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THE EFFECTS OF HATCHERY STRESSORS ON GROWTH, AGGRESSION, AND CANNIBALISM OF JUVENILE LUMPFISH

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ABSTRACT

Due to the high demand for cleanerfish in salmonid ocean farming operations, increasing lumpfish hatchery production and rearing efficiency are of great importance. Juvenile lumpfish are cannibalistic which is controlled, to some extent, through frequent size grading of the fish, however, cannibalism still occurs. Understanding and mitigating factors that exacerbate aggressive behaviors in juvenile lumpfish, which can lead to cannibalism, would help achieve the goal of increasing juvenile production in the hatchery. We hypothesize that lumpfish cannibalism is linked to a specific ontogenetic period related to fish size rather than age and can be exacerbated by various stressors such as stocking density and photoperiod.

To test this hypothesis, we subjected two different size classes of juvenile lumpfish (5-g and 11-g) to varying stocking densities (40 g/L, 65 g/L, or 90 g/L) under different photoperiod regimes (ambient, constant low light, or constant bright light) for an 8-week duration in winter 2022. Fish growth, survival, and aggression were measured biweekly, and stocking densities adjusted to baseline levels biweekly by removing any necessary fish.

Light significantly affected the overall percent growth (two-way ANOVA, $P < 0.001$) of 5-g lumpfish. In the ambient light treatment, 5-g fish grew 31.5 % faster than fish in the low light treatment ($P < 0.01$) and 28.5 % faster than fish in the high light treatment ($P < 0.05$). Stocking density did not have a significant effect on overall percent growth (two-way ANOVA, $P = 0.06$). Unlike the 5-g lumpfish, neither stocking density (two-way ANOVA, $P = 0.331$) nor light (two-way ANOVA, $P = 0.105$) significantly affected the overall percent growth of the 11-g fish. In support of our hypothesis, we did find a significant interaction between stocking density and light on aggressive behavior in 5-g fish (Binomial Generalized Linear Mixed Model, $P < 0.05$). Fish in the low light 40 g/L treatment had significantly less fin nipping than those in the low light

65 g/L and high light 40 g/L treatments. Fin nipping occurrence amongst the 5-g fish ranged from 38.40 % ± SE 10.40 % to 60.00 % ± SE 5.00 %. Fin nipping amongst the 11-g fish was not significantly affected by either stocking density or light (Binomial Generalized Linear Mixed Model, $P > 0.5$). For 11-g fish, fin nipping occurrence ranged from 4.80 % ± SE 8.2 % to 36.7 % ± SE 32.1 %. A trend towards increased fin nipping in smaller fish was observed (~45 % fin nipping occurrence in 5-g fish vs ~ 20 % in 11-g fish), indicating that cannibalism may be even greater when fish are < 5-g but decreases as the fish grow.

Manipulating lighting and stocking density (up to 90 g/L) can be used to suppress or increase growth rates in small lumpfish, depending on a hatchery's desired outcome, without resulting in an increase in fish aggression. However, these variables are less effective tools for controlling growth in larger juveniles. Future studies should focus on how these variables affect lumpfish < 5-g as there are indications that aggression is most severe at this size class.

PROLOGUE

The Progression of Aquaculture

Roughly 17 % of animal protein consumed worldwide is caught from the sea (Costello et al., 2020), and the demand for protein is only projected to increase as populations grow (FAO, 2022). Capture fisheries production yields have plateaued since the 1990s. Coupled with global population growth and an increased demand for marine protein, it is clear that capture fisheries are unable to meet the demand for seafood alone (Costello et al., 2020; FAO, 2022). Inversely, aquaculture has grown steadily, contributing to the market demand for seafood. As of 2020, aquaculture production accounted for 56 % of the global production of seafood, surpassing wild capture fisheries for the first time (FAO, 2022). Aquaculture is continuously growing with production rates increasing roughly 3.3 % annually in the past few years (FAO, 2022). Of all the aquatic organisms produced, finfish comprise the largest proportion. Though the vast majority of finfish aquaculture is inland based, roughly eight million tonnes of finfish are produced from marine and coastal aquaculture, with Atlantic salmon (*Salmo salar*) comprising the largest proportion (FAO, 2022).

Salmonid Net Pen Farms and Sea Lice

Globally, in 2020, more than two million tonnes of farmed salmon were produced, comprising ~32 % of marine farmed finfish (FAO, 2022). As of 2018 in the U.S., Atlantic salmon was the leading farmed marine finfish species, with an estimated 36.4 million pounds grown worth $66.5 million (NOAA, 2019). Almost all salmon production takes place in marine net pens with only a few land-based operations. However, Atlantic salmon, as well as steelhead trout (*Oncorhynchus mykiss*), reared in ocean cages face a considerable and costly hurdle.

Salmonids are vulnerable to ectoparasitic copepods and, in particular, sea lice. Sea lice are naturally occurring but can be prevalent when concentrated host populations exist as is the case when fish are contained in ocean farms (Costello, 2006). The ectoparasitic copepod latches onto the salmonid, rasping at the flesh (Boxaspen, 2006) and exposing the fish to secondary infections that may lead to mortality (Johansen et al., 2011). In 2015 in Norway alone, the largest salmonid producer, it was estimated that sea lice cost farmers more than $477 million USD in profits (Iversen et al., 2017). Sea lice are not only detrimental to salmonid aquaculture but also to the surrounding environment, as these naturally occurring parasites latch on and feed on wild salmonids as well as their farmed counterparts (Costello, 2006). The two main species that impact salmonids are *Lepeophtheirus salmonis* and *Caligus spp.* (Johnson et al., 2004; Boxaspen, 2006). Sea lice infestations are a serious matter that when not treated adequately can result in the transmission of the infestation to neighboring pens, farms, or surrounding species. Sea lice abundance is affected by many factors, such as temperature, species fecundity, growth rates, and host immune responses (Costello, 2006).

To combat sea lice, various methods of delousing salmonids have been developed and used, including the use of various chemicals such as pesticides, hydrogen peroxide, and emamectin benzoate, as well as hot water and freshwater showers or fallow periods, in which a pen is not used. Additional methods to combat sea lice have developed over the years, such as physical barriers in the form of skirts attached to pens, genetic breeding programs, seeking to breed fish that are less susceptible to sea lice, and the development of medicated feeds (Liu & Bjelland, 2014; Grøntvedt et al., 2018; Imsland & Reynolds, 2022). However, many of these treatments come at a cost. The use of chemical combatants such as hydrogen peroxide and emamectin benzoate have led to sea lice developing resistance to such treatments (Aaen et al.,

2015) as well as being harmful to the environment (Haya et al., 2005). As a result, these chemicals have been banned in most countries, including the U.S. In addition to the loss of efficacy of medical treatments (Lees et al., 2008; Jones et al., 2013), the use of thermal and freshwater delousing strategies is stressful to the fish and can lead to lower fish survival and growth, as well as the lice developing resistance to these mechanical treatments (Robinson, 2019). Physical barriers such as skirts can be extremely costly and pens must be kept in deep water to accommodate the skirt (Grøntvedt et al., 2018; Stien et al., 2018). Genetic breeding programs and medicated feed strategies also can be extremely costly as well as can take years and multiple generations to develop. Another relatively new method of delousing salmonids, currently used in Europe and Atlantic Canada, that may be more profitable and environmentally friendly, is the use of cleanerfish. Cleanerfish are fish that perform services for other fish such as the removal of ectoparasites and dead tissue (Feder, 1966; Losey et al., 1999). Starting in the 1990s, wrasse, including ballan wrasse (*Labrus bergylta*) and goldsinny wrasse (*Ctenolabrus rupestris*), were used as the primary fishes to delouse salmonids, such as Atlantic salmon and steelhead trout, in net pens (Bjordal, 1991). However, in waters colder than 6 °C, wrasse were not effective delousers (Sayer & Reader, 1996), resulting in a decrease in wrasse being used as cleanerfish. As such, research into the use of lumpfish (*Cyclopterus lumpus*), a colder water species, as cleanerfish began.

Presently in the salmonid aquaculture industry, lumpfish are the most commonly used cleanerfish (Powell et al., 2018a). Since the emergence of promising data showcasing the lumpfish's ability to effectively delouse salmon in sea pens (Imsland et al., 2014a,b,c; Imsland et al., 2015a,b; Imsland et al., 2018), the demand for lumpfish has risen exponentially (Powell et al., 2018a), creating a substantial lumpfish market. In 2020 alone, roughly 34 million lumpfish were farmed in Norway, the largest producer of lumpfish (Directorate of Fisheries, 2021). New research also has highlighted the cleaning efficiency of lumpfish in addition to ways to optimize their cleaning through size and personality selection/behavior (Imsland et al., 2019; Whittaker et al., 2021), breeding (Imsland et al., 2021; Boissonnot et al., 2022), and deployment time (Engebretsen et al., 2023). The use of lumpfish is a "green" and attractive alternative to other sea lice mitigation methods as lumpfish implementation does not reduce survival or growth of the salmonids reared in the pens (Imsland et al., 2014a,b,c; Imsland et al., 2015a,b; Imsland et al., 2018; Powell et al., 2018a). Additionally, lumpfish can be more cost effective compared to previously used mitigation efforts as they do not cause harm to the salmonids, on average are cheaper than wrasse, and will continue to delouse during the colder months unlike wrasse (Powell et al., 2018a).

Lumpfish Ecology

Lumpfish belong to the Order Scorpaeniformes and Family Cyclopteridae. Lumpfish, also referred to as lumpsuckers, can be easily identified by their gray to greenish coloration, lack of scales, presence of bony tubercules, and as their name implies, a suction type disk located on the ventral side (Davenport, 1985; Davenport & Thorsteinsson, 1990). This disk is formed from a set of modified pelvic fins that allow lumpfish to adhere to various substates (Cox & Anderson, 1922).

Lumpfish are found both in the eastern and western sides of the Atlantic Ocean, from Greenland to France on its eastern side as well as from Greenland to New Jersey on its western side. Lumpfish are semi-pelagic fish (0-80 m but have been recorded at depths up to 380 m), that as adults, tend to live offshore, preferring cooler (0-6 °C) waters; however, during spawning season, adults will migrate to the coastlines (Goulet et al., 1986; Collette & Klein-MacPhee, 2002; Kennedy et al., 2016). Adults primarily feed upon a wide range of organisms including small crustaceans, ctenophores, polychaetes, seagrass, insects, small fish, and fish eggs (Davenport, 1985; Davenport & Rees, 1993) and live approximately 10 to 15 years. In the wild, males reach maturity in two to three years, while females take three to four years to reach maturity (Albert et al., 2002; Hedeholm et al., 2014). There is evidence of predation of adult lumpfish by sharks, seals, and sperm whales in deeper waters, and in shallow waters predation of male lumpfish by gulls, eagles, and otters (Thorsteinsson, 1983).

Adults are sexually dimorphic, with males tending to be smaller than females. The sexual dimorphism of lumpfish becomes even more distinct during the spawning season. Males become bright pink to red in coloration, contrasted to the typical blue-green of the females. Spawning occurs generally in early spring. Females are batch spawners and lay two to three egg masses over the course of 8-14 days (Davenport, 1985), with a mean realized fecundity of 32,526 eggs per egg mass (Powell et al., 2018b). After spawning, females migrate back to deeper waters, while males remain inshore guarding the egg masses for 6-10 weeks until hatching (Davenport, 1985). Males can guard multiple egg masses, and by creating funnel-like depressions in the eggs, assist in gaseous exchange and waste removal from the eggs (Powell et al., 2018b). Development time for lumpfish eggs is highly dependent on temperature and, to a lesser degree, salinity, accounting for the variability of 6-10 weeks as increases in temperature lead to more rapid egg development (Kjørsvik et al., 1984; Davenport, 1985).

Larval and juvenile lumpfish inhabit the upper 0.5 m of the water column and associate highly with macroalgae (Daborn & Gregory, 1983; Moring, 1989). The diet of larval and juvenile lumpfish is primarily comprised of surface plankton and various invertebrates that also associate with this macroalgae (Daborn & Gregory, 1983). Passively feeding and reassociating with macroalgae rather than actively swimming, lumpfish are able to divert energy into somatic growth, growing rapidly in their early life history stages (Killen et al., 2007).

Lumpfish Aquaculture

Lumpfish slated for use as cleanerfish in the salmonid aquaculture industry are reared in hatcheries until roughly 25 g, upon which they are implemented into salmonid pens. Once female lumpfish are ready to spawn, eggs can be gently extracted by massaging the abdomen of the female (Wittwer & Treasurer, 2018; Fairchild et al., 2021). Milt is obtained by euthanizing the male lumpfish and extracting and macerating the testes. The milt then is passed through a sieve to filter out any testicular tissue (Wittwer & Treasurer, 2018; Fairchild et al., 2021). Upon obtaining the gametes, either the dry or wet method can be used (Fairchild et al., 2021). The dry method involves combining the gametes without seawater first, then adding seawater. The wet method combines the gametes while in seawater. Once fertilized, the eggs clump together.

Once fertilized eggs are obtained, they are placed into incubators. While incubator designs can vary, the most common set-up involves the upwelling of water through the incubator with an outlet located at the top (Jonassen et al., 2018; Fairchild et al., 2021). Upwelling acts to aid in gaseous exchange and the removal of wastes (Goulet et al., 1986). Eggs are incubated for roughly 280-300 degree days (Jonassen et al., 2018). During this time, eggs are inspected and disinfected as needed depending on the presence or absence of fungus (Fairchild et al., 2021). In Norway, eggs are disinfected with a buffodine solution bath (Treasurer, 2018).

