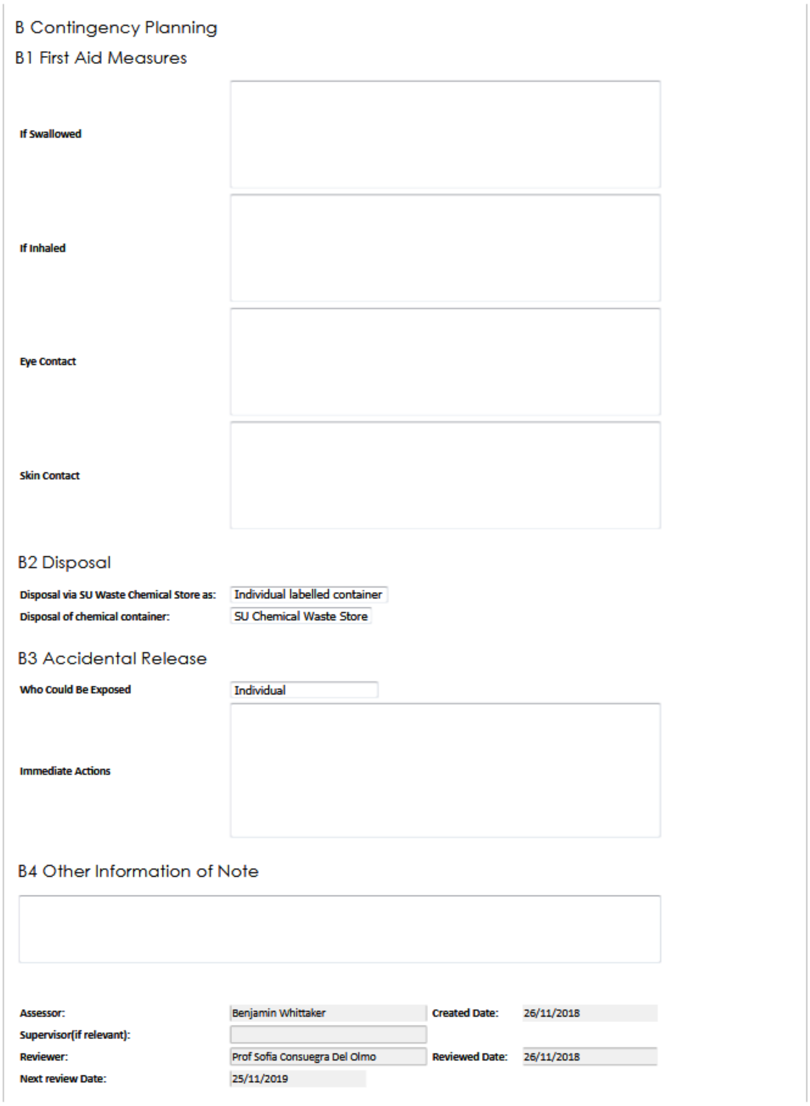
Body	Laboratory coat	**Eye/Face Protection**	Safety Glasses
Gloves	None	**Respiratory Protection**	Not Needed
Extraction Required	Open Bench		

Other (If you have answered 'Other' to any of the above, please give details here):

A13 Effects of Exposure to the Substance

Single Exposure	Serious ☐	Not Serious ☐	Unknown ☐
Repeated Low Exposure	Serious ☐	Not Serious ☐	Unknown ☐
Adverse Effect Could Be	Long term ☐	Short term ☐	Unknown ☐
Harmful to Reproductive System	Yes ☐	No ☐	Unknown ☐
Workplace Exposure limits apply	Yes ☐	No ☐	

Single exposure – significantly higher than in normal usage (e.g. spill etc.)
Repeated Low Exposure i.e. during normal usage of chemical
Serious = Long term effect on health (>1hr)
Long term = Impacts on-going health (sensitises, carcinogens, mutagens, Poisons)
Short term = No long term effects past initial treatment



B Contingency Planning

B1 First Aid Measures

If Swallowed

If Inhaled

Eye Contact

Skin Contact

B2 Disposal

Disposal via SU Waste Chemical Store as:	Individual labelled container
Disposal of chemical container:	SU Chemical Waste Store

B3 Accidental Release

Who Could Be Exposed	Individual
Immediate Actions	

B4 Other Information of Note

Assessor:	Benjamin Whittaker	**Created Date:**	26/11/2018
Supervisor(if relevant):			
Reviewer:	Prof Sofia Consuegra Del Olmo	**Reviewed Date:**	26/11/2018
Next review Date:	25/11/2019		

