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Improving Larval and Juvenile Lumpfish, Cyclopterus lumpus, Aquaculture: Nutrition and Growing Conditions

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IMPROVING LARVAL AND JUVENILE LUMPFISH, *CYCLOPTERUS LUMPUS*, AQUACULTURE: NUTRITION AND GROWING CONDITIONS

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ABSTRACT

Salmonids play a major role in aquaculture globally and their production is expected to increase as the human population rises and wild fish stocks decline. Sea lice infestation is the largest economic burdens to salmonid net pen aquaculture with hundreds-of-millions of dollars lost due to the treatment, labor, and loss of biomass associated with these parasitic copepods. Chemotherapeutics were originally used to treat these infestations, but their effects on nontarget species resulted in them being banned in many countries. Many salmonid farmers now utilize cleanerfish technology to resolve this financial stressor. Lumpfish (*Cyclopterus lumpus*) are efficient delousers in salmonid aquaculture and are produced and used by the tens-of-millions in European countries and Atlantic Canadian provinces. However, standard protocols for raising these fish do not exist. Several knowledge gaps remain on the proper conditions for rearing Lumpfish. The goal of this thesis was to address some of these gaps by evaluating nutritional and rearing conditions for juvenile Lumpfish and providing guidelines for facilities to utilize.

Few experiments have focused on the nutritional requirements of juvenile Lumpfish. Many Lumpfish facilities use feeds that are specifically marketed to Lumpfish, but there is a general lack of understanding of the nutritional requirements of juvenile Lumpfish. To address this, two diet trials were performed to determine the effects of varying protein and lipid concentrations, as well as plant versus fish meal-based protein sources, on the growth, survival, and aggression of juvenile Lumpfish. Six experimental diets and two commercial diets were tested that varied in their protein/lipid concentrations and protein source. Fish were fed five times per day at 3 % body weight. In general, protein and lipid concentrations did not influence growth, survival, or fish aggression of juvenile Lumpfish. However, fish fed an experimental diet

had significantly faster and greater growth than those fed a commercial diet even though both diets had the same protein/lipid concentrations (55/15). This was likely due to ingredient differences between the two diets. The use of plant-based protein, however, did influence the growth of juvenile Lumpfish. Fish fed diets with plant-based protein showed significantly suppressed growth.

Lumpfish have a ventral suction disk that they use to adhere to surfaces, including the sides and bottoms of the tanks they are grown in. This makes cleaning quite laborious and may increase stress in these fish as they encounter cleaning implements. To improve tank design for culturing juvenile Lumpfish, two trials were performed in which tank color and tank bottom type were evaluated. In the "Tank Color Trial," six tank colors were tested (red, green, grey, black, white, and blue) to determine if color impacted the growth, survival, and aggression of juvenile Lumpfish. In the "Tank Bottom Trial," six bottom treatments were tested (rough-dark, rough-light, smooth-dark, smooth-light, false bottom, and control) that varied in their texture, color, and bottom type to determine if these surfaces affected Lumpfish adhesion, and therefore cleaning efficiency, as well as fish growth, survival, or aggression. Neither color nor tank bottom influenced the growth, survival, or aggression of juvenile Lumpfish. However, tank bottom did influence the occurrence of Lumpfish adhesion and cleaning efficiency. "Rough bottomed" treatments deterred Lumpfish adhesion significantly more than "smooth bottomed" treatments. Light colored, smooth bottomed treatments resulted in less Lumpfish adhesion than dark, smooth bottomed treatments. The false-bottom treatment resulted in the least amount of Lumpfish adhesion and fastest cleaning times.

Juvenile Lumpfish are territorial and bite at the fins of other individuals. Density dependent interactions are not well understood with this species, therefore the effects of stocking

density for different size classes of fish needs to be evaluated. Facilities generally use the amount of surface area allotted to the Lumpfish as a proxy for how dense to grow their fish, but if densities are too high, cannibalism may ensue. To determine the effects of stocking density on juvenile Lumpfish and to determine if fish aggression occurred at specific ontogenetic period, two stocking density trials were performed, one using 2 g fish and one using 13 g fish. Fish were stocked at four different rearing densities (40, 60, 70, and 90 g/L) with growth, survival, and fish aggression measured. Stocking density was negatively correlated with growth but did not influence survival or fish aggression. As stocking density increased, growth decreased. Also, there was no difference in aggression rates between the two size classes of fish, supporting the null hypothesis that there is no ontogenetic shift in fish aggression.

Lumpfish are a hardy species but outgrow their usefulness as cleanerfish on salmonid farms. Facilities can use the results from these trials to increase or suppress the growth of their fish to better meet the financial and temporal needs of their operations. Lumpfish facilities can feed their fish diets utilizing plant-based protein and increase rearing densities to suppress their growth. To increase growth, more fish meal-based protein should be used in the Lumpfish feeds as well as using lower stocking densities. However, this may result in higher expenses with more expensive feed ingredients being used and less individuals being produced under these lower densities. Lumpfish operations can manipulate these nutritional and rearing densities without impacting survival or fish aggression. If facilities are producing juveniles in large quantities, it will be vital to increase cleaning efficiency and decrease cleaning implement-fish interactions in the Lumpfish rearing tanks. When budgets and resources are limited, facilities can use light colored or possibly textured bottoms to limit the adhesion of juvenile Lumpfish. But to maximize cleaning efficiency and minimize cleaning interaction, false-bottomed tanks may be more

efficient. With the demand for Lumpfish increasing, rearing protocols need to be refined so that the culture of these fish can be as efficient as possible. These foundational results can help improve the economic gain and animal welfare of juvenile Lumpfish.

INTRODUCTION

Salmonid Aquaculture Production

Global population growth has increased the demand for marine protein. In 2017, the global consumption of marine protein increased to 138.7 million metric tons, with the per capita consumption increasing to 20.3 kg (FAO, 2020). By 2050, the human population is projected to reach 9.3 billion, an amount that wild fisheries cannot support (Merino et al., 2012). Total wild harvest for 2018 was 87.5 million metric tons with only 10.9 million metric tons represented by inland fisheries (FAO, 2020). Roughly 34.2% of fisheries are currently fished over sustainable levels, and 59.6% of fisheries are fished at maximum sustainable yield, meaning that any increase in capture would result in unsustainable harvesting (FAO, 2020). Also, 63% of fishing stocks still require rebuilding to prevent collapse of vulnerable species (Worm et al., 2009).

In addition to human-consumption, marine fisheries are exploited for non-food-source products. For example, 20.1 million metric tons of the wild harvest for 2018 were for non-food uses such as the production of fish meal and fish oil supplements (FAO, 2020). Fish oil supplements are taken for brain and cardiac health by many people around the world. The aquaculture and agriculture industries incorporate fish meal and fish oil into animal feeds. This fish meal provides the necessary nutrients required for efficient growth under intensive rearing conditions (Tacon et al., 2009). However, the impacts on forage fisheries and further effects on food webs is uncertain. For example, the Peruvian Anchovy (*Engraulis ringens*) stocks, a major species used in fish meal production, experienced a severe crash in the 1980s and has had minimal success in rebounding. Although wild capture fisheries have plateaued, aquaculture has

increased (FAO, 2020). To supply the demand for fish meal and fish oil, is seafood by-products are utilized such as the skeletons, guts, and skin of fishes destined for human consumption (Shepherd and Jackson, 2013).

Out of the 161.9 million metric tons of fish taken in 2018, wild capture only accounted for 54% of the total. Aquaculture production made up the remaining 46% with 74.5 million metric tons produced (FAO, 2020). China is the global leader in aquaculture production. In 2018, China produced 43.1 million metric tons of finfishes, representing nearly 58 % of the total finfish production globally (FAO, 2020). Grass Carp (*Ctenopharyngodon idellus*), Silver Carp (*Hypophthalmichthys molitrix*), Common Carp (*Cyprinus carpio*), and Bighead Carp (*Hypophthalmichthys nobilis*) represent almost 33 % of the finfish production globally (10.5, 8.8, 7.7, and 5.8 %, respectively). While not common in the US, Asian countries raise carp for consumption. These fishes are easy and inexpensive to produce which is why they dominate aquaculture production (FAO, 2020).

Salmonids play a major role in the marine aquaculture sector; salmonids represent over 6% of global aquaculture production and 19% of internationally traded fish products (FAO, 2020). Production also occurs for stock enhancement to boost natural salmonid populations. To compensate for the severe decline of wild stocks on both the US Pacific and Atlantic coasts, Atlantic Salmon (*Salmo salar*) and Pacific Salmon fisheries rely on stock enhancement from hatcheries to keep populations from collapsing (Trushenski et al., 2015). Along the Eastern coast of the United States, this decline in wild Atlantic Salmon stocks has been attributed to overfishing, physical interruptions of spawning routes by dams, and climate change that alters prey abundance (Mills et al., 2013). Global grow out production of salmonids for food fish is increasing, in part to satisfy the US market demand for Atlantic Salmon, the most imported

finfish. Atlantic Salmon and Rainbow Trout (*Oncorhynchus mykiss*) production reached 3 million metric tons (2,226,000 and 774,000 metric tons respectively) in 2018, making salmonids a top finfish cultured globally (FAO, 2020). Norway, the global leader of salmonid aquaculture, produced approximately 860,000 metric tons of fish in 2019 alone (Directorate of Fisheries, 2020). In 2018, approximately 326,000 metric tons of Atlantic Salmon, valued at $3.4 billion, was imported into the US (USDA, 2019), dwarfing what the US produced. Total US production of Atlantic Salmon was only 14,696 metric tons in 2017, valued at $61.4 million (NMFS, 2020). This seafood trade deficit, in which more seafood is imported than exported or produced domestically, reached $16.8 billion in 2018 (NMFS, 2020). To surmount this seafood trade deficit, economically feasible, domestic salmon production must increase.

Sea Lice and Salmonid Farming

One of the biggest challenges to increasing salmonid production is managing infestations of sea lice, especially *Lepeophtheirus salmonis* and *Caligus spp*. Sea lice are ectoparasitic copepods with flattened bodies and appendages to adhere to their host. Females create two egg strands that can each hold up to 300 eggs (Pike and Wadsworth, 1999). *L. salmonis* has 10 life stages including two nauplius stages, one copepodid stage, four chalimus stages, two pre-adult stages, and an adult stage. The two nauplius stages and the copepodid stage are non-feeding, while the other seven life stages can all be found on the skin of fish. The chalimus stages are sessile, feeding on the flesh of their host, while the pre-adult and adult stages are free moving across the host's skin. The life stages of *Caligus spp.* are similar but lack a pre-adult stage. High densities of copepodid larvae are found in shallow estuarine areas where salmonid migrations occur (Costello, 2006). The free-living planktonic stages (nauplius 1, nauplius II, and copepodid)

of these crustaceans often make their way onto the skin of wild fishes, but the high densities in salmon farms provide ideal feeding grounds for these parasites (Boxaspen, 2006). The level of infestation of these parasites can differ based on host species. In a study performed by Bui et al. (2018), Chinook Salmon had less severe infections compared to Atlantic Salmon and Naïve Sea Trout, which both showed similar levels of infection. These copepods usually congregate around the head but can be found anywhere along the body of the host. Sea lice graze on their hosts, removing mucus, skin, and tissues beneath using rasping mouthparts. They have a range of effects on their hosts including skin loss, bleeding, necrosis of tissues, increased mucus discharge, changes in mucus layer biochemistry, and, in extreme cases, mortality. Wounds created by sea lice also give rise to secondary bacterial or fungal infections (Costello, 2006). The development of lice has a strong correlation with temperature. As temperature increases, lice infestation rates increase as well. With ocean temperatures rising due to climate change, especially in the Gulf of Maine, lice infestation is predicted to become more severe along with the resulting negative economic impact (Costello, 2006).

The economic loss on salmonid farms due to sea lice infestations is immense. The amount of salmonid biomass lost to infestation varies from 3.62 to 16.55% per farm resulting in a loss of $0.46 per kilogram (Abolofia et al., 2017). This biomass loss is caused by indirect fish mortality from the sea lice. Although direct mortality is rare, infested fish experience energetic and osmoregulatory stress that may lead to death later in the growth cycle (Vollset et al., 2018). In 2006, 1.5 million metric tons of salmonids were farmed worth $8.4 billion. Of this production value, 4 to 10% was lost because of costs related to sea lice infestations (Costello, 2009). Market value of the fish drops drastically due to the external lesions present on fish infected by the

copepods (Costello, 2009). Furthermore, parasitism by lice caused $436 million in damages globally in 2011 due to costs of treatment, labor, and loss of biomass (Abolofia et al., 2017).

Sea Lice Control and Cleanerfish Technology

Initially, sea lice infestations were controlled with chemotherapeutic treatments such as dichlorvos and hydrogen peroxide (Grant and Treasurer, 1993). However, hydrogen peroxide proved ineffective after a few years as lice developed resistance to the treatment and were able to reattach to the host (Treasurer and Grant, 1997). Cypermethrin and benzoate became commercially available in the late 1990s and these became the major treatments to control for sea lice. Compounds such as organophosphate azamethiphos and other chemotherapeutics also have been used successfully to treat sea lice infestations. Currently, the most widely used compound in treating for sea lice is emamectin benzoate which was introduced in the early 1990s. This chemical is still widely used in major foreign salmonid production operations (Bravo, 2008, Bloodworth et al., 2019). However, overuse has driven sea lice to develop resistance to these chemicals too (Grant and Treasurer, 1993; Denholm et al., 2002). Bakke et al. (2018) found that *L. salmonis* became resistant to deltamethrin, another commonly used chemotherapeutic. Resistant strains showed less skeletal muscle cell destruction when exposed to the chemotherapeutic (Bakke, 2018). There is also concern that these chemicals are not environmentally friendly. In northern New England, the American Lobster (*Homarus americanus*) fishery is the largest and most profitable with $684.3 million worth caught in 2018 (NMFS, 2020). Dounia et al. (2016) found that exposure to azamethipos resulted in hypoxia, metabolic disturbances, neuromuscular dysfunctions, and sometimes mortality in American Lobsters. Ninety-three percent (93%) mortality was observed in the Lobsters when exposed to

doses above 5 ug/L, and salmon farms treat fish at 100-150 ug/L (Dounia et al. 2016). The growing threats of chemical resistance by sea lice and chemical impacts on non-target species have resulted in many of these chemicals, including azamethipos, the main sea lice pesticide used in foreign sea lice farming, being banned in the United States (Dounia et al., 2016). As a result, the salmonid aquaculture industry has spurred research into non-chemical defenses to combat sea lice infestations. Selective breeding in Atlantic Salmon has been used to select for fish more resistant to sea lice (Robledo et al., 2019). However, resistance to sea lice is a polygenic trait and, thus, identifying the different variations in the quantitative trait loci (QTL) that result in resistance is a challenging task. Regions where these QTL occur are large and difficult to analyze due to the high occurrence of recombination in these regions. Also, the creation of a fully resistant strain through selective breeding can take generations (Robledo et al., 2019). Other methods like physical barriers (skirts or cages), lasers, manipulation of swim depth, and functional feeds also are being explored as treatments for sea lice infestations. A combination of these treatments may result in the best chance of sea lice prevention (Barrett et al., 2020). Growers also are exploring cleanerfish as a viable method (Treasurer, 2018a).

Cleanerfish technology is becoming more relevant in an age where consumers and environmentalists care about the processing that goes into the food that is grown around the world. “Cleaners” are organisms that remove dead tissue or ectoparasites off of other fish, the “clients,” and this symbiotic relationship occurs in nature. Fish in the families Gobiddae and Labridae feed almost exclusively on the materials removed from their client fish (Treasurer, 2018a). For example, Bluestreak Bleaner Wrasse (*Labroides dimidiatus*) cleans ectoparasitic gnathiid isopods off Blackeye Thicklip Wrasse (*Hemigymnus melapterus*) in the Great Barrier Reef. From dawn to dusk, there is a 4.5-fold decrease in the number of parasites found on *H.*

melapterus (Grutter, 1999). In another reef system, the Bluestreak Cleaner Wrasse sets up a "cleaning station" for longnose parrotfish (*Hipposcarus harid*) to have parasites picked off them. Longnose Parrotfish often return to the same "cleaning station" but will change cleaners if their last interaction resulted in poor or no cleaning by the cleanerfish (Bshary et al., 2002). Another study (Grutter et al., 2018) excluded cleanerfish (Wrasse species) from a reef and found the rate of infestation increased dramatically in their absence. The researchers discovered that these cleanerfish have both direct and indirect impacts on the infestation rates on reef fish (Grutter et al., 2018). The ecosystem service that cleanerfish provide in nature has since been duplicated by aquaculturists in salmonid farms.

Cleanerfish technology was first used to treat sea lice on commercial salmon farms in Norway in 1988 and in Scotland in 1990. In these early cases, Labrid Wrasses, namely the Ballan Wrasse (*Labrus bergylta*), were used to clean the salmon (Rae, 2002). Wrasses are still utilized today in salmonid aquaculture. One study by Grutter (1996) found that the Wrasses ate 4.8 ± 0.4 parasites (mostly gnathiid isopod larvae and some sea lice) per minute (Grutter, 1996). While these Wrasses are effective cleanerfish, their performance is temperature-dependent. Cleaning activity by Wrasses decreases during the colder months in northern latitudes, with wild Wrasse abundance dropping significantly when water temperatures fall below 7 ˚C (Deady et al., 1995). With Wrasse being limited to warmer temperatures, a colder-water form of sea lice mitigation is needed for aquaculture operations in more northern latitudes or during cold water months of the year.

Lumpfish Biology and Natural History

Lumpfish (*Cyclopterus lumpus*) is a cold-water species in the order Scorpaeniformes, family Cyclopteridae. This bony fish has distinct morphological features and is the only member of its genus. Lumpfish have a large dorsal ridge, with several bony tubercles lining the body, but their most unique feature is the presence of a large, ventral, muscular suction disc that is made of modified pelvic fins (Davenport and Thorsteinsson, 1990). These fish inhabit deep waters along 32,000 km of coastline on either side of the Atlantic Ocean (Davenport, 1985; Figure A). Adult Lumpfish migrate near the shore during spawning season, a period when sexual dimorphism is apparent (Cox and Anderson, 1922). Male Lumpfish turn bright orange, red, fuchsia, or develop hints of orange on their fins during spawning season. Males also create a nest and attract females for mating (Goulet et al., 1986). Females will lay 2-3 egg masses that males will protect until the larvae hatch 6-8 weeks later dependent on water temperature. The timing of the spawning season is highly temperature driven. Southwestern Gulf of Maine populations spawn in the months of March through May, and northeast Maine populations spawn from May to June (Cox and Anderson, 1922). Once the eggs hatch, the larvae disperse and stick to benthic or pelagic macroalgae. The movement of the seaweed in the water column provides the larvae protection from predation (Moring, 1989). The larvae spend much of their time associated with

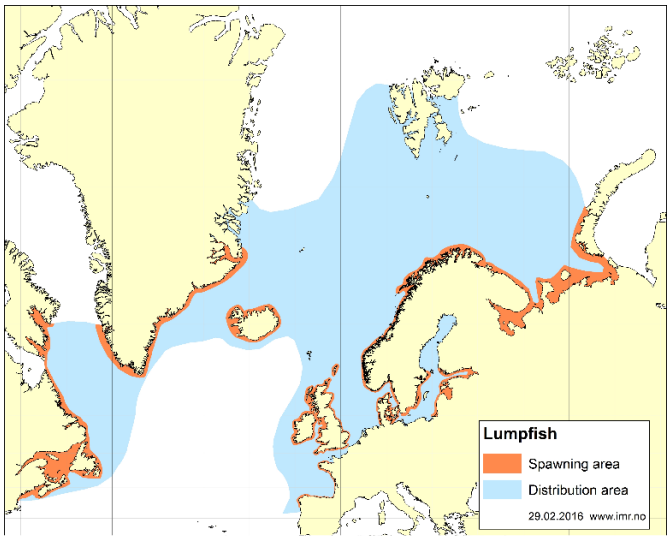

Figure A. The geographical range of Lumpfish, including major spawning areas. (Institute of Marine Research; https://www.hi.no/en/hi/temasider/species/Lumpfish)

and adhered to the macroalgae, allowing for much of their energy expenditure to be invested in growth (Killen et al., 2007). As a result, growth is fast with fish reaching 35 to 70 mm within the first year (Martin-Robichaud, 1991). Lumpfish reach sexual maturity within 2-3 years for males, and 3-4 years for females (Albert et al., 2002). However, under cultured conditions, female Lumpfish can reach maturity in under two years (Powell et al., 2018). As larvae, Lumpfish are gape-limited compared to adults, feeding on small invertebrates and sometimes other fish larvae (Tully and O'Ceidigh, 1989). Adult Lumpfish consume jellyfish, crustaceans, polychaetes, and other benthic invertebrates (Cox and Anderson, 1922). As Lumpfish grow older, they become more pelagic and associate less with structure. In the wild, Lumpfish live for 10 to 15 years and can reach total lengths of 30 ± 10 cm for males, and 42 ± 10 cm for females (Davenport and Thorsteinsson, 1989). While not well known throughout their range, Lumpfish biomass is thought to be high; in the Barents Sea alone, mean annual abundance was estimated at 53-132 million individuals from 1980 to 2012 (Eriksen et al., 2014). Many aspects of the ecology of wild Lumpfish populations remains poorly studied. Gaps in knowledge remain regarding Lumpfish distribution, abundance, population genetics, nutritional needs, and other aspects of their behavior (Powell et al., 2018).

Lumpfish as Cleanerfish

Until the 20th century, there was little economic value for Lumpfish - they were regarded as "trash fish" and as bycatch were used for bait or animal feed (Stevenson and Baird, 1988). Lumpfish exploitation began in the mid to late 20th century when they became targeted for their roe. Fisheries in Norway and Iceland began in the 1940s-1950s, followed by Canada in the 1970s, and then Greenland in the 1990s. Throughout the history of the Lumpfish fishery, Iceland

has been the dominant country, bringing in about 50% of the total landings since 1976 (Kennedy et al., 2019). The major fleets consist of small vessels (<15 m) and catch fish using large-mesh bottom set gillnets. Fleet size in these countries has been in decline, with peak catches near 7,257 metric tons of roe in 1987, followed by a decrease to about 2,268 metric tons in 2016 (Kennedy et al., 2019). Only the roe is harvested from the fish; the carcass yields little economic value (Kennedy et al., 2019). In 2006, 4 million kg of Lumpfish roe, worth about $60 million, was harvested for caviar and was sold by supermarkets for $36-72 per kg (Johannesson, 2006). Currently, there is a growing demand for Lumpfish for their use in salmonid aquaculture. However, there is a small demand for lumpfish fillets. Iceland supports a smoked Lumpfish fillet market, with male meat being valued over female. This fishery is small compared to other major fisheries and represents only a small portion of the economic value of Lumpfish (ICES, 2019). Today, the highest value for Lumpfish is as cleanerfish in salmonid aquaculture operations. As a result, Lumpfish fisheries have shifted from harvesting fish for roe to capturing adults to supply broodstock for Lumpfish hatcheries, as well as collecting wild egg masses (Powell et al., 2017). Production of Lumpfish in commercial hatcheries has been increasing exponentially in recent years. In 2016, global Lumpfish production increased to 30 million juveniles, with Norway producing 20 million alone (Powell et al. 2017). In 2019, Norway's production increased to 42.7 million juvenile Lumpfish (Directorate of Fisheries, 2020). Currently, in addition to Norway, United Kingdom, Iceland and Canada produce Lumpfish commercially, and research-scale production of Lumpfish has begun in the US. This US production has been based primarily at the University of New Hampshire.

Lumpfish have proven to be effective cleanerfish in salmonid cages (Imsland et al., 2014a, 2014b, 2015a, 2015b, 2018, Eliasen et al., 2018). Imsland et al. (2014b) found that there

were clear signs of Lumpfish grazing on sea lice. The Lumpfish lowered the number of mature female sea lice on salmon, and 28% of the fish were found to have sea lice in their stomach contents. There were significantly fewer pre-adult and adult *L. salmonis* on salmon in cages where Lumpfish were present (Imsland et al., 2014b). Imsland et al. (2018) looked at different stocking densities of Lumpfish (4, 6, and 8% of the total number of salmon in the cages) in salmon cages to see if higher Lumpfish densities would result in higher cleaning efficiency. The researchers found that the 8% density significantly reduced the amount of mature female *L. salmonis* on the salmon (Imsland et al., 2018a). Eliasen et al. (2018) found that after examining the stomach contents of 5,511 Lumpfish, 743 (13.5%) had sea lice in them. While in sea cages, Lumpfish have been classified as "strongly opportunistic," feeding mostly on salmon pellets but also hydrozoans, crustaceans, *Mytilus edulis*, and sea lice; on the last sampling day, 33-38% of the fish had ingested sea lice (Imsland et al., 2015a). However, while being classified as opportunistic predators, the high frequency of mature female sea lice in Lumpfish stomachs may suggest that the fish prefer larger lice (Imsland et al., 2014a, 2015b). The efficiency of sea lice removal by Lumpfish may be linked to maternal and paternal traits. Imsland et al. (2016b) tested the cleaning capabilities of nine different families of Lumpfish. One family, in particular, was found to have the most cleaning behavior with 15% of fish consuming sea lice and 43-92% lower sea lice averages in sea cages stocked with fish from this family. The other families showed little to no sea lice consumption and preferred competing with salmon for pellets. To eliminate this competition with salmon for pellets, studies have shown that Lumpfish can quickly switch from salmon pellets to specialized feed blocks (Imsland et al., 2018c). Also, in light of Lumpfish welfare, they must have suitable substrate to adhere to. Whether the substrate is more natural like kelp or smooth, plastic surfaces, Lumpfish need something to adhere to when resting overnight

(Imsland et al., 2015b). These experiments demonstrate the capability of Lumpfish as a cold-water cleanerfish. While several countries have developed hatcheries to supply salmon farmers with Lumpfish, there isn't a single standard operating procedure (SOP) for growing these fish (Powell et al., 2017).

Lumpfish Aquaculture

Currently Lumpfish aquaculture relies on acquiring adult fish for broodstock or wild eggs masses to rear to produce the cleanerfish necessary to stock salmon cages. This methodology may not be sustainable for wild populations but nonetheless it is necessary for commercial Lumpfish production until hatcheries are able to maintain self-supporting broodstock populations (Powell et al., 2017). Once broodstock are obtained, maturation may be accelerated by altering rearing conditions (i.e., photoperiod, temperature) (Jonassen et al., 2018). Hatcheries generally adopt a photoperiod of 17 hours of daylight (Jonassen et al., 2018), however, there is evidence suggesting that exposure to continuous light (24 hours of daylight) can result in faster fish growth and earlier maturation (Imsland et al., 2018b). Female Lumpfish, which produce anywhere from 100,000-400,000 eggs (Powell et al., 2018), are strip spawned. Males are not stripped spawned like other cultured fish species because the process is quite difficult with the testes positioned deep in the body. Instead, males typically are euthanized, and their gonads removed and macerated, then combined with the eggs in sea water. Fertilized eggs are incubated in upwelling systems such as standard salmon hatchery trays with water flowing from the bottom to the top (Jonassen et al., 2018). During incubation, eggs must be monitored for fungi and other pathogens which are kept at bay in nature by male fish who tend the eggs by fanning and puffing water at the eggs to keep the eggs clean (Goulet et al., 1986). Under ideal conditions, hatching

usually occurs after 290–300 degree days. Hatching success is temperature dependent with eggs incubated under cold (4-6 °C) and warm (10 °C) water regimes showing lower hatching success (46 and 50 %, respectively) compared to eggs exposed to a gradient temperature regime with temperatures gradually increasing from 4-10 °C as incubation commences (75% hatching success) (Imsland et al., 2019).

Once the larvae hatch, they quickly adhere to any available surface and move very little, except when feeding (Brown et al., 1992). Larvae are first fed 3-5 days post hatch (dph) after their yolk sac has been absorbed on either a microparticulate diet, enriched *Artemia*, or a combination of the two. Studies have shown that fish fed *Artemia* for the first 7-21 dph have faster growth and lower mortality than fish started on formulated dry feed (Belova, 2015). Larvae and juveniles are most commonly fed, by hand or via autofeeders, diets marketed specifically for Lumpfish (e.g., Skretting Clean Assist). However, generally, there is a lack of knowledge on the exact nutritional requirements for Lumpfish (Jonassen et al., 2018). Further, cultured Lumpfish have been known to develop cataracts. In salmonids, cataract development has been linked to malnutrition (Breck et al., 2005), so it is possible that commercial Lumpfish feeds are not nutritionally sufficient. A recent study done by Jonassen et al. (2017) showed similar cataract development in Lumpfish caused by osmotic imbalances. Willora et al. (2020) recently found that juvenile Lumpfish may benefit from using a mixture of plant-based protein and fish meal in their feeds. However, beyond this one study, no others exist focused on dietary needs of juvenile Lumpfish.

Juvenile Lumpfish are territorial (Imsland et al., 2015b) and show feeding hierarchy. Therefore, frequent grading of the fish is needed to minimize mortality and promote adequate growth of all individuals. Smaller individuals are often forced to the bottom of tanks, while

larger individuals remain towards the top of the water column where feed is more abundant. One strategy to minimize cannibalism is to increase tank surface area by adding panels and other fixtures in the tanks. Further, with limited surface area, and the powerful suction disk of the Lumpfish, fish may adhere to tank bottoms making it difficult to clean the tanks unless false or conical bottomed tanks are used (Jonassen et al., 2018). Many Lumpfish facilities are retrofitted Cod or other cold-water species hatcheries, so tailoring them to suit Lumpfish behavior may reduce tank cleaning effort. It is believed that Lumpfish are attracted to dark colors (green, dark grey, or black) and not as much to lighter colors, however, there is little experimental evidence supporting this (Jonassen et al., 2018). The attractiveness (or conversely, repulsion) of a color may help in tank cleaning by discouraging Lumpfish from sticking to the tank bottoms.

For cleanerfish use, juvenile Lumpfish are on-grown until 25 g, the size when they become effective consumers of sea lice (Imsland et al., 2016a) and are deployed into salmonid cages. Growth rates during the on-growing phase are significantly influenced by temperature. Nytro et al. (2014) showed a stepwise increase in growth rate with increasing temperatures from 4 to 16 °C. In high density tanks, oxygen saturation is kept at 110 %. Despite the use of these high-density systems where surface area is limited, the effects of stocking density on Lumpfish growth and survival remain unknown (Jonassen et al., 2018). Additionally, anecdotal evidence suggests that cannibalism increases during certain life stages, and, in particular, the highest fish aggression has been observed in 5g fish. Therefore, understanding the effects of stocking densities are paramount for hatcheries and grow-out facilities.

Despite global production of Lumpfish approaching 50 million annually, several gaps in Lumpfish culture protocol still remain. This thesis addresses some of these knowledge gaps in an effort to make production more efficient and sustainable, all while considering animal welfare.