Upon hatching, lumpfish larvae have a functional mouth, eyes, and well-developed digestive system (Brown, 1986; Brown et al., 1997). This well-developed digestive system allows for hatcheries to begin feeding the larvae a microparticulate diet, avoiding having to culture live feed, although some hatcheries do provide enriched *Artemia* as a first feed (Fairchild et al., 2021). Just as their wild counterparts participate in passive feeding strategies, lumpfish reared in hatcheries will associate highly with any smooth surface including tank sides and bottom. “Furniture” suspended in the tank, such as PVC panels, increases tank surface area and allows for greater fish density (Fairchild et al., 2021). Temperatures for growing juvenile lumpfish range from 4 ºC-16 ºC. When reared in this optimal temperature range, juvenile lumpfish are potentially large enough (25 g) for implementation into salmonid farms within six months of hatching (Nytrø et al., 2014).

It is during this young juvenile rearing period in the hatchery that cannibalism, the act of eating all or part of an individual of the same species irrespective to its development stage (Smith & Reay, 1991), occurs. From observations during the 2019 and 2020 lumpfish rearing seasons at the University of New Hampshire (UNH) Coastal Marine Laboratory (CML), this aggressive period seems to be a finite phase occurring when the fish are roughly 5-g. Size grading, the act of sorting and separating the fish by size into homogenous populations, is a standard protocol that hatcheries use to minimize cannibalism; however, despite this frequent and labor-intensive process, cannibalism in lumpfish persists and poses one of the largest causes of mortality in the hatchery. We question whether confounding stressors, such as density, light, and size disparity, that can contribute to cannibalism in teleosts (reviewed in Pereira et al. 2017), may affect lumpfish aggression and, if understood better, could be controlled to minimize lumpfish loss in the hatchery.

INTRODUCTION

Finfish aquaculture currently comprises around 66 % of the world's aquaculture production (FAO, 2022). Atlantic Salmon (*Salmo salar*) comprise roughly a third of all marine cultured fish, the largest percentage of any other marine species produced (FAO, 2022). This booming aquaculture sector, however, faces one considerable and costly hurdle. Atlantic salmon, as well as steelhead trout (*Oncorhynchus mykiss*), reared in ocean cages are vulnerable to ectoparasitic copepods and, in particular, to sea lice. This naturally occurring pest feeds upon salmonids, rasping at their flesh, and leaving the fish open to secondary infections (Boxaspen, 2006; Johansen et al., 2011). In order to manage sea lice infestations, cleanerfish are one tool used to delouse salmonids reared in net pens. The most commonly used cleanerfish in the salmonid aquaculture industry are lumpfish (*Cyclopterus lumpus*) (Powell et al., 2018a). In order to meet the demand for lumpfish in salmonid ocean farms, lumpfish aquaculture production has risen exponentially (Powell et al., 2018a). As of 2021 in Norway, the largest producer of lumpfish, roughly 27 million lumpfish were sold to Atlantic salmon farms, compared to the ~3 million fish sold in 2015 (Directorate of Fisheries, 2022).

Though lumpfish are fast growing and relatively easy to culture, lumpfish aquaculture faces a considerable hurdle in that juvenile lumpfish are cannibalistic (Nytrø et al., 2014; Powell et al., 2018a). Cannibalism is one of the largest sources of mortality in the hatchery. Lumpfish exhibit Type I cannibalism, in which the individual is not fully consumed (as opposed to Type II in which the individual is completely consumed) (Hecht & Appelbaum, 1988; Powell et al., 2018b), and begins with fin nipping. Aggression in juvenile lumpfish also has been observed, as they tend to be highly territorial, and in some instances, hierarchies can be established, forcing smaller juveniles to the bottom of the tank (Jonassen et al., 2018). While cannibalism does occur in wild lumpfish populations, it does not occur on the same scale as in hatchery production (Ingólfsson & Kristjánsson, 2002). This naturally occurring behavior is likely amplified in aquaculture. In intensive rearing, there are heightened density-dependent interactions leading to cannibalistic behavior that can be exacerbated by several factors. These factors may include food availability, shelter availability, and light intensity (Duk et al., 2017; Lopes et al., 2018; Hans et al., 2020). For the purpose of this study, we focus on stocking density and light intensity.

Whether for land- or sea-based systems, balancing stocking density to maximize both space efficiency and production, yet minimize negative density-dependent effects that impact fish welfare, is a challenge. High stocking densities are known to affect metabolic processes. Fish reared in crowed environments often exhibit lower growth rates (Montero et al., 1999). Conversely, lower stocking densities can lead to increased growth rates, as fish have more room to grow (Ntanzi, 2014). Behavioral patterns also can be influenced by stocking density leading to the formation of hierarchies, aggression, and cannibalism (Bagley et al., 1994; Ellis et al., 2002). Even when the amount of feed is not a limiting factor, high stocking densities, as mentioned before, facilitate higher chances of individual interactions, which may promote cannibalism (Baras et al., 2000) as cannibalism can be a density dependent population regulator (Polis, 1981). For example, in a study conducted on the effects of stocking density on Asian seabass (*Lates calcarifer*), stocking density had a significant effect on cannibalism with the number of aggressive interactions increasing with increasing stocking density (Khan et al., 2021). Additionally, high stocking densities can facilitate higher chances of individual interactions, increasing the likelihood of disease transmission (Ellis et al., 2002).

Lighting regime, including intensity and duration, is another factor manipulated in aquaculture facilities that individually or compounded with other stressors, affects fish production. Overall, the effects of light on the early life history stages of fish are variable and are species and age specific (Zhukinskij, 1986; Schreck et al., 1990), but some commonalities occur. Light plays a key role in various fishes' biological processes including, but not limited to growth rate, body pigmentation, and hormone secretion (Lopes et al., 2018). Varying photoperiods and light intensities have been linked to changes in growth rates (Schreck et al., 1990). Light also influences activity levels in fish and ultimately can impact their feeding behavior (Schreck et al., 1990), further affecting individual growth rates. Light also influences body pigmentation in fishes. Light intensity can affect pigmentation in the eyes of larval and juvenile fish (Bolla & Holmefjord, 1988; Boeuf & Le Bail, 1999) as well as overall body pigmentation. Light levels also affect the secretion of the hormone melatonin (Ekström & Meissl, 1997; Falcon et al., 2010). In the wild, melatonin production is tied to photoperiod and the animal's circadian rhythm, fluctuating as the seasons change to reflect the changes in light/dark patterns (Falcón et al., 2007). Melatonin increases as light levels decrease (Ekström & Meissl, 1997; Falcon et al., 2010), however, many aquaculture facilities have constant lighting (24-hrs Light: 0-hrs Dark) in order to increase fish growth rates and decrease time to market. This leads to a reduction in melatonin which can result in increased aggression in fish (Munro, 1986), and may ultimately lead to an increase in cannibalism. Therefore, lower light intensities may aid in mitigating aggression and cannibalism.

There are few published works detailing standard operating protocols for lumpfish hatcheries, with some information published in *Cleaner fish biology and aquaculture applications* (Treasurer, 2018) and *Lumpfish Hatchery Handbook* (Fairchild et al., 2021). What we do know is that lumpfish are visual feeders; and as such, most intensive indoor fish production facilities keep lights on 24-hrs/day to promote fast fish growth and shorter time to harvest for salmonid pen implementation (~25 g). However, lumpfish tend to grow more quickly compared to other cold-water fishes as their suction cup allows for passive feeding and in turn the allocation of energy stores to growth (Treasurer, 2018). Large (>150 g) lumpfish often do not consume sea lice and can outgrow their usefulness as cleanerfish (Imsland et al., 2016). As such, some lumpfish hatcheries are interested in retarding lumpfish growth rates to extend the period in which lumpfish act as cleanerfish and to time lumpfish size more closely with salmonid farm needs. Limiting light in the hatchery may be beneficial, both to reduce cannibalism and to slow fish growth (e.g., at Dalhousie University, lighting regime is kept at a low intensity 24-hr/day, yet lumpfish cannibalism still occurs (G. McBriarty, pers. comm.)).

The most efficient juvenile stocking density for fish output is not known for commercial lumpfish production, though 10 to 43 g/L is reportedly what some hatcheries use (Treasurer, 2018). Preliminary studies at UNH have shown no negative impacts on survival of growing out 13-g lumpfish in densities as high as 90 g/L, though growth was shown to be negatively impacted by increased densities (Spada, 2021).

Due to the high demand for cleanerfish in the salmonid aquaculture industry, it is important to increase juvenile lumpfish rearing efficiency in the hatchery by decreasing mortality due to aggressive behaviors. To our knowledge, there is no published research focusing on cannibalism in cultured juvenile lumpfish nor on mitigation efforts. Therefore, the aim of this study was to evaluate the effects of different light regimes and stocking densities on the growth, survival, and aggression of juvenile lumpfish. Additionally, we hypothesized that this cannibalistic period is linked to a specific ontogenetic period related to fish size rather than age, thus two different juvenile lumpfish sizes were evaluated.

METHODS AND MATERIALS

Fish Culture

Juvenile lumpfish were sourced from the 2021 cohort cultured and reared at the University of New Hampshire Coastal Marine Laboratory (CML). Fertilized lumpfish eggs for the 2021 cohort were obtained from Memorial University in Newfoundland, Canada. Upon receipt, egg masses were placed in incubation chambers that were suspended over the larval rearing tanks. Incubation chambers were outfitted to have constant upwelling to aid in gaseous exchange and waste removal. Eggs were monitored for infections and developmental stage throughout incubation. Eggs began to hatch after approximately 252 degree days upon which larvae swam into the larval tanks.

Once hatched, fish were fed a diet of *Artemia* (premium grade, Brine Shrimp Direct) for 13 days enriched with Skretting Ori-green for 24 hours. Upon harvest, *Artemia* were placed in cold storage in order to slow down their metabolic rate and consumption of the enrichment. Newly hatched fish were fed 6 L of *Artemia* three times daily (8:00 am, 1:00 pm, and 6:00 pm) with *Artemia* concentrations ranging from approximately 300 to 1000 *Artemia* per mL. At 13 days post hatch (dph), fish were co-fed 4 g of 0.2 mm Skretting Gemma Wean microparticulate diet three times daily (8:00 am, 10:00 am, and 12:00 pm) in addition to the enriched *Artemia*. At 17 dph, weaning began and larvae were then fed the microparticulate diet every hour in addition to the *Artemia* three times daily until 25 dph. From 26 to 30 dph, *Artemia* feedings were reduced to twice daily (1:00 pm and 6:00 pm) and then again at 31 dph to once daily (6:00 pm). Fish were fully transitioned onto the microparticulate diet at 36 dph and were fed eight times per day beginning at 8 am and every hour until 6 pm. All fish were graded with an adjustable bar grader to the desired initial fish sizes prior to stocking out the experiment. From the 2021 cohort, approximately 130 dph 5-g and 11-g fish were used (Table 1).

Table 1. Total number of juvenile lumpfish used in each lighting treatment per fish size. Due to lack of specimens, ambient trials on 11-g fish were not conducted. Ambient lighting 12-h light: 12-h dark = average lux 113.63 (±14.75), Low constant = average lux 21.63 (±5.07), High constant = 302.25 (±14.39).

	Light Intensity Treatment		
Fish Size	Ambient	Low Constant	High Intensity Constant
5-g	518	437	472
11-g	N/A	170	147

Experimental Design and Set Up

To evaluate the effects of fish size, stocking density, and light intensity, three separate flow-through, ambient temperature and salinity filtered seawater systems were used at the CML, each comprising an individual light intensity treatment (ambient photoperiod, low light intensity (simulating crepuscular periods), or high light intensity (constant 24 h-light) (Figure 1). The low and high light intensity seawater systems were enclosed by PVC frames wrapped with panda film to block out ambient laboratory lighting. The low light enclosure was outfitted with dimmable, 5-watt outdoor string lights with an average lux of 21.63 (±5.07), while the high intensity enclosure was outfitted with four clip-on spotlight lamps equipped with LED 100 watt lightbulbs with an average lux output of 302.25 (±14.39). The ambient photoperiod seawater system was not enclosed and was exposed to overhead laboratory lighting. Laboratory lighting was programmed to come on at 5:00 am and shut off at 7:00 pm to simulate a natural lighting regime. Average ambient lux was 113.63 (±14.75). Triplicate 3-L tanks for all three stocking densities (40 g/L, 65 g/L, and 90 g/L) and fish sizes (5-g, and 11-g) were randomly assigned to each photoperiod treatment, with the exception that 11-g fish were not subjected to ambient lighting due to not enough fish of that size on hand.

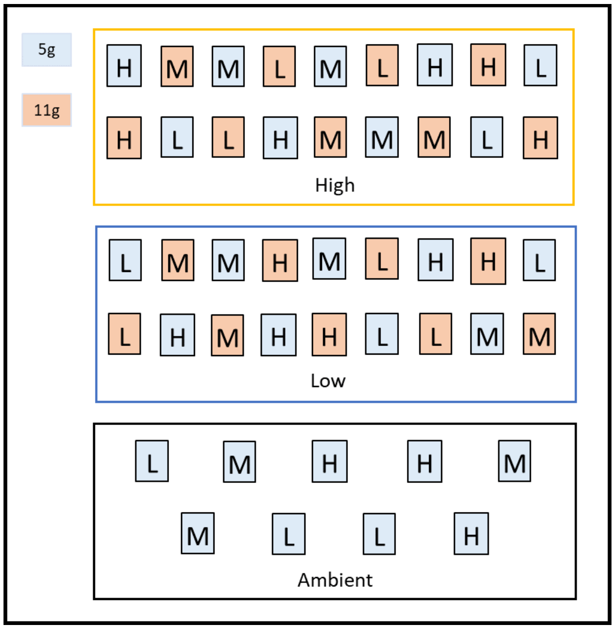

Figure 1. Experimental design of juvenile lumpfish experiments: L= low 40 g/L density; M = medium-65 g/L density; H = high 90 g/L density; Ambient = ambient lighting; Low = low, constant light intensity; High = bright, constant light intensity; Blue boxes = 5-g fish, Orange boxes = 11-g fish.