SCRIPTS

```
#Load libraries#
library(dplyr)
library(tidyr)
library(ggplot2)
library(lme4)
library(lmerTest)
library(readxl)
library(rcompanion)
library(gridExtra)
library(cowplot)
library(ggpubr)
library(MuMIn)
# Get Data#
SizeAnalysis <- read_excel("SizeAnalysis.xlsx")
View(SizeAnalysis)
attach(SizeAnalysis)
#Clean up for figure area#
cleanup=theme(panel.grid.major = element_blank(),panel.grid.minor =
element_blank(),panel.background = element_blank(),axis.line =
element_line((color="black")))
#
#Remove fish of Undetermined sex#
MF<- SizeAnalysis %>%
  filter(Sex == "M"|Sex == "F")
View(MF) #it worked
#
MF$Size<-MF$TotalLength
##Sucker Area##
#Full Model#
SA.mod<-lmer(SuckerArea~Sex*Stock*Size+(1|Tank),REML=FALSE,data=MF)
#Random?#
SA.mod2<-lm(SuckerArea~Sex*Stock*Size,REML=FALSE,data=MF)
anova(SA.mod,SA.mod2,test="Chisq") #No
#Assumptions#
par(mfrow=c(2,2))
plot(SA.mod2) #ok
par(mfrow=c(1,1))
#Reduction#
dredge(SA.mod2, options(na.action="na.fail"))
#Reduced model#
SA.mod3<-lm(SuckerArea~Size,REML=FALSE,data=MF)
#NUll#
SA.mod4<-lm(SuckerArea~1,REML=FALSE,data=MF)
anova(SA.mod3,SA.mod4) #Yes
#Refit#
SA.mod5<-lm(SuckerArea~Size,REML=TRUE,data=MF)
summary(SA.mod5)
#Plot#
ggsa<-ggplot(data=MF,aes(Size,SuckerArea))+
  geom_smooth(method=lm,se=FALSE)+
  geom_point(size=2.75,alpha=.8)+
  scale_color_manual(values=c("dodgerblue2","red1"))+
  xlab("Total length (mm)")+
  ylab('Sucker Area (' ~ mm^2*')')+
  cleanup
ggsa
#
```

```
##Mouth Length##
#Full Model#
ML.mod<-lmer(MouthLength~Sex*Stock*Size+(1|Tank),REML=FALSE,data=MF)
#Random?#
ML.mod2<-lm(MouthLength~Sex*Stock*Size,REML=FALSE,data=MF)
anova(ML.mod,ML.mod2,test="Chisq") #No
#Asumptions#
par(mfrow=c(2,2))
plot(ML.mod2) #ok
par(mfrow=c(1,1))
#reduction#
dredge(ML.mod2,options(na.action="na.fail"))
#Reduced#
ML.mod3<-lm(MouthLength~Sex+Size,REML=FALSE,data=MF)
#Null lRT
ML.mod4<-lm(MouthLength~1,REML=FALSE,data=MF)
anova(ML.mod3,ML.mod4, test="Chisq") #Yes
#Refit
ML.mod6<-lm(MouthLength~Sex+Size,REML=TRUE,data=MF)
summary(ML.mod6)
#Plot#
ggml<-ggplot()+
geom_smooth(data=MF,aes(Size,MouthLength,group=Sex,color=Sex),method
=lm,se=FALSE)+
geom_point(data=MF,aes(Size,MouthLength,group=Sex,color=Sex),size=2.
75,alpha=.8)+
  scale_color_manual(values=c("dodgerblue2","red1"))+
  scale_shape_manual(values=c(15,17))+
  xlab("Total Length (mm)")+
  ylab('Mouth Length (mm)')+
  cleanup
ggml
#
##Caudal Area##
CA.mod<-lmer(CaudalArea~Sex*Size*Stock+(1|Tank), REML=FALSE,
data=MF)
#Random?#
CA.mod2<-lm(CaudalArea~Sex*Size*Stock, REML=FALSE, data=MF)
anova(CA.mod,CA.mod2,test="Chisq")# No
#Asumptions#
par(mfrow=c(2,2))
plot(CA.mod2) #ok
par(mfrow=c(1,1))
#Reduction
dredge(CA.mod2,options(na.action="na.fail"))
#LRT Null#
CA.mod3<-lm(CaudalArea~1,REML=FALSE,data=MF)
anova(CA.mod2,CA.mod3) #yes
#refit
CA.mod3<-lm(CaudalArea~Sex*Size*Stock, REML=TRUE, data=MF)
summary(CA.mod3)
#
#Plot#
ggca<-ggplot(data=MF,aes(Size,CaudalArea,group=Sex,color=Sex))+
  geom_smooth(method=lm,se=FALSE)+
  geom_point(size=2.75,alpha=.8)+
  scale_color_manual(values=c("dodgerblue2","red1"))+
  xlab("Total Length (mm)")+
  ylab('Caudal Area (' ~ mm^2*')')+
  cleanup
ggca
```

```
##Hump Area##
#Full Model#
HA.mod<-lmer(HumpArea~Sex*Stock*Size+(1|Tank),REML=FALSE,data=MF)
#Random?
HA.mod2<-lm(HumpArea~Sex*Stock*Size,REML=FALSE,data=MF)
anova(HA.mod,HA.mod2,test="Chisq")# no
#Assumptions?
par(mfrow=c(2,2))
plot(HA.mod2) #ok
par(mfrow=c(1,1))
#Model Selection#
dredge(HA.mod2,options(na.action="na.fail"))
#Reduced Model#
HA.mod3<-lm(HumpArea~Sex+Size,REML=FALSE,data=MF)
#Null LRT#
HA.mod4<-lm(HumpArea~1,REML=FALSE,data=MF)
anova(HA.mod3,HA.mod4) #Yes
#Refit
HA.mod4<-lm(HumpArea~Sex+Size,REML=TRUE,data=MF)
summary(HA.mod4)
#Plot#
ggha<-ggplot(data=MF,aes(Size,HumpArea,group=Sex,color=Sex))+
  geom_smooth(method=lm,se=FALSE)+
  geom_point(size=2.75,alpha=.8)+
  scale_color_manual(values=c("dodgerblue2","red1"))+
  xlab("Total Length (mm)")+
  ylab('Hump Area (' ~ mm^2*')')+
  cleanup
ggha
#
##Flag Length##
#Full Model#
FL.mod<-lmer(FlagLength~Sex*Size*Stock+(1|Tank),REML=FALSE,data=MF)
#Random?
FL.mod2<-lm(FlagLength~Sex*Size*Stock,REML=FALSE,data=MF)
anova(FL.mod,FL.mod2,test="Chisq") #no
#Assumptions#
par(mfrow=c(2,2))
plot(FL.mod2) #ok
par(mfrow=c(1,1))
#reduction#
dredge(FL.mod2,options(na.action="na.fail"))
#Reduced#
FL.mod3<-lm(FlagLength~Sex,REML=FALSE,data=MF)
#LRT Null#
FL.mod4<-lm(FlagLength~1,REML=FALSE,data=MF)
anova(FL.mod3,FL.mod4) #yes
#refit
FL.mod5<-lm(FlagLength~Sex,REML=TRUE,data=MF)
summary(FL.mod5)
#Plot#
ggfl<-ggplot()+
geom_smooth(data=MF,aes(Size,FlagLength,group=Sex,color=Sex),method=
lm,se=FALSE)+
geom_point(data=MF,aes(Size,FlagLength,group=Stock,color=Sex),size=2
.75,alpha=.8)+
  scale_color_manual(values=c("dodgerblue2","red1"))+
  xlab("Total Length (mm)")+
  ylab('Flag Length (mm)')+
  cleanup
ggfl
```

```
##Combine plots##
ggfl
ggha
ggml
ggca
legend_a<-get_legend(ggml+theme(legend.position="bottom"))
sd<-plot_grid(ggha+theme(legend.position="none"),
              ggfl+theme(legend.position="none"),
              ggml+theme(legend.position="none"),
              ggca+theme(legend.position="none"),
              legend_a,
              ncol=2, nrow =3,
              rel_heights = c(1, 1, 0.2))

sd
```