Unsustainability is defined as deviating from ecological norms, usually by the hands of humans (Kuhlman and Farrington, 2010). With wild fish stocks declining, aquaculture serves as a sustainable source of producing animal protein while putting less stress on wild populations. Sustainability also pertains to the production of fish. Growing fish in conditions that are healthy and natural are ideal to maximize animal welfare. Garcia de Leaniz (2021) created a 4-point Likert scale of five behavioral indicators and 12 physical indicators of some of the welfare issues concerning Lumpfish. Behavioral indicators include loss of appetite, erratic swimming, lethargy, aggression, and gasping. Physical indicators include skin lesions, fin erosion, mortality, diseases, operculum erosion, eye condition, sucker condition, body/eye darkening, condition factor, malformations, growth, and blood parameters. All of these indicators were considered during the raising of Lumpfish for this thesis research and some indicators are directly. Chapter 1 focuses on the nutritional requirements of Lumpfish as it relates to protein and lipid concentrations. Chapter 2 looks at tank designs that aid in Lumpfish rearing and cleaning efficiency. Chapter 3 evaluates how stocking density affects the growth, survival, and aggression of juvenile Lumpfish.

CHAPTER 1: THE EFFECT OF PROTEIN AND LIPID CONCENTRATIONS ON THE GROWTH, SURVIVAL, AND AGRESSION OF JUVENILE LUMPFISH (*CYCLOPTERUS LUMPUS*)

Introduction

Lumpfish (*Cyclopterus lumpus*) are used as an effective mitigation technique for sea lice infestations on salmonid farms (Imsland et al., 2014a, 2014b, 2015a, 2015b, 2018, Eliasen et al., 2018). Norway is currently the leading producer of Lumpfish and currently almost 50 million juveniles are produced annually specifically for cleanerfish use (Directorate of Fisheries, 2020). Despite the millions of juveniles being raised, several knowledge gaps still exist on how to optimize growth in these fish. Though this is a relatively new sector of aquaculture, these knowledge gaps should be filled to avoid economic loss through slower growth rates and loss of biomass. Issues of animal welfare may be a factor as well with lack of understanding of the specific nutritional requirements of these fish.

Upon hatching in the wild, larval Lumpfish feed on Halacrid Mites or Harpacticoid Copepods primarily (Daborn and Gregory, 1983). Larvae and juveniles associate with macroalgae, feeding on the surface plankton and invertebrates associated with the flora (Ingolfsson and Kristjansson, 2002). Motile crustaceans larger than 0.5 mm dominate their diets, since there is a high abundance of these organisms amongst the algae (Ingolfsson and Kristjansson, 2002). As adults, Lumpfish are omnivorous, feeding on large planktonic and benthic organisms, as well as plant material like sea grass. Small crustaceans, polychaetes, ctenophores, small fish, insects, and fish eggs have all been found in the stomach contents of

adult fish (Davenport, 1985). Imsland et al. (2016) found that smaller Lumpfish had a stronger affinity for naturally occurring food items, including sea lice, more so than their larger counterparts while deployed in sea cages. Imsland et al. (2015a) determined that as Lumpfish age, their diet preferences broaden in scope, taking advantage of any available food source within salmonid net pens.

Under hatchery settings, larval Lumpfish begin feeding exogenously three to four days-post-hatch (dph) after their yolk sac is absorbed (Brown et al., 1997). Larvae are fed either a formulated feed, live feed (enriched *Artemia*), or a combination of the two, starting at 3 to 5 dph (Brown et al., 1992). Larvae intially fed *Artemia* only, rather than a formulated dry feed, have greater growth (Benfey and Methven, 1986, Belova, 2015) and higher survival (Belova, 2015). Larval Lumpfish have well developed eyes, mouths, and stomachs after hatching, which may justify feeding a formulated feed right away (Brown at al., 1997). Fish fed live feed are weaned onto pelleted diets for the ongrow phase. Early production of Lumpfish relied on feeding juveniles and adults pelleted diets that were originally designed for Cod and other cold-water, marine species (Jonassen et al. 2018). While some facilities still use these diets (Jonassen et al. 2018), specific feeds are currently marketed for Lumpfish, like the CLEAN Assist Cleanerfish Europa diet feed made by Skretting (Stavanger, Norway). This is the diet used by the University of New Hampshire for the Lumpfish raised at the Coastal Marine Laboratory (CML). Though Lumpfish diets exist, there is still poor understanding on the specific nutritional needs of these fish. These nutritional knowledge gaps may be leading to malnutrition (Jonassen et al., 2018). Breck et al. (2005) showed the relationship between malnutrition and the development of caratacts in salmonids. Jonassen et al. (2017) demonstrated that this relationship may hold true for Lumpfish with current feeds resulting in osomtic imbalances and cataracts. Many studies

have examined rearing Lumpfish for their use as cleanerfish in salmonid ocean farms (Imsland et al., 2014a, 2014b, Nytro et al., 2014, Imsland et al., 2015a, 2015b, 2018a, 2018b). During these experiments, juvenile Lumpfish were fed a variety of diets including crushed salmon pellets, AgloNorse formulated feed, Gemma Micro (Skretting), natural plankton, and *Artemia* (Imsland et al., 2014a, 2014b, Nytro et al., 2014, Imsland et al., 2015a, 2015b, 2018a, 2018b). Despite all these studies, none have focused on juvenile nutrition, therefore, this remains an area requiring further study.

Though little information exists on the specific nutritional requirements for Lumpfish, much is known about marine, cold-water, omnivorous, juvenile fish nutrition. Omnivorous fish, like Lumpfish, do not require as much dietary protein as carnivorous fish (Gatlin, 2010) which is advantageous for hatcheries since protein is the most expensive component of fish feed. However, the protein requirements for juvenile fish are generally higher than for other fish life stages due to the fast juvenile growth rates (Craig and Helfrich, 2017). Juvenile fish may also utilize more protein due to their lower catabolic adaptability (Dabrowski, 1986), or the ability of an organism to adapt fuel oxidation to fuel availability (O'donnell et al., 2013). This results in higher nitrogen loss during the digestion of proteins (Dabrowski, 1986). Proteins provide energy, amino acids, and aid in the structure and function of functional proteins like enzymes, hormones, and structural proteins (Prabu et al., 2017). Marine species usually require 40-45 % protein levels in their feeds (Craig and Helfrich, 2017). Up to 65 % of the protein in feeds can be lost to the environment through ammonia secretion via the gills and through solid wastes (Craig and Helfrich, 2017). Therefore, protein levels higher than 45 % can lead to eutrophication of surface waters due to excess nitrogen (Craig and Helfrich, 2017). Within these protein sources, a series of amino acids are essential for fish growth and include: arginine, histidine, isoleucine, leucine,

lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Meeting the minimum levels of these 10 essential amino acids is vital and may result in reduced growth if not met (Gatlin, 2010). Diets can be made with either plant protein (e.g., soybean meal) or animal protein (e.g., fish meal). Diets made with plant protein sources can be low in essential amino acids, like methionine, and therefore, require supplementation of these amino acids (Craig and Helfrich, 2017). Animal protein is considered higher quality but is also more expensive than plant protein (Prabu et al., 2017). However, as opportunistic omnivores, juvenile Lumpfish can grow and perform better when fed diets that are a 50:50 mix of fish meal and plant-based meal than diets that are strictly plant-based protein or fish meal (Willora et al., 2020).

Carbohydrates are the least expensive component of fish feed (Craig and Helfrich, 2017), but are also the least digestible for fish (Gatlin, 2010). Omnivorous fish can utilize carbohydrates much more efficiently than carnivorous fish and usually have soluble carbohydrate levels ranging from 25-45 % (Gatlin, 2010). Coldwater fish are limited in their ability to use carbohydrates as an energy source, with excess carbohydrates affecting the livers and natural microbiota in the guts of fish (Lall, 2000). Adding carbohydrates may be a poor energy source for some species but adding them to feeds provide the pellets with durability and also makes them less dense, causing the pellets to float (Gatlin, 2010, Craig and Helfrich, 2017). Carbohydrates also can improve growth and are the precursors for some amino acids (Prabu et al., 2017).

Lipids make up 7-15 % of fish feeds and contain roughly twice the energy density of both carbohydrates and proteins (Craig and Helfrich, 2017). Marine fish require more highly unsaturated fatty acids in the form of omega-3 fatty acids as their lipid sources (Gatlin, 2010, Craig and Helfrich, 2017). Fats are key energy sources for fish but aid in the structure of cell

membranes (Prabu et al., 2017). Larval fish require the most lipids in their diet (Dabrowski, 1986). Dabrowski et al. (1986) demonstrated this by showing that the fatty acid content in larval Striped Bass (*Morone saxatilis*) and herring declines as they age. Some suppliers cut costs and increase lipid levels in fish feeds to lower the use of more expensive components like proteins (Craig and Helfrich, 2017). However, too much excess fat can build up in the liver and affect fish health, as well as the quality and shelf life of the feeds (Craig and Helfrich, 2017).

Minerals can be classified as macro- or microminerals depending on the quantity needed in the fish feed. Common macrominerals in feeds include calcium, phosphorus, magnesium, chloride, sodium, potassium, and sulfur. Phosphorus is the most important macromineral supplemented in fish diets since fish cannot absorb it through their gills in the form that is present in the water (Gatlin, 2010, Craig and Helfrich, 2017). Microminerals are less abundant in fish feeds but are important for fish growth and feed efficiency. Common microminerals used in fish feeds include cobalt, chromium, iodine, iron, copper, zinc, selenium, and manganese (Gatlin, 2010, Craig and Helfrich, 2017). Vitamins in fish diets are either fat-soluble, stored in the body lipids of a fish, or water-soluble (Gatlin, 2010). Fat soluble vitamins include vitamins A, D, E, and K; water-soluble vitamins include B vitamins, inositol, choline, and vitamin C (Gatlin, 2010, Craig and Helfrich, 2017). While broad fish nutritional needs are understood, the exact nutritional requirements for cultured fishes are species specific and details for Lumpfish nutritional needs are unknown or may not be publicly available.

The purpose of this study was to evaluate experimental diets composed of varying protein and lipid concentrations, and different protein sources to determine which formulation would result in the fastest growth, highest survival, and lowest fish aggression in juvenile Lumpfish. It was hypothesized that (1) higher-protein, higher-lipid diets would accelerate growth and

decrease mortality and fish aggression in juvenile Lumpfish, and (2) animal protein diets would outperform plant protein diets.

Methods

Growth and survival of juvenile Lumpfish fed diets differing in protein/lipid concentrations and protein sources were evaluated at the CML in two separate experiments, herein referred to as "Trial 1" and "Trial 2." Trial 1 took place over the course of 10 weeks in which six experimental diet treatments were tested in quadruplicate, and two commercially available diets (Skretting Europa and BioTrout) were tested in triplicate (Tables 1.1 - 1.4). All diets were 2.0 mm in size. The experimental diets were made by the USDA Aquaculture Research Service at the Bozeman Fish Technology Center, in Bozeman, Montana in February 2019 and were freezer stored when not in use. The experimental diets were made by grinding all ingredients to <200 μm in an air-swept pulverizer. The diets were then put into an extruder at 127 °C for about 25 sec. These pellets were then put into a pulse bed dryer at 102 °C for 20 min with a 10 min cooling period following. A vacuum-coater was then used to top-coat all oil onto the pellets. The CLEAN Assist Cleanerfish Europa diet made by Skretting (Stavanger, Norway) served as a control as it is the standard diet fed to the general Lumpfish population at UNH. The BioTrout diet made by Bio-Oregon (Longview, Washington) is a salmonid feed and would be available to Lumpfish while out in salmon cages. Based on the results from Trial 1, three diets resulting in lower fish growth metrics (overall percent growth, growth rate, weight gain, and specific growth rate) were eliminated. Prior the start of Trial 2, the four experimental diets and one commercially available diet were tested by the USDA National Cold Water Marine Aquaculture Center (Franklin, ME) to see if they were still nutritionally intact. Since the

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels had not decreased since the diets were manufactured, the diets were deemed safe for testing and then retested with juvenile Lumpfish in triplicate in Trial 2 over the course of eight weeks (Tables 1.1 - 1.4).

Fish from the general population of cultured, juvenile Lumpfish reared at the CML were graded to the desired size and stocked into 10 L aquaria in a flow-through, ambient temperature and salinity seawater system, exposed to a 12-hour light: 12-hour dark photoperiod (Figure 1.1). Fish in both trials were fed at 3 % body weight (based on the total weight of the tank), over the course of five feedings per day (8am, 10am, 12pm, 2pm, and 5pm). All fish were sampled biweekly for growth and aggression. Growth was measured by taking the wet weights of each individual to the nearest 0.1 g. Fish aggression was tabulated using a scale from 0 to 5, with 0 representing a fish with an undamaged caudal fin, and 5 a fish with severe damage to the caudal peduncle (see Appendix A). Mortalities were recorded, weighed, scored for aggression, and removed daily. Excess feed in the tanks was removed via siphons as needed. Water temperature and salinity were measured daily (Figures 1.2 and 1.3).

Data Analysis:

All data were analyzed using Excel 2019 and JMP Pro 15. Overall percent growth, weight gain, specific growth rates (SGR), and feed conversion ratios (FCR) were calculated for each trial using the following formulas:

Overall percent growth = ((Final Weight – Original Weight)/ Original Weight) x 100 %

Weight gain = Final Weight – Initial Weight

SGR = (ln(Final Weight) – ln(Initial Weight))/ Days Tested

FCR = Total Amount Fed/ (Final Total Tank Weight – Initial Total Tank Weight)

One-way ANOVAs and Tukey's tests were used to compare the mean percent growth, mean growth rates, mean weight gain, mean SGR, and mean FCR between the treatments overall. Chi-squared tests were used to compare mean occurrence of fin nips between the treatments.

Figure 1.1. The experimental, flow-through seawater system used in both diet trials.

Table 1.1. The testing parameters used for each of the diet trials.

Parameter	**Trial 1**	**Trial 2**
Testing Period	5/18/20 – 6/27/20	3/27/21 – 5/12/21
Initial Fish Size ± 1 s.d. (g)	8.54 ± 1.10	8.39 ± 1.35
Number of Treatments	8	5
Body Weight/Feed Percentage (%)	3	3
Temperature Range (°C)	8-19	4-11
Salinity Range (ppt)	33-36	27-33
Number of Fish per Tank	15	10
Initial Fish Age (days post hatch)	251	198
Initial Stocking Density ± 1 s.d. (g/L)	12.81 ± 0.51	8.39 ± 0.20

Table 1.2. The protein/lipid concentrations and protein source for each of the diet treatments.

Diet	**Protein/Lipid Concentration (%)**	**Trial 1**	**Trial 2**
1	50/15	Tested	Omitted
2	55/10	Tested	Tested
3	50/20	Tested	Tested
4	55/20	Tested	Tested
5	55/15	Tested	Tested
6	50/10	Tested	Omitted
7	47/24 (BioTrout)	Tested	Omitted
8	55/15 (Skretting Europa)	Tested	Tested

Table 1.3. The dietary formulation and composition, as percent of the diet dry weight, of the experimental diets (1-6) used in both Trial 1 and 2. Because complete formulation of the commercial diets tested (7 and 8) is proprietary information, only diet ingredients (not quantities) are known. All diets are considered to be nutritionally complete, but some information (like quantities) is not provided by the diet manufacturers.

Diet	**4**	**5**	**2**	**3**	**1**	**6**	**7***	**8****
Protein/Lipid Concentration (%)	55/20	55/15	55/10	50/20	50/15	50/10	47/24	55/15
Ingredient (% of diet dry)								
SeaPro 75, Bio-Oregon Proteins	30.00	30.00	30.00	27.27	27.27	27.27		
Chicken 42 - ADF	20.00	20.00	20.00	18.18	18.18	18.18		
Squid meal	5.28	5.28	5.28	4.80	4.80	4.80		
Blood meal-AP301	3.00	3.00	3.00	2.73	2.73	2.73		
Menhaden fish oil	11.37	6.38	1.39	12.04	7.05	2.06	Present	Present
Wheat gluten meal	5.02	4.08	3.14	2.77	1.80	0.83	Present	
Wheat flour	18.50	24.41	30.34	24.38	30.31	36.25	Present	Present
Lecithin - Yelkinol AC dry lecithin	3.00	3.00	3.00	3.00	3.00	3.00	Present	
Stay-C 35	0.15	0.15	0.15	0.15	0.15	0.15		
Vitamin premix ARS 702	1.00	1.00	1.00	1.00	1.00	1.00	Present	Present
Monocalcium Phosphate	1.10	1.10	1.10	1.50	1.50	1.50		
Choline Cl 50%	1.00	1.00	1.00	1.00	1.00	1.00	Present	Present

DL-Methionine	0.00	0.00	0.00	0.11	0.12	0.13	Present	
Lysine HCl	0.00	0.00	0.00	0.38	0.39	0.39		
Threonine	0.00	0.00	0.00	0.09	0.10	0.11		
Taurine	0.50	0.50	0.50	0.50	0.50	0.50		
Trace mineral premix ARS 1440	0.10	0.10	0.10	0.10	0.10	0.10	Present	Present
Sum with oil:	100.00	100.00	100.00	100.00	100.00	100.00		
Formulated nutrient content (% diet dry weight basis)								
Crude Protein	55.00	55.00	55.00	50.00	50.00	50.00		
Crude Fat	20.00	15.00	10.00	20.00	15.00	10.00		
Digestible protein	52.17	52.02	51.88	47.25	47.11	46.96		
Digestible energy (cal/g)	5198	4877	4556	5020	4698	4376		
Total Phosphorus	0.86	0.87	0.87	0.89	0.90	0.90		
Available phosphorus	0.55	0.55	0.55	0.55	0.55	0.55	Present	Present
Ala	2.97	2.96	2.95	2.67	2.66	2.65		
Arg	3.52	3.51	3.51	3.17	3.16	3.15	Present	
ASP	4.89	4.89	4.88	4.43	4.42	4.42		
Glu	8.98	8.89	8.81	7.88	7.78	7.69		
Gly	2.45	2.44	2.44	2.20	2.20	2.19		
His	1.39	1.38	1.36	1.24	1.22	1.21	Present	

Ile	2.22	2.21	2.19	1.98	1.97	1.96		
Leu	4.46	4.45	4.43	4.00	3.99	3.97		
Lys	3.89	3.88	3.88	3.82	3.82	3.82	Present	
Met	1.34	1.33	1.32	1.30	1.30	1.30		
Phe	2.39	2.38	2.36	2.13	2.12	2.10		
Ser	2.36	2.35	2.34	2.11	2.10	2.09		
Tau	0.50	0.50	0.50	0.50	0.50	0.50		
Thr	2.28	2.27	2.26	2.14	2.14	2.14		
Tyr	1.76	1.76	1.75	1.58	1.57	1.55		
Val	2.82	2.82	2.81	2.54	2.53	2.52		
Pro	2.46	2.36	2.25	2.04	1.93	1.82		

*Also contains: Poultry meal, fish meal, soybean meal, feather meal, poultry oil, water, mold inhibitor, astaxanthin, vitamin E, ethoxyquin, vitamin C, hydrogenated vegetable oil, virocam, HT mix, panaferd-AX, luctarom, potato starch, canthaxanthin, monoammonium, propylene glycol, yeast autolysate dehydrated, zinc proteinate, guar gum, dadex defend, nasmix, nucleotide, sodium butyrate.

**Also contains: Fish meal, mold inhibitor, vitamin C, vitamin E, ethoxyquin

Table 1.4. The dietary formulation and composition for the commercial diets used in both Trial 1 and 2.

	Commercial Diet	
Ingredients	**BioTrout 2.0 mm**	**Skretting Europa 2.0 mm**
Crude Protein (Minimum %)	47	55
Crude Fat (Minimum %)	24	15
Crude Fiber (Maximum %)	1.8	2
Phosphorus (Mininmum %)	1	2

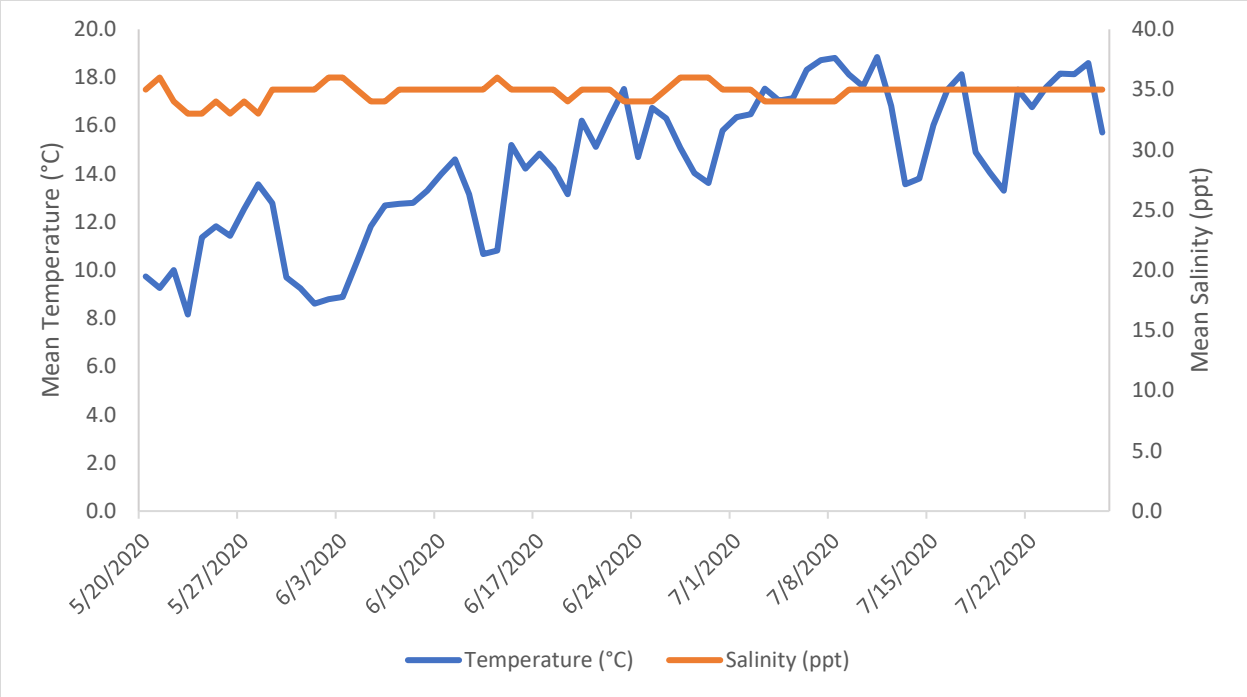

Figure 1.2. Mean daily temperature and salinity during Trial 1.

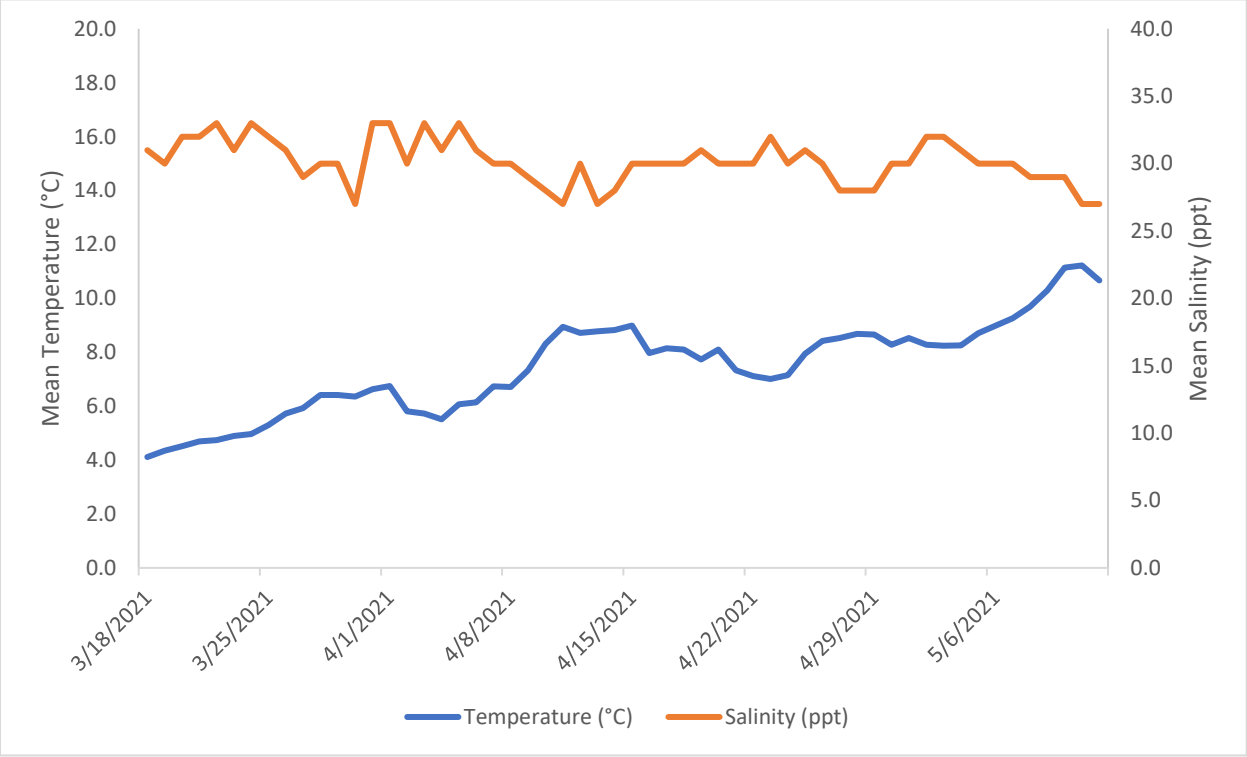

Figure 1.3. Mean daily temperature and salinity during Trial 2.

Results

Trial 1

Fish survival was not impacted by diet in Trial 1, with only two mortalities reported. One fish died in the 55/20 treatment on 7/9/20 and another fish diet in the 47/24 (BioTrout) treatment on 7/27/20.

Overall mean fish growth rates varied from 0.47 ± 0.18 g/day to 0.96 ± 0.36 g/day depending on the diet treatment (Table 1.5, Figure 1.4). Fish fed the 55/20 and 55/15 diets had significantly faster growth than fish fed the 50/15, 55/15 (Europa), and 47/24 (BioTrout) diets (one-way ANOVA, $p < 0.0001$, Table 1.5, Figure 1.4). Overall mean percent growth varied from 394.00 ± 30.03 % to 781.45 ± 36.35 % and was affected by diet. Fish in the 55/15 treatment had significantly higher overall mean percent growth (781.45 ± 36.35 %) than fish in in the 47/24 (BioTrout) and 55/15 (Europa) treatments (394.00 ± 30.03 % and 632.86 ± 33.09 %, respectively, one-way ANOVA, $p < 0.0001$) but did not have greater growth than fish in the remaining diet treatments (Figure 1.5). Overall mean weight gain ranged from 32.57 ± 2.23 g to 67.30 ± 3.14 g and was also affected by diet. Fish fed the 55/20 and 55/15 experimental diets had significantly higher weight gain than fish fed the 50/15, 47/24 (BioTrout), and 55/15 (Europa) diets (one-way ANOVA, $p < 0.0001$, Table 1.5, Figure 1.6). Fish fed the BioTrout diet had significantly lower weight gain than fish in any other diet treatment (one-way ANOVA, $p < 0.0001$, Table 1.5, Figure 1.6). The BioTrout treatment was the only plant-based protein, salmonid diet, therefore, growth was also analyzed excluding this treatment. When the 47/24 (BioTrout) diet, a plant protein-based diet, was removed from analyses, fish in the 55/15 treatment had significantly higher weight gain than fish in the 50/15 and 55/15 (Europa) treatments (one-way ANOVA, $p = 0.0039$, Table 1.5, Figure 1.7). Mean specific growth rates

were also impacted by diet and varied from 0.023 ± 0.001 g/day to 0.031 ± 0.0001 g/day. Fish fed the 55/15 diet had a significantly higher specific growth rate than fish fed the 47/24 (BioTrout) and 55/15 (Europa) diets, however, no differences existed between the experimental treatments (one-way ANOVA, $p < 0.0001$, Table 1.5, Figure 1.8). Feed conversion ratios ranged from 1.10 ± 0.01 to 1.54 ± 0.09. The 47/24 (BioTrout) treatment had a significantly higher FCR than all other treatments (one-way ANOVA, $p < 0.0001$, Figure 1.9).

Diet did not affect the occurrence of fish aggression ($X^2(7, N = 448) = 0.39, p = 0.9997$). Mean percentage of fin nipping occurrence ranged from 1.00 ± 0.91 % in the 55/15 treatment to 14.22 ± 7.79 % in the 55/15 (Europa) treatment (Table 1.5, Figure 1.10). Because final mean severity of fish nipping was low and only ranged from 0.01 ± 0.01 to 0.19 ± 0.12 (Table 1.5) and only 2.9 % of fish had fin nipping damage above a level 1 (See Appendix A), the severity of fin nipping data were not analyzed. Thirteen fish out of 448 fish sampled showed fin damage above a level 1: one fish in the 55/10 treatment, one fish in the 50/20 treatment, two fish in the 55/20 treatment, one fish in the 50/10 treatment, one fish in the 47/24 (BioTrout) treatment, and four fish in the 55/15 (Europa) treatment.

Trial 2

The five diets evaluated in Trial 2 did not affect survival, growth, or aggression in juvenile Lumpfish (Table 1.5), Fish survival was 100% throughout the eight-week period.

Overall mean fish growth rate for each diet treatment varied from 0.64 ± 0.36 g/day to 0.72 ± 0.38 g/day and was not influenced by diet (one-way ANOVA, $p = 0.7863$, Table 6, Figure 1.11). Diet also did not impact overall mean percent growth, which ranged from 424.41 ± 34.07

% to 486.98 ± 88.70 % (one-way ANOVA, p = 0.6909, Figure 1.12). Overall mean weight gain was also unaffected by diet, varying from 35.96 ± 3.46 g to 40.14 ± 6.99 g (one-way ANOVA, p = 0.7863, Figure 1.13). Mean specific growth rates varied from 0.025 ± 0.0001 g/day to 0.027 ± 0.0002 g/day and were not affected by diet either (one-way ANOVA, p = 0.6772, Figure 1.14). Feed conversion ratios were not influenced by diet as well and varied from 0.75 ± 0.07 to 0.82 ± 0.07 (one-way ANOVA, p = 0.7178, Figure 1.15).

Diet did not impact fish aggression in Trial 2. Only two out of the 120 fish sampled showed evidence of fin damage. Therefore, fish aggression analyses were not performed.

Trial 1

Table 1.5. The results of the different testing parameters measured for Trial 1. Different superscript letters denote statistical differences between treatments in each column.