A total of 1,427 fish were stocked out into the experiment on 12 January 2022 for 5-g fish, and 317 fish were stocked on 2 February 2022 for 11-g fish (Table 1). Due to the quantity of fish needed for each tank, batch weighing was used to stock out the 5-g fish tanks. Small batches of fish were weighed, counted, and stocked into the tank until the desired mass for the target stocking density was achieved. For 11-g fish treatments, individual fish were weighed, counted, and stocked into the tank until the desired mass for the target stocking density was achieved.

System Monitoring and Daily Survival Evaluation

Fish were hand fed 5 times daily (at 8:00 am, 10:00 am, 12:00 pm, 2:00 pm, 5:00 pm) at 3 % body weight per fish/day so feed was not a limiting factor. All fish were fed a Gemma Wean diet (Skretting) that was increased in size in accordance with fish growth. At the beginning of the experiment, 5-g fish initially were fed a 0.8-mm diet, transitioned to 1.2-mm at 2 weeks, and then to a 1.8-mm feed at 6 weeks. Eleven-gram fish were fed a starting diet size of 1.2-mm, transitioned to 1.8-mm at 2 weeks, and to 2.0-mm feed at 6 weeks. Water quality, fouling, and fish mortality were monitored daily in each tank. Temperature (ºC) and salinity (ppm) were measured with an Extech Waterproof Thermometer and a Fisherbrand Handheld Analog Clinical Refractometer, respectively. Temperature was measured from each tank, while salinity was measured from one designated tank. Tanks were cleaned as needed to remove excess feed that could lead to fouling and poor water quality. Tanks were cleaned with extreme care in order to minimize stress to the fish by using a small siphon to remove any excess feed. Tank walls were cleaned on a biweekly basis when sampling occurred. Any mortalities were removed daily, weighed, and a fin nipping score was assigned and recorded, using a fin nipping scale reproduced from Spada (2021), ranging 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 (Figure 2).

0: "Healthy Fish"

- Caudal fin is free of bites and 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

Figure 2. Fin nipping scale used to quantify juvenile lumpfish aggression. Reproduced from Spada (2021). The scale ranges from 0-5, increasing in fin damage as the number increases. 0 represents a completely intact and healthy caudal fin, while 5 represents a fish with no caudal fin and severe damage to the caudal peduncle.

Biweekly Sampling

All tanks were sampled biweekly for eight weeks for total biomass, growth (wet weight, nearest 0.1 g), and aggression. In tanks that housed 5-g fish, a subsample of the population (20 fish) was used to estimate tank biomass by multiplying the average weight of fish by the number of fish in the tank. Whereas, in tanks containing 11-g fish, all fish were sampled individually from each tank in order to obtain an absolute tank biomass. Each sampled fish was weighed. Fin nipping occurrence was recorded as either a 1 for presence or 0 for absence of fin nipping and each fish assigned a fin nipping score (Figure 2). The fish then was placed in a holding container in order to prevent duplicate sampling. After each tank was sampled, density was recalculated and restored to baseline density treatments by removing fish as needed from each tank. Removed fish were returned to the general lumpfish population tanks in the CML. Remaining experimental lumpfish then were placed back into their respective tanks and returned to the experimental system. After 8 weeks, a final sampling was conducted following the aforementioned protocol, subsampling 20 fish (or the entire tank in the event there were less than 20 fish) from the 5-g tanks, while all fish from the 11-g tanks were sampled. Final sampling occurred on 9 March 2022 for 5-g fish and on 30 March 2022 for 11-g fish. Fish were weighed and assigned a fin nipping score. Once sampled, fish were returned back to the general lumpfish population tanks in the CML.

Growth rate was calculated by dividing the tank weight gain between biweekly sampling dates by the 14 days in between the sampling: (Current Weight – Previous Weight)/14 days. Overall percent growth was calculated by subtracting the initial tank weight from the final tank weight, divided by the original weight and multiplying by 100: ((Final Fish Mass – Initial Fish Mass)/ Initial Fish Mass) x 100 %).

Statistical Analysis

All data were analyzed using Excel 365 and R (v 4.2.1, R Core Team 2022). A two-way ANOVA was used to analyze the effects of light and stocking density on the final survival for both size classes. A two-way ANOVA was used to analyze the effects of light and stocking density on the overall percent growth per fish for both size classes. A linear mixed model was used to analyze the effects of light, stocking density, and time on the growth rates between bi-weekly sampling for both size classes over the 8 week experiment. For all linear mixed models, replicates were accounted for as a random variable in the model. Finally, a binomial generalized mixed model was used to analyze the effects of light, stocking density, and time on the fin nipping occurrence throughout the experiment for both size classes of lumpfish. For all binomial generalized mixed models, replicates were accounted for as a random variable in the model.

RESULTS

5-g Juveniles

Water temperature ranged from 2.00 ºC ± SE 0.06 to 6.97 ºC ± SE 0.03, while salinity ranged from 30 ppt to 37 ppt (Figure 3). Neither stocking density (two-way ANOVA, $P = 0.135$) nor light intensity (two-way ANOVA, $P = 0.135$) had an effect on survival of 5-g fish. There also was no significant interaction between stocking density and light (two-way ANOVA, $P = 0.332$) on the survival of 5-g fish. Fish survival was 98.9 % throughout the experiment with only 15 individuals dying out of the total 1,427 fish used in the experiment (Table 2). Of the 15 mortalities, nine were due to a loss in flow that occurred once overnight, while the remaining six likely died as a result of cannibalism as fin nipping was observed on the caudal fin.

Light significantly affected the overall percent growth (two-way ANOVA, $P <0.001$) of 5-g lumpfish, with overall percent growth ranging from 92.60 ± SE 4.91 % to 170.65 ± SE 13.99 % per fish (Figure 4). Stocking density did not have a significant effect on overall percent growth (two-way ANOVA, $P = 0.06$). Additionally, there was no significance in the interaction between stocking density and light (two-way ANOVA, $P = 0.100$). In the ambient light treatment, 5-g fish grew 31.5 % faster than fish in the low light treatment ($P < 0.01$) and 28.5 % faster than fish in the high light treatment ($P < 0.05$) (Figure 4).

Time significantly affected growth rates of 5-g fish (Linear Mixed-Model, $P<0.001$). Neither stocking density (Linear Mixed-Model, $P = 0.845$) nor light (Linear Mixed-Model, $P = 0.557$) significantly affected the growth rates of 5-g lumpfish. There also was no significant interaction between stocking density and light (Linear Mixed-Model, $P = 0.924$). Growth rates ranged from 0.247 g/day ± SE 0.026 to 0.313 g/day ± SE 0.025 (Figure 5).

There was a significant interaction between stocking density and light on aggressive behavior in 5-g fish (Binomial Generalized Linear Mixed Model, $P < 0.05$). Fish in the low light 40 g/L treatment had significantly less fin nipping than those in the low light 65 g/L and high light 40 g/L treatments (Figure 6). Fin nipping occurrence ranged from 38.40 ± SE 10.40 % to 60.00 ± SE 5.00 % (Figure 6). A significant interaction between light and time was also found (Binomial Generalized Linear Mixed Model, $P < 0.05$). At week 4, fin nipping was significantly higher in high light treatments than low light treatments ($P < 0.05$). Overall, all treatments over time resulted in a similar percentage of fin nipping. Fin nipping severity scores ranged from 0 to 1 for at least 90 % of the population in all treatments with scores of 2 to 3 accounting for ~10 % or less of the population in all treatments (Figure 7).

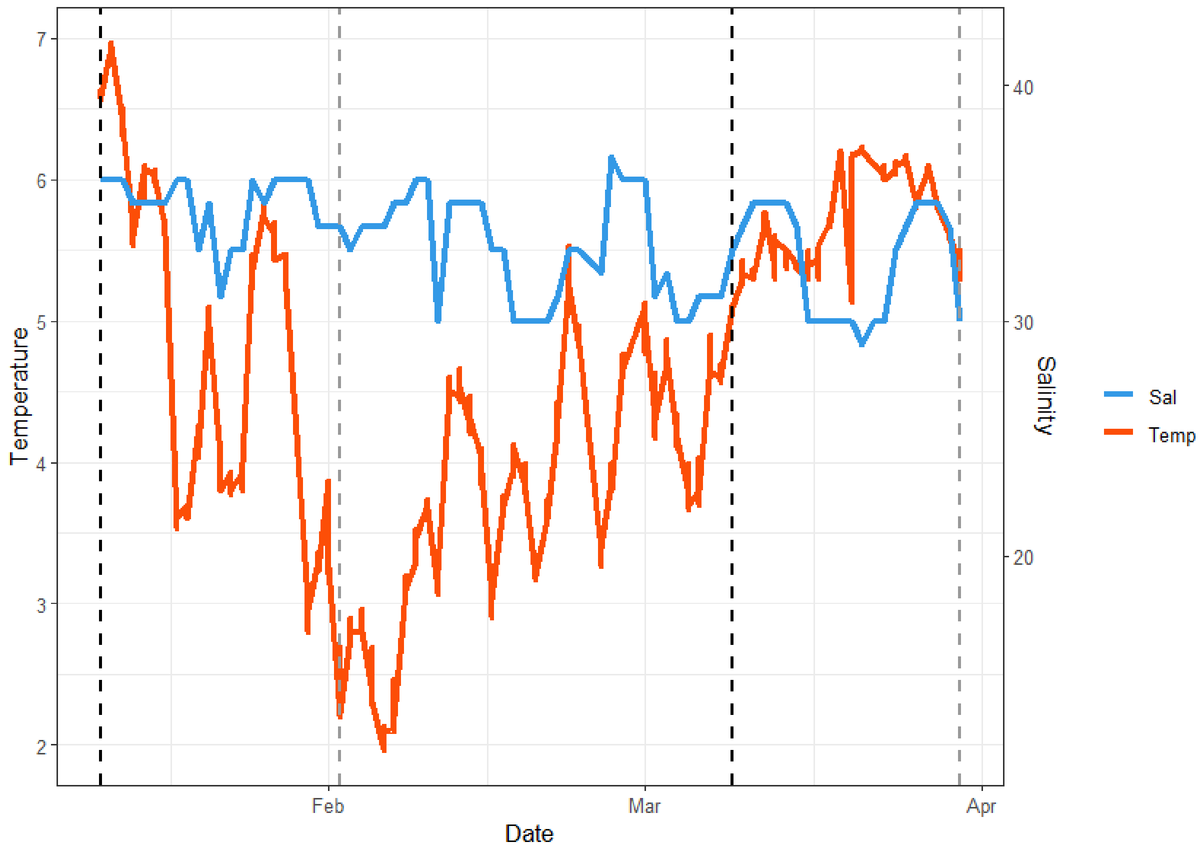

Figure 3. Mean temperature and salinity over the duration of the 8-week experimental periods for both 5-g and 11-g lumpfish. Water temperature ranged from 2.00 ºC ± SE 0.06 to 6.97 ºC ± SE 0.03 for the 5-g lumpfish trial, while salinity ranged from 30 ppt to 37 ppt. For the 11-g lumpfish trial, water temperature ranged from 1.97 ºC ± SE 0.06 to 6.23 ºC ± SE 0.03, while salinity ranged from 29 ppt to 37 ppt. Experiment start date is represented by vertical lines. Dotted black lines = 5-g experimental window. Dotted grey lines = 11-g experimental window.

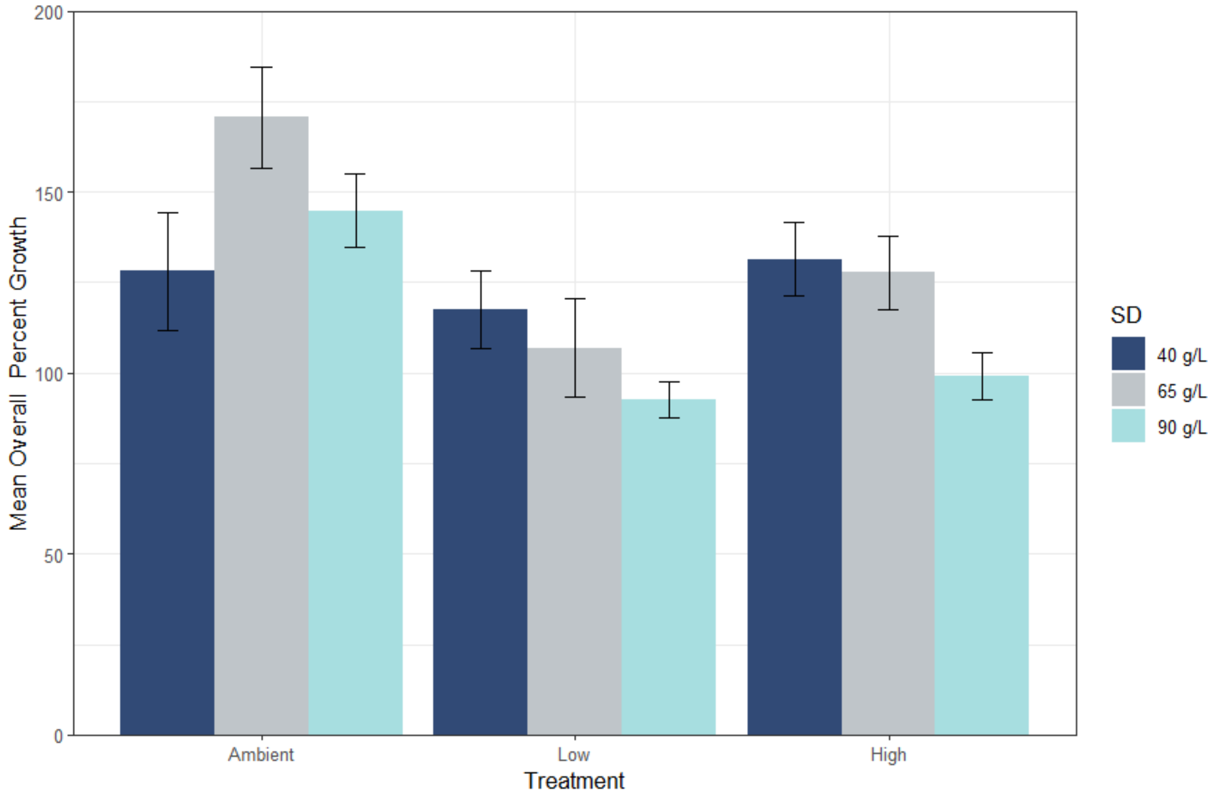

Figure 4. Mean overall percent growth (± standard error) of 5-g lumpfish subjected to varying combinations of stocking densities (SD: 40 g/L, 65 g/L, and 90 g/L) and light intensity treatments (ambient, low constant, high constant) over eight weeks.