Growth Rate

```
library(dplyr)
library(tidyr)
library(ggplot2)
library(lme4)
library(MuMIn)
library(lmerTest)
library(readxl)
library(ggResidpanel)
rm=list()
cleanup=theme(panel.grid.major = element_blank(),panel.grid.minor =
element_blank(),panel.background = element_blank(),axis.line =
element_line((color="black")))
#
#Data#
TotalSGR <-read_excel("Experiments/DataChapter2/2ndR/TotalSGR.xlsx")
View(TotalSGR)
#median#
mediansgr= TotalSGR$SGR
median(meadiansgr)
PIT<-as.factor(PIT)
#Remove unsexed#
MF<- TotalSGR %>%
  filter (Sex == "M"| Sex =="F")
View(MF)
#check n of sex
nsex<- MF %>%
  group_by(Sex) %>%
  summarise(no_rows=length(Sex))
nsex
#Full model#
SGRmodel<-lm(SGR~Country*Sex+Tank,REML=FALSE,data=MF)
#Assumptions#
par(mfrow=c(2,2))
plot(SGRmodel) # Looks ok
par(mfrow=c(1,1))
#Model reduction#
dredge(SGRmodel)
#Reduced#
SGRmodel.2<-lm(SGR~Country + Sex,REML = FALSE,data = MF)
#Null LRT
```

```
nullsgr<-lm(SGR~1,REML=FALSE,data=MF)
anova(nullsgr,SGRmodel.2) #yes
#Best model#
SGRmodel.3<-lm(SGR~Country + Sex,REML = TRUE,data = MF)
summary(SGRmodel.3)
#
#Start weight#
StartWeights<- Weight %>%
  group_by(Tank) %>%
  filter(Time =="1")
View(StartWeights)
m1<-aov(LumpWeight ~ Tank, data=StartWeights)
summary(m1)
m2<-aov(LumpWeight ~ Stock, data=StartWeights)
summary(m2)
#
#Violin Plot#
sumDataSGR<-MF %>%
  group_by(Country,Sex) %>%
  summarise(meanSGR=mean(SGR),
            sdSGR=sd(SGR))
View(sumDataSGR)
ggplot()+
  geom_violin(data=MF,aes(Country,SGR,fill=Country),alpha =.4)+
  scale_color_manual(values=c("UK"="dodgerblue3","Iceland"="red2"))+
  scale_fill_manual(values=c("UK"="dodgerblue3","Iceland"="red2"))+
  scale_shape_manual(values=c("M"=15,"F"=17))+
  xlab("Stock")+
  geom_hline(data=MF,aes(yintercept=5.349),
  linetype="dashed",color="black")+
  scale_x_discrete(breaks=c("Iceland","UK"),
  labels=c("Northern","Southern"))+
  ylab("Specific Growth Rate (%)")+
  theme(legend.position = "none")+
  cleanup
```

Swimming Activity

```
#Average Tank Behaviour#
sumdat<-BehaviourLMM %>%
  group_by(Week, Cam, Session, Stock) %>%
  summarise(meanswim = mean(No.Swimming),
           sdswim = sd(Prop.Swimming),
           meancling = mean(No.Clinging),
           sdcling = sd(No.Clinging))

#Median#
medianswim= sumdat$meanswim
median(medianswim)
#Proportion
sumdat$Prop<-cbind(sumdat$meanswim,sumdat$meancling)
View(Prop)
#Full model
Activity.1<-glmer(Prop ~  Stock + Session +Stock:Session + Cam +
(1|Week), family = "binomial",REML = FALSE, data = sumdat)
#random#
Activity.2<-glmer(Prop ~  Stock + Session +Stock:Session + (1|Week),
family = "binomial",REML = FALSE, data = sumdat)
```

```
anova(Activity.1,Activity.2,test="Chisq")# no - remove tank
#reduction#
dredge(Activity.2, options(na.action="na.fail"))
#reduced#
Activity.3<-glmer(Prop ~  Stock + (1|Week), family = "binomial",REML
= FALSE, data = sumdat)
#assumptions#
resid_panel(Activity.3) #ok
#null lRT
nullact<-glmer(Prop ~  1 + (1|Week), family = "binomial",REML =
FALSE, data = sumdat)
anova(nullact,Activity.3) #yes
#Refit
Activity.4<-glmer(Prop ~  Stock + (1|Week), family = "binomial",REML
= TRUE, data = sumdat)
summary(Activity.4)
#
#Figure#
ggplot()+
  geom_violin(data=sumdat,aes(Stock,meanswim,fill=Stock),alpha =.4)+
  scale_color_manual(values=c("UK"="dodgerblue3","Iceland"="red2"))+
  scale_fill_manual(values=c("UK"="dodgerblue3","Iceland"="red2"))+
  scale_shape_manual(values=c("M"=15,"F"=17))+
  xlab("Stock")+
  geom_hline(data=MF,aes(yintercept=12.386),
  linetype="dashed",color="black")+
  scale_x_discrete(breaks=c("Iceland","UK"),
  labels=c("Northern","Southern"))+
  ylab("Average Porportion Swimming (%)")+
  theme(legend.position = "none")+
  cleanup
```