Diet Treatment (protein/ lipid)	Final Overall Percent Growth (± one standard deviation, %)	Mean Growth Rate (± one standard deviation, g/day)	Mean Weight Gain (± one standard deviation, g)	Mean Specific Growth Rate (± one standard deviation, g/day)	Mean Feed Conversion Ratio (± one standard deviation)	Final Mean Occurrence of Fin Nipping (± one standard deviation, %)	Final Mean Severity of Fin Nips (± one standard deviation)	Final Percent Survival (%)	Final Mean Fish Weight (± one standard deviation, g)
50/15	690.86 (±61.44)^{ab}	0.82 (± 0.35)^{b}	57.05 (± 3.62)^{b}	0.030 (±0.001)^{ab}	1.14 (± 0.06)^{b}	1.67 (± 1.18)	0.02 (± 0.01)	100.00	65.33 (± 3.47)
55/10	718.55 (± 30.55)^{ab}	0.86 (± 0.42)^{ab}	60.06 (± 1.83)^{ab}	0.030 (± 0.001)^{ab}	1.16 (± 0.02)^{b}	3.67 (± 1.83)	0.05 (± 0.02)	100.00	68.42 (± 1.73)
50/20	684.77 (± 62.59)^{ab}	0.85 (± 0.32)^{ab}	59.18 (± 5.14)^{ab}	0.029 (± 0.001)^{ab}	1.16 (± 0.07)^{b}	5.33 (± 1.39)	0.08 (± 0.02)	100.00	67.82 (± 5.13)
55/20	752.26 (± 36.20)^{ab}	0.95 (± 0.36)^{a}	66.34 (± 1.84)^{a}	0.031 (± 0.001)^{ab}	1.14 (± 0.07)^{b}	6.33 (± 4.31)	0.09 (± 0.06)	97.78	75.18 (± 2.06)
55/15	781.45 (± 36.35)^{a}	0.96 (± 0.36)^{a}	67.30 (± 3.14)^{a}	0.031 (± 0.001)^{a}	1.10 (± 0.01)^{b}	1.00 (± 0.91)	0.01 (± 0.01)	100.00	75.92 (± 3.28)
50/10	705.51 (± 77.48)^{ab}	0.87 (± 0.33)^{ab}	60.69 (± 6.88)^{ab}	0.030 (± 0.001)^{ab}	1.13 (± 0.06)^{b}	4.33 (± 3.65)	0.06 (± 0.04)	100.00	69.30 (± 7.06)
47/24 (BioTrout)	394.00 (± 30.03)^{c}	0.47 (± 0.18)^{c}	32.57 (± 2.23)^{c}	0.023 (± 0.001)^{c}	1.54 (± 0.09)^{a}	8.00 (± 1.99)	0.13 (± 0.04)	97.78	40.84 (± 2.17)
55/15 (Europa)	632.86 (± 33.09)^{b}	0.78 (± 0.27)^{b}	54.90 (± 3.09)^{b}	0.028 (± 0.001)^{b}	1.23 (± 0.06)^{b}	14.22 (± 7.79)	0.19 (± 0.12)	100.00	63.58 (± 3.19)

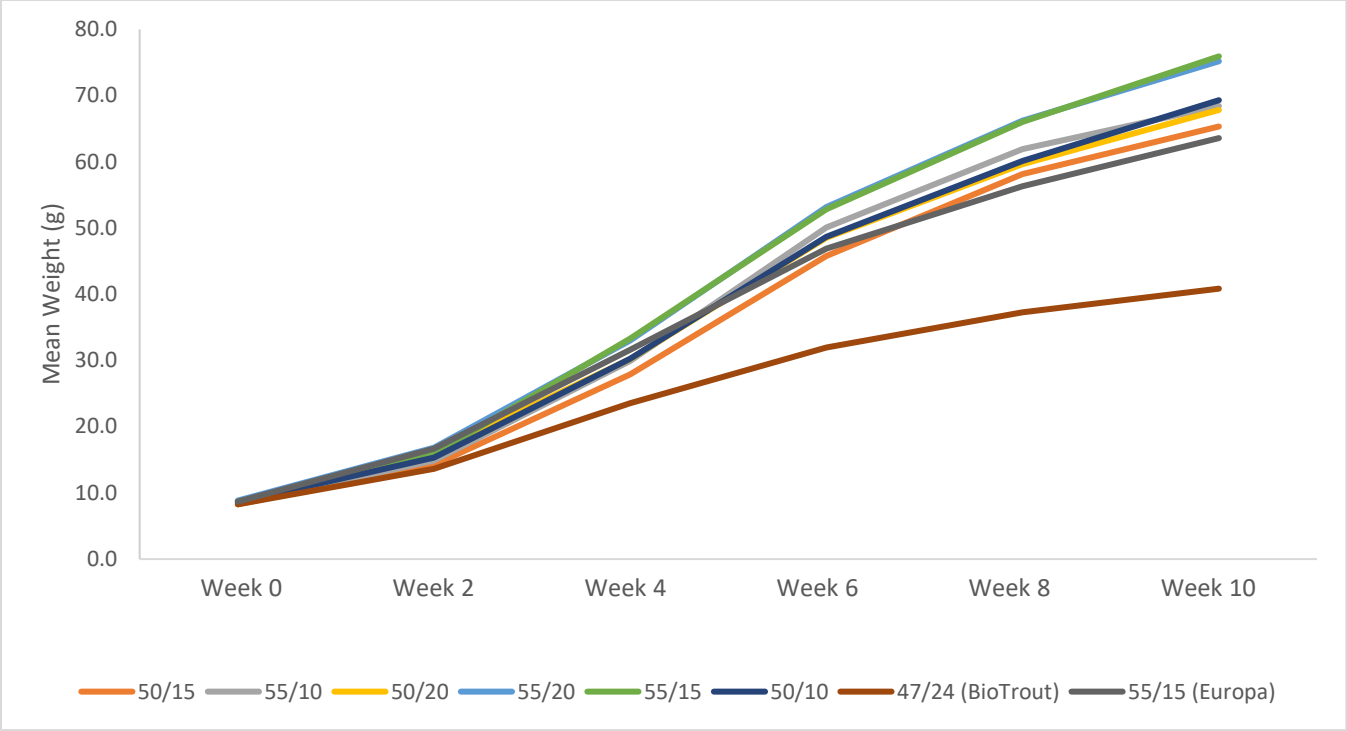

Figure 1.4. Mean weights of the juvenile Lumpfish over time in each diet treatment in Trial 1, including the BioTrout treatment.

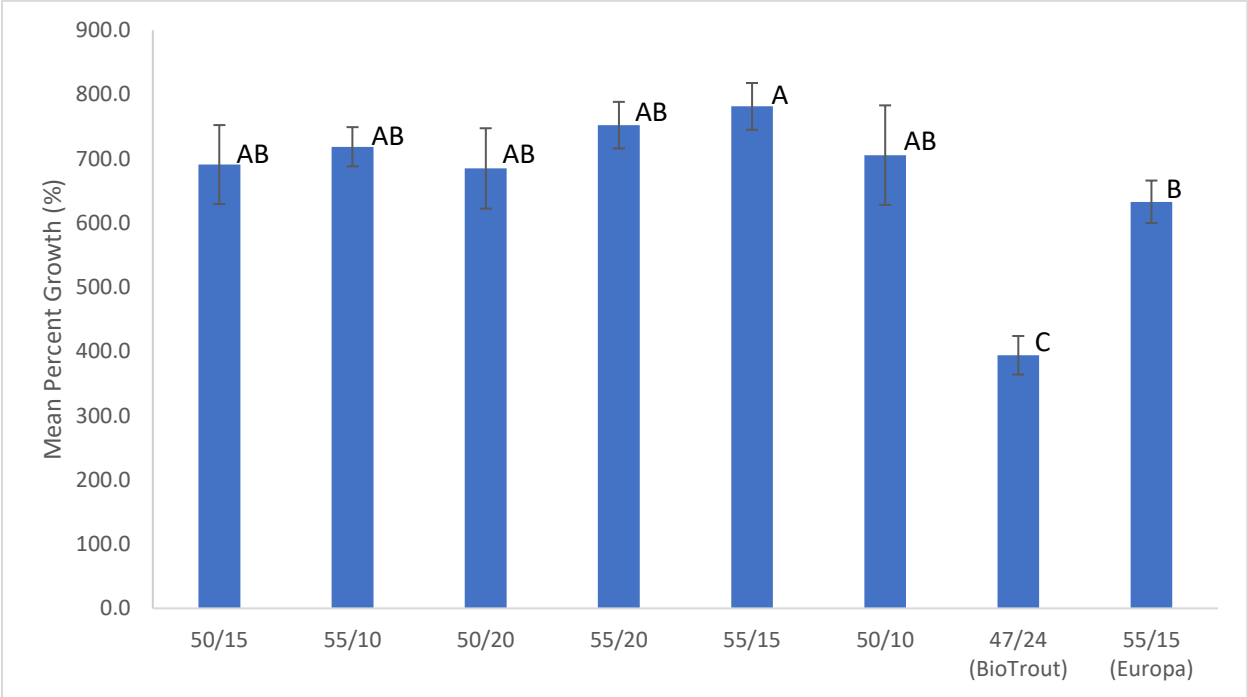

Figure 1.5. Overall mean percent growth (± one standard deviation) of juvenile Lumpfish in each diet treatment in Trial 1, including the BioTrout diet. Differing letters denote significant differences between treatments.

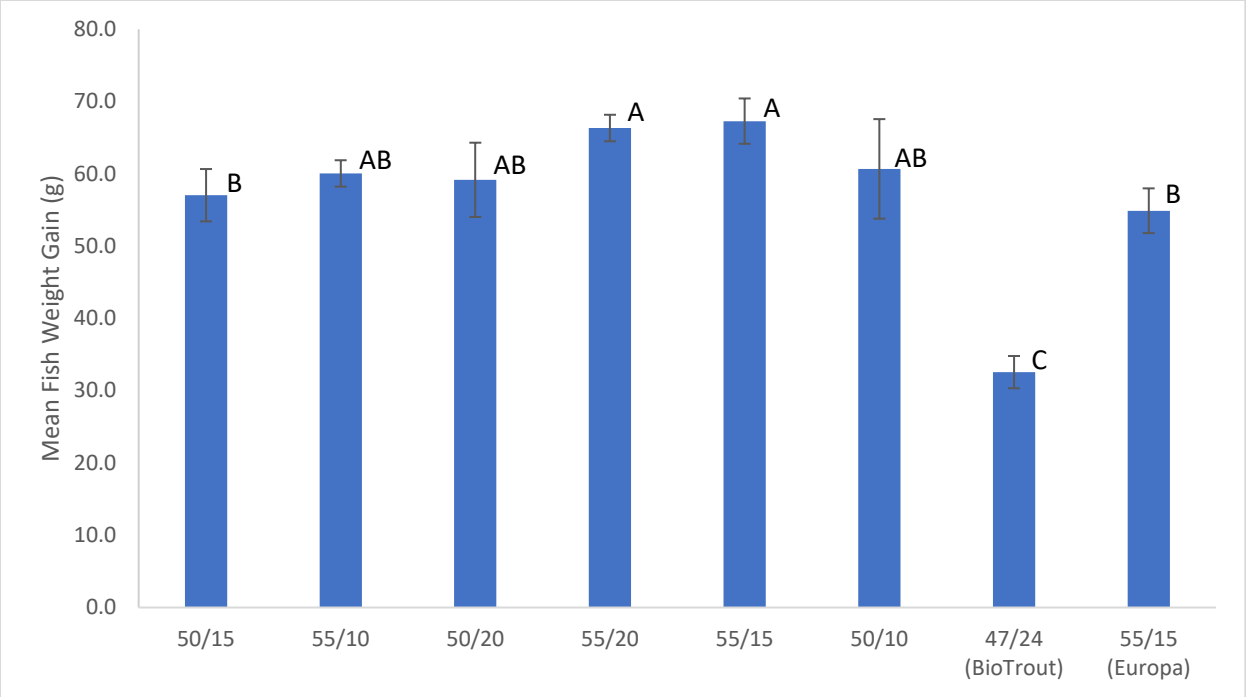

Figure 1.6. Overall mean weight gain (± one standard deviation) of juvenile Lumpfish in each diet treatment in Trial 1, including the BioTrout diet. Differing letters denote significant differences between treatments.

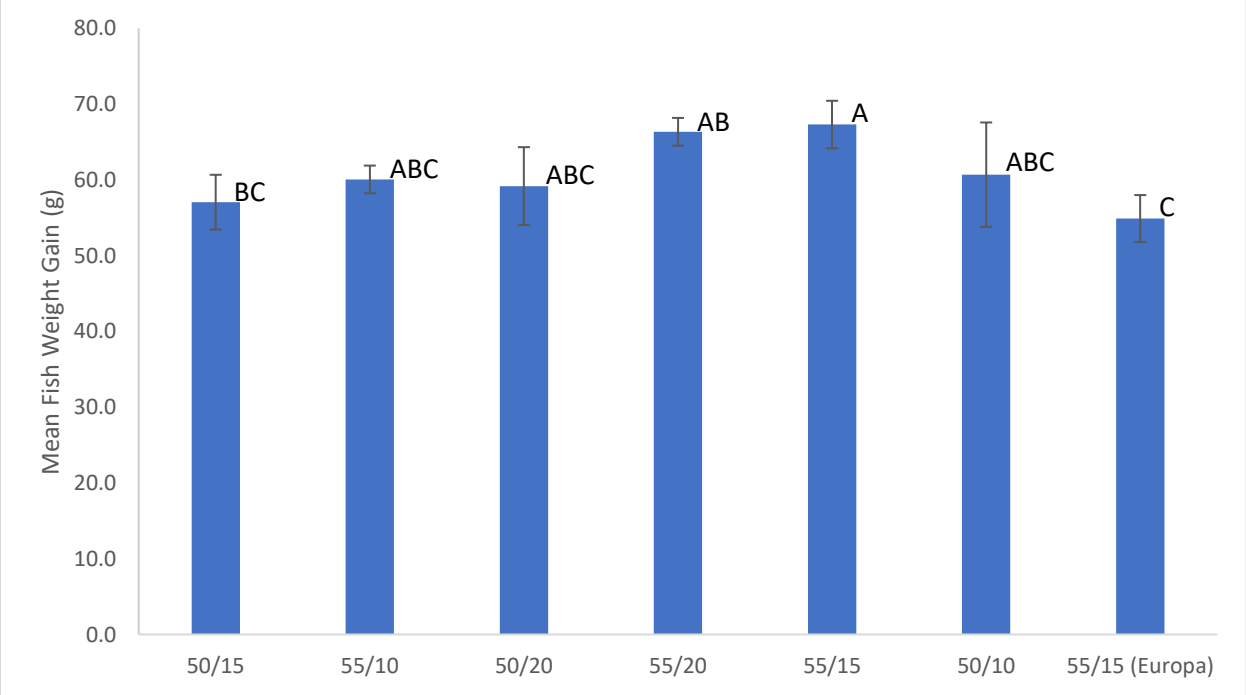

Figure 1.7. Overall mean weight gain (± one standard deviation) of juvenile Lumpfish in each diet treatment in Trial 1, excluding the BioTrout diet. Differing letters denote significant differences between treatments.

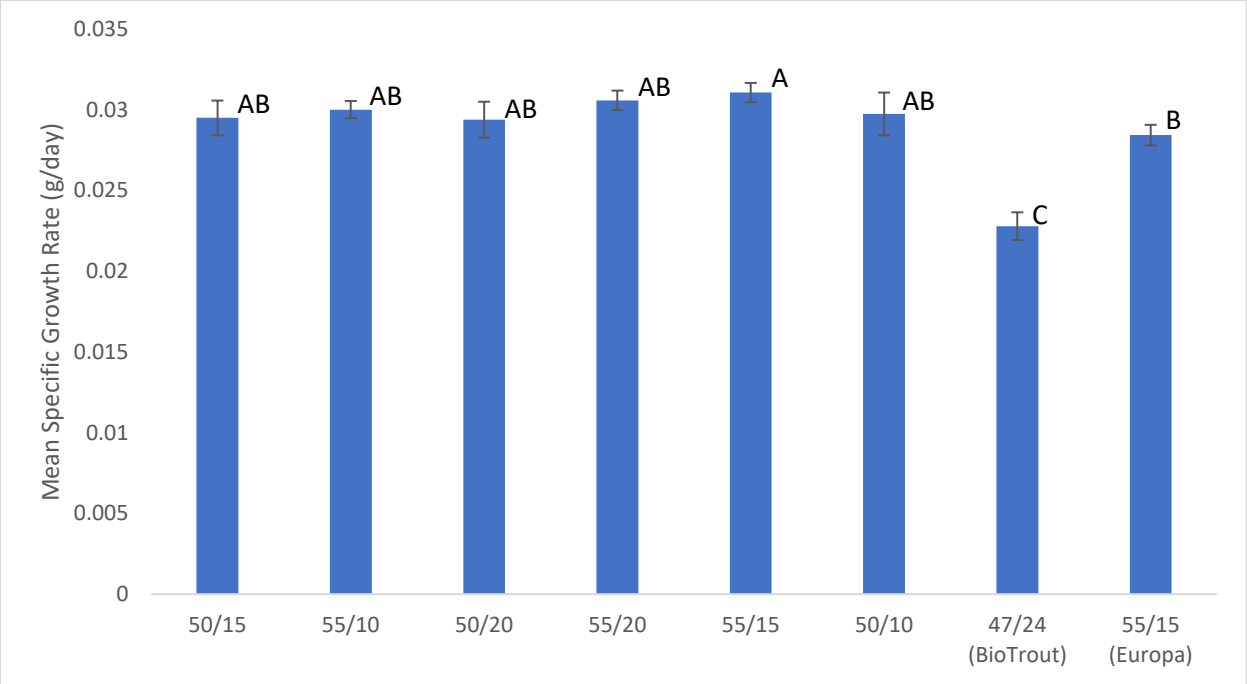

Figure 1.8. Overall mean specific growth rate (± one standard deviation) of juvenile Lumpfish in each diet treatment in Trial 1, including the BioTrout diet. Differing letters denote significant differences between treatments.

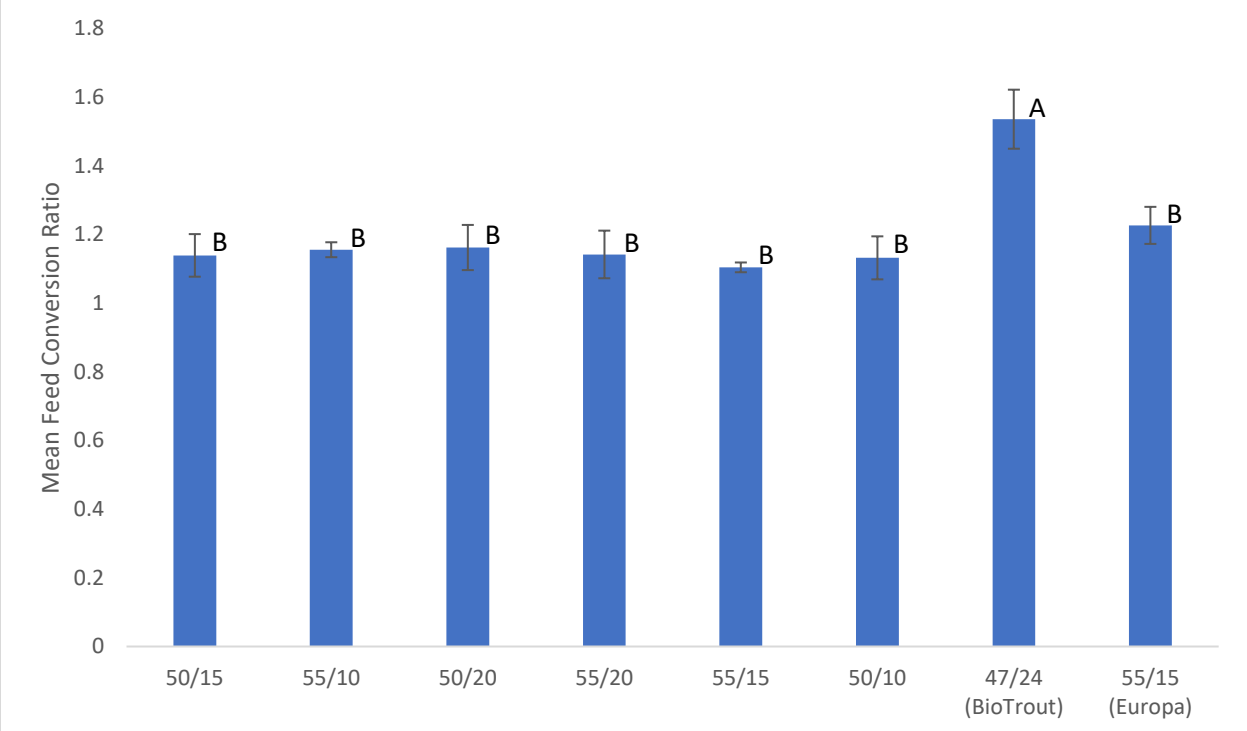

Figure 1.9. Overall mean feed conversion ratio (± one standard deviation) of juvenile Lumpfish in each diet treatment in Trial 1, including the BioTrout diet. Differing letters denote significant differences between treatments.

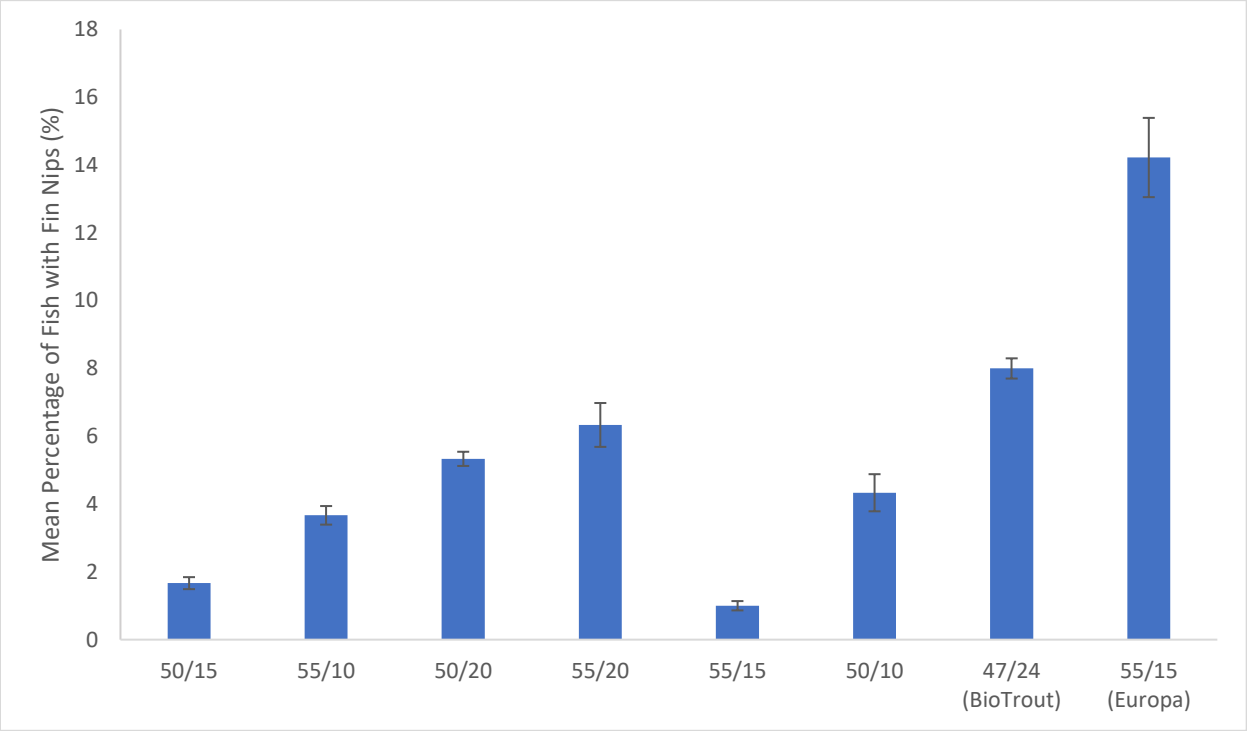

Figure 1.10. Mean occurrence (± one standard deviation) of juvenile Lumpfish aggression in each diet treatment in Trial 1, including the BioTrout treatment.

Trial 2

Table 1.6. The results of the different testing parameters measured for Trial 2.

Diet Treatment (protein/ lipid)	**Final Overall Percent Growth (± one standard deviation, %)**	**Mean Growth Rate (± one standard deviation, g/day)**	**Mean Weight Gain (± one standard deviation, g)**	**Mean Specific Growth Rate (± one standard deviation, g/day)**	**Mean Feed Conversion Ratio (± one standard deviation)**	**Final Percent Survival (%)**	**Final Mean Fish Weight (± one standard deviation, g)**
55/10	424.41 (± 34.07)	0.64 (± 0.36)	35.96 (± 3.46)	0.025 (± 0.001)	0.80 (± 0.05)	100.00	44.43 (± 3.59)
50/20	468.60 (± 38.66)	0.71 (± 0.43)	39.98 (± 4.08)	0.026 (± 0.001)	0.75 (± 0.07)	100.00	48.50 (± 4.26)
55/20	486.98 (± 88.70)	0.72 (± 0.38)	40.14 (± 6.99)	0.027 (± 0.002)	0.77 (± 0.10)	100.00	48.38 (± 6.93)
55/15	467.52 (± 20.44)	0.70 (± 0.39)	39.27 (± 1.29)	0.026 (± 0.001)	0.76 (± 0.02)	100.00	47.67 (± 1.32)
55/15 (Europa)	463.72 (± 54.54)	0.69 (± 0.30)	38.60 (± 4.68)	0.026 (± 0.001)	0.82 (± 0.07)	100.00	46.93 (± 4.76)

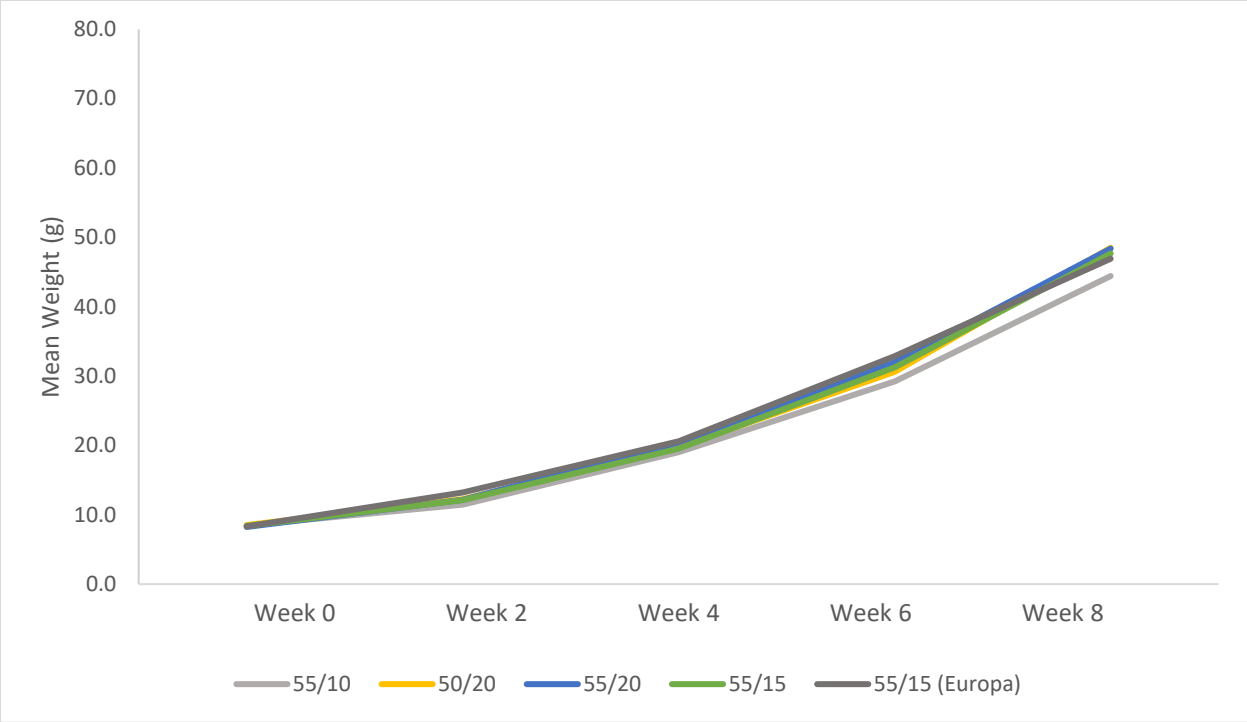

Figure 1.11. Mean weights of juvenile Lumpfish over time in each diet treatment in Trial 2.

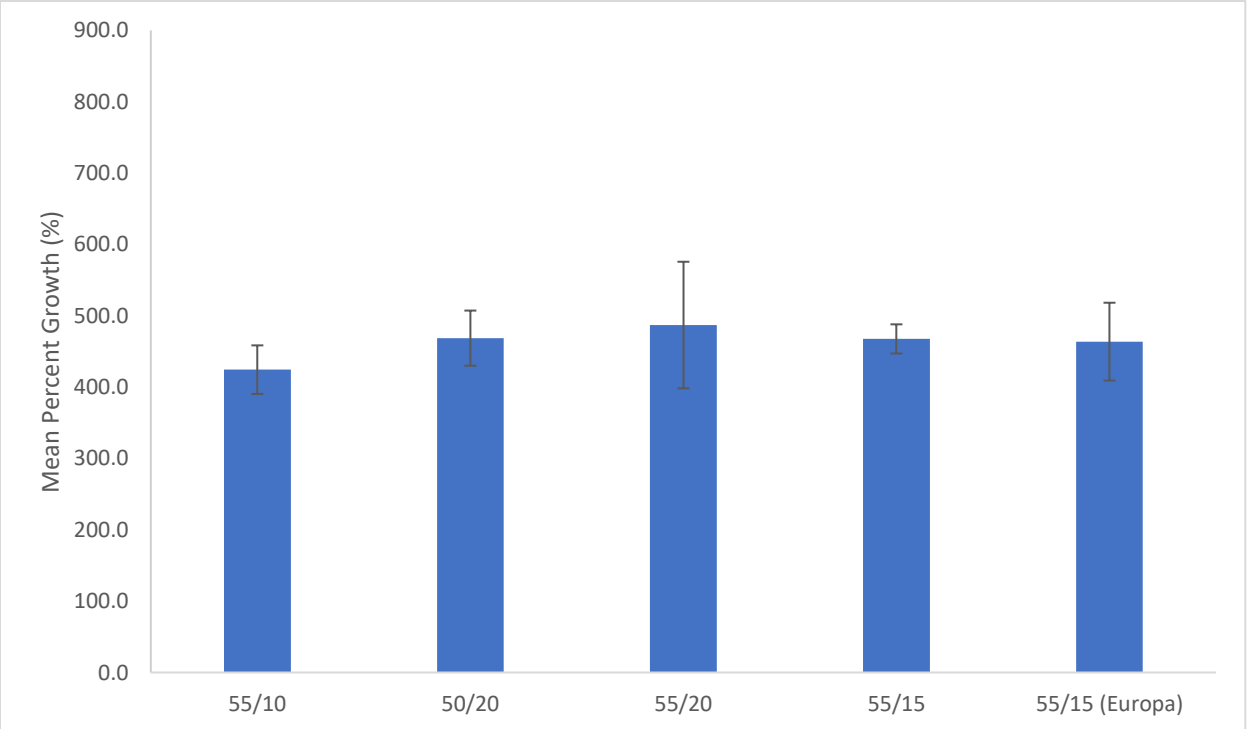

Figure 1.12. Overall mean percent growth (± one standard deviation) of juvenile Lumpfish in each diet treatment in Trial 2 at week 8.