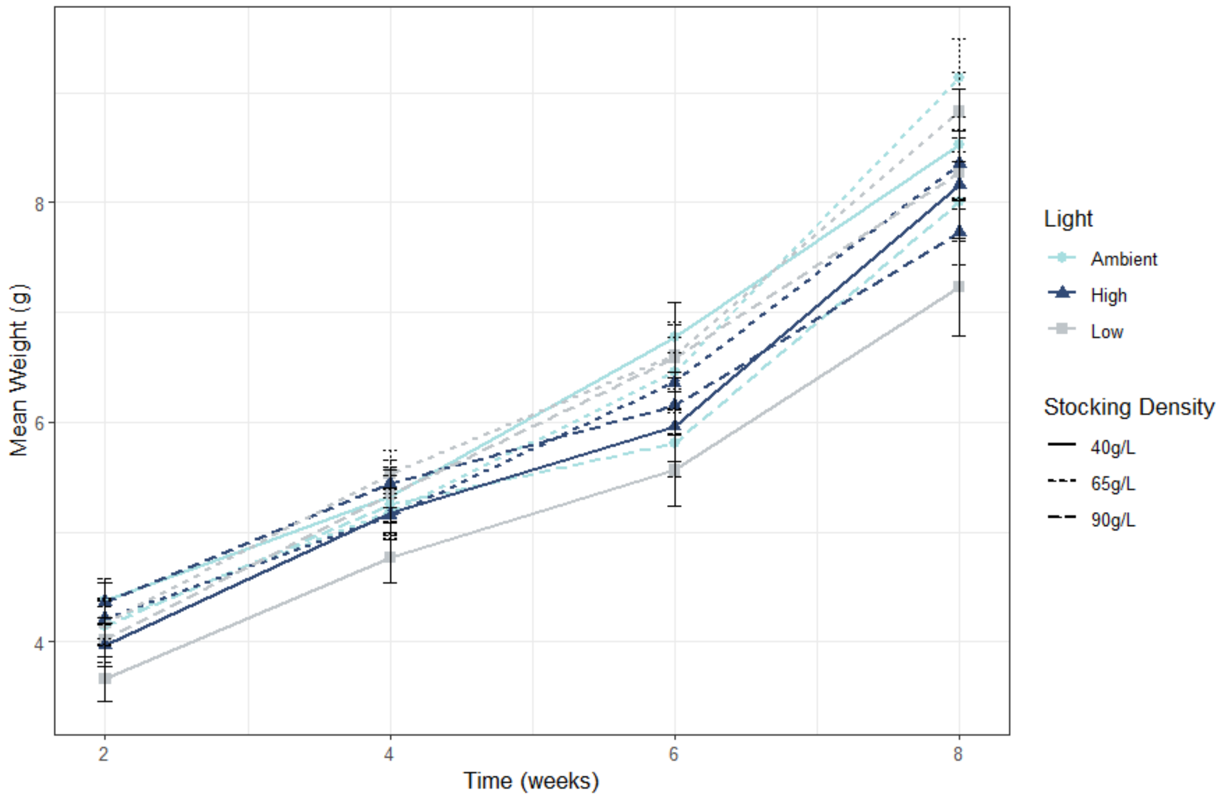

Figure 5. Mean weight (± standard error) of 5-g lumpfish subjected to varying combinations of stocking densities (40 g/L, 65 g/L, and 90 g/L) and light intensity treatments (ambient, low constant, high constant) over eight weeks. Stocking density treatment is represented by line type, while light treatment is represented by line color. Slope of lines indicate growth rates.

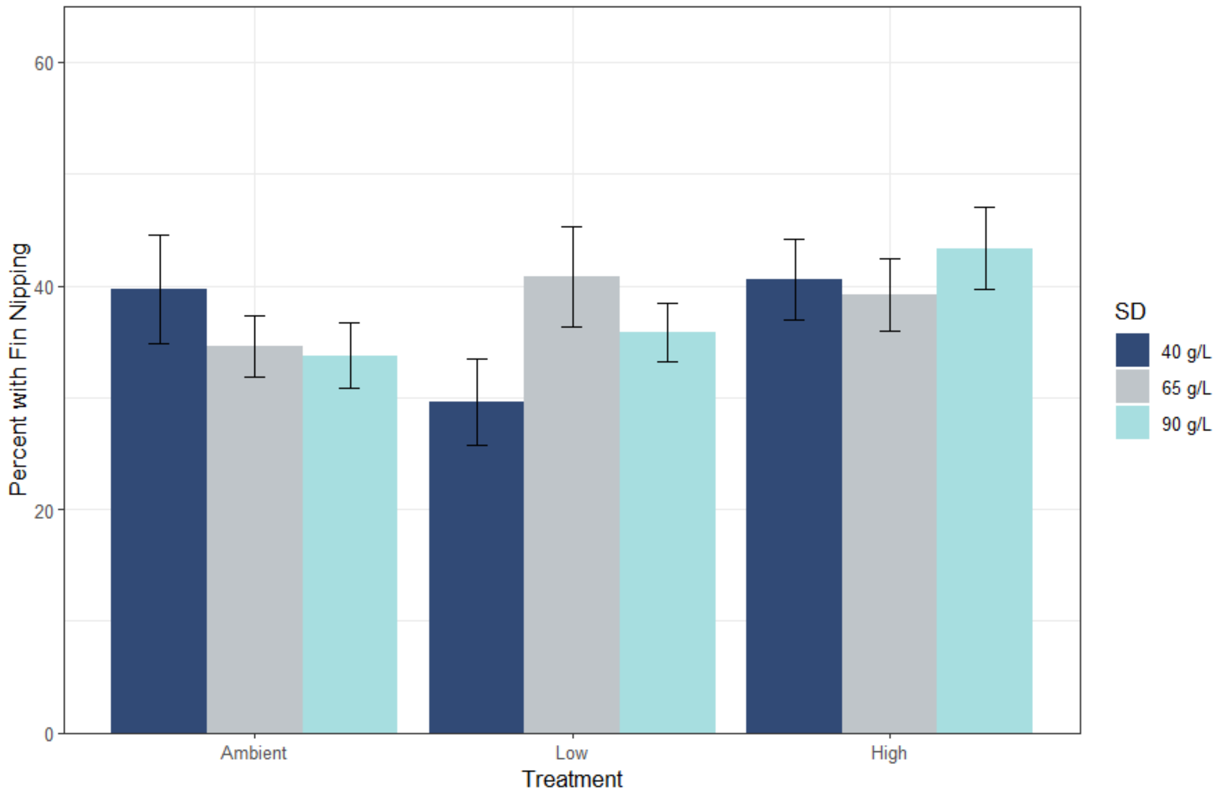

Figure 6. Overall mean percent fin nipping (± standard error) per treatment of 5-g fish. SD = Stocking density with three treatment levels, 40 g/L, 65 g/L, and 90 g/L. Stocking densities are grouped by light intensity treatment in which each light treatment has the three stocking densities.

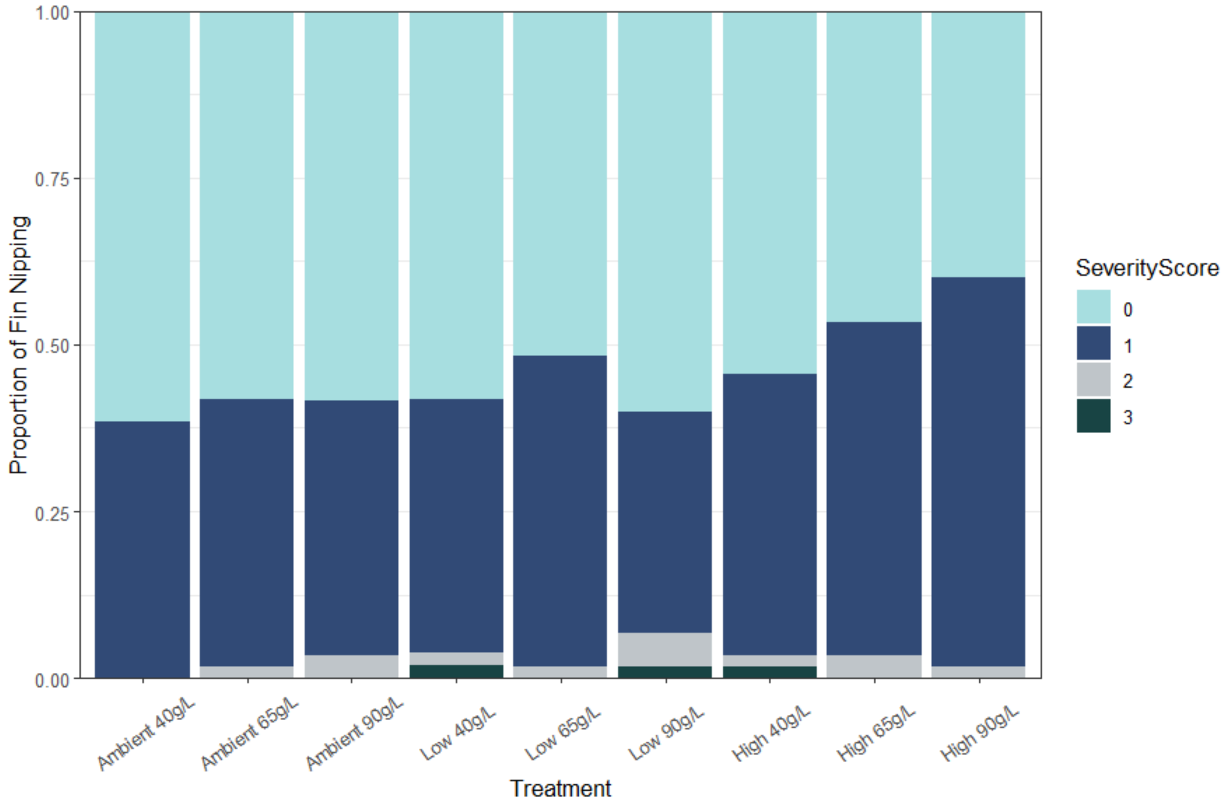

Figure 7. Proportion of fin nipping severity of 5-g lumpfish subjected to varying combinations of stocking densities (40 g/L, 65 g/L, and 90 g/L) and light intensity treatments (ambient, low constant, high constant) after eight weeks. Severity scores range from 0 to 5 with increasing caudal fin damage as the number increases. A ‘0’ represents a completely intact and healthy caudal fin, while a ‘5’ represents a fish with no caudal fin and severe damage to the caudal peduncle.

Table 2. Measured parameters (± standard error) for 5-g fish per treatment combination. Overall percent growth = ((Final Fish Mass – Initial Fish Mass)/ Initial Fish Mass) x 100 %). Fin nipping occurrence is the percentage of fin nipping observed in each tank averaged over four sampling periods for each treatment combination. Survival is the final survival after the 8 week experiment.

Light	Stocking Density (g/L)	Mean Overall Percent Growth (%)	Mean Percent Fin Nipping Occurrence (%)	Final Survival (%)
Ambient	40	108.40 (± 17.07)	38.36 (± 6.03)	90.93
Ambient	65	170.65 (± 13.99)	41.67 (± 1.67)	100
Ambient	90	144.89 (± 10.00)	41.67 (± 8.33)	100
Low	40	99.10 (± 10.12)	41.72 (± 9.79)	100
Low	65	106.93 (± 13.59)	48.33 (± 9.28)	98.55
Low	90	92.60 (± 4.91)	40.00 (± 5.77)	100
High	40	103.22 (± 4.81)	45.56 (± 3.84)	100
High	65	127.76 (± 10.14)	53.33 (± 1.67)	98.75
High	90	99.13 (± 6.34)	60.00 (± 2.89)	99.52

11-g Juveniles

Water temperature ranged from 1.97 ºC ± SE 0.06 to 6.23 ºC ± SE 0.03, while salinity ranged from 29 ppt to 37 ppt (Figure 3). Neither stocking density (two-way ANOVA, P = 0.178) nor light intensity (two-way ANOVA, P = 0.109) had an effect on survival of 11-g fish. There also was no significant interaction between stocking density and light (two-way ANOVA, P = 0.397) on the survival of 11-g fish. Fish survival was 99.7 % throughout the experiment with only 1 individual dying out of the total 317 fish used in the experiment (Table 3).

Neither stocking density (two-way ANOVA, P = 0.331) nor light (two-way ANOVA, P = 0.105) significantly affected the overall percent growth of the 11-g lumpfish. There also was no significant interaction between stocking density and light (two-way ANOVA, $P = 0.129$). For 11-g fish overall percent growth ranged from 154.55 ± SE 10.7 % to 219.53 ± SE 18.6 % (Figure 8).

There was a significant three-way interaction between light, stocking density, and time on the growth rates of 11-g fish (Linear Mixed-Model, $P < 0.01$). In the low light treatment, the 40 g/L stocking density was significantly different from the 90 g/L stocking density, with a growth rate 1.37 times faster (Figure 9). Growth rates ranged from 0.306 g/day ± SE 0.066 to 0.420 g/day ± SE 0.058 (Figure 9).