Survival Analysis

```
#Survival analysis#
#install.packages
library(survminer)
library(ggplot2)
library(ggpubr)
library(magrittr)
library(ggfortify)
library(GGally)
library(reReg)
library(coxme)
library(survival)
library(frailtypack)
#data#
survive <-
read.csv("~/Experiments/DataChapter2/2ndR/survivalanalysis.csv")
attach(survive)
View(survive)
#Cleanup#
cleanup=theme(panel.grid.major = element_blank(),panel.grid.minor =
element_blank(),panel.background = element_blank(),axis.line =
element_line((color="black")))
#Frailtypack#
#Shared frailty model#
```

```
Shared.mod<-frailtyPenal(Surv(Start,Time,Event)~ cluster(Tank) +
Country + InitialWeight,n.knots=8, kappa=10000,  recurrentAG=FALSE,
data=survive, cross.validation =TRUE, maxit=350)
print(Shared.mod)
summary(Shared.mod)
Cmeasures(Shared.mod)
#Check Hazard ratio Plots#
plot(Shared.mod,type.plot="Survival")
plot(Shared.mod,type.plot="Hazard",conf.bands=TRUE)
plot(Shared.mod,type.plot="Survival",level=0.95,conf.bands=TRUE)
#Curve for stocks#
fit<-survfit(Surv(Time,Event)~cluster(Tank)+Country,data=survive)
ggsurvplot(fit,conf.int=TRUE,palette=c("#FF0000","#0000FF"),xlab=("T
ime (Days)"),ylab=("Survival Probability (%)"),legend=c("none"))
#Check Tanks#
fit2<-
survfit(Surv(Time,Event)~Country+frailty(Tank,df=2),data=survive)
ggsurvplot(fit2,legend.labs=c("1","2","3","4","5","6","7","8"),palet
te =
c("#FF0000","#FF0001","#FF0002","#FF0003","#0010FF","#0100FF","#0001
FF","#0000FF"), xlab=("Time(Days)"),ylab=("Survival Probability
(%)"),legend=c("none"))
```