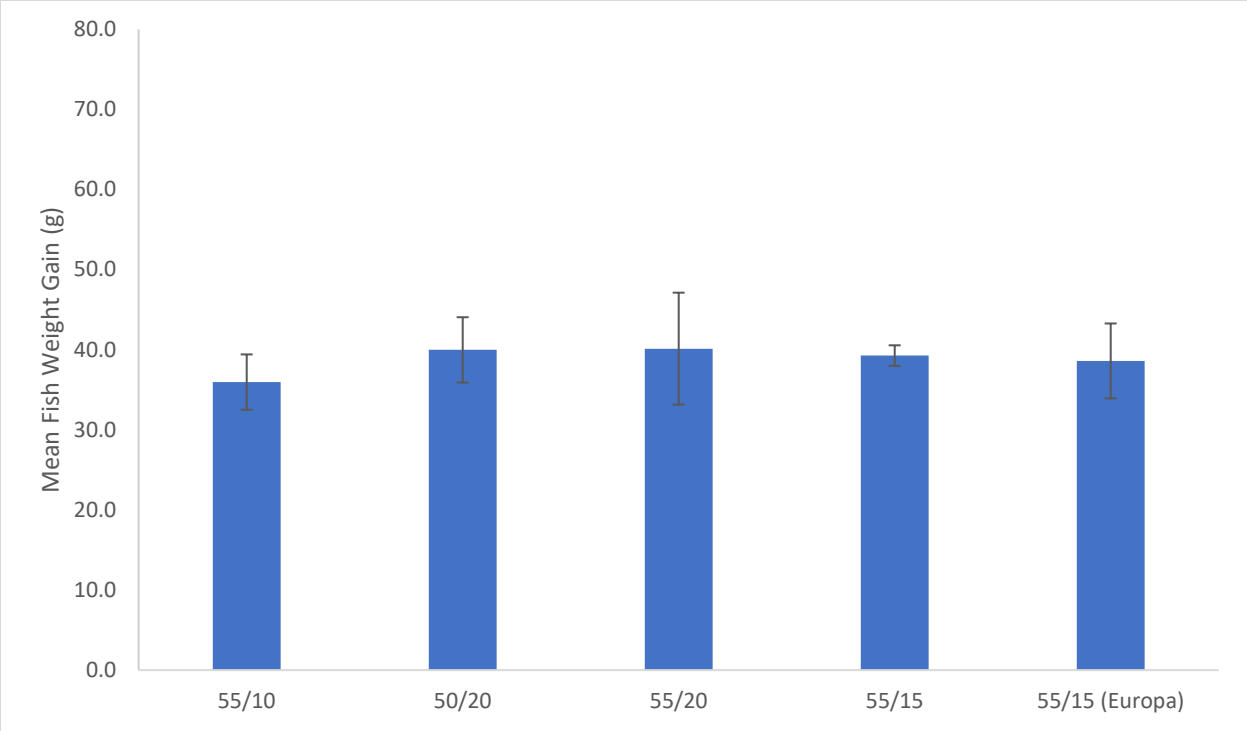

Figure 1.13. Overall mean weight gain (± one standard deviation) of juvenile Lumpfish in each diet treatment in Trial 2.

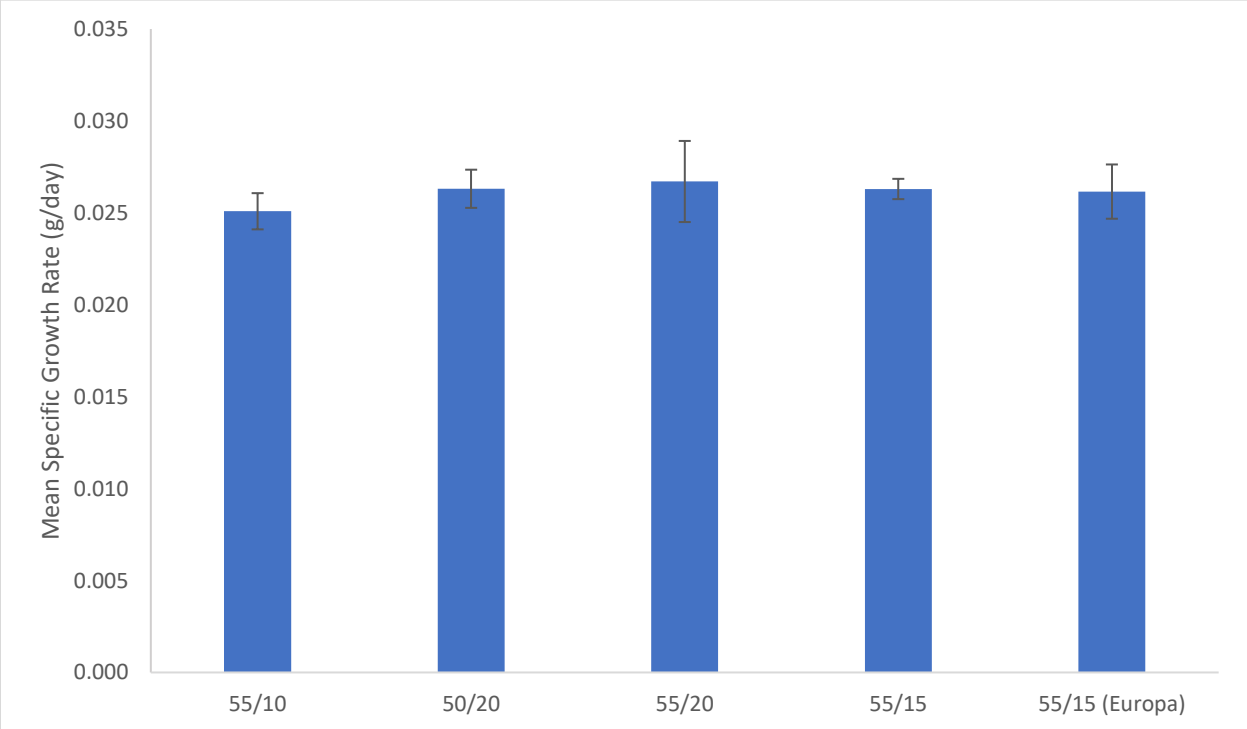

Figure 1.14. Overall mean specific growth rate (± one standard deviation) of juvenile Lumpfish in each diet treatment in Trial 2.

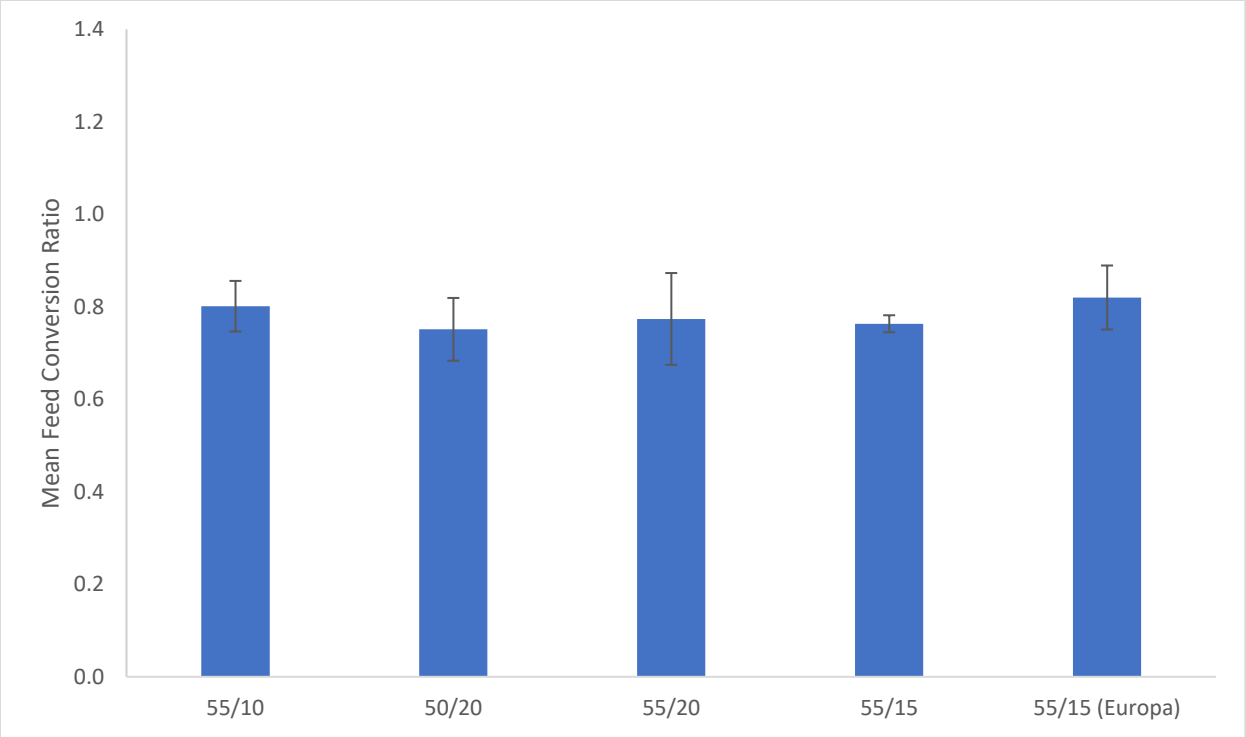

Figure 1.15. Overall mean feed conversion ratio (± one standard deviation) of juvenile Lumpfish in each diet treatment in Trial 2.

Discussion:

Even though juvenile Lumpfish were fed diets that varied in protein source (plant vs. animal protein), and protein (50-55 %) and lipid (10-20 %) concentrations, survival was unaffected (Table 1.5 and 1.6). Studies on other juvenile fish species have shown similar results. Tuan and Williams (2007) found juvenile Malabar Grouper (*Epinephelus malabaricus*) survival was unaffected when the fish were fed diets that varied in crude protein (44-60 %) and lipid (7-23 %) concentrations. Altering the digestible protein (37, 42, and 47 %) and digestible lipid (7 and 14 %) levels in the feed of juvenile Rockfish (*Sebastes schlegeli*) also did not affect mortality (Lee et al., 2002). Catacutan et al. (2008) found similar results when feeding juvenile Mangrove Snapper (*Lutjanus argentimaculatus*) different dietary treatments (protein: 35, 42.5, and 50 %, lipid: 6 and 12 %). Survival of juvenile Largemouth Bass (*Micropterus salmoides*)

and Nile Tilapia (*Oreochromis niloticus*) was unaffected when fish were fed diets that varied in their protein source (soybean vs. fish meal, Tidwell et al., 2005, Thompson et al., 2012). The lack of mortality seen in either of the diet trials may have been attributed to the short testing period. Trial 1 ran for ten weeks and Trial 2 for eight. The effects of malnutrition can take up to several months to become apparent, and the fact that the diets were not completely devoid of the essential macronutrients needed for fish means that impacts on survival are unlikely to be seen (Hardy, 2001). Diet studies of other finfish species show contradictory results. Li et al. (2010) found that the survival of Blunt Snout Bream (*Megalobrama amblycephala*) fingerlings significantly decreased with increasing protein levels (27, 31, and 35 %), while increasing lipid levels (4, 7, and 10%) had no effect. When juvenile White Sea Bass (*Atractoscion nobilis*) where fed diets that varied in dietary lipid levels (2.6, 7.4, 11.6, 15.3, and 19.4 %), fish fed the 2.6 % lipid diet showed significantly lower survival than fish in the other treatments (Lopez et al., 2009). As opportunistic omnivores (Davenport, 1985), juvenile Lumpfish are hardy, able to take advantage of a broad array of prey items and dietary conditions.

For the most part, juvenile Lumpfish growth was unaffected by the different protein and lipid concentrations, especially when fed any of the experimental diets. This is because all the experimental diets showed ample growth in both trials. In Trial 1, the fish fed the experimental diets showed around 700 % overall mean percent growth over the 10-week testing period. In Trial 2, the fish fed the experimental diets, and the Europa diet, showed around 400 % growth over the 8-week testing period. With these diets showing good growth in either trial, they are likely nutritionally suitable for Lumpfish. The only significant differences were seen in Trial 1 where the 55/15 treatment showed significantly higher overall mean percent growth, mean growth rate, mean weight gain, and mean specific growth rate than the two commercial diets

tested. The significantly lower growth in the 47/24 BioTrout treatment than any of the other diets may be a result of the primary protein source which is soybean meal rather than the fish meal incorporated in all the other diets tested. Although there were two diet treatments with the same protein/lipid concentrations (55/15: one an experimental diet and the other the commercial Europa diet), fish fed the experimental diet had significantly higher growth metrics in Trial 1. These differences were not observed in Trial 2, however. These growth differences may have been due to differences in dietary ingredient compositions, though the exact discrepancy remains unknown as the Europa formulation is proprietary information. A more likely explanation for the differences between the two trials when comparing the 55/15 experimental diet to the Europa diet is the effects of temperature. In Trial 1, the temperature ranged 8-19 °C whereas in Trial 2 the temperatures ranged from 4-11 °C. With Trial 1 taking place at much higher temperatures, it is likely that the juvenile Lumpfish had a faster metabolism. Metabolism often increases with temperature for finfishes (Gillooly et al., 2001). Schurmann and Steffensen (2005) found that the active metabolic rates of Atlantic Cod, another cold-water finfish species, increased as temperature increased. With the increased temperatures in Trial 1, fish in this trial had a higher metabolism and therefore faster growth which is why the 55/15 experimental diet had significantly higher growth than the Europa diet. In both trials, there were no differences between the experimental diets tested regarding the metrics tested, except for the significantly higher mean growth rate in fish fed the 55/15 experimental diet compared to fish fed the 50/15 diet (Tables 1.5 and 1.6, Figures 1.4 - 1.8). Also, there was no impact on the feed conversion ratios or fish aggression in fish fed any of the diets except when the 47/24 BioTrout treatment was included in analyses (Tables 1.5 and 1.6, Figures 1.4 -1.15). The lack of significant growth differences in fish from the dietary treatments is unlike other juvenile finfish dietary studies. In

general, the majority of studies on juvenile cultured fishes found that as protein levels increase, so does growth (Stefanussen et al., 1993, Aksnes et al., 1996, Catacutan et al., 2001, Morais et al., 2001, Lee et al., 2002, Tuan and Williams, 2007, Li et al., 2010, Chatzifotis et al., 2012). These studies also show that lipid levels generally do not affect the growth of the juvenile fish (Stefanussen et al., 1993, Aksnes et al., 1996, Catacutan et al., 2001, Morais et al., 2001, Lee et al., 2002, Tuan and Williams, 2007, Li et al., 2010, Chatzifotis et al., 2012). Morais et al. (2001) fed juvenile Atlantic Cod (*Gadus morhua*) diets that varied in their protein (48 and 58 %) and lipid (12 and 16 %). The researchers found that fish fed the 58/16 treatment resulted in the highest specific growth rate and feed conversion efficiency compared to the other dietary treatments (Morais et al., 2001). Atlantic Halibut (*Hippoglossus hippoglossus*) fed diets that varied in their protein, carbohydrate, and lipid concentrations showed significantly higher growth in fish fed diets with increased protein levels, however, there was no effect of lipid concentration on growth (Aksnes et al., 1996). Stefanussen et al. (1993) found that there was faster growth in juvenile Common Wolffish (*Anarhichas lupus*) when fed dry pellets rather than moist pellets. The dry pellets had lower carbohydrate levels and higher protein concentrations, resulting in higher fat and protein contents in the fillets of the juvenile Wolffish (Stefanussen et al., 1993). One study suggests that low protein diets can result in higher growth. Hillestad and Johnsen (1994) reported that juvenile Atlantic Salmon (*Salmo salar*) grew 27 % greater the lowest protein concentration (35 %) than the highest protein concentration (42 %). Other studies found no effect on growth when manipulating protein and lipid concentrations. Eliason et al. (2007) found no significant differences in the specific growth rates of juvenile Rainbow Trout (*Oncorhynchus mykiss*) when fed three difference diets that varied in their protein and lipid contents (55/10, 45/15, and 35/20). In another study using Atlantic Cod, researchers found no significant

differences in growth rates for juvenile Cod fed diets with varying protein (49, 54, 58, and 63 %) and lipid (11, 16, 20, 23, and 28 %) concentrations (Grisdale-Helland et al., 2008). Like other cold-water finfishes intensively grown in cultured settings, Lumpfish are resilient to a variety of dietary treatments. Juvenile Lumpfish can be fed diets with 50-55 % protein concentration and 10 - 20 % lipid concentration without impacting growth, mortality, or aggression.

Feeding juvenile Lumpfish diets with plant-based protein affected their growth. Fish fed the 47/24 BioTrout treatment showed the significantly lowest values of all growth metrics measured (Tables 1.5 and 1.6, Figures 1.4 - 1.9). BioTrout contains mostly soybean meal as the primary protein source. It is possible that the use of plant protein, rather than animal protein, is the reason for the reduced growth of fish in this treatment. Berge et al. (1999) found that feeding juvenile Atlantic Halibut diets with a mixture of soy protein concentrate and fish meal did not impact specific growth rates. Penn et al. (2011) found that feeding juvenile Atlantic Salmon diets with high levels of pea protein concentrate resulted in lower growth, reduced enzyme activity in the intestines, and the development of enteropathy. Feeding Sharp-Snout Sea Bream (*Diplodus puntazzo*), a temperate species, diets with high levels of pea protein concentrate also resulted in lower growth (Nogales-Merida et al., 2016). Though this study uses a warm-water species, Thompson et al. (2012) found that juvenile Nile Tilapia cannot effectively utilize diets that are primarily soybean protein (>75 % of protein total) as seen through the significantly lower percent weight gain values for fish in these treatments. However, they also determined that diets containing a 50:50 mix of soybean and fish meal were suitable for Nile Tilapia (Thompson et al., 2012). Reigh and Ellis (1992) fed juvenile Red Drum (*Sciaenops ocellatus*), another warm-water species, six dietary treatments that varied in their soy protein (SP) to fish protein (FP) ratios (100 % FP, 100 % SP, 75 % SP and 25 % FP, 75 % FP and 25 % SP, 50 % SP and 50 % FP, and 100

% SP with a methionine supplement). The researchers found that fish fed diets with 50 % or greater fish protein had significantly higher growth than fish fed the 75 SP:25 FP treatment (Reigh and Ellis, 1992). Though in general it seems that fish fed diets with higher protein concentrations performed better, protein or lipid concentration does not affect juvenile Lumpfish growth under cultured settings. This may be because Lumpfish are opportunistic feeders and are able to thrive under a variety of dietary conditions. However, the diet fed to these fish may be dependent on the temporal needs of the salmonid farms. If Lumpfish hatcheries are producing fish at a rapid pace, it may be beneficial for the growers to utilize the specialized Lumpfish diets currently marketed. To cut costs, facilities can decrease dietary fishmeal concentrations to 50% since this did not significantly affect juvenile Lumpfish growth, survival, or aggression in our study. Morais et al. (2001) grew Atlantic Cod under varying protein (48 and 58 %) and lipid (12 and 16 %) concentrations and determined that the 48/16 diet was the best compromise between cost, growth, and feed utilization. While the BioTrout diet uses soy protein as the primary protein source, recent studies have shown that partial replacement of fish meal with plant-based protein sources do not have ill effects on growth in juvenile Lumpfish. Willora et al. (2020) found that feeding juvenile Lumpfish a 52 % protein and 14 % lipid diet where 50 % of the protein was fish meal and the other 50 % was a 1:1 mix of soy protein and pea protein concentrate did not impact growth, muscle fiber cellularity, or chemical composition in juvenile Lumpfish. Fish fed this treatment were significantly longer, wider (fish height), and heavier compared to fish in the other treatments. The fact that Lumpfish are opportunistic omnivores in the wild may explain why the fish fed the 50 % fish meal, 50 % plant-based meal treatment grew the best (Willora et al., 2020). Therefore, growers may be able to cut costs and create feeds that are more sustainable, with less reliance of fishmeal, without impacting the growth of juvenile Lumpfish. However, feeding

Lumpfish diets where the majority of the protein is plant-based may also be beneficial, despite the depression of growth seen during Trial 1. Lumpfish outgrow their usefulness as cleanerfish, with smaller juveniles being more effective delousers (Imsland et al., 2016a, Jonassen et al., 2018). BioTrout is a salmonid diet that the Lumpfish would likely encounter while in the net pens. Imsland et al. (2015a) found that the majority of the stomach contents of Lumpfish stocked in net pens were formulated pellet fragments. If the juvenile Lumpfish feed on plant-based protein, salmonid feeds, they might remain for longer at the optimal size for maximizing cleaning efficiency, which is 50-180 g (Herrmann et al., 2021). However, feeding Lumpfish diets formulated for salmonids may be at the cost of animal welfare. These fish may not receive enough nutrients, or too much of some for that matter, which may result in emaciation or death.

While both trials produced similar results, they were not without their limitations. These limitations should be improved upon for future studies. Stocking density was likely a limitation in Trial 1. Fifteen fish were stocked per tank during this trial while only ten fish were stocked per tank in Trial 2. The stocking density was lowered for Trial 2 because we believed stocking density was limiting the growth of the juvenile Lumpfish. Higher stocking densities can lower the growth of finfishes (Enache et al., 2011, Liu et al., 2016, Arifin et al., 2019, Long et al., 2019; Chapter 3 of this thesis) which was likely what occurred in the first trial. The differences in temperatures between the two trials also proved to be a confounding factor. Trial 1 occurred at a much higher temperature range than Trial 2 (Table 1) and because of this, the fish showed faster and greater growth. However, fish in Trial 1 showed less efficient feeding as observed by the higher feed conversion ratios. This may indicate that the fish were eating more at these higher temperatures but were not digesting and utilizing the feed as efficiently. To better compare the results of the two experiments, they should have been performed at the same time of

year when temperature ranges were similar. Another limitation was the scale of the experiment. Fish were held in 10 L aquaria for the entirety of experimentation (Figure 1). While this is effective in providing replication in a laboratory setting, it does not accurately mimic the holding tanks that these fish would be held in at hatcheries and grow-out facilities. Performing these trials using larger holding tanks may yield more accurate results. Another limitation was in the measurement of fish aggression. While the use of the fin nipping scale (see Appendix A) was useful, using a total length to caudal fin length ratio in conjunction with the scale may give more accurate readings in terms of the level of caudal fin erosion. For future studies, a wider range of protein and lipid concentrations should be tested. In this study we only tested a small range (47-55%) of protein levels. It is possible that decreasing protein levels below 50 % will have ill effects on fish growth, as seen in other studies (Catacutan et al., 2001, Chatzifotis et al., 2012, Lee et al., 2002, Li et al., 2010, Tuan and Williams, 2007) but this remains unknown for juvenile Lumpfish. Skretting is beginning to include 55-62 % protein concentrations in their line of Lumpfish feeds, suggesting that higher protein levels benefit Lumpfish (Skretting, 2019). However, these data need experimental validation. Three lipid concentrations were tested (10, 15, and 20 %) but adding even more variation may help to reveal the nutritional requirements of Lumpfish. Adding more variation in terms of protein and lipid concentrations may help to create a matrix with different feeding scenarios. For example, if one wants to cut costs and use a lower protein concentration, say 50 %, what lipid concentration should be included in the diet to maximize growth of the juvenile fish? Conversely, a matrix could provide information on the diet formulations that depress growth if growers are attempting to keep fish at the most efficient cleaning size for a longer duration. For future studies, more variations in protein sources should be explored. While fish meal is regarded as highly nutritious and effective for aquaculture,

production of this protein source may not be sustainable (Miles and Chapman, 2006). Small pelagic species, including Gulf Menhaden (*Brevoortia patronus*), Japanese Anchovy (*Engraulis japonicus*), Capelin (*Mallotus villosus*), Atlantic Herring (*Clupea harengus*), and Chilean Jack Mackerel (*Trachurus murphyi*), are harvested by the fish meal industry (Shepherd and Jackson, 2013). Though, a large percentage of fish meal and fish oil is produced using the by-products of human seafood production, we do not exactly know the impacts we are having on these forage fisheries and the greater impact on the food webs that they feed so future emphasis should be placed on testing alternative protein sources. While, in this study, soybean protein resulted in slower juvenile Lumpfish growth, other protein sources exist such as byproducts of fisheries and aquaculture, food wastes, insect meal, poultry meal, cottonseed meal, and corn meal (Hua et al., 2019). Exploring these different protein sources could help make Lumpfish production more sustainable.

Conclusion:

Though feeds are marketed specifically for Lumpfish, the exact nutritional requirements of these fish remain unknown. Protein and lipid concentrations tested in this study did not affect the growth, survival, or aggression in juvenile Lumpfish. Lumpfish facilities may be able to reduce feed costs by lowering protein and lipid concentrations without affecting the performance of the juveniles. However, feeding these fish diets composed of only plant-based protein likely depresses growth. Salmonid, plant-based protein diets are available to Lumpfish once the Lumpfish are stocked into ocean net pens. This may be advantageous for salmonid farmers to suppress Lumpfish growth, extending the duration that the fish are of optimal cleaning size. Having an even mix of plant-based protein and fish meal in Lumpfish feeds may promote faster

growth in omnivorous, juvenile Lumpfish (Willora et al., 2020). More studies, using a wider range of protein and lipid concentrations, as well as utilizing other protein sources, should be performed. These studies, along with this one, will provide further insight into the nutritional needs of Lumpfish and how different diet formulations affect fish growth rates. This information will help guide commercial Lumpfish facilities with choosing the appropriate diet for the desired outcome needed to match the needs of the salmonid farms better.

CHAPTER 2: THE EFFECT OF TANK REARING DESIGN ON CULTURING JUVENILE LUMPFISH (*CYCLOPTERUS LUMPUS*): FISH GROWTH, SURVIVAL, AND TANK CLEANING EFFICIENCY

Introduction

Lumpfish (*Cyclopterus lumpus*) are effective at treating sea lice infestations (Imsland et al., 2014a, 2014b, 2015a, 2015b, 2018, Eliasen et al., 2018), and are now being produced by the tens of millions globally for this purpose, with Norway leading the way (Directorate of Fisheries, 2020). Regardless of nearly 50 million Lumpfish raised annually, Lumpfish production is a relatively new sector in aquaculture and as such there remain knowledge gaps on the specific culturing protocols and environmental conditions needed for these fish. These gaps may be leading to economic loss, through increased labor costs and loss of biomass, and issues of animal welfare, including issues of water quality and added stress on the animals during cleaning periods.

Upon hatching, Lumpfish employ their powerful ventral suction disk to adhere to surfaces, including the walls and bottoms of the larval and grow out tanks, making it difficult to clean excess food and fecal matter without causing harm or stress to the fish (Jonassen et al., 2018). For example, at the University of New Hampshire's Coastal Marine Laboratory (CML), Lumpfish are grown in flat-bottomed, flow through tanks with internal, central standpipes. Waste removal is performed by siphoning water through a screen where any larval and small juvenile Lumpfish sucked up are collected. These cleaning periods may cause unintended physical harm to the fragile, young fish. Further, many Lumpfish facilities elsewhere are former Atlantic Cod

(*Gadus morhua*) and other cold-water fish hatcheries that have been retrofitted for growing Lumpfish (Jonassen et al., 2018). For instance, commercial Lumpfish hatcheries and grow out facilities in countries like Norway also use large flat-bottomed tanks but implement large conical bottom tanks too (Jonassen et al., 2018). As an example, Johnson and Chen (2006) determined that conical bottoms with a 60° slope were a low maintenance solution for waste removal in recirculating aquaculture systems (RAS) when growing Rainbow Trout (*Oncorhynchus mykiss*). Optimizing tank design to maximize growth while also minimizing issues pertaining to water quality, tank cleaning, and fish stress is important for any hatchery. Two attributes that may aid in achieving this for Lumpfish production include considering tank color and tank bottom type.

Lumpfish can change colors and match the color of the substrate that they adhere to (Powell et al., 2018). As a result, light intensity, photoperiod, and tank color may affect the growth and survival of these fish (Powell et al., 2018). Davenport and Bradshaw (1995) found that altering these conditions affected the melanin concentrations of juvenile Lumpfish. At the CML, juveniles are raised in tanks with black sides and pale blue bottoms. Dark gray PVC panels are hung in the tanks to provide more surface area for the Lumpfish to adhere to. We observe that there are more fish adhered to the panels and sides of the tanks than the bottom of the tanks, leading us to believe that Lumpfish may prefer darker surfaces.

In the wild, Lumpfish larvae often associate with the smooth surfaces of macroalgae (Killen et al., 2007). Further, divers report frequently finding Lumpfish attached to smooth surfaces such as metal chains and the concrete pillars of piers and docks (UNH divers, pers. comm.). In a study testing suitable hide types for Lumpfish stocked in salmon net-pens, Lumpfish adhere to smooth plastic vertical surfaces rather than to stones or car tires (Imsland et al., 2015b) as well as to the natural floating seaweeds. Given this information, it is possible that

Lumpfish prefer smoother surfaces to rougher ones, providing hatcheries with a tool to deter the adhesion of larvae and juveniles to specific tank areas. Additionally, using false-bottom tanks may discourage Lumpfish from occupying the tank bottom while also minimizing routine tank cleaning. McMillian et al. (1998) tested the waste removal of three false-bottom tanks and one control (solid bottom) tank. The researchers determined that the false-bottom tanks were more efficient at waste removal than the control tank (McMillian et al., 1998).

The purpose of this study was to investigate tank design elements for larval and juvenile Lumpfish that would discourage fish from occupying the tank bottom and thus reduce interactions with fish during routine maintenance like tank cleaning, while not impeding fish growth and survival. It was hypothesized that (1) fish grown in dark-colored environments (black, blue, and green) would have the greatest growth and survival and lowest fish aggression compared to those grown in lighter colored environments (red, gray, white) and (2) fish would avoid tank bottoms that were rough, light, and false-bottomed compared to smooth and dark.

Methods

To evaluate a better tank design for juvenile Lumpfish, two experiments were conducted assessing the effects of tank color and tank bottom type, herein referred to as 'Tank Color Trial' and 'Tank Bottom Trial' at the CML.

For both experiments, fish from the general population of cultured, juvenile Lumpfish reared at the CML were graded to the desired size (see Table 2.1) and stocked into the 3.8 L, plastic experimental tank treatments in a flow-through, ambient temperature and salinity seawater system, subjected to a 12-hour light: 12-hour dark photoperiod (Figure 2.1).

For the Tank Color Trial, six color treatments (red, green, grey, black, white, and blue) were tested in triplicate (Figure 2.2a). The results from the Tank Color Trial were used to inform the Tank Bottom Trial treatments; the tank color treatment that resulted in the best growth was used as the base color (i.e., control treatment) in the Tank Bottom Trial. For the Tank Bottom Trial, six bottom treatments were tested: rough-dark, rough-light, smooth-dark, smooth-light, false bottom, and control. Dark treatments had dark-blue paint coated on the bottom of the tanks, while light treatments had white paint. Both paints were Krylon Fusion All-In-One enamel-based paint and primer. Rough treatments had a texture additive ("Stone" additive made by Homax) combined with the paint, while the smooth treatments did not. Texture additive was slowly sprinkled onto fresh paint with another coat of paint sprayed on after. The false bottom treatment had aquaria with 1 mm fiberglass mesh bottoms rather than solid bottoms. The control treatment was an unmodified tank (Figure 2.2b). All experimental tanks were soaked for 48 hours to allow any possible harmful chemicals to leach out.

Fish were fed at 2 % and 7 % body weight/day (based on the total weight of all fish in each tank), in the Tank Color and Tank Bottom Trials, respectively, three times per day (morning, afternoon, evening). Growth was measured biweekly (wet weights to the nearest 0.1g); for the Tank Color Trial, each individual fish was weighed, whereas for the Tank Bottom Trial, fish were weighed in batches. Fish aggression was measured biweekly in the Tank Color Trial. Fin damage of each fish was assessed using a scale from 0 to 5, with 0 representing a fish with an undamaged caudal fin, and 5 a fish with severe damage to the caudal peduncle (see Appendix A). Fish aggression was not monitored in the Tank Bottom Trial since individual fish were too small to evaluate without causing likely harm. Both trials ran for 8 weeks.