Stocking density and light did not significantly affect the occurrence of fin nipping in 11-g fish (Binomial Generalized Linear Mixed Model, $P > 0.5$). Fin nipping occurrence ranged from 4.80 ± SE 8.2 % to 36.7 ± SE 32.1 % (Figure 10). Due to the relatively low incidence of acute fin nipping in 11-g fish, severity of fin nipping was not analyzed. Less than 1 % of fish sampled (4 out of 962 fish) had fin nipping scores above a 1, with the highest score being only a 2 (Figure 11).

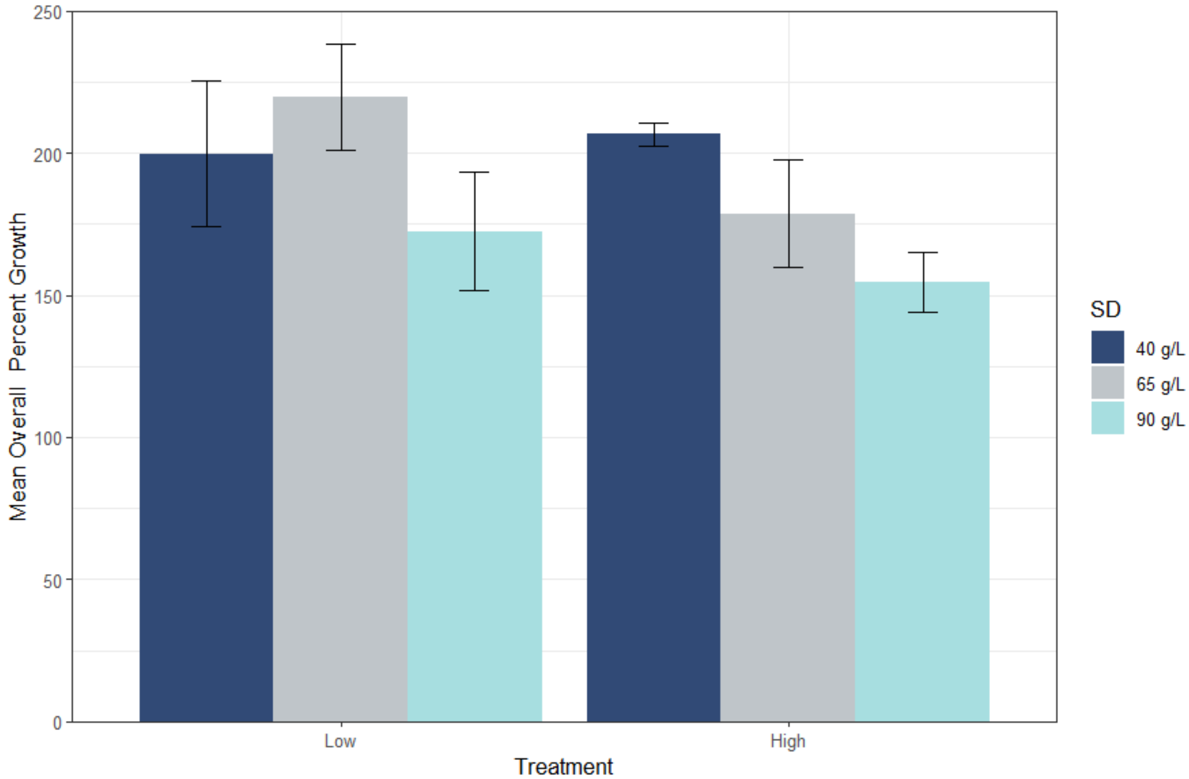

Figure 8. Mean overall percent growth (± standard error) of 11-g lumpfish subjected to varying combinations of stocking densities (SD: 40 g/L, 65 g/L, and 90 g/L) and light intensity treatments (low constant, high constant) over eight weeks.

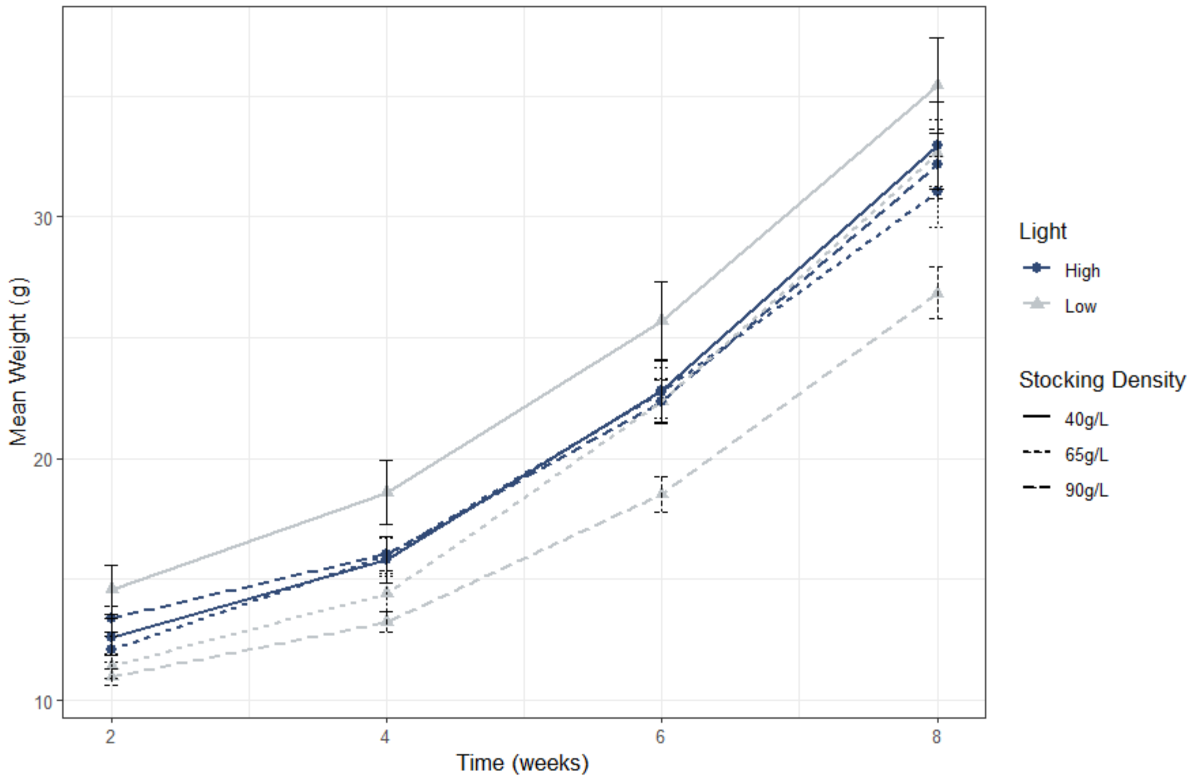

Figure 9. Mean growth (± standard error) over time of 11-g lumpfish subjected to varying combinations of stocking densities (40 g/L, 65 g/L, and 90 g/L) and light intensity treatments (low constant, high constant). Stocking density treatment is represented by line type, while light treatment is represented by line color. Slope of lines indicate growth rates.

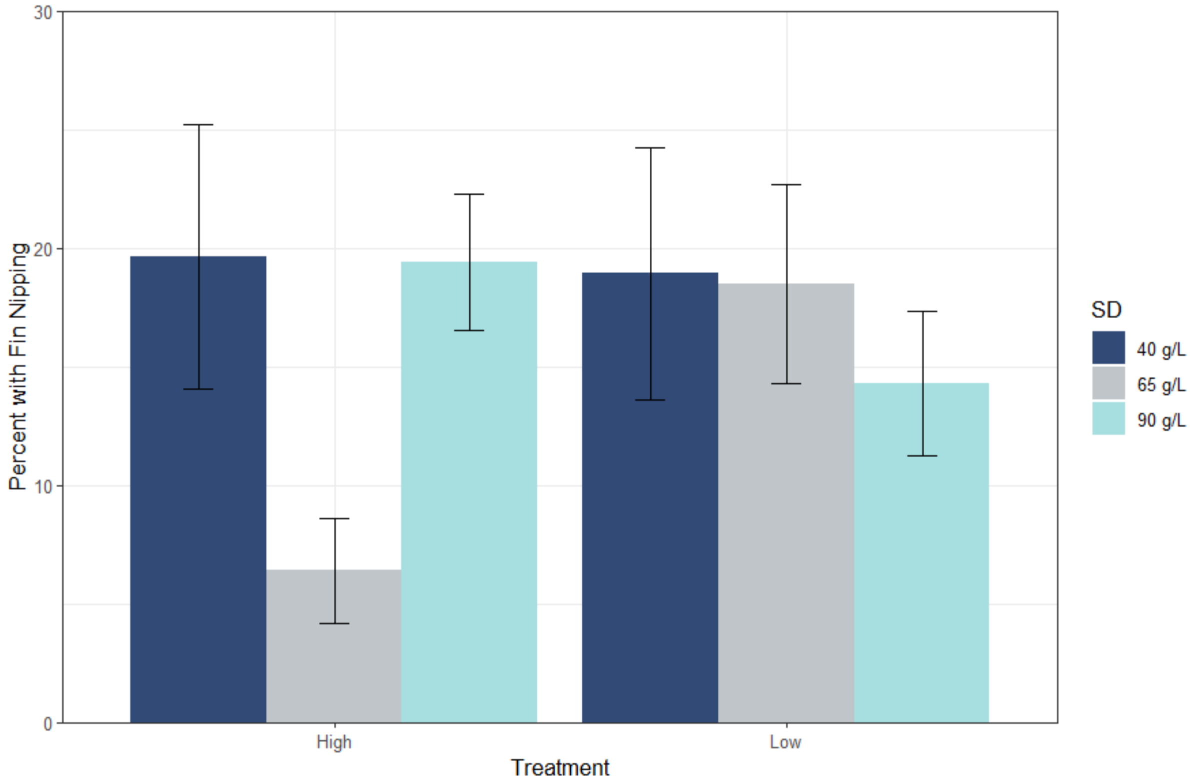

Figure 10. Overall mean percent fin nipping (± standard error) of 11-g lumpfish subjected to varying combinations of stocking densities (SD: 40 g/L, 65 g/L, and 90 g/L) and light intensity treatments (low constant, high constant) over eight weeks.

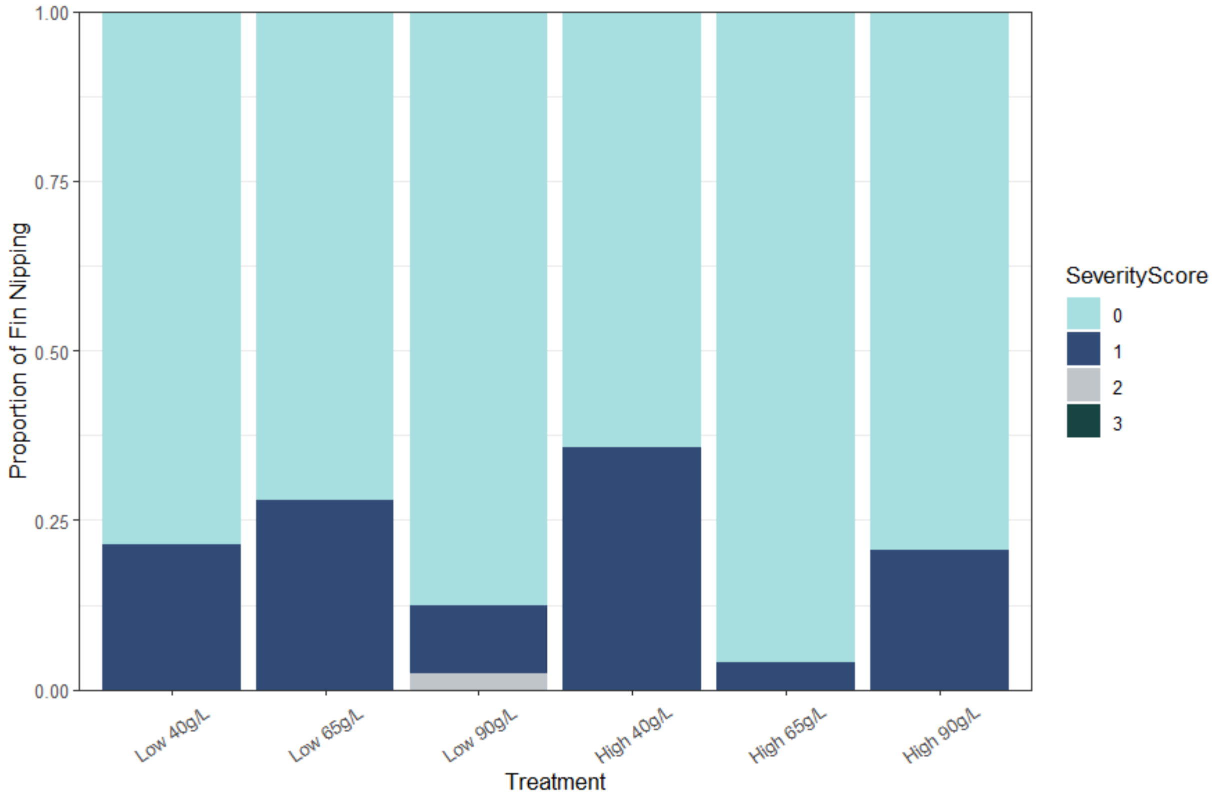

Figure 11. Proportion of fin nipping severity of 11-g lumpfish subjected to varying combinations of stocking densities (40 g/L, 65 g/L, and 90 g/L) and light intensity treatments (low constant, high constant) after eight weeks. Severity scores range from 0 to 5 with increasing caudal fin damage as the number increases. A ‘0’ represents a completely intact and healthy caudal fin, while a ‘5’ represents a fish with no caudal fin and severe damage to the caudal peduncle.

Table 3. Measured parameters (± standard error) for 11-g per treatment combination. Overall percent growth = ((Final Fish Mass – Initial Fish Mass)/ Initial Fish Mass) x 100 %). Fin nipping occurrence is the percentage of fin nipping observed in each tank averaged over four sampling periods for each treatment combination. Survival is the final survival after the 8 week experiment.