Personality Covariates & Repeatability Analysis

```
#Start#
#Load Library#
rm=list()
library(ggplot2)
library(readxl)
library(lme4)
library(lmerTest)
library(rptR)
library(dplyr)
library(rcompanion)
library(MuMIn)
library(pscl)
library(scales)
#Get Data#
Personality <- PersonalityDataFinal
View(Personality)
attach(Personality)
#Clean up Figures#
cleanup=theme(panel.grid.major = element_blank(),panel.grid.minor =
element_blank(),panel.background = element_blank(),axis.line =
element_line((color="black")))
#
Fish_ID<-as.factor(Fish_ID)
#
###Activity###
#
##Crossing Frequency##
#Full Model#
CrossFreq.mod<-glmer(Crossing_Freq ~ Sex + Origin + SGR + Tank + Day
+ (1|Fish_ID), family="poisson",data=Personality, REML=FALSE)
#Model Reduction#
dredge(CrossFreq.mod)
#Reduced#
```

```
CrossFreq.mod2<-glmer(Crossing_Freq ~ Sex + SGR + Origin +
(1|Fish_ID), data=Personality, family = "poisson", REML=FALSE)
#Null LRT#
nullcrossfreq<-
glmer(Crossing_Freq~1+(1|Fish_ID),family="poisson",data=Personality)
anova(nullcrossfreq,CrossFreq.mod2, test="Chisq") #Yes
#Best#
CrossFreq.mod3<-glmer(Crossing_Freq ~ Sex + SGR + Origin +
(1|Fish_ID), family = "poisson", data=Personality, REML=TRUE)
summary(CrossFreq.mod3) # Sex*, SGR**
#Repeatability#
plotNormalDensity(Crossing_Freq) #Gaussian
CrossFreqrep<-rptPoisson(Crossing_Freq ~ Sex + SGR + (1|Fish_ID)
,grname = "Fish_ID", data=Personality, nboot=1000, npermut= 1000)
summary(CrossFreqrep) #Repeatable!#
print(CrossFreqrep)
#
##Crossing Time##
#Full Model#
CrossTime.mod<-
lmer(Crossing_Time~Sex+Origin+SGR+Tank+Day+(1|Fish_ID), REML=FALSE,
data=Personality)
#Model Reduction#
dredge(CrossTime.mod)
#Reduced model#
CrossTime.mod2<-
lmer(Crossing_Time~Sex+SGR+(1|Fish_ID),REML=FALSE,data=Personality)
#Assumptions#
#Assumptions#
resid_panel(CrossTime.mod2) #normality?
#Cook's#
plot(cooks.distance(CrossTime.mod2))
#Transform#
lgCrossing_Time <- log10(Crossing_Time+0.1)
Personality$lgCrossing_Time <- log10(Crossing_Time +0.1)
lgCrossTime.mod2<-lmer(lgCrossing_Time~Sex+SGR+(1|Fish_ID), REML =
FALSE, data=Personality)
#Null LRT#
nullcrosstime<-lmer(lgCrossing_Time~1+(1|Fish_ID), REML = FALSE,
data=Personality)
anova(nullcrosstime,lgCrossTime.mod2, test="Chisq") #Yes
#Best#
CrossTime.mod3<-lmer(lgCrossing_Time~Sex+SGR+(1|Fish_ID), REML =
TRUE, data=Personality)
summary(CrossTime.mod3) #none
##rptR##
lgCrossSpeed<-rptGaussian(lgCrossing_Time ~ (1|Fish_ID),grname =
"Fish_ID", data=Personality, nboot=1000, npermut= 1000)
summary(lgCrossSpeed) #Repeatable
print(lgCrossSpeed)
#
##Swimming Time##
#Full Model#
Swim.mod<-
lmer(Swim_Total~Sex+Origin+SGR+Tank+Day+(1|Fish_ID),REML=FALSE,data=
Personality)
#Reduction#
dredge(Swim.mod)
#Reduced model#
Swim.mod2<-
lmer(Crossing_Time~(1|Fish_ID),REML=FALSE,data=Personality)
```

```
#Assumptions#
resid_panel(Swim.mod2) #normality?
#Cook's#
plot(cooks.distance(Swim.mod2))
#Transform#
SqSwim_Total <- sqrt(Swim_Total+0.1)
Personality$SqSwim_Total <- sqrt(Swim_Total+0.1)
SqSwim.mod2<-lmer(SqSwim_Total~(1|Fish_ID), REML = FALSE,
data=Personality)
#Assumptions#
resid_panel(SqSwim.mod2) #better
#Cook's#
plot(cooks.distance(SqSwim.mod2))#ok
#Null LRT#
#Is null#
anova(Swim.mod,SqSwim.mod2,test="Chisq") #no
#
Swim.mod3<-lmer(SqSwim_Total~(1|Fish_ID), REML = TRUE,
data=Personality)
summary(Swim.mod3)
#Repeatability#
Swimrep<-rptGaussian(SqSwim_Total ~ (1|Fish_ID),grname = "Fish_ID",
data=Personality, nboot=1000, npermut= 1000)
summary(Swimrep) #Repeatable
print(Swimrep)
#
###Aggression###
#
##Bite Freq##
#Full Model#
Bite.mod<-glmer(Bite_Freq~Sex+Origin+SGR+Tank+Day+(1|Fish_ID),family
= "poisson", REML=FALSE, data=Personality)
#Reduction#
dredge(Bite.mod)
#Reduced model#
Bite.mod2<-glmer(Bite_Freq~Day+(1|Fish_ID),family = "poisson",
REML=FALSE, data=Personality)
#Null LRT#
nullbit<-glmer(Bite_Freq~1+(1|Fish_ID),family = "poisson",
REML=FALSE, data=Personality)
anova(nullbit,Bite.mod2,test="Chisq") #no
Bite.mod3<-glmer(Bite_Freq~Day+(1|Fish_ID), REML = TRUE,
family="poisson",data=Personality)
summary(Swim.mod3) #none
#Repeatability#
Biterep<-rptPoisson(Bite_Freq ~ (1|Fish_ID),grname = "Fish_ID",
data=Personality, nboot=1000, npermut= 1000)
summary(Biterep)
print(Biterep)
#
###Extraversion###
#
##Mirror Frequency##
#Full Model#
#glmer#
Mirror.mod<-
glmer(Mirror_Freq~Sex+Origin+SGR+Tank+Day+(1|Fish_ID),family =
"poisson", REML = FALSE, data=Personality)
#Reduction#
dredge(Mirror.mod)
#Reduced Model#
```

```
Mirror.mod2<-glmer(Mirror_Freq~Day+(1|Fish_ID),family = "poisson",
REML = FALSE, data=Personality)
#Null LRT#
nullmirrorfreq<-glmer(Mirror_Freq~1+(1|Fish_ID),family = "poisson",
REML = FALSE, data=Personality)
anova(nullmirrorfreq,Mirror.mod2) #sig
#
Mirror.mod3<-glmer(Mirror_Freq~Day+(1|Fish_ID),family = "poisson",
REML = TRUE, data=Personality)
summary(Mirror.mod3) #Day***
#Repeatability#
Mirrorrep<-rptPoisson(Mirror_Freq ~ Day + (1|Fish_ID),grname =
"Fish_ID", data=Personality, nboot=1000, npermut= 1000)
summary(Mirrorrep) #Repeatable
print(Mirrorrep)
#
##Mirror Time##
#Full model#