In addition to growth, the number of fish attached to the bottom of the tanks and cleaning time were assessed in the Tank Bottom experiment. At around 3 pm daily (to standardize fish counts), the number of fish attached to the bottom of each tank was recorded. Aquaria in the Tank Bottom Trial were cleaned weekly and the total amount of time (min) it took to siphon each treatment was recorded. Mortalities were counted, weighed, and removed daily in each experiment. In the Tank Color Trial, any mortalities also were scored for aggression. Water temperature and salinity were measured daily (Figures 2.3 and 2.4).

Data Analysis:

All data were analyzed using Excel 2019 and JMP Pro 15. Overall percent growth was calculated by comparing the original fish mean weights to the final fish mean weights: ((Final Weight – Original Weight)/ Original Weight) x 100%. Mean growth rates were calculated by taking the weight gain between each biweekly sampling period and dividing it by the number of days the fish were allowed to grow (14 days): (Final Weight – Original Weight)/14 days. The growth rate from each biweekly sampling period was averaged to get an overall mean growth rate for each treatment. For both tank trials, one-way ANOVAs and Tukey's tests were used to compare the final mean overall percent growth and mean growth rates between the treatments. Chi-squared tests were used to compare mean occurrence of fin nips between the tank color treatments. One-way ANOVAs and Tukey's tests were used to compare final mean number of fish attached to the bottom and mean cleaning time between the treatments in the tank bottom modification trial.

Figure 2.1. The experimental, flow-through seawater system used in both trials.

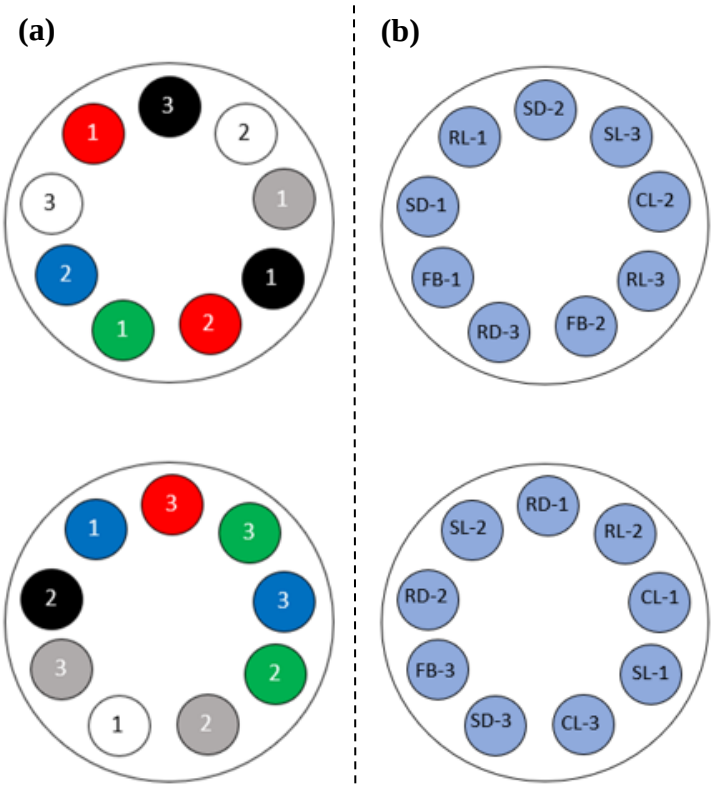

Figure 2.2. The experimental setups for the Tank Color Trial (a) and the Tank Bottom Trial (b). Experimental units were 3.8 L plastic aquaria (represented by the small circles) plumbed into a flow-through, seawater system (represented by the larger circles). Numbers and letters refer to treatment replicate. RD=rough dark; RL=rough light; SD=smooth dark; SL=smooth light; FB=false bottom; C=control.

Table 2.1. The testing parameters used for each of the tank modification trials.

Parameter	**Tank Color Trial**	**Tank Bottom Trial**
Testing Period	5/11/20 – 7/6/20	11/24/20 – 1/19/21
Mean Initial Fish Size ± 1 s.d. (g)	5.79 ± 0.44	0.35 ± 0.08
Number of Treatments	6	6
Body Weight/Feed Percentage (%)	2	7
Temperature Range (°C)	7-18	4-8
Salinity Range (ppt)	31-36	31-37
Number of Fish per Tank	15	50
Initial Stocking Density (g/L)	22.9	4.6
Initial Fish Age (days post hatch)	244	85
Growth Sampling	Individual Weights	Batch Weights
Mortality	Measured	Measured
Fish Aggression	Measured	Not Measured
Number of Fish Attached to Bottom	Not Measured	Measured
Cleaning Time (min)	Not Measured	Measured

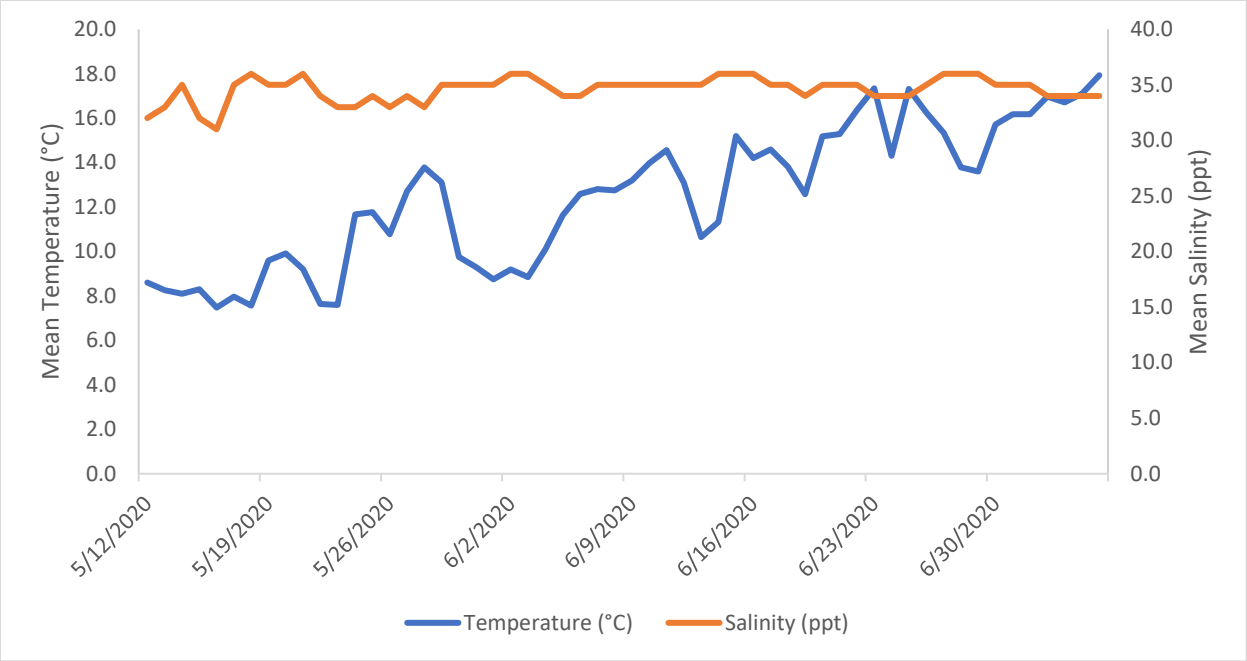

Figure 2.3. Mean daily temperature and salinity during the Tank Color Trial.

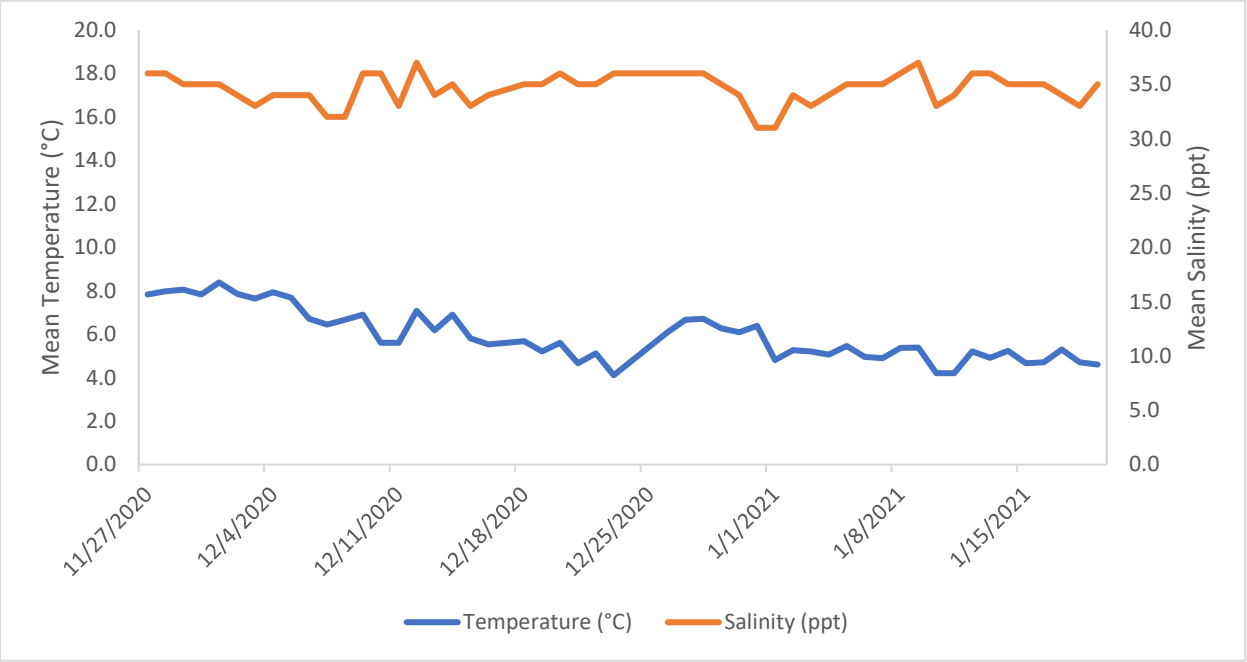

Figure 2.4. Mean daily temperature and salinity during the Tank Bottom Trial.

Results

Tank Color Trial

Fish survival was not impacted by tank color despite a total of 16 mortalities. Thirteen out of 15 fish died overnight in one of the white color replicates due to a disruption in water flow after 28 days. As a result, this replicate was removed from the experiment and subsequent analyses. Apart from that event, only four other fish died throughout the trial: one fish in the white treatment on day 18, and two fish in the blue treatment and one in the grey treatment on day 42. With these four mortalities in mind, there were no significant differences in survival between the color treatments (one-way ANOVA, p = 0.6327, Table 2.2).

Overall mean fish growth rates for each color treatment varied from 0.50 ± 0.22 g/day to 0.58 ± 0.32 g/day and did not differ significantly between the treatments (one-way ANOVA, p = 0.0670, Table 2.2, Figure 2.5). Final mean percent growth varied from 471.8 ± 24.4 % to 584.5 ± 70.8 % (Figure 2.6). Fish in the blue treatment had significantly higher overall mean percent growth (584.5 ± 70.8 %) than fish in in the black treatment (471.8 ± 24.4 %, one-way ANOVA, p = 0.0199, Figure 2.6).

Tank color did not impact the occurrence of fish aggression ($X^2(5, N = 253) = 0.18$, $p = 0.9993$). Though not significant, fish in the black treatment had the highest mean percentage of fin nipping occurrence (20.56 ± 9.32 %) and fish in the grey treatment had the lowest (1.11 ± 1.28 %, Figure 2.7). Because treatment did not have an effect on fish aggression, the severity of fin nipping data were not analyzed. Only 14 out of the 253 fish sampled showed fin damage above a level 1 (see Appendix A) after 8 weeks. Three fish in the red treatment, three fish in the

blue treatment, five fish in the black treatment, two fish in the green treatment, and one fish in the white treatment had damage scores above level 1.

Tank Bottom Trial

In the Tank Bottom Trial, blue colored tanks were used as the control treatment since they yielded the highest final overall percent growth after eight weeks in the Tank Color Trial (Figure 2.6) and thus the wall color for all tank bottom treatments.

Even though there was a total of 111 mortalities, survival was not impacted by bottom type during the trial. Ninety percent (100 out of 111 fish) died due to a cutoff of waterflow in one rough-dark and one smooth-light treatment after 11 and 21 days, respectively. These replicates were removed from the experiment and subsequent analyses. Not factoring these major die offs, there were no significant differences in survival between the treatments (one-way ANOVA, $p = 0.3584$). The smooth-light treatment had four more mortalities: two on day 33, one on day 43, and one on day 46. The control treatment had one mortality after 33 days. The smooth-dark treatment had a total of three mortalities that occurred on days 39, 41, and 47, respectively. The rough-light treatment had a total of two mortalities that occurred after 43 and 49 days, respectively. The last mortality occurred on day 54 in the false bottom treatment (Figure 2.8).

Daily mean growth rates of fish did not vary significantly amongst the treatments, with all being 0.02 ± 0.01 g/day (one-way ANOVA, $p = 0.9001$, Table 2.3, Figure 2.9). Mean overall percent growth ranged from 259.1 ± 67.4 % to 509.2 ± 162.1 % and did not vary significantly between the treatments (one-way ANOVA, $p = 0.0708$, Table 2.3, Figure 2.10).

Mean percentage of fish attached to the bottom ranged from 0.79 ± 0.75 % to 10.00 ± 1.07 % (Table 2.3, Figure 2.11). There was a significantly higher percentage of fish attached to the bottom of smooth bottomed tanks (smooth-dark, smooth-light, and control; 7.42 ± 2.58 %) compared to the percentage of fish attached to the rough bottomed and false bottom treatments (1.24 ± 0.45 %, one-way ANOVA, $p = 0.0152$, Figure 2.12). The control treatment had the significantly highest percentage of juveniles attached (10.00 ± 1.07 %) to the bottom than any of the other treatments. The rough-light and the rough dark treatments had the lowest percentage of juveniles attached (0.79 ± 0.75 % and 1.28 ± 0.87 %, respectively, Table 2.3, Figure 2.11).

Cleaning time also was impacted by bottom type and ranged from 1.54 ± 1.18 min/cleaning event to 2.85 ± 1.34 min/cleaning event (Table 2.3). In general, rough treatments took longer to clean than the other treatments, however the only significant difference in cleaning time was between the false bottom treatment (1.54 ± 1.18 min) and the rough-light treatment (2.85 ± 1.34 min, one-way ANOVA, $p = 0.0047$, Figure 2.13).

Tank Color Trial

Table 2.2. The results of the different testing parameters measured for the Tank Color Trial. Different superscript letters denote statistical differences between treatments in each column.

Color Treatment	**Final Mean Percent Growth (± one standard deviation, %)**	**Mean Growth Rate (± one standard deviation, g/day)**	**Final Mean Occurrence of Fin Nipping (± one standard deviation, %)**	**Final Mean Severity of Fin Nips (± one standard deviation)**	**Final Percent Survival (%)**	**Final Mean Fish Weight (± one standard deviation, g)**
Red	491.28 (± 70.77)[ab]	0.53 (± 0.30)	15.56 (± 10.18)	0.22 (± 0.17)	100.00	35.91 (± 1.31)
Blue	584.53 (± 30.22)[b]	0.55 (± 0.28)	9.30 (± 3.59)	0.21 (± 1.00)	95.56	36.03 (± 1.00)
Black	471.80 (± 4.69)[b]	0.50 (± 0.22)	17.78 (± 16.78)	0.50 (± 0.05)	100.00	34.22 (± 1.52)
Green	515.08 (± 6.62)[ab]	0.53 (± 0.27)	13.33 (± 6.67)	0.20 (± 0.12)	100.00	35.26 (± 1.88)
White	528.60 (± 14.41)[ab]	0.58 (± 0.32)	9.68 (± 7.15)	0.14 (± 0.10)	96.67	38.92 (± 3.25)
Gray	561.11 (± 24.39)[ab]	0.56 (± 0.29)	0	0	97.78	36.70 (± 1.83)

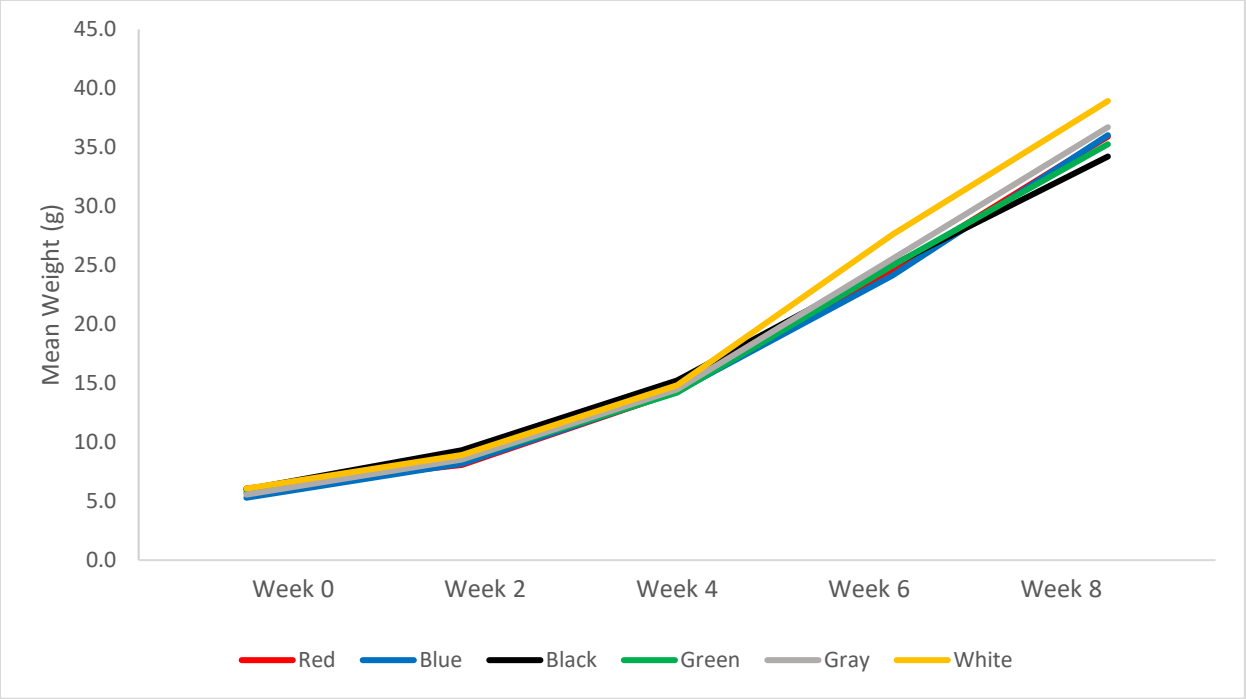

Figure 2.5. Mean weights of the juvenile Lumpfish over time in each tank color treatment.

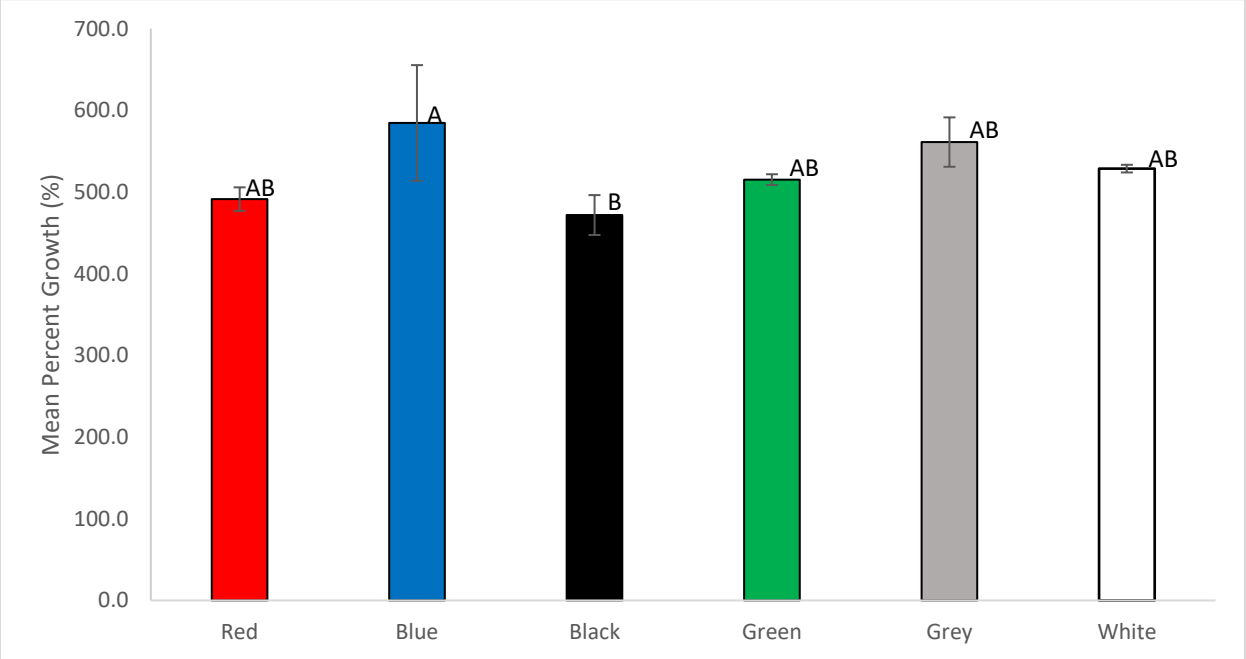

Figure 2.6. Final overall mean percent growth (± one standard deviation) of juvenile Lumpfish in each tank color treatment. Differing letters denote significant differences between treatments.

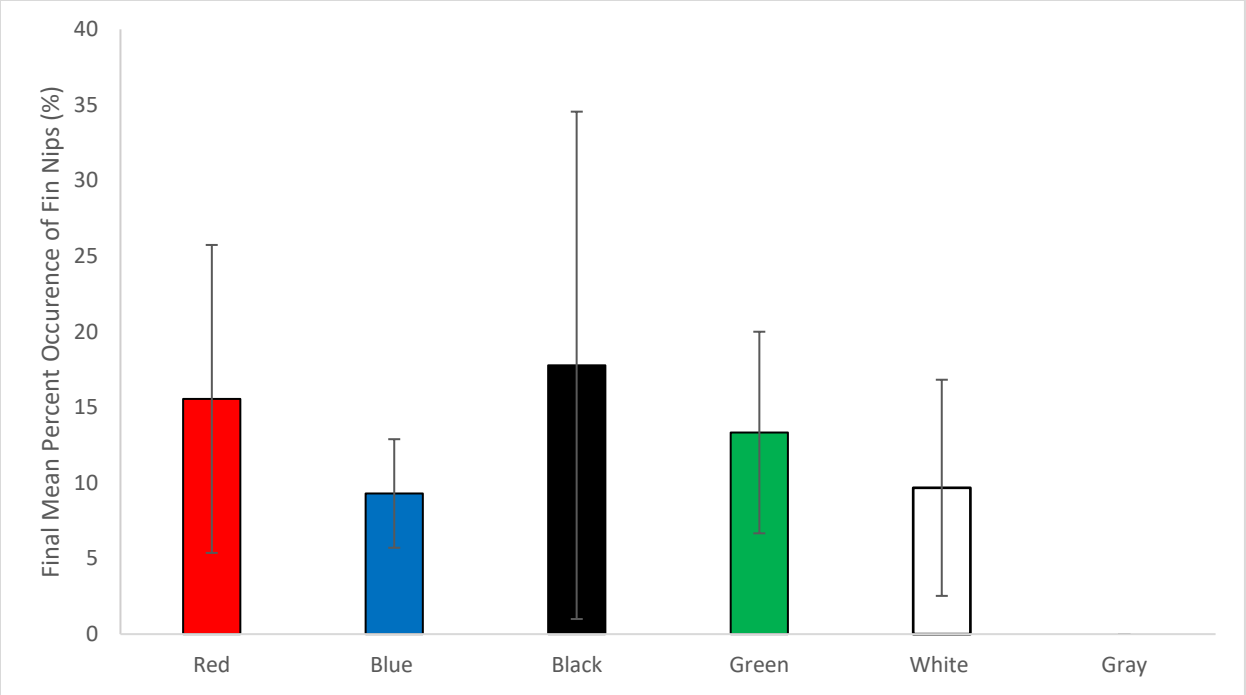

Figure 2.7. Final overall mean percent occurrence (± one standard deviation) of juvenile Lumpfish aggression in each tank color treatment.

Tank Bottom Experiment

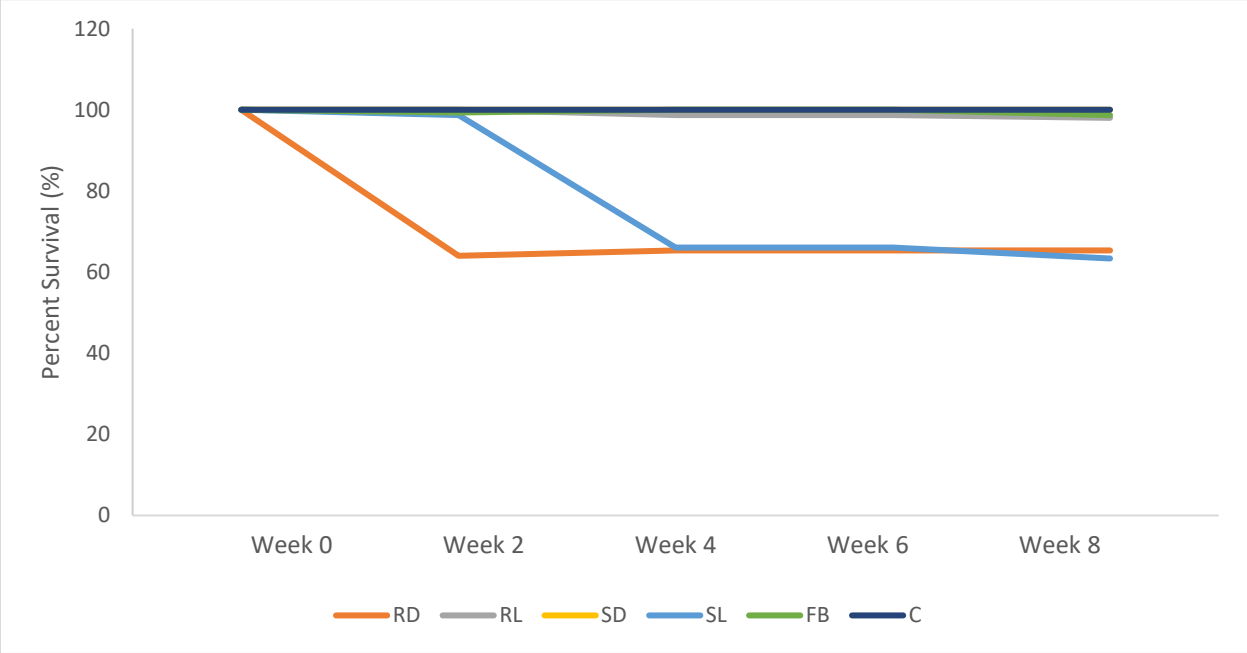

Figure 2.8. Fish survival in each tank bottom treatment at each biweekly weigh-in.

Table 2.3. The results of the different testing parameters measured for the Tank Bottom Trial. RD=rough dark; RL=rough light; SD=smooth dark; SL=smooth light; FB=false bottom; C=control. Different superscript letters denote statistical differences between treatments in each column.

Bottom Treatment	**Mean Percent Growth (± one standard deviation, %)**	**Mean Growth Rate (± one standard deviation, g/day)**	**Mean Percentage of Juveniles Attached to the Bottom (± one standard deviation, %)**	**Mean Cleaning Time (± one standard deviation, min)**	**Final Percent Survival (%)**	**Final Mean Fish Weight (± one standard deviation, g)**
RD	259.10 (± 67.36)	0.02 (± 0.01)	1.28 (± 0.87)^{d}	2.77 (± 1.22)^{ab}	98.00	1.59 (± 0.29)
RL	312.86 (± 64.70)	0.02 (± 0.01)	0.79 (± 0.74)^{d}	2.85 (± 1.34)^{a}	98.00	1.47 (± 0.20)
SD	341.31 (± 42.64)	0.02 (± 0.01)	7.42 (± 1.63)^{b}	2.15 (± 1.20)^{ab}	100.00	1.57 (± 0.13)
SL	427.22 (± 31.38)	0.02 (± 0.01)	4.83 (± 0.55)^{c}	1.62 (± 0.74)^{ab}	95.00	1.57 (± 0.37)
FB	509.17 (± 162.14)	0.02 (± 0.01)	1.68 (± 1.40)^{d}	1.54 (± 1.18)^{b}	98.67	1.57 (± 0.21)
C	401.77 (± 20.03)	0.02 (± 0.01)	10.00 (± 1.07)^{a}	1.65 (± 0.83)^{ab}	99.33	1.51 (± 0.12)

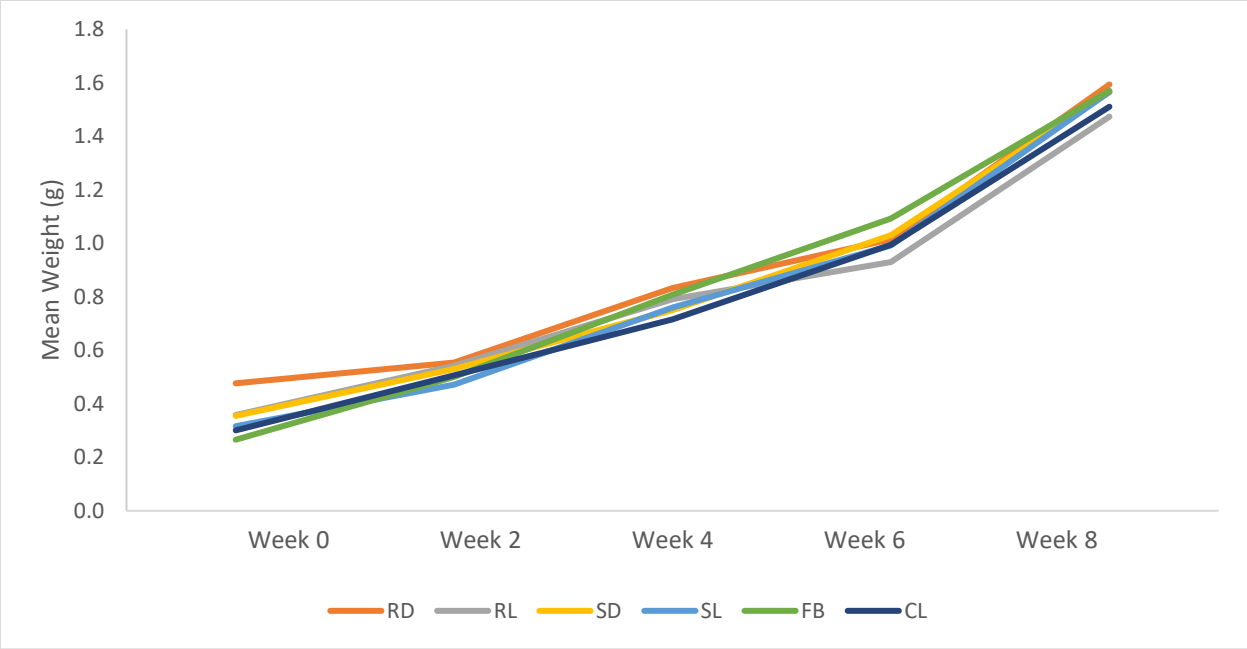

Figure 2.9. Mean weights of the juvenile Lumpfish over time in each tank bottom treatment. RD=rough dark; RL=rough light; SD=smooth dark; SL=smooth light; FB=false bottom; C=control.