Light	**Stocking Density (g/L)**	**Mean Overall Percent Growth (%)**	**Mean Percent Fin Nipping Occurrence (%)**	**Final Survival (%)**
Low	40	35.80 (± 4.52)	23.33 (± 14.53)	100
Low	60	38.86 (± 0.27)	26.98 (± 14.32)	100
Low	90	31.58 (± 0.42)	13.03 (± 9.19)	100
High	40	43.92 (± 2.67)	36.67 (± 18.56)	96.88
High	65	36.54 (± 2.80)	4.76 (± 4.76)	100
High	90	34.91 (± 0.57)	20.20 (± 7.07)	100

DISCUSSION

Growth and Survival

Although it is well documented that stocking density can play a considerable role in the growth of fishes reared in aquaculture (Baldwin, 2011; Policar et al., 2013; Manley et al., 2014; Ntanzi, 2014; Karnatak et al., 2021), in the case of lumpfish, this may be a more complicated story. Our findings indicate that rearing juvenile lumpfish at stocking densities as high as 90 g/L exhibit no significant effect on overall percent growth for either 5-g or 11-g fish and minimal effect to growth rates of 11-g fish. Though it is likely that stocking density does play a role in the growth of juvenile lumpfish reared in the hatchery under some conditions, other factors such as light may play a more important role. Light significantly affected the overall percent growth of 5-g juveniles, independent of stocking density, but not of 11-g juveniles. For 5-g juveniles, lumpfish grown in the ambient light treatment tended to grow faster and had significantly higher overall growth than those in the low or high light treatments, while there was no effect of light on the overall growth of 11-g juveniles. However, growth rates for 11-g juveniles were significantly affected by both stocking density and light over time. The difference in significance for both growth metrics - growth rates and overall percent growth - of both fish size classes may be attributed to resetting stocking densities in the experimental tanks after sampling tanks biweekly. By reducing the stocking density of the tanks back to their initial treatment level, it is likely that growth rates increased because the fish had more room to grow and lower density-dependent interactions. Additionally, as fish grow, the shape of their growth curve changes from exponential to linear, eventually forming a sigmoidal curve (Hopkins, 1992) compared to a snapshot of information of an aggregated amount of time represented by the overall percent growth of fish. It is also important to note that the practice of reducing stocking densities over time is standard practice in hatcheries.

Overall, high stocking densities and low light resulted in slower and reduced growth. Though not significant, trends of lower growth were observed in both low and high 90 g/L treatments for both 5-g and 11-g fish. Survival throughout the experiment was not affected by either stocking density or light, indicating that while 40 g/L is the reported standard stocking density for commercial lumpfish facilities, densities up to 90 g/L could be used regardless of light intensity without increasing mortality, allowing for greater efficiency and higher lumpfish production. Additionally, stocking densities as high as 95 g/L have been recorded in the 2021 lumpfish cohort reared at the CML; however, the effects of such a high density on juvenile lumpfish survival and aggression are not known at this time as these parameters were not measured consistently in the general lumpfish rearing tanks (see Appendix A).

Previously reported works that have looked at the relation between stocking density and growth have primarily focused on schooling species such as: Nile tilapia (*Oreochromis niloticus*) (Ntanzi, 2014), Thai climbing perch (*Anabas testudineus*) (Hossain, 2012), minor carp (*Labeo bata*) (Karnatak et al., 2021), Atlantic Salmon (*Salmo salar*) (Liu et al., 2017), and gilthead seabream (*Sparus aurata*) (Montero et al., 1999), with fewer studies focusing on benthic fishes such as Japanese flounder (*Paralichthys olivaceus*) (Bolasina et al., 2006) and African catfish (*Clarias gariepinus*) (Kaiser et al., 1995) or relatively inactive species such as lumpfish (*Cyclopterus lumpus*) (Spada, 2021). In the majority of these studies, researchers found that fish growth was inversely related to stocking density with higher stocking densities resulting in lower growth.

In the only other known juvenile lumpfish density study (Spada, 2021), the effects of four stocking densities (40, 60, 70, and 90 g/L) on the growth and aggression of 2-g and 13-g lumpfish were researched. The effects of lighting were not examined, and instead all stocking density treatments were subjected to the same ambient lighting (12-h light: 12-h dark) rather than the three light intensities (ambient, low constant light, and high constant light) tested in the current study. Similarly to the current study, Spada's (2021) research was conducted over an 8-week period for both size classes of lumpfish and was conducted using the same ambient temperature and salinity flow through system used in this study. Spada (2021) found that in both 2-g and 13-g lumpfish, stocking density did affect overall percent growth of the fish. For 2-g fish, growth rates also were negatively affected by stocking density, while 13-g growth rates were not (Spada, 2021). However, in the present study, this relationship was only observed for 11-g lumpfish growth rates, while neither 5-g lumpfish growth metrics were significantly affected by stocking density. The difference in observed results from Spada (2021) and our current research may be due to intrinsic differences in broods, different temperature ranges, different fish sizes, and even different staff (Dutta, 1994). Diversity in broods could possibly contribute a great deal of variation to the growth of fish, offering a possible explanation to the differences seen between the current study and research conducted by Spada (2021) (Dutta, 1994). In fact, a significant difference was found between cohorts of fish used by Spada (2021) (2019 UNH lumpfish cohort) and the current study (2021 UNH lumpfish cohort) with fish growth significantly affected by an interaction between cohort and days post hatch (Generalized Linear Mixed Model, $P < 0.05$) (see Appendix A). Stocking density also had a significant effect on the growth of both lumpfish cohorts (Generalized Linear Mixed Model, $P < 0.01$) (see Appendix A); however, for both analyzed metrics, various other factors such as temperature, tank variation, and water quality also may have contributed to the variation seen between the 2019 and 2021 cohorts. The highest density recorded for the 2019 lumpfish cohort was 72.61 g/L, while the highest density for the 2021 lumpfish cohort was 95.46 g/L, indicating that stocking densities higher than 90 g/L may be feasible or even beneficial to further reducing fish growth depending on the desires of the hatchery. Spada's (2021) 2019 lumpfish cohort was approximately 266 dph for 2-g fish and 163 dph for 13-g fish when implemented into the experiment and had been reared at densities of 10 g/L for 2-g fish and 40 g/L for 13-g fish prior to being stocked into the experiment. Fish used in the current study were younger - approximately 130 dph when implemented into the experiment - and reared at lower densities of approximately 6 g/L for 5-g fish and 5 g/L for 11-g fish prior to the start of the study (see Appendix A).

The genetics of a brood can have major implications in how fast or slow a fish may grow. Depending on the parents, a brood may inherit various genes that can either aid or hinder growth. These genes could be related to hormone expression and/or behavioral cues that may also influence behavior (Dutta, 1994). Temperature also is known to affect the growth of fish (Dutta, 1994). In Spada (2021) for 2-g fish, water temperature ranged from 3-7 ºC, while for 13-g fish, water temperature ranged from 9-19 ºC. Water temperature for the current study ranged from 2-7 ºC. While higher temperatures can result in higher growth, accounting for variation in growth between 13-g fish and 11-g fish, temperature ranges for 2-g and 5-g fish were almost identical. Additionally, Spada's (2021) 2-g fish had drastically higher overall percent growth (169.69 % to 306.51 %) than that of the current study's 5-g fish (92.60 % to 170.65 %). While this difference in overall percent growth cannot be attributed to temperature differences, variation in growth rates can. Smaller fish have a larger scope for growth, growing at rates much faster than their larger counterparts (Hecht & Pienaar, 1993; Baras & Jobling, 2002). As juvenile fish grow, their growth rates shift from exponential to linear, eventually developing into a sigmoidal curve (Hopkins, 1992). This shift may account for the difference observed between 2-g and 5-g fish. Either individually or collectively, these differences between studies may account for the differences seen in the results. Lastly, differences in staff and experience may account for variation between lumpfish cohorts. As more and more lumpfish have been raised at the CML, our lumpfish rearing skills also have been honed and we have strived to produce larger fish faster. Further, during Spada's (2021) research, outside factors such as the COVID-19 pandemic affected staffing, possibly resulting in differences in lumpfish cohort growth.

Similarly to stocking density, light also has been well documented in its effects on growth of various fishes (Dutta, 1994). Depending on the fish, light may have more or less of an impact on growth due to variation in circadian rhythms, feeding strategy (visual or not), or life stage (Bolla & Holmefjord, 1988; Boeuf & Le Bail, 1999; Boeuf & Falcôn, 2001; Downing & Litvak, 2001). Atlantic salmon reared in continuous light grew larger than counterparts reared in natural light: dark settings (Hansen et al., 2017). However, African catfish had reduced growth rates as light period increased (Britz & Pienaar, 1992). Atlantic salmon, a fish the relies heavily on seasonality in addition to living higher in the water column and being a visual feeder (Hansen et al., 2017), is much more likely to be affected by light rather than an African catfish, a fish that is primarily nocturnal and relies heavily on tactile, chemical, and electrical signaling (Britz & Pienaar, 1992). In addition to duration, wavelength and intensity also can elicit various effects on a fish's growth (Fiksen et al., 2002; Ruchin, 2004; Volpato et al., 2013; Tian et al., 2015). Research conducted on juvenile blunt snout bream (*Megalobrama amblycephala*) found that higher light intensities increased growth, but only to a certain point (Tian et al., 2015). At light intensities higher than 1600-lx, poor growth was observed (Tian et al., 2015). As blunt snout bream are a herbivourous fish, they tend to inhabit lower levels of the water column (Zhen et al., 2018), and as such, would naturally not be exposed to high intensities of light.

In the case of lumpfish, it is likely that light plays a larger role in the growth of smaller juveniles. For 5-g fish, overall percent growth tended to be higher in ambient lighting compared to fish subjected to low or high light intensities. This may be due to both the high and low light treatments' intensities being outside of the optimal intensity for juvenile lumpfish growth. Wild larval and juvenile lumpfish inhabit the upper 0.5 m of the water column, and while they are visual feeders, as juvenile lumpfish grow, they begin to move deeper into colder waters (Daborn & Gregory, 1983; Moring, 1989). This transition from the upper levels of the water column to the dark cold depths is one that may give insight on the relationship of light and juvenile lumpfish growth. Intensities that are too high could result in a number of energetically costly behaviors such as increased foraging behavior and stress responses (Boeuf & Falcôn, 2001; Tian et al., 2015). While increased foraging in the wild may result in increased growth, in a hatchery setting, foraging outside of feeding times may be an energetically costly behavior. As lumpfish grow, it is likely that the effects of light on growth lessen, as no significance was observed on the overall percent growth of 11-g fish. The significant interaction between stocking density, light, and time on the growth rates of 11-g fish does indicate that light still does have an effect on growth in 11-g fish though it is likely less than the effects on smaller individuals.

Fin Nipping Occurrence and Severity

In aquaculture, aggression and ultimately cannibalism is a common issue that hatcheries face (Britz & Pienaar, 1992; Baras & Jobling, 2002; Ellis et al., 2002; Carvalho et al., 2013; Duk et al., 2017), and something that industry stakeholders recognize as an issue when rearing lumpfish (Garcia de Leaniz et al., 2022). Aggression and cannibalism can result from various stressors such as stocking density, light, and intrinsic behaviors (Duk et al., 2017; Lopes et al., 2018; Hans et al., 2020).

Stocking density increases the chances of individual interactions, possibly promoting cannibalism, as cannibalism can be a density dependent population regulator (Polis, 1981; Baras et al., 2000). This relation of stocking density with cannibalism, however, does not appear to hold true in the case of lumpfish. Our findings showed that in stocking densities of 90 g/L, occurrence of fin nipping was not significantly different from that of 40 g/L. While stocking density does have the potential to promote cannibalism, whether it does or does not, can depend on various factors. Generally, high stocking densities will result in higher instances of cannibalism (Baras & Jobling, 2002), however, in some instances, increasing stocking density can reduce cannibalism by disrupting the formation of dominance hierarchies (Smith & Reay, 1991; Duk et al., 2017). With higher stocking densities, the amount of available surface area to effectively form territories is less, in turn potentially decreasing aggression (Baras & Jobling, 2002; Duk et al., 2017). Lumpfish are known to attempt to form hierarchies, forcing smaller individuals to the bottom of the tank (Jonassen et al., 2018). It is possible that a stocking density as high as 90 g/L is able to effectively disrupt the aggressive behavior of small 5-g lumpfish. For 11-g fish, stocking density did not appear to have any effect on occurrence of fin nipping, though further research is needed to determine if under ambient lighting, the effects of stocking density may differ. In previous work done by Spada (2021), stocking density (40, 60, 70, and 90 g/L) did not affect the occurrence of fin nipping. However, in the current study, stocking density did have a significant effect but it in combination with light, suggesting that the compounded effect of light with stocking density, in some instances, can affect fin nipping occurrence. Combinations of low light and low stocking density resulted in the lowest incidence of fin nipping, however, this was not significantly different from the occurrence of fin nipping in low light 90 g/L treatments. Additionally, high light in combination with 90 g/L treatments resulted in the highest instance of fin nipping.