MirrorTime.mod<-
lmer(Mirror_Time~Sex+Origin+SGR+Tank+Day+(1|Fish_ID),REML=FALSE,data
=Personality)
#reduction#
dredge(MirrorTime.mod)
#Reduced Model#
MirrorTime.mod2<-
lmer(Mirror_Time~(1|Fish_ID),REML=FALSE,data=Personality)
#Assumptions#
resid_panel(MirrorTime.mod2) #normality?
#Cook's#
plot(cooks.distance(MirrorTime.mod2))#ok
#Transform#
lgMirror_Time <- log10(Mirror_Time+0.1)
Personality$lgMirror_Time <- log10(Mirror_Time+0.1)
lgMirrorTime.mod2<-
lmer(lgMirror_Time~(1|Fish_ID),REML=FALSE,data=Personality)
#Assumptions#
resid_panel(lgMirrorTime.mod2) #better
#Cook's#
plot(cooks.distance(lgMirrorTime.mod2))#ok
#Null LRT#
#Is null#
nullmirrortime<-lmer
anova(MirrorTime.mod,lgMirrorTime.mod2,test="Chisq") #no
#
summary(lgMirrorTime.mod2)
#Repeatability#
plotNormalDensity(Mirror_Time) #Poisson
MirrorTimerep<-rptGaussian(lgMirror_Time ~(1|Fish_ID),grname =
"Fish_ID", data=Personality, nboot=1000, npermut= 1000)
summary(MirrorTimerep) #Repeatable!
print(MirrorTimerep)
#
###Neuroticism###
##Shelter Time##
#full model#
Shelter.mod<-
lmer(Shelter_Time~Sex+Origin+SGR+Tank+Day+(1|Fish_ID),REML=FALSE,dat
a=Personality)
dredge(Shelter.mod)
#reduced model#
```

```
Shelter.mod2<-
lmer(Shelter_Time~Day+(1|Fish_ID),REML=FALSE,data=Personality)
#Assumptions#
resid_panel(Shelter.mod2) #normality?
#Cook's#
plot(cooks.distance(Shelter.mod2))
#Transform#
SQShelter_Time <-sqrt(Shelter_Time)
Personality$SQShelter_Time <- sqrt(Shelter_Time)
SQShelter.mod2<-
lmer(SQShelter_Time~Day+(1|Fish_ID),REML=FALSE,data=Personality)
#Assumptions#
#Assumptions#
resid_panel(SQShelter.mod2) #better
#Cook's#
plot(cooks.distance(SQShelter.mod2)) #ok
#Null LRT#
nullshelter<-
lmer(SQShelter_Time~1+(1|Fish_ID),REML=FALSE,data=Personality)
anova(nullshelter,SQShelter.mod2) #yes
#Best Model#
Shelter.mod2<-lmer(Shelter_Time~Day+(1|Fish_ID),REML=TRUE,
data=Personality)
summary(Shelter.mod2) #none
#Repeatability#
Shelterrep<-rptGaussian(Shelter_Time ~ (1|Fish_ID),grname =
"Fish_ID", data=Personality, nboot=1000, npermut= 1000)
summary(Shelterrep) #Repeatable!
print(Shelterrep)
#
##Perimeter##
#Full Model#
Perimeter.mod<-
lmer(Perimeter_Time~Sex+Origin+SGR+Tank+Day+(1|Fish_ID),REML=FALSE,d
ata=Personality)
#reduction#
dredge(Perimeter.mod)
#reduced model#
Perimeter.mod2<-
lmer(Perimeter_Time~Origin+(1|Fish_ID),REML=FALSE,data=Personality)
#Assumptions#
resid_panel(Perimeter.mod2) #ok
#Cook's#
plot(cooks.distance(Perimeter.mod2)) #ok
#Transform#
lgPerimeter_Time <- log10(Perimeter_Time+0.1)
Personality$lgPerimeter_Time <-log10(Perimeter_Time+0.1)
lgPerimeter.mod2<-
lmer(lgPerimeter_Time~Origin+(1|Fish_ID),REML=FALSE,data=Personality
)
#Assumptions#
resid_panel(lgPerimeter.mod2) #better
#Cook's#
plot(cooks.distance(lgPerimeter.mod2)) #ok
#Null LRT#
nullperimeter<-
lmer(Perimeter_Time~1+(1|Fish_ID),REML=FALSE,data=Personality)
anova(nullperimeter,lgPerimeter.mod2,test="Chisq") #Yes
#Best#
Perimeter.mod3<-
lmer(lgPerimeter_Time~Origin+(1|Fish_ID),REML=TRUE,data=Personality)
```

```
summary(Perimeter.mod3)
#Repeatability
Perimeterrep<-rptGaussian(lgPerimeter_Time ~ (1|Fish_ID),grname =
"Fish_ID", data=Personality, nboot=1000, npermut= 1000)
summary(Perimeterrep)
View(Personality)
print(Perimeterrep)
#
###Openness###
##Novel Freq##
#Full Model#
Novel.mod<-
glmer(Novel_Freq~Sex+Origin+SGR+Tank+(1|Fish_ID),REML=FALSE,family="
poisson", data=Personality)
#Reduction#
dredge(Novel.mod)
#reduced model#
Novel.mod2<-
glmer(Novel_Freq~Origin+(1|Fish_ID),REML=FALSE,family="poisson",
data=Personality)
#Null LRT#
nullnovel<-
glmer(Novel_Freq~1+(1|Fish_ID),REML=FALSE,family="poisson",
data=Personality)
anova(nullnovel,Novel.mod2) #yes
#Best#
Novel.mod3<-
glmer(Novel_Freq~Origin+(1|Fish_ID),REML=TRUE,family="poisson",
data=Personality)
summary(Novel.mod3) #Origin***
#Repeatability#
Novelrep<-rptPoisson(Novel_Freq ~ Origin + (1|Fish_ID),grname =
"Fish_ID", data=Personality, nboot=1000, npermut= 1000)
summary(Novelrep) #Yes
print(Novelrep)
#
##Novel latency##
#full model
Novelate.mod<-
lmer(Novel_Latency~Sex+Origin+SGR+Tank+Day+(1|Fish_ID),REML=FALSE,
data=Personality)
#reduction#
dredge(Novelate.mod)
#reduced model#
Novelate.mod2<-lmer(Novel_Latency~(1|Fish_ID),REML=FALSE,
data=Personality)
#Assumptions#
resid_panel(Novelate.mod2) #normality?
#Cook's#
plot(cooks.distance(Novelate.mod2))
#Transform#
sqNovel_Latency <- sqrt(Novel_Latency)
Personality$sqNovel_Latency <-sqrt(Novel_Latency)
sqNovelate.mod2<-
lmer(sqNovel_Latency~Origin+(1|Fish_ID),REML=FALSE,data=Personality)
#Assumptions#
resid_panel(sqNovelate.mod2) #better
#Cook's#
plot(cooks.distance(sqNovelate.mod2)) #ok
#null LRT#
```

```
nulllate<-
lmer(sqNovel_Latency~1+(1|Fish_ID),REML=FALSE,data=Personality)
anova(nulllate,sqNovelate.mod2, test="Chisq") #no
#
Novelate.mod3<-lmer(Novel_Latency~(1|Fish_ID),REML=FALSE,
data=Personality)
summary(Novelate.mod3)
#Repeatability#
Novelaterep<-rptGaussian(Novel_Latency ~ (1|Fish_ID),grname =
"Fish_ID", data=Personality, nboot=1000, npermut= 1000)
summary(Novelaterep)
print(Novelaterep)
#
```