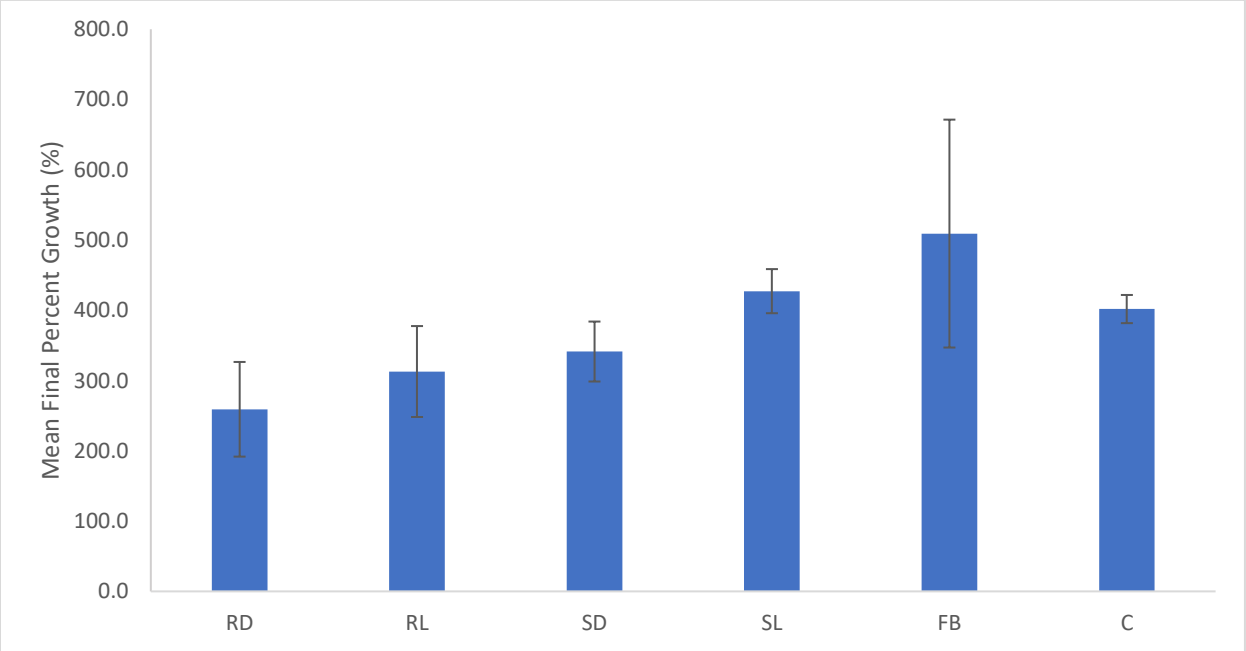

Figure 2.10. Final overall mean percent growth (± one standard deviation) of juvenile Lumpfish in each tank bottom treatment. RD=rough dark; RL=rough light; SD=smooth dark; SL=smooth light; FB=false bottom; C=control.

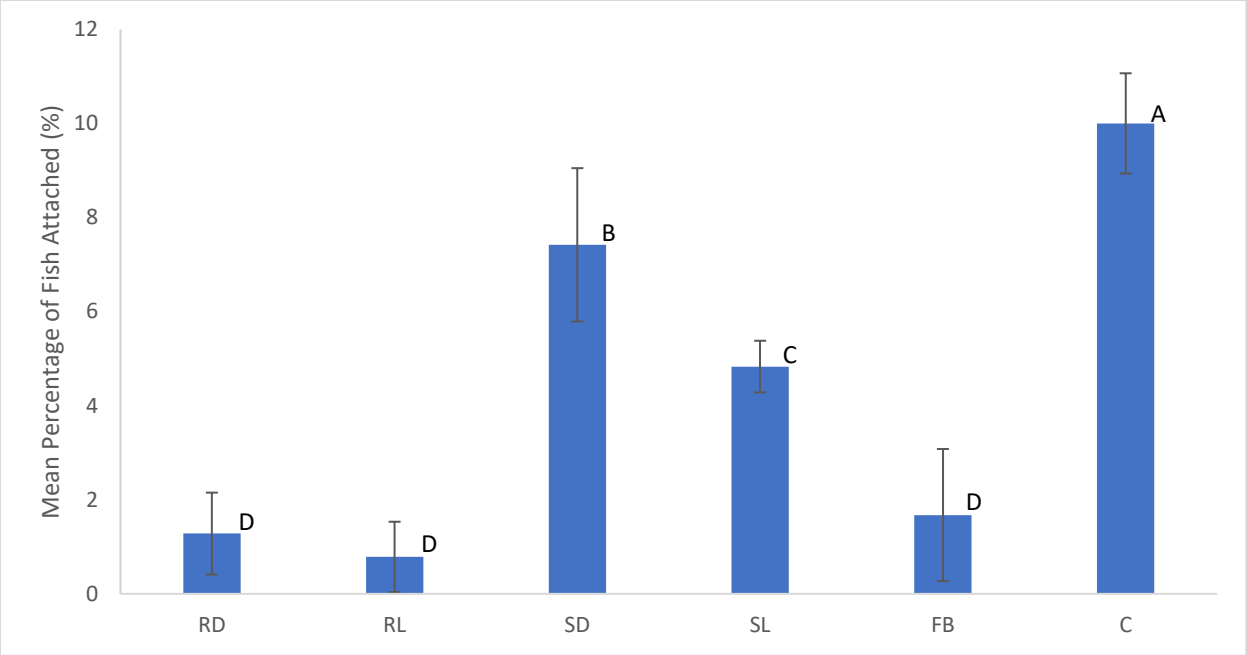

Figure 2.11. Mean percentage of juvenile Lumpfish attached (± one standard deviation) to the bottom of each tank bottom treatment. Differing letters denote significant differences between treatments. RD=rough dark; RL=rough light; SD=smooth dark; SL=smooth light; FB=false bottom; C=control.

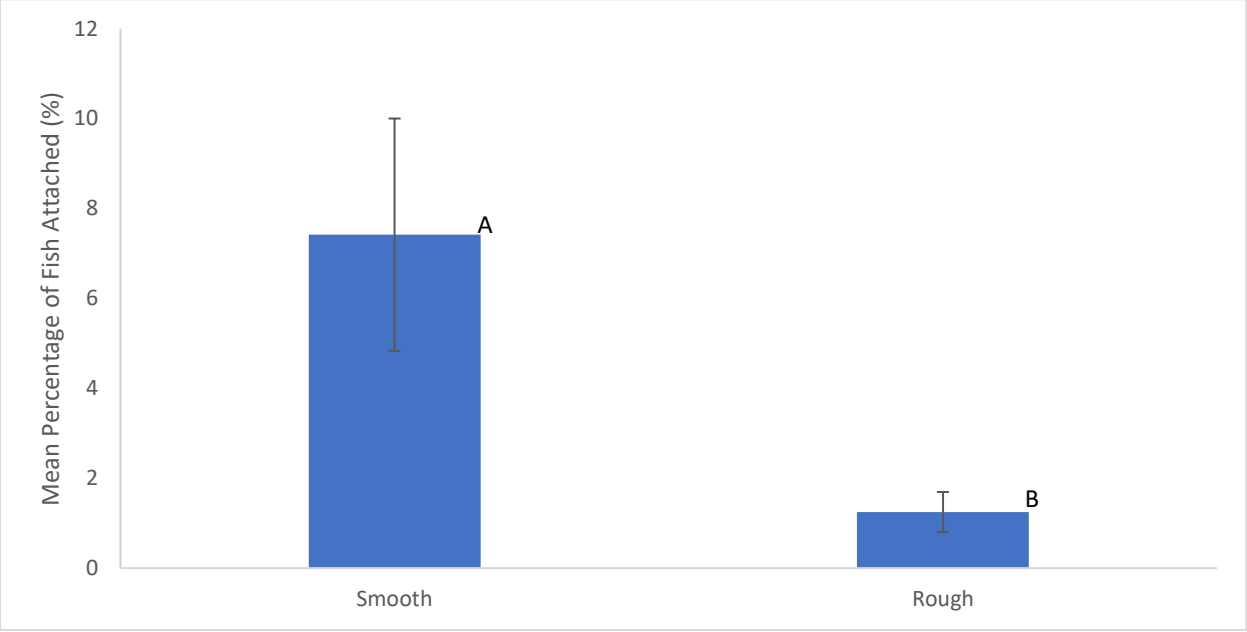

Figure 2.12. Mean percentage of juvenile Lumpfish attached (± one standard deviation) to the bottom of each treatment grouping; “smooth bottomed” (smooth dark, smooth light, and control) and “rough bottomed” (rough dark, rough light, and false-bottom).

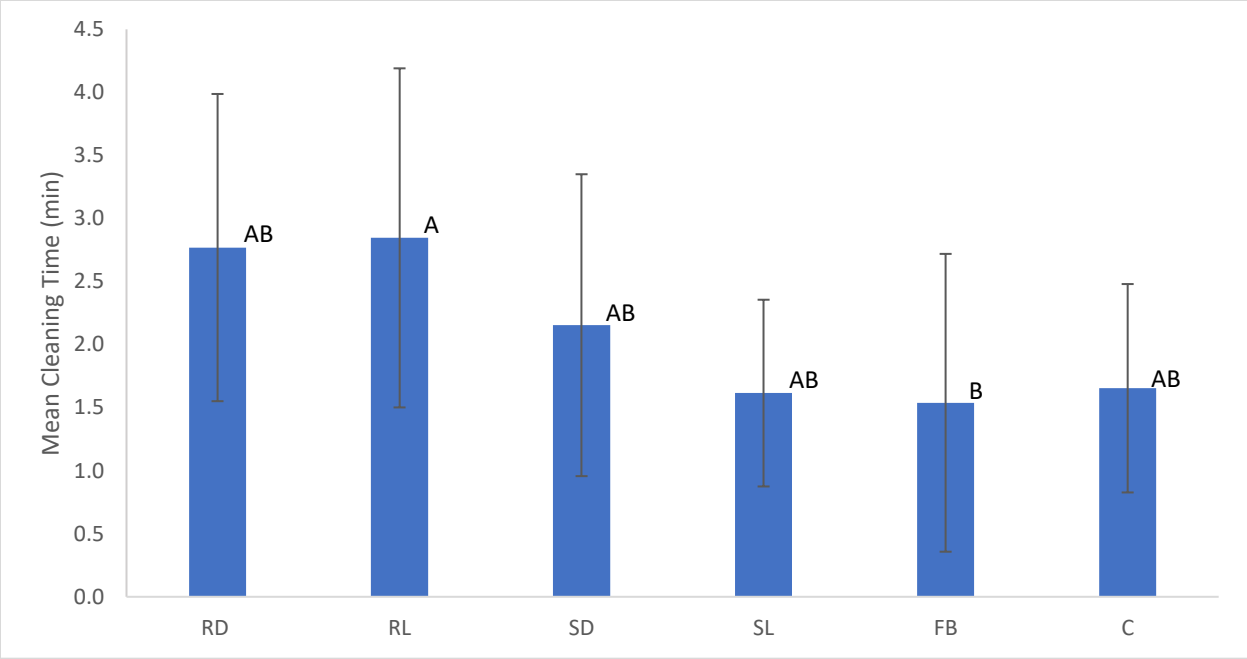

Figure 2.13. Mean cleaning time (± one standard deviation) for each of the tank bottom treatments per cleaning event. Differing letters denote significant differences between treatments. RD=rough dark; RL=rough light; SD=smooth dark; SL=smooth light; FB=false bottom; C=control.

Discussion

Any of the colors evaluated would be suitable for Lumpfish rearing tanks as fish had good growth (around 500 % overall mean percent growth) over the 8-week testing period. Survival, growth, and fish aggression were all unaffected for juvenile Lumpfish during the Tank Color Trial (Table 2.2). There were no significant differences between the mean growth rates of each treatment (Table 2.2, Figure 2.5). Looking at final overall mean percent growth, the only significant difference that existed was between the blue and black treatments (Figure 2.6). There were also no significant differences in fish aggression metrics between the color treatments (Table 2.2, Figure 2.7). This was surprising since tank color can affect larval and post-larval fishes in many ways including survival, growth, feed conversion, stress, health, body color, and behavior. This means that any of these colored tanks would be suitable for Lumpfish growth.

Tank color has been a focal issue related to fish vision and providing contrast to the fish feed (McLean, 2021). When Dolphin larvae (*Coryphaena hippurus*) were grown in black or tan tanks, larvae survival was improved by 130 % in tan tanks. Researchers concluded that the contrast provided by the black tanks allowed the Dolphin larvae to see and capture rotifers more efficiently (Ostrowski, 2011). Kesbic et al. (2016) grew juvenile European Sea Bass (*Dicentrarchus labrax)* in red, blue, green, and yellow tanks. While survival was unaffected by tank color, growth metrics were influenced by the color treatments. Fish grown in the yellow treatment had significantly higher relative and specific growth rates than fish in the red and green treatments (Kesbic et al., 2016). Coho Salmon (*Oncorhynchus kisutch*) grown in bisected tanks of black vs. white, blue vs. white, and dark grey vs. light grey preferred and were less aggressive in darker environments (Gaffney et al., 2016). However, in other species, color did not affect fish survival or growth. For example, Imanpoor and Abdollahi (2011) found that growing Caspian Kutum (*Rutilus Kutum*) in black, blue, red, yellow, or white tanks did not significantly affect specific growth rates. Survival rates of Eurasian Perch larvae (*Perca fluviatilis*) did not vary when fish were grown in either grey or black wall-colored tanks (Jentoft et al., 2006). Similarly, juvenile Pot-Bellied Seahorses (*Hippocampus abdominalis*) thrive under a variety of color treatments including clear, white, red, blue, black, yellow, orange, and green with growth and survival is unaffected by tank color (Martinez-Cardenas and Purser, 2007). Banan et al. (2011) concluded that juvenile Beluga Sturgeon (*Huso huso*) were unaffected by tank color (white, red, blue, black, and green) in terms of weight gain, specific growth rate, condition factor, and survival. These studies further add to the idea that the impacts of tank color on the rearing of finfishes is species dependent. Like Eurasian Perch, Pot-Bellied Seahorses, and Beluga Sturgeon, juvenile Lumpfish seemed unaffected by tank color.

Most fish are thought to be either dichromatic or trichromatic which refers to the number of different types of cone cells in the eye (Cheney and Marshall, 2009). These cone cells are used for distinguishing different wavelengths or light. Some fish can detect wavelengths ranging from ultraviolet to red (Hawryshyn, 1992). Hurst (1953) performed a rudimentary experiment to determine if fish can distinguish different wavelengths of color. Bluegill sunfish (*Lepomis macrochirus*) were kept in 189.2 L aquaria and were presented with two boxes that were illuminated with red or green light. If fish went to the box illuminated with the red light, they were given food, but if the fish went to the box with the green light, they were not. The bluegills preferred the red boxes over the green boxes at a ratio of 94/7 (Hurst, 1953). Cheney et al. (2009) determined that fish that are trichromatic and ultraviolet sensitive are better at determining different colors than fish that are dichromatic. They also determined that trichromatic fish are better at distinguishing mimic species from model species in their environments (Cheney et al., 2009). Therefore, the range of light that fish can see is species specific, however, it is also dependent on the fish's position in the water column (Hawryshyn, 1992). One must also consider environmental factors that influence light availability. Because light availability decreases with water depth (Liu et al., 2007), deep sea species have adapted larger eyes and higher receptor densities to detect what little light is present. Even with these adaptations, special resolution is limited to tens of centimeters (NeiBe et al., 2020). Light availability also decreases with the amount of turbidity that species are exposed to. Temperate regions tend to experience more turbidity than tropical ones, so species in deep, temperate waters (like Lumpfish) may be more visually impaired than tropical fish (Utne-Palm, 2002). Adult Lumpfish inhabit the deep (greater than 868 m), temperate waters of the North Atlantic for most of the year (Davenport, 1985; Parin et al., 2002), traveling near shore only during the spawning season (Cox and Anderson, 1922).

Though not well studied, one can postulate that because Lumpfish inhabit deep, temperate waters, they lack the visual capabilities of species inhabiting the photic zone. However, in a recapture study performed by Foss et al. (2020) with 30-80 g Lumpfish using small mesh cages at 4-5 m in depth, the highest recapture rate was seen in cages where blue light was shined on the cages, rather than red or yellow, suggesting that Lumpfish utilize lower wavelengths of light. The studies above focused primarily on the vision of large juvenile and adult fish. Small juvenile Lumpfish were tested during these trials and therefore further exploring the visual capabilities and preferences of Lumpfish (of all life stages) will help hatcheries in cleaning tanks, lowering costs of labor and loss of biomass. However, the results from this study as well as the one performed by Foss et al. (2020), may indicate that lumpfish have stronger visual capabilities for colors lower on the wavelength spectrum. This may be the reason why the blue color treatment resulted in the highest growth on average.

Juvenile Lumpfish were not only unaffected by the tank colors we tested but they mostly were unaffected by the rearing tank bottom types we evaluated too. Tank bottom type did not influence fish survival (Table 2.3). There was no significant difference between the growth rates and final overall mean percent growth of fish reared in the different tank bottom treatments (Table 2.3, Figures 2.9 and 2.10). This differences in growth did not exist because of the high variability in the overall mean percent growth data. While some treatments showed poor growth, others, like the false-bottom, treatment showed good growth over the 8-week testing period. But because of the high variability within each treatment, no significant differences were found. Studies exploring different bottom colors and textures, and their effects on finfishes are limited. One study using Atlantic Cod larvae found similar results in which after 55 days there were no significant differences in fish survival or growth (dry wright and standard length) reared in light

(beige) or dark (black) bottomed tanks with black walls (Monk et al., 2008). However, most studies focus on whether or not to utilize complex benthic habitats. For instance, Tuckey and Smith (2001) found that while growth was unaffected in Southern Flounder (*Paralichthys lethostigma*), survival was higher in tanks with no sand compared to tanks with sanded bottoms. When Senegalese Sole (*Solea senegalensis*) were grown in tanks with similar walls and plain cement, cement blended with silica fume (an additive used to increase the durability of concrete), or epoxy coated bottoms, higher specific growth rates were obtained from fish in the cement blended with silica fume bottoms (Almansa et al., 2017). Tupper and Boutilier (1997) grew cunner (*Tautogolabrus adspersus*) in four benthic habitat types (rocky reef, cobble, seagrass, and sand), and discovered that post-settlement survival varied with habitat type and was positively correlated to habitat complexity, but growth did not. The impact of bottom type on the survival and growth of cultured species is dependent on the behavior and resilience of the species. While one may infer that benthic species, like Flounder and Lumpfish, may have their survival and growth altered by substrate type, this is not always the case.

The main goal of this study was to determine a bottom type that would limit larval and juvenile Lumpfish adhesion to the bottom of their tanks to increase cleaning efficiency. Only focusing on texture, the treatments can be separated into “smooth bottomed” (smooth dark, smooth light, and control) and “rough bottomed” (rough dark, rough light, and false-bottom) treatments. When grouping the treatments into these categories, significantly fewer juvenile Lumpfish adhered to rough bottomed treatments (1.25 ± 0.45 %) compared to smooth bottom treatments (7.42 ± 2.58 %, one-way ANOVA, $p = 0.0152$, Figure 2.12). Furthermore, the greatest adhesion occurred in the control treatment than all the other treatments tested (10.00 ± 1.07 %, Table 2.3, Figure 2.11). These results are consistent with Imsland et al.’s (2015b)

findings in which Lumpfish preferred adhering to smooth plastic surfaces rather than to rougher surfaces like car tires while out in salmon pens. However, this distinction is not as prevalent in other benthic species. Reig et al. (2010) grew Sole (*Solea senegalensis*) in plastic, concrete, and sanded bottoms with various textures and colors. They determined that there was no clear hierarchy in terms of texture and color, however, Sole seem to prefer light sand of whatever texture and rough plastic of whatever color (Reig et al., 2010). Lumpfish prefer smooth surfaces rather than rough ones. This is likely because smoother surfaces allow for more effective suction by juvenile Lumpfish. The smooth bottoms may be less abrasive on the ventral surfaces of Lumpfish as well. If Lumpfish hatcheries want to limit the quantity of larval and juvenile fish adhered to the bottom of their tanks, then rough textures should be utilized. However, this may be at the cost of cleaning efficiency. Though not significant, it took longer to clean both the rough dark (2.77 ± 1.22 min) and rough light (2.85 ± 1.34 min) treatments compared to all other treatments (Figure 2.13). The rough light treatment resulted in significantly higher cleaning times than the false-bottom treatment (1.54 ± 1.18 min, Table 2.3). Furthermore, we noted anecdotally but did not quantify, it was more difficult to siphon the rough bottomed tanks and they did not get cleaned as well as the smooth- or false-bottomed tanks. With the high growth, high survival, low fish adhesion, and low cleaning time, it seems that the false-bottom treatment is the ideal choice for culturing small juvenile Lumpfish. However, implementing false-bottomed tanks into a facility may not be feasible for some companies and changing the bottom texture of tanks may result in drawbacks associated with tank cleaning. However, separating the smooth-bottomed treatments by color indicates a possible solution. The smooth light treatment had significantly lower Lumpfish adhesion (4.83 ± 0.55 %) than the smooth dark and control treatments (7.42 ± 1.63 % and 10.00 ± 1.07 %, respectively), which in themselves are darker than the smooth light

treatment (Table 2.3, Figure 2.11). Lighter bottomed tanks seem to deter Lumpfish more readily than their counterparts. Therefore, altering bottom color may be a more realistic solution for Lumpfish facilities with limited budgets or resources, achieved by painting the bottom of existing tanks with a light colored, nontoxic marine paint.

While these trials provide tank design suggestions for Lumpfish facilities, there were limitations to these studies. The first limitation concerns the scale of these trials. Small (3.8 L) buckets (Figure 2.1) were used as the experimental units due to a lack of space in the research facility and because these buckets could be altered easily to create the various bottom treatments. However, these small volumed tanks do not simulate commercial aquaculture facilities. Future studies should utilize larger tanks to replicate hatchery conditions better. Though not a major goal of the Tank Color Trial, measuring fish aggression in the various color treatments could have been performed more accurately. The use of the fin-nipping scale (see Appendix A) was useful but adding caudal fin length measurements in conjunction with the scale would likely produce more accurate results. For the Tank Bottom Trial, there were some limitations to measuring fish adhesion to tank bottoms and cleaning time. Lumpfish adhesion was only measured once per day. An attempt was made to use time lapse video cameras (Brinno) as another way of measuring adhesion, but poor lighting made video analysis futile. Therefore, if this experiment were to be repeated, better lighting schemes should be utilized if cameras are being used, which would allow for more accurate evaluating of bottom tank use by the fish. If not, Lumpfish adhesion should be checked manually at least 3-5 times per day. Consistency in cleaning time could have been improved. To measure cleaning time, one person recorded the amount of time it took them to clean each treatment; multiple people performed this task throughout the experiment. To standardize the cleaning measurements, only two people should

be involved in cleaning the tanks with exclusive roles: one person cleans the tanks, and the other person times the activity. This study was conducted in a flow-through seawater system. If recirculating aquaculture systems are used, additional water quality measurements beyond just temperature and salinity should be compared between the treatments including ammonia, nitrate, and nitrite. Furthermore, it would be interesting to repeat this experiment similar to Gaffney et al.'s (2016) study in which tanks were bisected into two different bottom types. Bisecting the tanks would allow Lumpfish to choose which texture or color they preferred during experimentation, further helping in deciding which bottom type to use when culturing larval and juvenile Lumpfish.

Conclusion

Based on these trials, Lumpfish are a resilient species, able to be grown in multiple tank colors and bottom types. Neither juvenile Lumpfish survival nor growth were impacted by the tank modifications tested in this study, however, we propose recommendations for improving cleaning efficiency for Lumpfish hatcheries. For those with limited resources or budgets, modifying tank bottom color can be an effective method to reduce juvenile Lumpfish adhesion. Light bottomed tanks proved to be the best at deterring Lumpfish adhesion out of the smooth bottomed tanks during the Tank Bottom Trial. For facilities with more funds and/or resources, altering bottom texture or installing false-bottom tanks should be considered. While rough bottomed treatments proved to be effective at deterring Lumpfish from sticking, this was at the cost of cleaning efficiency. The false-bottom treatment resulted in high survival, high growth, low Lumpfish adhesion, and low cleaning time when compared to the other treatments. With these metrics in mind, as well as the fact that false-bottom tanks are efficient at waste removal

(McMillian et al., 1998), Lumpfish hatcheries and grow-out facilities should consider false-bottom tanks to maximize animal welfare and profits. With increased cleaning efficiency, labor costs should be lower and fish production higher with fewer fish interactions during cleaning events and increased water quality.

CHAPTER 3: THE EFFECTS OF STOCKING DENSITY ON THE GROWTH, SURVIVAL, AND AGRESSION OF JUVENILE LUMPFISH (*CYCLOPTERUS LUMPUS*)

Introduction

Density dependence refers to the effects of population density on the performance of a species (Rose et al., 2002). Keeping fish under very dense conditions can have ill effects on aquaculture operations, resulting in stunted fish growth, increased mortality, and increased aggression (Rose et al., 2002, Baldwin, 2011). Generally, higher stocking densities are often met with increased stress in fishes (Baldwin, 2011). These density dependent effects are also seen in Lumpfish (*Cyclopterus lumpus*, Jonassen et al., 2018), a species that is used as a biological delouser for the salmonid aquaculture industry (Imsland et al., 2014a, 2014b, 2015a, 2015b, 2018, Eliasen et al., 2018). With their cleaning capabilities in mind, these fish are now produced by the tens of millions, with Norway leading the way (Directorate of Fisheries, 2020). Almost 50 million juvenile Lumpfish are produced annually. However, cleanerfish technology and the culture of Lumpfish is a relatively new area of aquaculture. Publicly available standard operating procedures for rearing Lumpfish do not exist, and there are several knowledge gaps on culturing protocols, including the effects of rearing densities. These gaps may be resulting in losses of biomass, increased labor costs, and issues of animal welfare for Lumpfish hatcheries, ultimately resulting in economic loss.

Commercial Lumpfish hatcheries usually grow juvenile fish at stocking densities ranging from 10-43 g/L until they are ready for use as cleanerfish in salmonid farms (Treasurer, 2018a), however, it is not known whether these densities are good for Lumpfish growth and survival.

There are no published studies specifically focused on evaluating stocking densities for juvenile Lumpfish. Researching stocking density in cultured Lumpfish is vital since this species is territorial. Juveniles bite each other's caudal fins, often resulting in death. Lumpfish also develop social hierarchies through territorialism in which smaller, less dominant individuals are pushed towards the bottom of the tank during feeding events. To prevent all aggressive behaviors and feeding hierarchies, frequent size grading of fish is needed to separate small and large individuals (Jonassen et al., 2018). Juvenile Lumpfish seem to be most aggressive when 5-7 g. Therefore, there may be an ontogenetic shift in behavior as Lumpfish grow, meaning that as the fish develop, there are shifts in their behavior. Hatcheries attribute increased cannibalism to a lack of surface area in their holding tanks. Lumpfish are not a schooling species but rather use their ventral suction disk to adhere to surfaces. Therefore, unlike other cultured species, tank volume is not as important in rearing these fish. If enough surface area isn't provided to the Lumpfish, fish aggression will increase (Jonassen et al., 2018). Several strategies have been developed to combat this issue. Hanging plastic panels in the water column is something that has been utilized at CML but is also widely used at other facilities. Hatcheries sometimes use "doughnut" tanks where a circular wall is inserted into the middle of the tank to provide more surface area. However, these solutions are not perfect and are often temporary (Jonassen et al., 2018). It is important to minimize aggression in cultured species to maximize production, profit, and ensure a high standard of animal welfare. For example, under intense culture, Lumpfish are prone to suction cup deformities, fin damage, and body damage, all of which can increase emaciation in these fish (Rabadan et al., 2021).

Lumpfish are not unique in their display of territorialism and aggressive behaviors. These behaviors have been documented in several other cultured finfish species including Three-spine

Tticklebacks (*Gasterosteus aculeatus*), Pumpkin Seed Sunfish (*Lepomis gibbosus*), Mozambique Tilapia (*Oreochromis mossambicus*), Mangrove Killifish (*Kryptolebias marmoratus*), Blue Gourami (*Trichogaster trichopterus*), Siamese Fighting Fish (*Betta splendens*), and Paradise Fish (*Macropodus opercularis*, da Silva et al., 2021). These aggressive behaviors may be linked to chemical communication between individuals (Giaquinto and Volpato, 1997, Bayani et al., 2017, Gauy et al., 2019, da Silva et al., 2021) and the presence of social hierarchies. Some studies suggest that more dominant individuals release "dominance pheromones" that are used to tell the status, and therefore aggressiveness, of the sender (da Silva et al., 2021). As a result, social hierarchies are formed amongst individuals (da Silva et al., 2021). Social hierarchies in finfish are well studied and documented and show that larger, more dominant fish outcompete other individuals for space and resources (Knights, 1987, Ashley, 2007, Cubitt et al., 2008, Boscolo et al., 2011, Folkedal et al., 2017, Carbonara at al., 2019). Knights (1987) found that there was a size hierarchy in cultured American Eels (*Anguilla anguilla*). Larger individuals attacked more frequently, ate more, and had higher growth rates and survival (Knights, 1987). On the contrary, Boscolo et al. (2011) found that grouping Nile Tilapia (*Oreochromis niloticus*) into similar size classes resulted in more aggressive behavior and a breakdown of social hierarchy, which resulted in a decrease in animal welfare in the form of increased fin erosion (Boscolo et al., 2011). Social hierarchies also exist in Lumpfish aquaculture. Intraspecific territorialism occurs when members of the same species compete for space (Ferreira et al., 2001). Lumpfish are highly territorial with more dominant Lumpfish occupying the upper water column of their holding tank near the feeding stations, resulting in smaller, less dominant individuals on the bottom where food is less available. Because of this hierarchy, frequent size grading is needed to maximize growth of juvenile Lumpfish (Jonassen et al., 2018). The ill effects of social hierarchies and aggression in

finfish are well documented and are often associated with elevated stocking densities at their holding facilities.

The purpose of this study was to evaluate the effects of varying stocking densities on cultured Lumpfish and to determine if these effects are size specific. We hypothesized that (1) increasing stocking density would decrease growth, increase mortality, and increase fish aggression in juvenile Lumpfish, and that (2) an ontogenetic shift in juvenile Lumpfish behavior would occur with larger juveniles showing less aggressive behaviors than smaller juveniles.