As previously stated, light can, either individually or compounded with stocking density, increase aggression and ultimately cannibalism (Munro, 1986; Britz & Pienaar, 1992; Baras & Jobling, 2002; Carvalho et al., 2013; Duk et al., 2017). Previous research has shown that when reared in dark conditions, African catfish showed significantly less cannibalism (Appelbaum & Kamler, 2000). As African catfish are a nocturnal species, it is that likely that they may experience stress in high light, resulting in cannibalism. Similar results were observed with Nile tilapia with higher light intensities resulting in higher aggression (Carvalho et al., 2013). While not a nocturnal fish, Nile tilapia are known to be highly territorial and aggressive (Barreto et al., 2011). When combined with being a visual feeder, high light intensities would likely facilitate higher instances of aggression (Outa et al., 2014). These findings align with the current study in that 5-g fish subjected to high light treatments had significantly higher instances of fin nipping than low light treatments at week 4 sampling supporting my hypothesis of light aiding in the mitigation of cannibalism. It should however be noted that during the 8-week experiment, temperature was not constant, and in some cases, temperature spikes were observed. Variation in temperature may impact juvenile lumpfish metabolic rates, promoting feeding and possibly aggression as a result of higher cortisol levels (Remen et al., 2022). Though fish sizes were not analyzed together due to a difference in light treatment levels, a trend of higher fin nipping occurrence was observed in 5-g fish compared to 11-g fish (~45 % fin nipping occurrence in 5-g fish vs ~20 % in 11-g fish) suggesting that as juvenile lumpfish grow in size, an ontogenetic shift may take place, reducing aggression and instances of cannibalism. The roughly 45 % fin nipping occurrence observed in 5-g fish treatments is comparable to reported fin nipping occurrence in hatcheries with reports ranging from 50-93 % (Johannesen et al., 2018; Gutierrez Rabadan et al., 2021).

Fin nipping severity was not analyzed for either 5-g or 11-g fish due to the relatively low scores observed. Overall, more than 90 % of the scores observed were either a 0 or a 1 for both 5-g and 11-g fish. Though the observed scores were relatively low, when coupled with other welfare issues, a severity score of a 1 may still be detrimental (Garcia de Leaniz et al., 2022). In a recent publication by Gutierrez Rabadan et al., an operational welfare score index was developed and tested for lumpfish. The fin nipping scale used by Gutierrez Rabadan et al. ranged from 0-2, with zero being a fish with no fin damage, while a two represented a fish with what was deemed severe damage. Based off of the welfare index developed by Gutierrez Rabadan et al., a score of a two in the current study would be considered severe enough damage, as such damage can reduce the fish's ability to resist secondary infections. Further research is needed to determine the possible implications of fin nipping in lumpfish populations, as a score of a one may still illicit detrimental effects either within the hatchery or upon implementation into salmonid pens. Having a damaged fin of any score may prove to limit mobility, potentially affecting the lumpfish's ability to delouse salmonids and or resist strong currents that may pass through the pen (Gutierrez Rabadan et al., 2021).

Limitations of This Study

Though the current study does provide insight into the effects of stocking density and light on the growth and aggressive behavior of juvenile lumpfish, due to limitations, its results are not all encompassing and should be interpreted and integrated as such. First, only a subset of treatment levels was examined. Only two size classes of juvenile lumpfish were assessed in the current study, providing a snapshot of fish culture from 5 to 31 g, rather than an examination of the stressors throughout the entire juvenile production cycle of lumpfish. Additionally, only three stocking densities were assessed. It is possible that the stocking densities used in the current study (40, 65, and 90 g/L) are already at the high end of spectrum, and stocking densities beyond 90 g/L may prove to be detrimental to juvenile lumpfish. Future research should assess a larger range of stocking densities and their effects on the growth, aggression, and cannibalism of juvenile lumpfish. Second, due to limiting space in the research facility, the experiment was conducted in small (3-L) tanks. This is not reflective of the size and scale at which commercial lumpfish hatcheries operate so the effects observed in this study may present differently in larger tanks. Replication of this experiment at a larger scale with additional replicates, may be able to shed light on the observable trends in both 5-g and 11-g growth metrics that were not found to be statistically significant in the current study. Third, other parameters also may have affected the results of the current study. Research conducted by Spada (2021) indicates that tank color may impact overall percent growth as well as the occurrence of fin nipping with blue tanks resulting in faster growth and trends of higher fin nipping occurring in black tanks. Clear tanks, a color not tested by Spada (2021), were used in this study, so it remains unknown if they affected the results. In addition to tank color, personality and growth potential were not assessed. Recent findings indicate that lumpfish personality may impact aggression (Whittaker et al., 2021). The personality and in turn level of aggressiveness that a lumpfish has may even play a role in which lumpfish are better delousers (Whittaker et al., 2021). Growth potential may also have affected the results of the current study. Just as there is variation in growth between broods, there may also variation within a brood (Dutta, 1994).

Finally, availability of food sources in the current study also should be considered when interpreting cannibalism metrics. Feed was administered five times daily at 3 % body weight per fish; however, it should be noted that in instances in a hatchery setting where feed may be less or even more, the effects of stocking density and light may be affected.

Conclusion

Increasing human populations and dwindling terrestrial space for animal protein production raise the question of how we will continue to feed the world. Currently, wild caught fisheries provide roughly half of the animal protein harvested from global waters (FAO, 2022). However, over the past 10 years, wild caught fisheries production stabilized while aquaculture production has steadily increased and has the potential to continue to do so (Costello et al., 2020; FAO, 2022). Increasing efficiency as well as the sustainability of aquaculture is vital to meeting the future demand for animal protein (FAO, 2022). By increasing the efficiency and sustainability of lumpfish production, salmonid production in turn can increase in efficiency and sustainability, aiding in the overall production of animal protein harvested from global waters.

In the case of lumpfish, while the cannibalistic nature of lumpfish may not be eliminated in intensive rearing, our research demonstrates that rearing juvenile lumpfish at stocking densities as high as 90 g/L, relative to 40 g/L, exhibits no effect on overall percent growth for either 5-g or 11-g fish and minimal effect to growth rates of 11-g fish. The slight reduction in growth rates (1.37 times slower) observed in 11-g fish subjected to low light and 90 g/L also may prove to be beneficial as lumpfish can outgrow their usefulness as cleanerfish once they reach a certain size (~150 g) so suppressing fish growth may be of interest to lumpfish hatchery managers. Depending on the needs of industry, light may be an additional tool used to reduce the overall percent growth of fish around 5 g in size. By keeping high stocking densities and lower light levels as fish grow, growth rates may be manipulated in order to achieve a slower growth rate and, in turn, potentially extending the window of time in which lumpfish consume sea lice. Additionally, our findings demonstrate that at high stocking densities of 90 g/L, occurrence of cannibalism in 5-g fish is not any higher than in individuals stocked at 40 g/L. Keeping hatchery lighting on either a low or ambient setting also can further aid in reducing the occurrence of cannibalism in tanks as well as potentially decreasing the costly energetic demands of the hatchery.

While not statistically significant, overall, 11-g fish had trends of less fin nipping occurring, indicating a possible ontogenetic shift. Further research is needed to confirm this potential shift by testing fish smaller than 5-g and larger than 11-g. With respect to the severity of fin nipping, more than 90 % of observed fin nipping incidences were either scored a 0 or a 1 for both 5-g and 11-g fish. Though observed scores were relatively low, when coupled with other welfare issues, a severity score of a 1 may still be detrimental (Garcia de Leaniz et al., 2022). Survival throughout the experiment was not affected by either stocking density or light, indicating that while 40 g/L is the reported standard stocking density for industry, densities up to 90 g/L could be used regardless of light intensity without increasing mortality, allowing for greater efficiency and higher lumpfish production. The highest density recorded for cohorts reared at the UNH CML was roughly 95 g/L, indicating that stocking densities higher than 90 g/L may be feasible or even beneficial to further reducing growth depending on the desires of the hatchery. Further research is needed to determine the possible implications of fin nipping in lumpfish populations in addition to examining the effects of other hatchery stressors on the growth and cannibalism of juvenile lumpfish. These findings add to the groundwork for the optimization of lumpfish rearing in hatcheries, increasing fish production along with fish welfare.

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Appendix A

Lumpfish have been reared annually at the University of New Hampshire (UNH) Coastal Marine Laboratory (CML) since 2019, producing four cohorts of lumpfish. Daily record keeping of these fish, at a minimum, included data such as date, fish age, water temperature, salinity, and mortality for each tank of lumpfish. Growth was estimated by weighing subsets of fish approximately every two weeks, however, not always consistently. Despite the inconsistencies, data for two cohorts of lumpfish, hatched in 2019 and 2021 and reared at the UNH CML, were mined to ascertain growth metrics, population size, and tank densities. Mean weights of fish were based on weights of subsets of 15 fish. When fish from an entire tank were culled or moved, all fish were counted yielding a final population size. Back calculating using the daily mortality counts yielded the initial population size. Tank densities were calculated by dividing the fish biomass (mean fish weight x total number of fish) by the tank volume.

All data were analyzed using Excel 365 and R (v 4.2.1, R Core Team 2022). Weight data were log transformed in order to achieve normality. A generalized linear mixed model was used to analyze the effects of cohort, density, and days post hatch on the weight for both 2019 and 2021 cohorts. Tank variation was accounted for as a random variable in the model. A significant effect of density was found on the weights of both cohorts (Generalized Linear Mixed Model, $P < 0.001$). Additionally, a significant interaction between cohorts and days post hatch was found on fish weights (Generalized Linear Mixed Model, $P < 0.05$). The 2021 cohort had significantly higher weights than the 2019 cohort (Generalized Linear Mixed Model, $P < 0.001$). These data illustrate the intrinsic variation in growth that can occur between cohorts reared in similar conditions.

Table A1. Measured parameters (± standard error) for 2019 and 2021 lumpfish cohorts reared at the UNH CML grouped by DPH (Days Post Hatch) up to the last sampling point in which all juveniles either reached or slightly surpassed 25 grams (the size at which lumpfish are stocked into salmonid ocean pens).

Cohort	DPH Range	Mean Density (g/L)	Density Ranges (g/L)	Mean Weight (g)	Weight Ranges (g)
2019	50-150	1.81 (± 0.23)	(0.64-2.99)	0.62 (± 0.08)	(0.26-1.22)
2021	50-150	5.16 (± 0.83)	(1.13-8.53)	6.86 (± 1.82)	(0.86-17.41)
2019	151-250	9.88 (± 1.78)	(0.80-35.61)	8.20 (± 1.70)	(1.04-36.41)
2021	151-250	12.20 (± 0.91)	(9.39-14.94)	18.06 (± 4.46)	(7.32-30.41)
2019	251-350	39.95 (± 4.39)	(12.39-75.46)	79.04 (± 9.97)	(14.82-158.07
2021	251-350	43.74 (± 4.77)	(20.02-94.05)	164.61 (± 12.53)	(60.96-232.01)

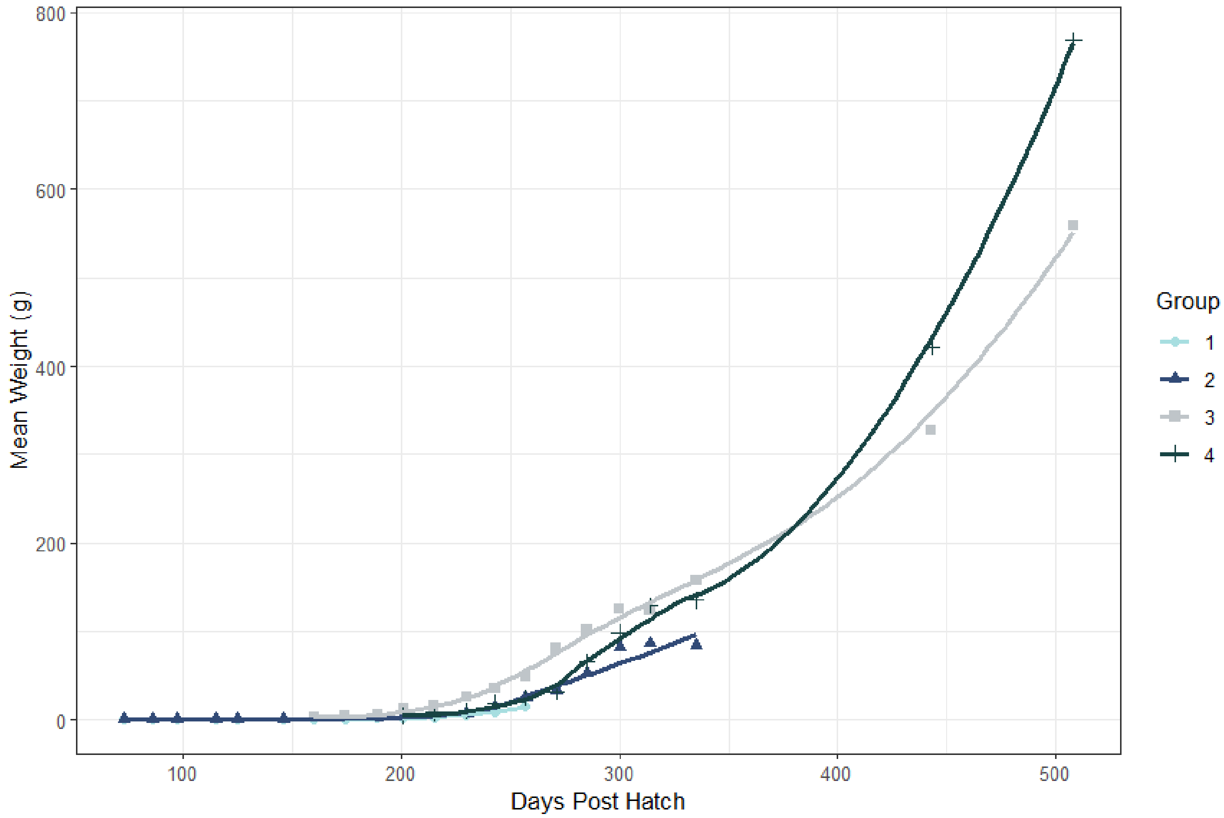

Figure A1. Mean weight of lumpfish by age (days post hatch) for the 2019 cohort reared at the UNH CML. Fish were reared in four tanks, represented by Groups 1 through 4 and denoted by both differing point shape and line color. Mean weights/group ranged from 0.26 g to 768.07 g. Locally weighted smoothing regression lines were fitted to the data for better trend visualization.