Cleaner-Client Analysis

```
#Start#
#Load Library#
rm=list()
library(ggplot2)
library(readxl)
library(lme4)
library(lmerTest)
library(rptR)
library(dplyr)
library(MuMIn)
library(fmsb)
library(radarchart)
library(scales)
library(gridExtra)
library(cowplot)
library(ggpubr)
library(lattice)
library(ggResidpanel)
#Get Data#
read_excel("F:/Chapter3/PersonalityPCAData")
attach(PersonalityPCAdata)
View(PersonalityPCAdata)
#Clean up Figures#
cleanup=theme(panel.grid.major = element_blank(),panel.grid.minor =
element_blank(),panel.background = element_blank(),axis.line =
element_line((color="black")))
#Rescale#
Agg<-rescale(Bite,to=c(0:1))
PersonalityPCAdata$Agg<-Agg
Opp<-rescale(Novel,to=c(0:1))
PersonalityPCAdata$Opp<-Opp
#
#PCA of Personalities!#
#Activity#
#Make Data Frame#
Actmatrix<-
data.frame(row.names=Fish_ID,Crossing_Freq,Crossing_Time,Swim_Total)
View(Actmatrix) #looks ok
#PCA#
ActPCA<-prcomp((Actmatrix),scale=TRUE)
plot(ActPCA$x[,1],ActPCA$x[,2])
#Look at variation#
pca.var<-ActPCA$sdev^2
pca.var.per<-round(pca.var/sum(pca.var)*100,1)
```

```
barplot(pca.var.per,main="Scree Plot",xlab="Principal
Component",ylab="Percent Variation")
#Make a ggplot#
ggAct<-data.frame(Sample = rownames(ActPCA$x),
                  X=ActPCA$x[,1],
                  Y=ActPCA$x[,2])
ggAct #looks ok
ggplot(data=ggAct,aes(X,Y))+
  geom_point()+
  xlab(paste("PC1 - ",pca.var.per[1],"%",sep=""))+
  ylab(paste("PC2 -",pca.var.per[2],"%",sep=""))+
  theme_bw()+
  cleanup
#PC1 = 61.1%, PC2=27.9%#
PersonalityPCAdata$ActPC1<-ActPCA$x[,1]
View(PersonalityPCAdata)
ActPC1
#PC1 loadings#
loadingscores<-ActPCA$rotation[,1]
CID_scores<-abs(loadingscores)
CID_score_ranked<-sort(CID_scores,decreasing=TRUE)
CID_score_ranked
Act<-rescale(PersonalityPCAdata$ActPC1,to=(0:1))
PersonalityPCAdata$Act<-rescale(PersonalityPCAdata$ActPC1,to=(0:1))
#
#Extraversion#
#Make Data Frame#
Extmatrix<-data.frame(row.names=Fish_ID,Mirror_Freq,Mirror_Time)
View(Extmatrix) #looks ok
#PCA#
ExtPCA<-prcomp((Extmatrix),scale=TRUE)
plot(ExtPCA$x[,1],ExtPCA$x[,2])
#Look at variation#
pca.var<-ExtPCA$sdev^2
pca.var.per<-round(pca.var/sum(pca.var)*100,1)
barplot(pca.var.per,main="Scree Plot",xlab="Principal
Component",ylab="Percent Variation")
#Make a ggplot#
ggExt<-data.frame(Sample = rownames(ExtPCA$x),
                  X=ExtPCA$x[,1],
                  Y=ExtPCA$x[,2])
ggExt #looks ok
ggplot(data=ggExt,aes(X,Y))+
  geom_point()+
  xlab(paste("PC1 - ",pca.var.per[1],"%",sep=""))+
  ylab(paste("PC2 -",pca.var.per[2],"%",sep=""))+
  theme_bw()+
  cleanup
#PC1 = 48%, PC2=27%#
PersonalityPCAdata$ExtPC1<-ExtPCA$x[,1]
#PC1 loadings#
loadingscores<-ExtPCA$rotation[,1]
CID_scores<-abs(loadingscores)
CID_score_ranked<-sort(CID_scores,decreasing=TRUE)
CID_score_ranked
PersonalityPCAdata$Ext<-rescale(PersonalityPCAdata$ExtPC1,to=(0:1))
#
#Neuroticism#
#Make Data Frame#
Neumatrix<-data.frame(row.names=Fish_ID,Perimeter_Time,Shelter_Time)
View(Neumatrix) #looks ok
```

```
#PCA#
NeuPCA<-prcomp((Neumatrix),scale=TRUE)
plot(NeuPCA$x[,1],NeuPCA$x[,2])
#Look at variation#
pca.var<-NeuPCA$sdev^2
pca.var.per<-round(pca.var/sum(pca.var)*100,1)
barplot(pca.var.per,main="Scree Plot",xlab="Principal
Component",ylab="Percent Variation")
#Make a ggplot#
ggNeu<-data.frame(Sample = rownames(NeuPCA$x),
                  X=NeuPCA$x[,1],
                  Y=NeuPCA$x[,2])
ggNeu #looks ok
ggplot(data=ggNeu,aes(X,Y))+
  geom_point()+
  xlab(paste("PC1 - ",pca.var.per[1],"%",sep=""))+
  ylab(paste("PC2 -",pca.var.per[2],"%",sep=""))+
  theme_bw()+
  cleanup
#PC1#
PersonalityPCAdata$NeuPC1<-NeuPCA$x[,1]
#PC1 loadings#
loadingscores<-NeuPCA$rotation[,1]
CID_scores<-abs(loadingscores)
CID_score_ranked<-sort(CID_scores,decreasing=TRUE)
CID_score_ranked
PersonalityPCAdata$Neu<-rescale(PersonalityPCAdata$NeuPC1,to=(0:1))
#
#
#Cleaner - Client Interactions#
##Chaser##
Chase.mod<-
lmer(Chase.Total~Act+Agg+Ext+Neu+Opp+Origin+SGR+Sex+Tank+(1|Day),REM
L=FALSE,data=PersonalityPCAdata)
#reduction#
dredge(Chase.mod)
#Best#
Chase.mod2<-
lmer(Chase.Total~Opp+(1|Day),REML=FALSE,data=PersonalityPCAdata)
#Assumptions#
resid_panel(Chase.mod2)#normality? linearity?
#Cook's#
plot(cooks.distance(Chase.mod2))
#Transform#
PersonalityPCAdata$SqChase.Total<-sqrt(Chase.Total+0.1)
SqChase.mod2<-
lmer(SqChase.Total~Act+Opp+(1|Day),REML=FALSE,data=PersonalityPCAdat
a)
#Assumptions#
resid_panel(SqChase.mod2)#better...
#Cook's#
plot(cooks.distance(SqChase.mod2)) #ok
##null LRT##
nullchase<-
lmer(Chase.Total~1+(1|Day),REML=FALSE,data=PersonalityPCAdata)
anova(nullchase,Chase.mod2,test="Chisq") #yes
Chase.mod3<-