Methods

To evaluate stocking densities of juvenile Lumpfish and to see if aggression is size specific, two trials using 2 g and 13 g Lumpfish, herein referred to as 'small' and 'large' juveniles, were conducted at the University of New Hampshire's Coastal Marine Laboratory (CML). Fish from the general population of cultured, juvenile Lumpfish reared at the CML were graded to the desired size and stocked into 3 L aquaria in a flow-through, ambient temperature and salinity seawater system, exposed to a 12-hour light: 12-hour dark photoperiod (Figure 3.1). Fish were stocked into each treatment using batch weights until the desired stocking density was reached. For each size-class of fish, four density treatments (40, 60, 70, and 90 g/L) were tested in triplicate over the course of eight weeks. The lowest density treatment, 40 g/L, was chosen as a baseline stocking density because this reportedly is the industry standard for commercial Lumpfish hatcheries (personal communication with Cooke Aquaculture, St George, New Brunswick, Canada). The highest density treatment, 90 g/L, was chosen to test the limits of Lumpfish growth, survival, and aggression when fish were subjected to extreme crowding

(Jonassen et al., 2018). Two intermediate densities (60 and 70 g/L) also were chosen to see if the industry standard of 40 g/L could be increased to grow juvenile Lumpfish more efficiently.

Protocols for evaluating small and large juveniles differed slightly (see Table 3.1). Small and large fish were fed at 5 % and 2 % body weight (based on the total weight of the tank), respectively, over the course of three feedings per day (morning, afternoon, evening). Growth and fish aggression were monitored biweekly; for small fish, a subsample of 20 fish was evaluated from each trial unit, whereas for large fish, all individuals were evaluated. Wet weights were recorded to the nearest 0.1, g. Fish aggression was tabulated using a scale from 0 to 5, with 0 representing a fish with an undamaged caudal fin, and 5 a fish with severe damage to the caudal peduncle (see Appendix A). After each biweekly sampling, fish were removed from each tank to maintain the original stocking densities. The total weight and stocking density were calculated for each experimental unit, and fish were removed and weighed so that the total weight and stocking density matched the original treatment values (Tables 3.2 and 3.3). Fish removed were placed into quarantine for two weeks and then placed back into the general population. Mortalities were recorded, weighed, scored for aggression, and removed daily from each trial. Excess feed in the trial tanks was removed via siphons as needed. Water temperature and salinity were measured daily (Figures 3.2 and 3.3).

Data Analysis:

All data were analyzed using Excel 2019 and JMP Pro 15. Overall percent growth was calculated by comparing the original fish mean weights to the final fish mean weights: ((Final Weight – Original Weight)/ Original Weight) x 100%). Mean growth rates were calculated by

taking the weight gain between each sampling period and dividing it by the number of days the fish were allowed to grow (14 days): (Final Weight – Original Weight)/14 days. The growth rate from each biweekly sampling period was averaged to get an overall mean growth rate for each treatment. Chi-squared tests were used to compare mean occurrence of fin nips between the treatments. One-way ANOVAs and Tukey's tests were used to compare the mean overall final percent growth, mean growth rates, and mean occurrence of fin nips between the treatments and between the trials.

Figure 3.1. The experimental flow-through seawater system used in both trials.

Table 3.1. The testing parameters used for each of the stocking density trials.

Parameter	**Small Juveniles**	**Large Juveniles**
Testing Period	2/10/21 – 4/7/21	6/2/20 – 7/28/20
Initial Fish Size ± 1 s.d. (g)	2.14 ± 0.61	12.87 ± 1.32
Number of Treatments	4	4
Body Weight/Feed Percentage (%)	5	2
Temperature Range (°C)	3-7	9-19
Salinity Range (ppt)	27-34	34-36
Initial Mean Number of Fish (40 g/L)	56	10
Initial Mean Number of Fish (60 g/L)	83	14
Initial Mean Number of Fish (70 g/L)	93	17
Initial Mean Number of Fish (90 g/L)	123	21
Initial Fish Age (days post hatch)	266	163
Growth Sampling	Subsample of 20 Fish	All Individuals Weighed
Stocking Density as fish/L (40 g/L)	19	3
Stocking Density as fish/L (60 g/L)	28	5
Stocking Density as fish/L (70 g/L)	31	6
Stocking Density as fish/L (90 g/L)	41	7

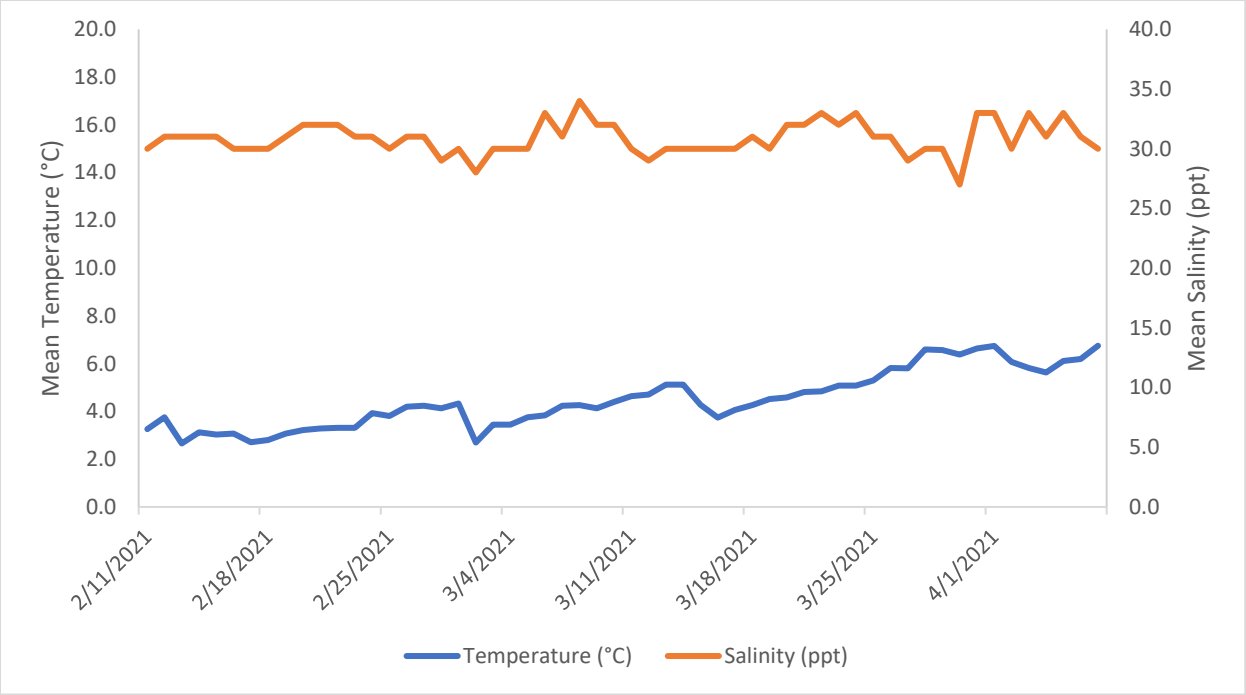

Figure 3.2. Mean daily temperature and salinity during the Small Juvenile Trial.

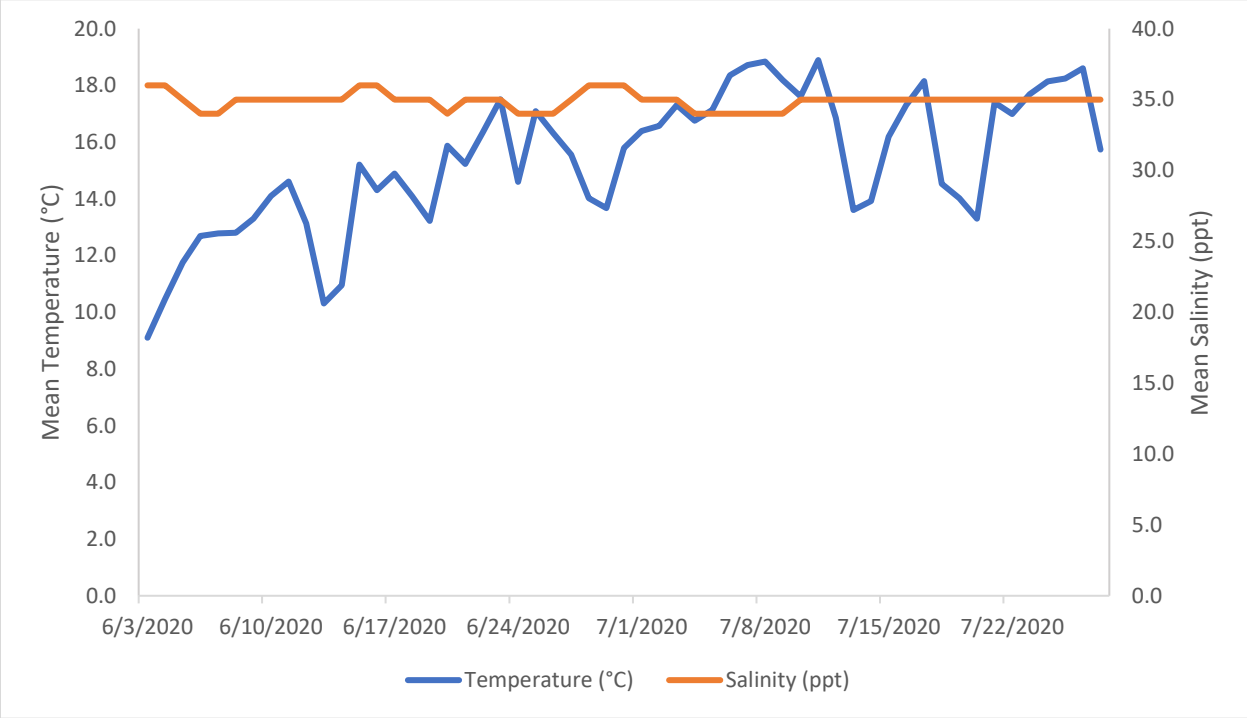

Figure 3.3. Mean daily temperature and salinity during the Large Juvenile Trial.

Results

Small Juveniles

Stocking densities tested did not affect small juvenile fish survival. Fish survival was 100% throughout the eight-week period (Table 3.6).

Lumpfish growth rates and final percent growth were affected by stocking density. Overall mean fish growth rates varied from 0.07 ± 0.04 g/day to 0.12 ± 0.06 g/day (Table 3.6, Figure 3.4). Overall mean percent growth varied from 169.69 ± 21.13 % to 306.51 ± 22.15 % (Table 3.4, Figure 3.5). For both growth metrics, the 40 g/L treatment resulted in significantly faster growth rates (0.12 ± 0.06 g/day) and significantly higher overall mean percent growth (306.51 ± 22.15 %) than juvenile Lumpfish in the higher density treatments. The 60 g/L treatment had significantly faster growth rates (0.09 ± 0.06 g/day, one-way ANOVA, $p < 0.0001$) and higher overall mean percent growth (245.51 ± 17.58 %) than the 70 and 90 g/L treatments (one-way ANOVA, $p = 0.0002$), which did not differ significantly from one another for both growth metrics (Table 3.6, Figures 3.4 and 3.5).

Stocking density did not impact the occurrence of fish aggression in the small juveniles ($X^2(3, N = 240) = 0.06$, $p = 0.9958$). Mean fin nipping occurrence ranged from a low of 0.83 ± 0.96 % in the 40 and 60 g/L treatments to a high of 3.75 ± 1.60 % in the 90 g/L treatment (Figure 3.6). Because density treatment did not affect fish aggression, the severity of fin nipping data were not analyzed. Only two out of the 240 fish sampled showed fin damage above a level 1 (see Appendix A) after eight weeks. One fish in the 40 g/L treatment and one in the 90 g/L treatment showed fin damage above a level 1.

Large Juveniles

Stocking densities tested also did not affect large juvenile fish survival. Fish survival was 100% throughout the eight-week period (Table 3.4).

Overall mean fish growth rates varied from 0.67 ± 0.15 g/day to 1.06 ± 0.27 g/day and was not affected by stocking density (one-way ANOVA, p = 0.0741, Table 3.4, Figure 3.7). Overall mean percent growth varied from 286.40 ± 30.15 % to 470.93 ± 54.88 % and was affected by stocking density. Fish in the 40 g/L treatment had significantly higher overall mean percent growth (470.93 ± 54.88 %) than fish in all other treatments. Also, fish in the 60 g/L treatment had significantly higher overall percent growth (376.71 ± 64.95 %) than the 90 g/L (286.40 ± 30.15 %, one-way ANOVA, p = 0.0348, Table 3.4, Figure 3.8).

The stocking densities tested did not impact the occurrence of fish aggression amongst the large juveniles ($X^2(3, N = 57) = 0.54, p = 0.9091$). Mean fin nipping occurrence ranged from a low of 1.39 ± 2.78 % in the 40 g/L treatment to a high of 21.40 ± 6.21 % in the 70 g/L treatment (Figure 3.9). Because treatment did not affect fish aggression, the severity of fin nipping data were not analyzed. Only one out of the 57 fish sampled showed fin damage above a level 1 (see Appendix A) after eight weeks. One fish in the 70 g/L treatment showed fin damage above a level one.

Small vs. Large Juveniles

Comparing the small versus large juveniles regarding the occurrence of fish aggression within each stocking density treatment revealed that the only significant difference was between the small and large juveniles in the 70 g/L treatment. Small juveniles in the 70 g/L treatment had

a significantly lower occurrence of fin damage (1.25 ± 1.60 %) than the large juveniles in the same treatment (21.40 ± 6.21 %, one-way ANOVA, p = 0.0008, Tables 3.4 and 3.6, Figure 3.10).

Small Juveniles

Table 3.2. The number of fish removed to maintain initial stocking density treatments at each sampling interval during the Small Juvenile Trial.

		Number of Fish Removed at Each Sampling Interval		
Stocking Density (g/L)	**Replicate**	**Week 2: 2/24/21**	**Week 4: 3/10/21**	**Week 6: 3/24/21**
40	1	11	14	11
40	2	7	18	9
40	3	13	18	8
60	1	13	20	14
60	2	10	27	12
60	3	8	28	10
70	1	11	32	13
70	2	0	35	8
70	3	6	35	10
90	1	1	46	19
90	2	14	35	19
90	3	8	33	23

Table 3.3. The results of the different testing parameters measured for the Small Juvenile Trial. Different superscript letters denote statistical differences between treatments in each column.

Stocking Density (g/L)	**Mean Percent Growth (± one standard deviation, %)**	**Mean Growth Rate (± one standard deviation, g/day)**	**Mean Occurrence of Fin Nipping (± one standard deviation, %)**	**Mean Severity of Fin Nips (± one standard deviation)**	**Final Percent Survival (%)**	**Final Mean Fish Weight (± one standard deviation, g)**
40	306.51 (± 22.15)[a]	0.12 (± 0.06)[a]	0.83 (± 0.96)	0.02 (± 0.02)	100.00	8.57 (± 0.48)
60	245.51 (± 17.58)[b]	0.09 (± 0.06)[b]	0.83 (± 0.96)	0.01 (± 0.02)	100.00	7.31 (± 0.36)
70	177.76 (± 25.22)[c]	0.07 (± 0.04)[c]	1.25 (± 1.60)	0.01 (± 0.02)	100.00	5.91 (± 0.28)
90	169.69 (± 21.13)[c]	0.07 (± 0.04)[c]	3.75 (± 1.60)	0.08 (± 0.05)	100.00	5.93 (± 0.28)

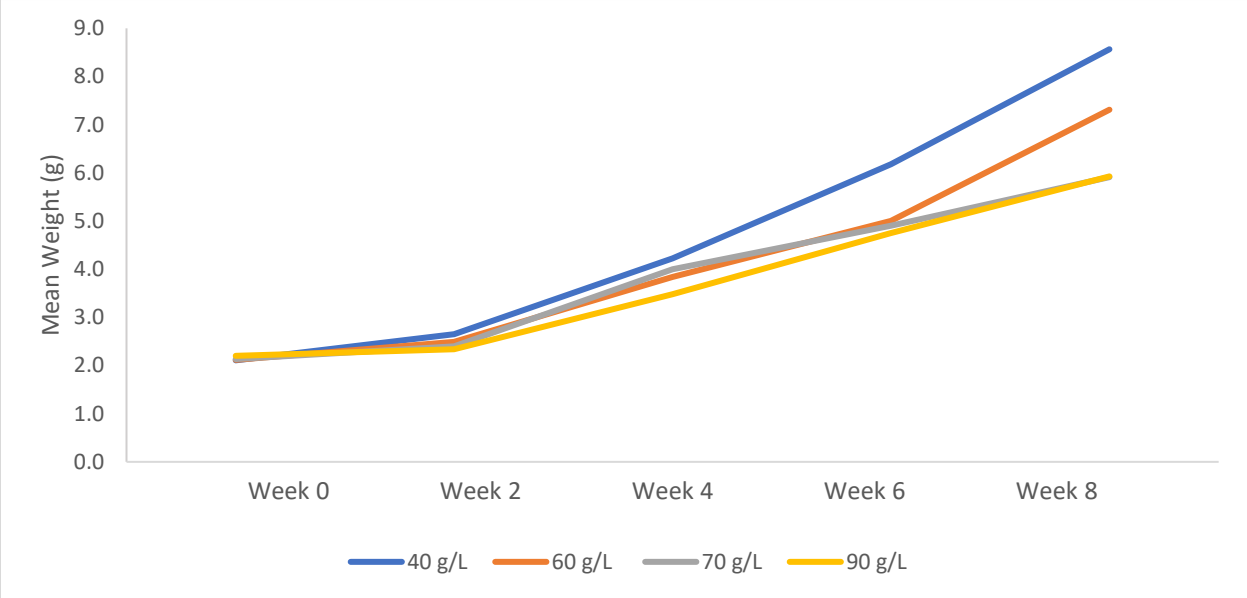

Figure 3.4. Mean weights of the small juvenile Lumpfish over time in each density treatment.

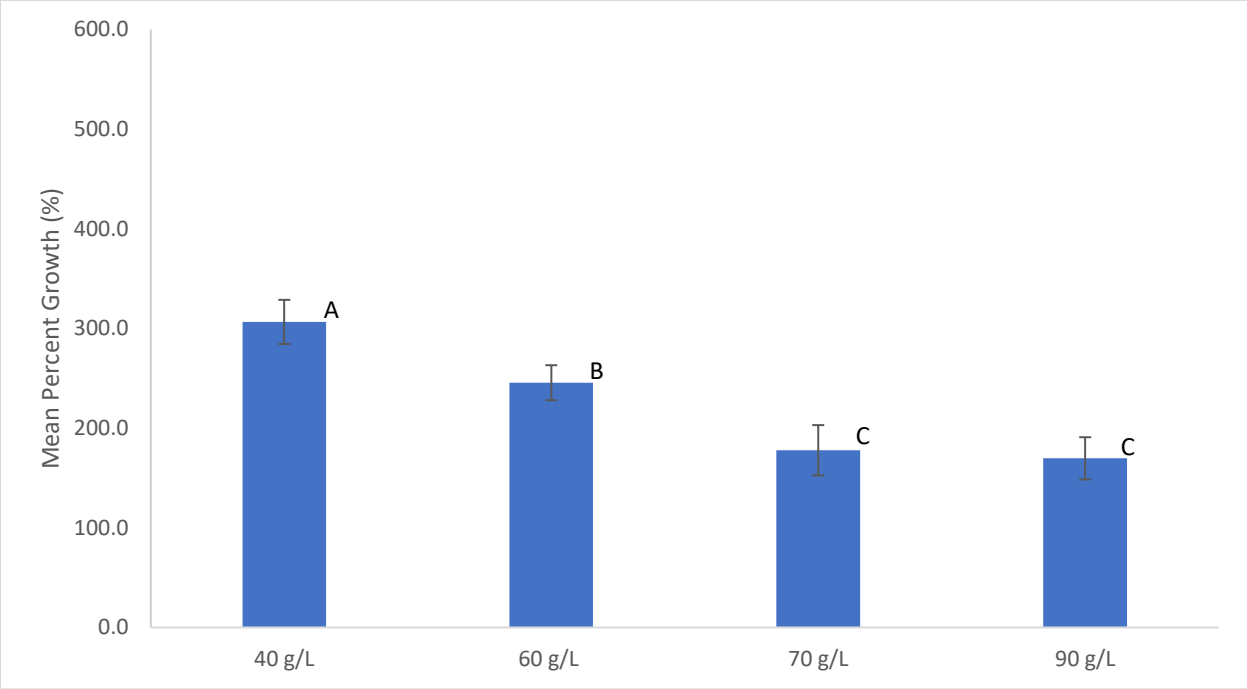

Figure 3.5. Overall mean percent growth (± one standard deviation) for each density treatment. Differing letters denote significant differences between treatments.

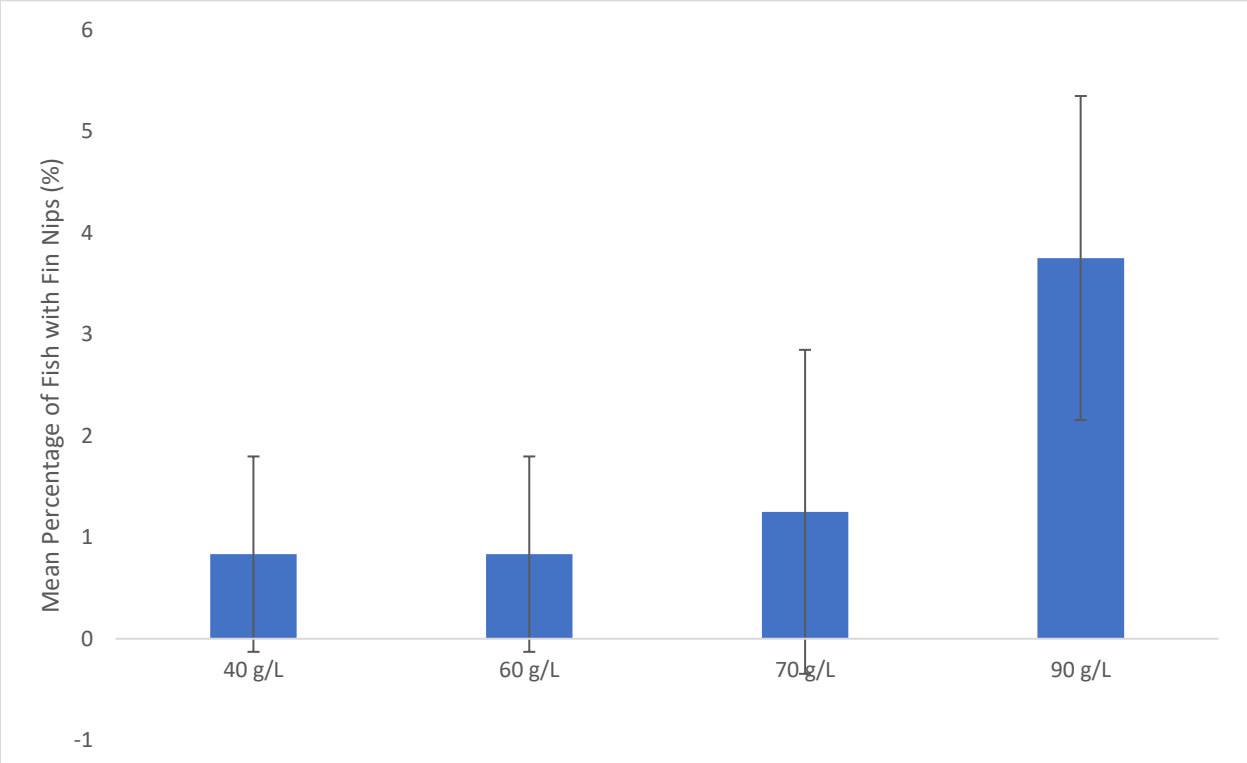

Figure 3.6. Mean occurrence (± one standard deviation) of fish aggression for each of the density treatments.

Large Juveniles

Table 3.4. The number of fish removed to maintain initial stocking density treatments at each sampling interval during the Large Juvenile Trial.

		Number of Fish Removed at Each Sampling Interval		
Stocking Density (g/L)	**Replicate**	**Week 2: 6/16/20**	**Week 4: 6/30/20**	**Week 6: 7/14/20**
40	1	5	3	1
40	2	4	2	1
40	3	4	2	0
60	1	5	3	1
60	2	5	3	1
60	3	6	4	1
70	1	5	4	1
70	2	6	4	2
70	3	5	4	2
90	1	6	5	2
90	2	7	5	3
90	3	6	5	3

Table 3.5. The results of the different testing parameters measured for the Large Juvenile Trial. Different superscript letters denote statistical differences between treatments in each column.

Stocking Density (g/L)	**Mean Percent Growth (± one standard deviation, %)**	**Mean Growth Rate (± one standard deviation, g/day)**	**Mean Occurrence of Fin Nipping (± one standard deviation, %)**	**Mean Severity of Fin Nips (± one standard deviation)**	**Final Percent Survival (%)**	**Final Mean Fish Weight (± one standard deviation, g)**
40	470.93 (± 54.88)[a]	1.06 (± 0.27)	1.39 (± 2.78)	0.01 (± 0.03)	100.00	72.07 (± 11.52)
60	376.71 (± 64.95)[b]	0.88 (± 0.24)	6.45 (± 7.46)	0.06 (± 0.07)	100.00	62.55 (± 4.53)
70	324.35 (± 89.66)[bc]	0.73 (± 0.14)	21.40 (± 6.21)	0.24 (± 0.08)	100.00	53.76 (± 13.61)
90	286.40 (± 30.15)[c]	0.67 (± 0.15)	13.73 (± 7.29)	0.14 (± 0.07)	100.00	50.24 (± 8.34)

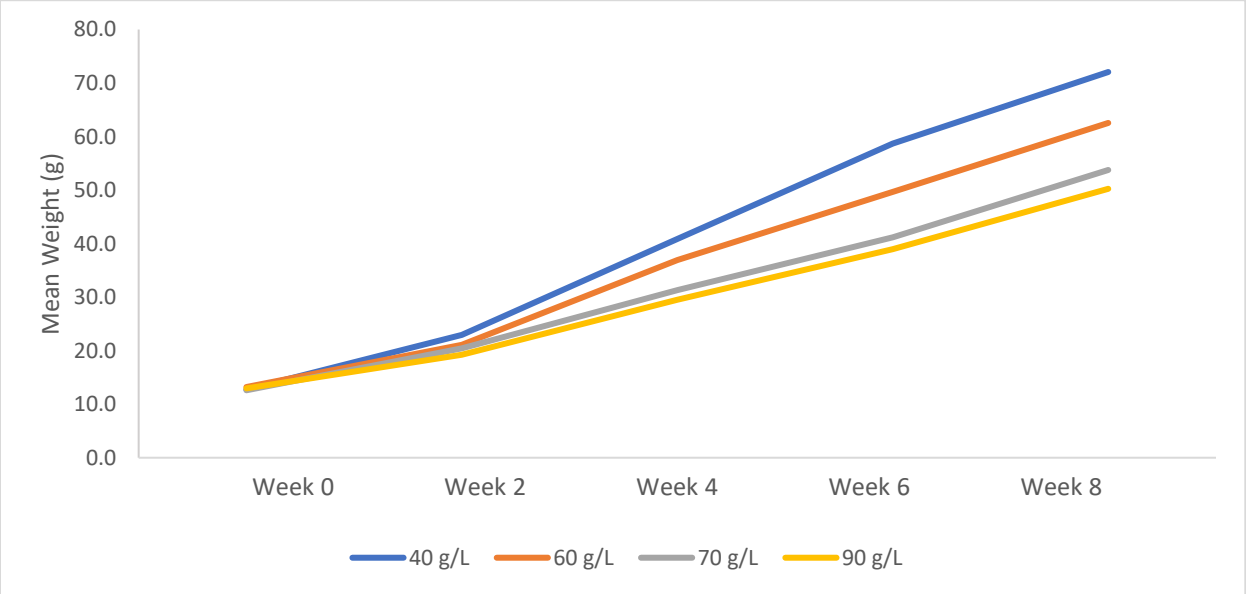

Figure 3.7. Mean weights of the large juvenile Lumpfish over time in each density treatment.

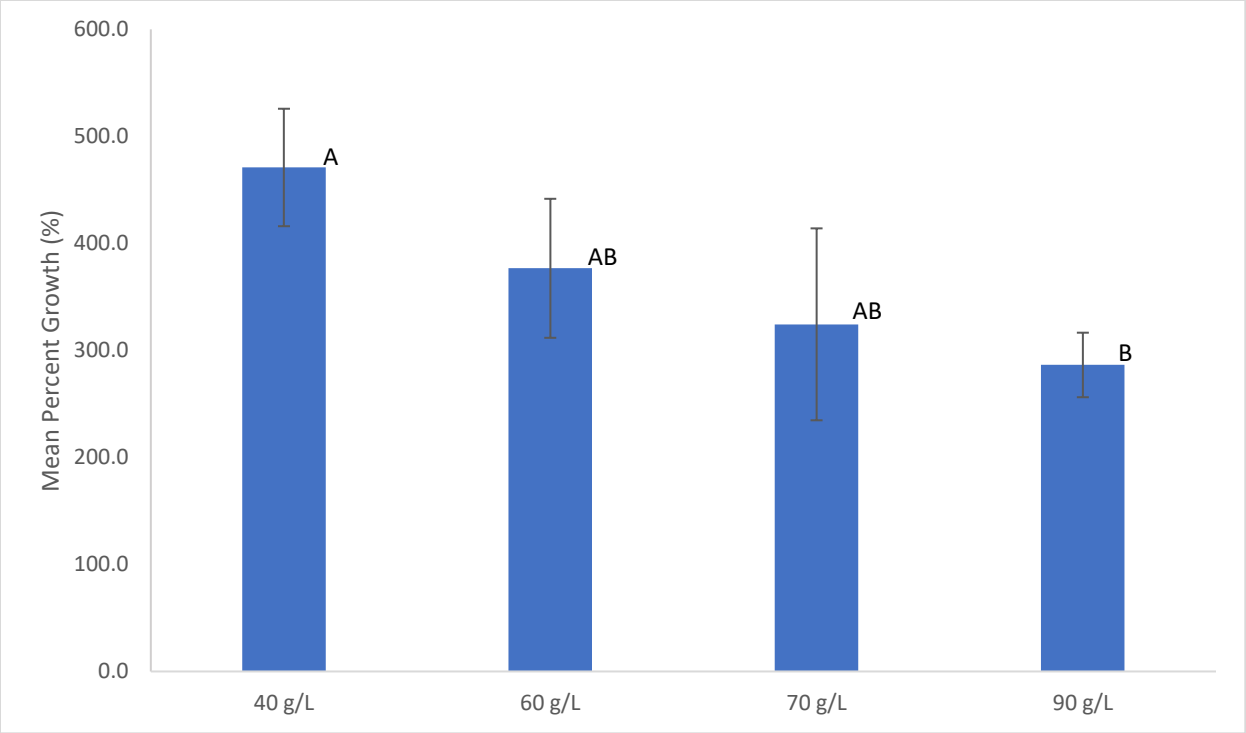

Figure 3.8. Overall mean percent growth (± one standard deviation) of large juvenile Lumpfish in each density treatment. Differing letters denote significant differences between treatments.

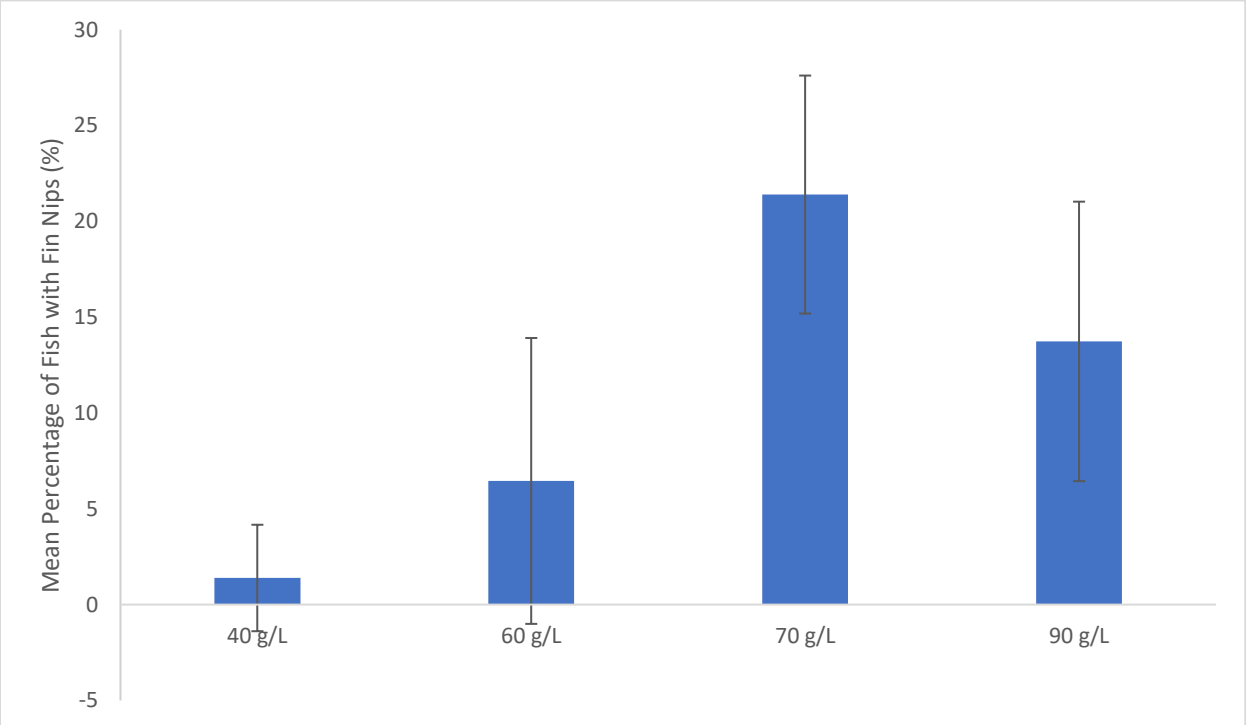

Figure 3.9. Mean occurrence (± one standard deviation) of large juvenile Lumpfish aggression in each density treatment.

Small vs. Large Juvenile Aggression

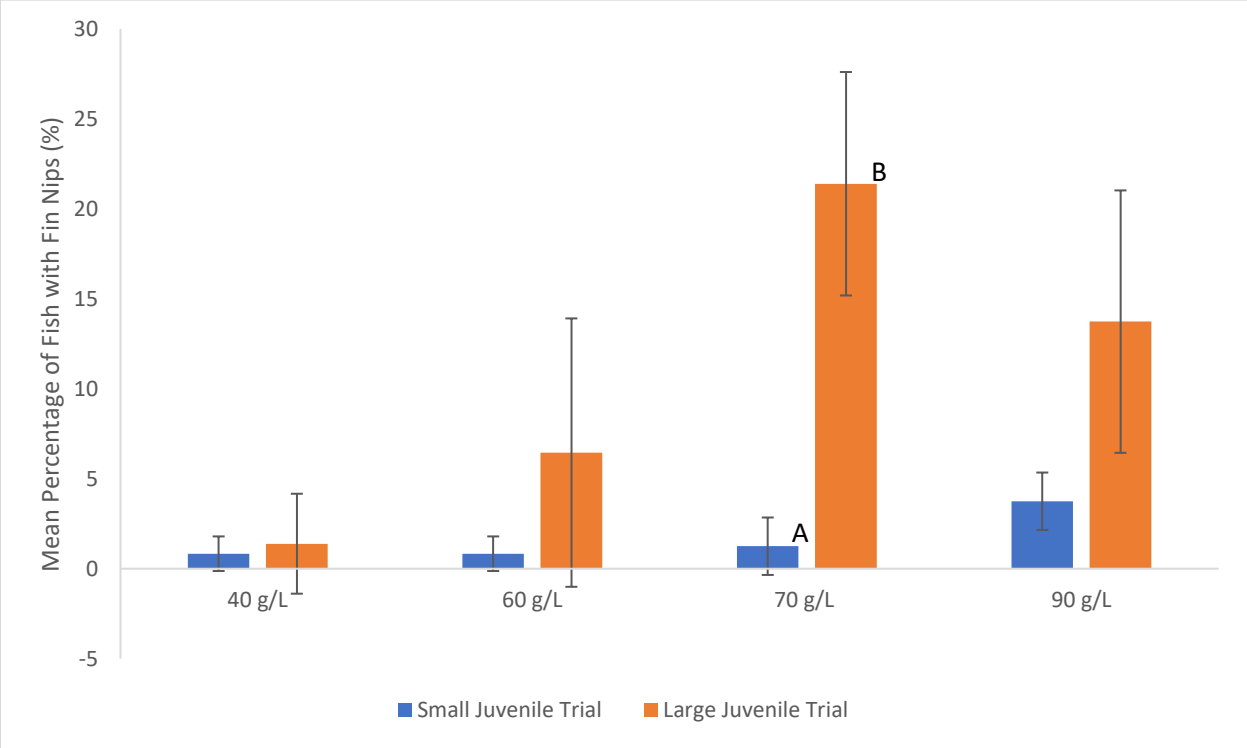

Figure 3.10. Mean occurrence (± one standard deviation) of small versus large juvenile Lumpfish aggression in each density treatment. Differing letters denote significant differences between treatments.

Discussion

There is well documented evidence of the negative effects of higher stocking densities on the growth, survival, and rate of aggression of intensively cultured finfish species (Huang and Chiu, 1997, Fairchild and Howell, 2001, North et al., 2006, Rowland et al., 2006, Enache et al., 2011, Miki et al., 2011, Liu et al., 2016, Arifin et al., 2019). Our hypothesis that increasing stocking density would decrease juvenile lumpfish growth, increase mortality, and increase fish aggression was partially supported based on the results of this study. While survival and fish aggression were unaffected by the different density treatments, there was an inverse relationship between growth and stocking density, with growth increasing as stocking density decreased.

The high survival seen in both trials is inconsistent with other finfish stocking density experiments. Rowland et al. (2006) performed a similar study using Australian freshwater Silver Perch (*Bidyanus bidyanus*), stocking fish at 12, 25, 50, 100, and 200 fish/L at 109.3 - 115.4 g. The researchers found that as stocking increased, survival decreased with the fish stocked in the 12 fish/L treatment showing significantly higher survival than the than fish in all other treatments. (Rowland et al., 2006). Stocking density had a significant effect on Tilapia fry survival with lower survival at higher densities (Huang and Chiu, 1997). The high survival rate seen in the juvenile Lumpfish is likely due to the hardiness of the species. From personal observations of raising Lumpfish at the UNH's CML, juveniles respond well to both handling stress and sudden changes in water quality. Other studies have confirmed the hardiness of this species when it comes to changes in water quality (namely dissolved oxygen and temperature, Nytro et al. 2014, Jorgensen at al. 2017). This resilience seems to apply to tank crowding as well.

In most cases, stocking density influenced the growth of both small and large juvenile Lumpfish. Growth rates were affected by stocking density for small juveniles but not large juveniles, with the 40 g/L treatment resulting in faster growth than all other treatments (Tables 3.4 and 3.6, Figures 3.4 and 3.7). For both trials however, the 40 g/L treatment had significantly higher overall mean percent growth than all other treatments, meaning that the fish in this treatment showed good growth while the other treatments showed slower growth (Tables 3.4 and 3.6, Figures 3.5 and 3.8). This suppression of growth at higher stocking densities is what was expected based on studies of other finfish species. Common Carp (*Cyprinus Carpio*) grown under two densities, 32 kg/L and 64 kg/L, had higher specific growth rate and mean biomass when reared at the lower density (Enache et al., 2011). The lower density also resulted in a lower feed conversion ratio, suggesting a higher growth efficiency (Enache et al., 2011). Survival of

larval Giant Gourami (*Osphronemus goramy*) was not impacted when grown under six densities (0.6, 1.2, 2.4, 4.8, 9.6, 19.2 individuals/L), but growth severely decreased with increasing stocking densities (Arifin et al., 2019). Long et al. (2019) raised juvenile Chinese Sturgeon (*Acipenser sinensis*) under a low (4.80 kg m^{-2}), medium (8.99 kg m^{-2}), and high (12.68 kg m^{-2}) stocking density. Fish in the high-density treatment had significantly suppressed growth, as well as significantly increased stress hormone levels (Long et al., 2019). In a similar study, Atlantic Salmon (*Salmo salar*) grown in a recirculating aquaculture system had significantly higher final weights and specific growth rates in the low stocking density (9.80 kg m^{-3}) than in the medium (19.62 kg m^{-3}) and high densities (28.79 kg m^{-3}, Liu et al., 2016). However, other studies found conflicting results. Stocking density did not affect the specific growth rates of adult Burbot (*Lota lota*) under cultured settings (Wocher et al., 2011). In a study performed by PaPoutsoglou et al. (1998), European Sea Bass (*Dicentrarchus labrax*) showed the highest specific growth rate and lowest feed conversion ratio in fish subject to the highest stocking density treatment. Other studies suggest that increasing stocking density benefits some finfish species. Brown et al. (1992) found that higher stocking densities resulted in higher mean weights, higher mean lengths, lower agonistic behavior, and more shoaling behavior in Arctic Charr (*Salvelinus alpinus*). Islam et al. (2006) had similar results when raising Sutchi Catfish (*Pangasius sutchi*). Higher densities resulted in higher growth and increased profit (Islam et al., 2006). Culture recommendations for fishes tend to be species-specific. Lumpfish growth is suppressed under more crowded conditions, but this scenario may be beneficial to hatcheries. Lumpfish show lower cleaning efficiency once they sexually mature, so the upper limit for keeping Lumpfish in salmon cages is around 400 - 500 g, before the fish reach sexual maturity (Jonassen et al., 2018). In conjunction with nearing sexual maturity, as these fish get larger, they change diet preferences

and become less effective delousers (Imsland et al., 2015a, Imsland et al., 2016b). Imsland et al. (2015a) found that smaller Lumpfish prefer natural food sources, like sea lice, while larger individuals forage on whatever is available to them in the net pen, including salmon feed. Juveniles are usually stocked into salmon cages at around 20 - 30 g in facilities in Norway. However, Lumpfish become the most effective delousers from 50 - 180 g (Herrmann et al., 2021). Based on the results of this study, Lumpfish hatcheries may be able to suppress or increase the growth of Lumpfish to better time Lumpfish production to the needs of the salmonid farms without affecting survival or animal welfare and to better coincide hatchery production with salmon farm needs. Using a 90 g/L density for growing Lumpfish is roughly double the industry standard (Treasurer, 2018a). Producing fish at this density may slow down growth but the number of individuals produced greatly increases. Hatcheries may be able to produce more juveniles for stocking into salmonid cages without influencing survival or animal condition, in the form of suction cup deformities, fin damage, and body damage (Rabadan et al., 2021).

For both small and large juveniles, stocking density did not impact fish aggression. Though not supported from these results, Lumpfish grown under high densities at CML show cannibalistic behavior, nipping at each other's caudal fins repeatedly. However, the rearing densities used at CML are not measured and, thus, are unknown and may be greater than 90 g/L at times. Because Lumpfish do not school and mostly adhere to tank surfaces with their suckers, surface area is more important than tank volume for rearing Lumpfish. Therefore, like flatfish (Merino et al., 2007) and other benthic fishes like Wolffishes (*Anarhichas spp*., Le François et al., 2021), Lumpfish hatcheries use the amount of surface area provided to the Lumpfish as a proxy for how dense to pack their tanks (personal observation, Jonassen et al., 2018). At the CML, Lumpfish were graded frequently into different size cohorts when cannibalism was

observed. Fish with damaged caudal fins were often found attached to the sides and bottom of the tanks, with undamaged fish attached to furniture close to the surface where food was easily accessible (personal observation). Lumpfish are territorial and establish social hierarchies, and it is likely that more dominant and aggressive fish inhabit areas of the tank where food is most accessible (Imsland et al., 2014b). Therefore, the lack of significance in Lumpfish aggression with increased densities are unexpected since this trend has been documented in other species. Social hierarchies are well established during the culture of Greater Amberjacks (*Seriola dumerili*), with most mortality occurring in small individuals cannibalized and picked on by bigger individuals (Miki et al., 2011). Fairchild and Howell (2001) cultured juvenile Winter Flounder (*Pseudopleuronectes americanus*), another demersal species, under 50, 100, 200, and 300 % stocking densities (ventral fish area to bottom tank area ratio). They found that there was no significant difference between the density treatments in terms of the degree of caudal fin damage the juvenile Winter Flounder incurred. However, fish size was inversely related to caudal fin damage, with smaller individuals showing more severe damage than large ones (Fairchild and Howell, 2001). North et al. (2006) found that fin erosion was correlated with increasing stocking density in cultured Rainbow Trout (*Oncorhynchus mykiss*). Though not significant, an interesting trend occurred in the occurrence of fin nipping amongst large juvenile Lumpfish in which the 70 g/L treatment had a higher percent occurrence of fish with fin damage (21.40 ± 6.21 %) than fish in the 90 g/L treatment (13.73 ± 7.29 %, Table 3.6, Figure 3.9). We expected more fish in the densest treatment to have fin damage, compared to fish in the lower density treatments, however, other studies have documented fish agression peaking at intermediate densities. Manley et al. (2014) stocked larval Spotted Seatrout (*Cynoscion nebulosus*) in 15, 30, and 60 fish/L treatments and found that aggressive behavior did not

increase further after 30 fish/L (Manley et al., 2014). African Catfish (*Clarias gariepinus*) stocked in 0.3, 0.6, and 1.2 fish cm^{-2} showed the signficantly highest mortality and rate of fish agression in the 0.6 fish cm^{-2} treatment (Kaiser et al., 1995). This agression peak at intermediate densities may be due to the fact that fish in higher densities have less room to swim and cannibalize their neighbors than fish in intermediate densities. Under intermediate densities, fish are able to target their conspecifics more readily, resulting in higher rates of fish agression or a possible plateau in aggressive behaviors. In some cultured species, it is suggested to raise fish under high densities because they disrupt social hiearchies and terrestorialism. Brown et al. (1992) found that Arctic Charr show less agression, higher growth, and more shoaling behavior when held under higher stocking densities. However, the Brown et al. (1992) and Manley et al. (2014) studies represent behaviors for schooling species, a behavior not seen in Lumpfish. Lumpfish operations may not have to be concerned as much about animal welfare regarding survival and fish agression when manipulating stocking desnities, however, stocking desnity impacts the growth of juvenile Lumpfish, which can be beneficial or detrimental to hatcheries and ongrow facilties based on their production goals.

The lack of significance in the fish aggression data does not support the second hypothesis that an ontogenetic shift occurs with smaller juvenile Lumpfish showing more aggressive behavior than larger ones. Aggressive behavior did occur, but neither trial showed levels that resulted in statistical significance. While we expected an ontogenetic shift to occur based on personal observations with aggression peaking when the juveniles reached 5-7 g, the results from these trials do not support such a claim. This size range was not specifically tested during either of these trials, but the juveniles in Trial 1 reached this size range during experimentation and yet, there was not an increase in aggressive behavior. Rousseau and Dufour

(2012) separate metamorphosis into two types: 1) first or larval metamorphosis in which dramatic changes in physiology and morphology are observed as fish transition from larvae to juveniles, and 2) secondary metamorphosis in which these changes are not as dramatic as seen with smoltification in salmonids. Metamorphosis is thought to be met with an increase of hormones and thyroid changes. These differing levels of hormones, specifically sex hormones, may be met with ontogenetic shifts in behavior that accompany the physiological and morphological changes seen in fish. Also, better mouth and eye development in fish as they age may be a cause of increased aggression (Rousseau and Dufour, 2012). Ontogenetic shifts in aggressive behaviors in which agression increases as juveniles age has been observed in Chum Salmon (*Oncorhynchus keta*) and damselfish species (*Parma spp.*, Ryer and Olla, 1991, Buckle and Booth, 2009). However, these are two species that exhibit schooling behavior, which is not the case for Lumpfish. Based on the sedentary nature of Lumpfish, it appears that the development of Lumpfish is not met with an onset of aggression. When comparing the occurrence of fish aggression between small and large juveniles within each density treatment, only fish in the 70 g/L treatment showed a significant difference. Small juveniles in the 70 g/L treatment had significantly less fin damage than their larger counterparts (Figure 3.10). While this is not definitive proof that an ontogenetic shift in aggression occurs in Lumpfish, it does indicate that larger Lumpfish had a higher occurrence of fin damage than smaller ones on average. This difference is likely due to the differences in temperature in which either trial took place. The Small Juvenile Trial took place at a temperature range of 3-7 °C, while the Large Juvenile Trial took place at a range of 9-19 °C. The Lumpfish in the Large Juvenile Trial likely had a higher metabolism due to the higher temperature range. Metabolic rate in finfishes often increases with temperature (Gilloly et al., 2001, Schurmann and Steffensen, 2005). Higher

metabolic rates are associated with higher activity rates (Gilloly et al., 2001), which is likely why the increase in aggression was seen in the large juveniles. There are several studies showing similar findings. Ros et al. (2006) found that aggression in Mozambique Tilapia (*Oreochromis mossambicus*) was correlated with increasing oxygen consumption. Castro et al. (2006) found a similar result in their study using Siamese Fighting Fish (*Betta splendens*). To better compare the two trials regarding the occurrence of fish aggression, these trials should have been performed at similar temperature ranges.

This experiment was not without its limitations and some improvements could be made for future experiments. The scale of the experiment was a major limitation. Due to limited laboratory space in the CML, fish were raised in 3 L aquaria which does not accurately simulate commercial aquaculture operations. Future experiments should consider raising juvenile Lumpfish in larger tanks, similar to the ones used in Lumpfish hatcheries. Another limitation was how fish agression was tabulated. While the fin nipping scale (see Appendix A) was a useful tool in estimating fin damage, more accurate methods could be implemented. Measuring the ratio of caudal fin size to total length of the Lumpfish to see how much was biten off could be used in conjucntion with the fin nipping scale to get more accurate estimations. Rabadan et al. (2021) utilized a more standardized fin damage scale that would be useful for future studies of this type. In this study, all rayed fins (dorsal, both pectoral, anal, and caudal) on juvnile Lumpfish were scored from 0-4, with 4 being the most severe damage. The researhers observed the “splitting” of the rays as one of the indicators of damage. A fin with a score of 0 had no damage to their fins. A fin with a score of 1 had damage of up to 25 % of the fin area. Fins with a scores of 2 had damage affected 25-50 % of the fin area. A fin with a score of 3 had damage of 50-75 % of the fin area. Fins with scored of 4 had damage of over 75 % of the fin area. The scores of all 5 fins

observed were culminated to get an overall score out of 20 for the fish observed (Rabadan et al., 2021) Also, observing other signs of agression between and damage to the juveniles should be considered. Lumpfish have large, bony tubercles along their sides which can be quite abrasive (Davenport and Thorsteinsson, 1990) and can cause damage to other individuals if juveniles are packed too densely into a system. Cataracts in Lumpfish have been observed under cultured settings (Garcia de Leaniz et al., 2021) and Lumpfish rubbing against each other may be one of the causes. Measuring these other physical damages may give a more accurate depiction on the effects of stocking density on Lumpfish, thus providing improvements to standard operating procedures. Mortality did not occur during this experiment, so the upper limit for stocking juvenile Lumpfish remains unknown. Increasing stocking densities to greater than 90 g/L may yield different results and should be examined to determine upper thresholds. More experiments testing combinations of other densities and fish sizes would be worthwhile and contribute to creating a matrix of input (stocking density) versus output (growth and production (i.e., cannibalism)) scenarios for facilities to utilize. Personal observations suggest that peak cannibalistic behavior may occur in Lumpfish around 5-7 g, however the results from these trials contradict this hunch. These observations need to be further verified experimentally to test their validaty. Also, comparing different sized juveniles using the same metrics as the one used in this study should be done at the same time of year. Trial 1 took place during the winter months, and Trial 2 during the summer months. Temperature can affect the growth and agression of fish (Baras and Jobling, 2002, Castro et al., 2006, Ros et al., 2006), so these trials should be performed at similar temperture ranges.

Conclusion

This study lays foundational knowledge for understanding the effects of rearing density on juvenile Lumpfish survival, growth, and aggression. Based on the results from these trials, increasing stocking desnity supresses growth but does not influence fish survival or agression. We propose manipulating stocking density to stunt or increase growth of Lumpfish may be a useful tool to match Lumpfish production to the seasonal needs of salmon farms. Lumpfish outgrow their usefulness as cleanerfish, so keeping them at the optimal cleaning size range for longer durations will make the removal of sea lice more efficient, saving farmers more money. Based on the growth rates from Trial 1, it would take 2 g Lumpfish held under 40 g/L roughly 192 days to reach 25 g, a size that Lumpfish are commonly stocked into salmonid cages. For fish held under 60 g/L it would take 256 days to reach this size, and for 70 and 90 g/L, 329 days. However, Trial 1 took place during the winter months, with ambient temperatures ranging from 3-6 °C. Therefore, these lower temperatures may have impacted growth rates. Using the growth rates from Trial 2, 2 g juveniles would reach 25 g more rapidly at each of the rearing densities; 40 g/L:22 days, 60 g/L:26 days, 70 g/L:32 days, and 90 g/L:34 days. Thus, temperature should be taken into account when projecting growth. Knowing the tradeoffs and effects between fish size and tank density and their outcome on fish growth could help to create a matrix including all these parameters. Creating this matrix and further adding to this data set will be key in deciding what stocking density a facility should use. Hatcheries may want to utilize a lower stocking density to promote faster growth. The results from these trials indicate that there is not an ontogenetic shift in aggressive behavior as Lumpfish develop. These results contradict personal observations in which cannibalistic behavior was assumed to be size related, with larval and small juveniles showing little to no fish aggression. Based on these trials, manipulation of

stocking density may not be at the expense of Lumpfish welfare, with no significant differences in the amount of fin damage observed between the density treatments. However, other stocking densities should be further examined, testing densities greater than 90 g/L to understand the upper limits for juvenile Lumpfish culture as Lumpfish are a hardy species and may be capable of being grown at much higher densities. The ability of Lumpfish to withstand a variety of growing conditions without negatively affecting their production increases their value as a cost-effective delousing tool for salmonid ocean farms. Being able to manipulate rearing densities based on the needs of salmonid farms make these fish a dynamic tool for a problem with great economic repercussions.

FINAL CONCLUSIONS

Standard operating protocols are not in place for juvenile Lumpfish culture with several knowledge gaps existing within the industry. The purpose of this thesis was to establish answers to these gaps by developing better practices regarding nutritional and rearing conditions. Six experiments took place during 2020-2021 to expand upon these protocols and practices. Two diet experiments occurred to understand the protein and lipid concentrations, as well as the effects of plant versus fish meal-based protein sources, suitable for juvenile Lumpfish feeds. Two experiments targeting tank design for juvenile fish occurred, one observing the effects of tank color on the culture of Lumpfish, and one manipulating tank bottoms to improve cleaning efficiency for Lumpfish hatcheries. Two stocking density experiments ensued to determine if an better rearing density existed for juvenile Lumpfish, and to observe if aggression in these fish was size related.

Dietary protein (50-55%) and lipid (10-20%) concentrations evaluated did not impact the growth, survival, or aggression of juvenile Lumpfish. However, compared to fish meal-based diets, fish fed a diet with primarily plant-based protein resulted in suppressed growth. Also, fish fed an experimental diet (55/15) had faster and greater growth than either of the commercial diets tested (Europa 55/15 and BioTrout 47/24). These differences were likely due to ingredient differences between the diets and reveal that commercial diets specifically tailored to Lumpfish can be improved. Hatcheries and grow-out facilities may be able to cut costs by decreasing protein and lipid concentrations in their feeds without impacting Lumpfish survival or aggression. While manipulating these concentrations may influence growth, this may be to the benefit of Lumpfish facilities. Lumpfish outgrow their usefulness as cleanerfish. Suppressing

growth of these fish can keep them at a more desirable size range for salmonid farms to increase cleaning efficiency. BioTrout is a salmonid feed and one that Lumpfish would likely encounter while deployed in sea cages. Feeding Lumpfish these plant-based protein salmonid diets out in the net pens can also keep these fish at the optimal size that maximizes cleaning efficiency. However, this may be at the expense of animal welfare for these salmonid feeds may lack nutrients or have excess of some nutrients for Lumpfish, resulting in the poor health and emaciation of these fish. Further studies exploring a wider range of protein concentrations, lipid concentrations, and protein sources should be performed to further develop the nutritional profile of Lumpfish. These studies would further add to the matrix of diet formulations that Lumpfish facilities can utilize to increase or decrease growth of these fish based on their financial restraints and the temporal needs of salmonid farms.

While tank color or tank bottom did not influence the growth, survival, or aggression of juvenile Lumpfish, different bottom types deterred Lumpfish adhesion and increased cleaning efficiency. Fewer Lumpfish occupied the bottom of rough bottomed tanks compared to smooth bottomed tanks. Using rough bottomed tanks may be at the expense of cleaning efficiency, however. While rough bottomed treatments did not take longer to clean than smooth ones, it seemed more laborious to clean the rough bottomed tanks and they appeared to not get as clean. Further, tanks with lighter colored bottoms resulted in less Lumpfish adhesion than dark colored ones. The false-bottom treatment seemed to be the best candidate for Lumpfish culture based on the low occurrence of Lumpfish adhesion and low cleaning times. However, facilities may not have the funds available to manipulate tanks in this way or purchase tanks with false bottoms. Therefore, facilities can utilize textured paints to cover the bottoms of their tanks or facilities can paint their tank bottoms lighter colors to discourage Lumpfish from sticking to them. All of these

strategies will increase cleaning efficiency and reduce cleaning gear-fish encounters during cleaning events, overall increasing profit of hatcheries by reducing cleaning labor and increasing biomass by creating healthier water conditions. Future studies should standardize cleaning and fish adhesion count methodologies. Also, tank bottom preference studies, using individual tanks with multiple bottom options, may reveal if a preference in bottom type exists for larval and juvenile Lumpfish.

Stocking density affected the growth of juvenile Lumpfish but not survival or fish aggression. As stocking density increased, growth decreased in both small and large juvenile fish. The lack of mortality and fish aggression seen in the juveniles further adds to the narrative that Lumpfish are a hardy species, capable of being grown in a variety of conditions. Hatcheries can manipulate rearing densities to suppress or increase growth in these fish based on the temporal needs of salmonid aquaculture operations. Raising fish at densities of 90 g/L or more will increase the number of individuals produced by a facility without impacting survival or aggression rates. Growth may be suppressed, but again, this may benefit Lumpfish facilities by keeping the Lumpfish at smaller, more cleaning efficient sizes for longer. Further stocking density studies testing the limits of these fish should be performed. This information would further help to develop a matrix of rearing density versus growth. A matrix of this type would allow facilities to choose stocking densities based on the timing of their operations.

Based on the results from these experiments it was discovered that Lumpfish are a hardy species, making them ideal for aquaculture. Hatcheries globally can manipulate the nutritional and growing conditions of these fish based on the timing needs of salmonid farms and the budgets and facility space of the Lumpfish facilities. Larger matrices covering all the parameters tested in these experiments could be created to see what financial and temporal goals facilities

need to meet. However, further research is required. Experiments further testing the limits of Lumpfish should be performed by using a wider range of topics, whether they be nutritional, density dependent, or observing more tank designs. Interactive effects should be explored as well. A combination of the variables tested may yield significant results that facilities can utilize to maximize (or possibly suppress) growth. Finally, these studies should be scaled up to validate the results in a more commercial-like setting.

Salmonids play a major role in aquaculture, a field that will only increase as wild fish populations continue to decrease and the human population continues to increase. Therefore, making salmonid aquaculture as sustainable as possible is vital. Lumpfish may be a hardy species, but this does not mean they are immune to stress. The culture of these fish is reliant on the collection of wild adults for broodstock, so developing protocols to best grow these fish for broodstocks is necessary, an area of research currently being focused by Lumpfish researchers. Also, with the use of chemotherapeutics decreasing across the world and an increasing consumption of marine protein, the use of other non-chemical delousers, including the use of Lumpfish as cleanerfish, is necessary. Global grow out production of salmonids for food fish is increasing, in part to satisfy the US market demand for Atlantic Salmon, the most imported finfish. Domestic production of salmonids is limited due to sea lice infestations, making the demand for Lumpfish high. Though with these high demands for Lumpfish, protocols for raising these fish vary from facility to facility. This thesis provides foundational information on how to improve the culture of Lumpfish for the sake of animal welfare, financial gain, and sustainability.

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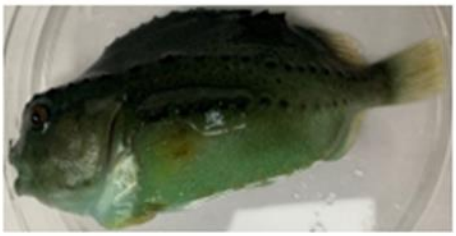
APPENDIX A

Fin Nipping Scale – Live Fish

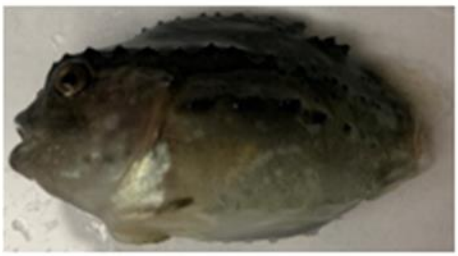


0: "Healthy Fish"
- Caudal fin is free of bites or wounds
- Caudal peduncle is intact
- Body is intact

1: "Minor Caudal Fin Damage"
- Caudal fin has a few nicks in it
- Caudal peduncle is intact
- Body is intact

2: "Moderate Caudal Fin Damage"
- Caudal fin is worn down
- Caudal peduncle is intact
- Body is intact

3: "Severe Caudal Fin Damage"
- Caudal fin is absent
- Caudal peduncle is intact
- Body is intact



4: "Minor Caudal Peduncle Damage"
- Caudal fin is absent
- Caudal peduncle is worn down
- Body is intact

5: "Severe Caudal Peduncle Damage"
- Caudal fin is absent
- Caudal peduncle is absent
- Body is beginning to be worn away

The fin nipping scale used to assess fish aggression in all trials. The scale ranges from 0-5, with 0 representing a healthy, undamaged fish, and 5 representing a fish with severe caudal fin and peduncle damage.

APPENDIX B

University *of* New Hampshire

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24-May-2018

Fairchild, Elizabeth A
Dept of Biological Sciences
Rudman Hall
Durham, NH 03824-2618

IACUC #: 180505
Project: Lumpfish Aquaculture and their Use as Cleaner Fish for Salmonids
Approval Date: 17-May-2018

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under pain or distress category D - *Animal use activities that involve accompanying pain or distress to the animals for which appropriate anesthetic, analgesic, tranquilizing drugs or other methods for relieving pain or distress are used.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,
Jessica A. Bolker, Ph.D.
Chair

cc: File