lmer(SqChase.Total~Act+Opp+(1|Day),REML=TRUE,data=PersonalityPCAdata
)
summary(Chase.mod2) #Opp***
#
```

```
#Inspection#
Inspect.mod<-
lmer(Inspect.Total~Act+Agg+Ext+Neu+Opp+Origin+SGR+Sex+Tank+(1|Day),R
EML=FALSE,data=PersonalityPCAdata)
dredge(Inspect.mod)
#best#
Inspect.mod2<-
lmer(Inspect.Total~Act+Opp+Sex+(1|Day),REML=FALSE,data=PersonalityPC
Adata)
#Assumptions#
resid_panel(Inspect.mod2) #normality?
#Cook's#
plot(cooks.distance(Inspect.mod2)) #ok
#Transform#
PersonalityPCAdata$lgInspect.Total<-log10(Inspect.Total+0.1)
lgInspect.mod2<-
lmer(lgInspect.Total~Act+Opp+Sex(1|Day),REML=FALSE,data=PersonalityP
CAdata)
#Assumptions#
resid_panel(lgInspect.mod2) #better
#Cook's#
plot(cooks.distance(lgInspect.mod2)) #ok
##null LRT##
nullinspect<-lmer(lgInspect.Total~1+(1|Day),data=PersonalityPCAdata)
anova(nullinspect,Inspect.mod2,test="Chisq")# no
#
Inspect.mod3<-
lmer(lgInspect.Total~Act+Opp+Sex+(1|Day),REML=TRUE,data=PersonalityP
CAdata)
summary(Inspect.mod3) #none
#
#Jolt Rate#
JoltRate.mod<-
lmer(Jolt.Rate~Act+Agg+Ext+Neu+Opp+Origin+SGR+Sex+Tank+(1|Day),REML=
FALSE,data=PersonalityPCAdata)
#Reductions#
dredge(JoltRate.mod)
#Best model#
JoltRate.mod2<-
lmer(Jolt.Rate~Agg+Ext+Opp+Origin+SGR+Tank+(1|Day),REML=FALSE,data=P
ersonalityPCAdata)
#Assumptions#
resid_panel(JoltRate.mod2) #normality?
#Cook's#
plot(cooks.distance(JoltRate.mod2)) #ok
#Transform#
PersonalityPCAdata$lgJolt.Rate<-log10(Jolt.Rate+0.1)
lgJolt.mod2<-
lmer(lgJolt.Rate~Agg+Ext+Opp+Origin+SGR+Tank+(1|Day),REML=FALSE,data
=PersonalityPCAdata)
#Assumptions#
resid_panel(lgJolt.mod2) #better
#Cook's#
plot(cooks.distance(lgJolt.mod2)) #ok
##LRT##
nullJolt<-lmer(lgJolt.Rate~1+(1|Day),data=PersonalityPCAdata)
anova(nullJolt,lgJolt.mod2,test="Chisq") #yes
Jolt.mod3<-
lmer(lgJolt.Rate~Agg+Ext+Opp+Origin+SGR+Tank+(1|Day),REML=TRUE,data=
PersonalityPCAdata)
summary(Jolt.mod3)
```

```
########
#Stacked bat plot#
Stackedplot$Fish<-as.factor(Stackedplot$Trial)
Stackedplot$Fish<-as.factor(Stackedplot$Ranking)
ggplot(data=Stackedplot, aes(x= Fish, y= Freqy, fill=Interaction))+
  geom_col(stat="identity")+
geom_hline(data=Stackedplot,aes(yintercept=25.7),linetype="dashed",c
olor="black")+
scale_fill_manual(values=c("firebrick3","cornflowerblue","palegreen3
"))+
  ylab("Total Number of Interactions")+
  xlab("Lumpfish ID")+
  theme(legend.position = "bottom")
#Radar Chart#
cols<-grDevices::col2rgb(c("red","darkorange","green"))
labs<-
c("Activity","Aggression","Extraversion","Neuroticism","Openness")
Interact2<-rescale(Interact,to=c(0,1))
summary(Interact2)
View(PersonalityPCAdata)
View(Interact2)
labs<-
c("Activity","Aggression","Extraversion","Neuroticism","Openness")
totscores<-list(
  "lower"=c(0.523,0.055,0.525,0.522,0.139),
  "mid"=c(0.611,0.190,0.444,0.581,0.138),
  "higher"=c(0.527,0.111,0.474,0.533,0.354)
)
totgg<-chartJSRadar(scores=totscores,labs=labs,colMatrix =
cols,showToolTipLabel = FALSE)
totgg
#CCI models#
gg1<-ggplot(data=PersonalityPCAdata,aes(Opp,Chase))+
  geom_smooth(method=lm, se=FALSE)+
  geom_jitter(size=2,alpha=.8)+
  xlab("Cleaner Openness Score")+
  ylab("Cleaner Chase Freq.")
gg2<-ggplot(data=PersonalityPCAdata,aes(Opp,Inspect))+
  geom_smooth(method=lm, se=FALSE)+
  geom_jitter(size=2,alpha=.8)+
  xlab("Cleaner Openness Score")+
  ylab("Cleaner Inspection Freq.")
gg3<-ggplot(data=PersonalityPCAdata,aes(Neu,Inspect))+
  geom_smooth(method=lm, se=FALSE)+
  geom_jitter(size=2,alpha=.8)+
  xlab("Cleaner Neuroticism Score")+
  ylab("Cleaner Inspection Freq.")
gg4<-ggplot(data=PersonalityPCAdata,aes(Act,Jolt))+
  geom_smooth(method=lm, se=FALSE)+
  geom_jitter(size=2,alpha=.8)+
  xlab("Cleaner Activity Score")+
  ylab("Client Jolt Freq.")
plot_grid(gg1, gg4, gg2, gg3, ncol=2, nrow=2, labels =
c('a','b','c.1','c.2'))

#CCI models#
gg1<-ggplot(data=PersonalityPCAdata,aes(Opp,Chase.Total))+
  geom_smooth(method=lm, se=FALSE)+
  geom_jitter(size=2,alpha=.8)+
  xlab("Lumpfish Boldness Score")+
  ylab("Total Chase Time (secs)")
```

```
gg2<-ggplot(data=PersonalityPCAdata,aes(Agg,Jolt.Rate))+
  geom_smooth(method=lm, se=FALSE)+
  geom_jitter(size=2,alpha=.8)+
  xlab("Lumpfish Aggression Score")+
  ylab("Jolt rate (per min)")
gg3<-ggplot(data=PersonalityPCAdata,aes(Ext,Jolt.Rate))+
  geom_smooth(method=lm, se=FALSE)+
  geom_jitter(size=2,alpha=.8)+
  xlab("Lumpfish Sociality Score")+
  ylab("Jolt rate (per min)")
gg4<-ggplot(data=PersonalityPCAdata,aes(SGR,Jolt.Rate))+
  geom_smooth(method=lm, se=FALSE)+
  geom_jitter(size=2,alpha=.8)+
  xlab("Lumpfish SGR")+
  ylab("Jolt rate (per min)")
plot_grid(gg1, gg4, gg2, gg3, ncol=2, nrow=2)
```