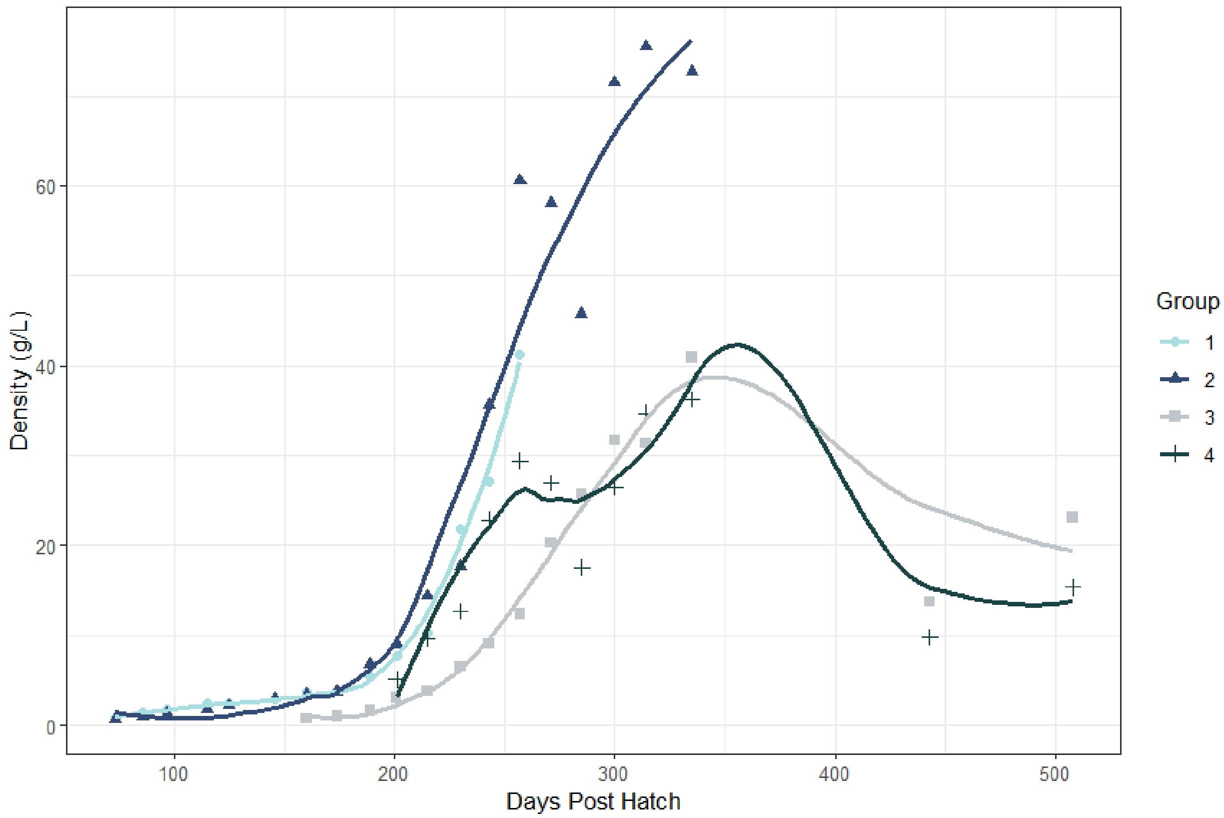

Figure A2. Rearing density of lumpfish by age (days post hatch) for the 2019 cohort cultured at the UNH CML. Fish were reared in four tanks, represented by Groups 1 through 4 and denoted by both differing point shape and line color. Mean rearing densities/group ranged from 0.64 g/L to 75.46 g/L. Locally weighted smoothing regression lines were fitted to the data for better trend visualization.

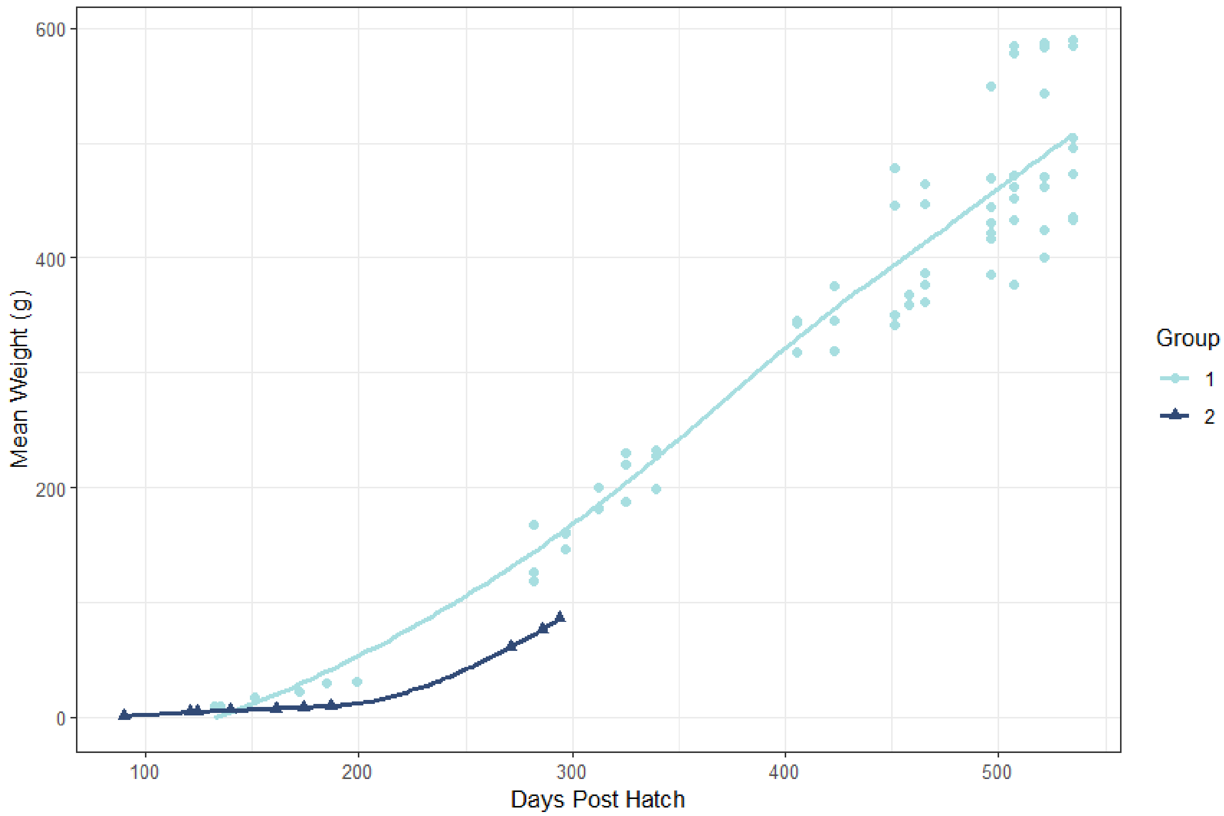

Figure A3. Mean weight of lumpfish by age (days post hatch) for the 2021 cohort reared at the UNH CML. Fish were reared in 2 tanks, represented by Groups 1 through 2 and denoted by both differing point shape and line color. Mean weights/group ranged from 0.86 g to 589.43 g. Locally weighted smoothing regression lines were fitted to the data for better trend visualization.

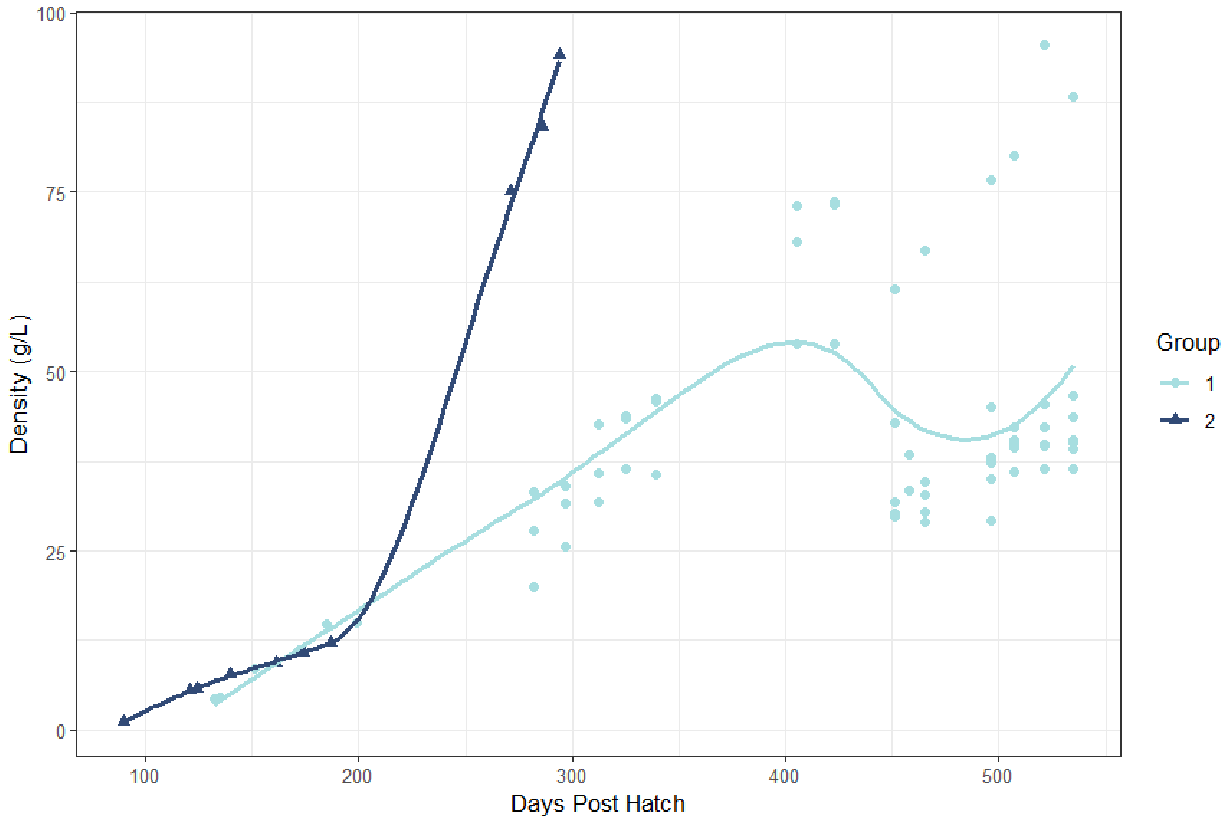

Figure A4. Rearing density of lumpfish by age (days post hatch) for the 2021 cohort cultured at the UNH CML. Fish were reared in two tanks, represented by Groups 1 through 2 and denoted by both differing point shape and line color. Mean rearing densities/group ranged from 1.13 g/L to 95.46 g/L. Locally weighted smoothing regression lines were fitted to the data for better trend visualization.

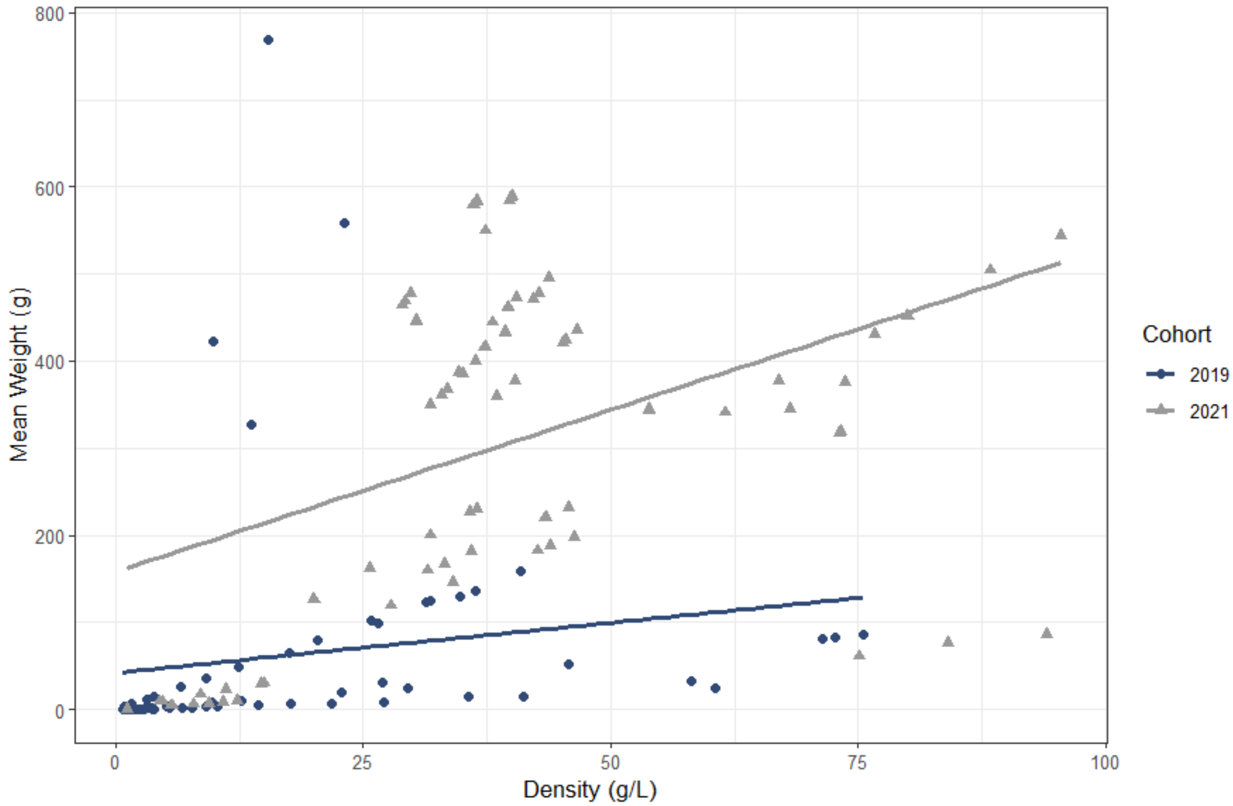

Figure A5. The relationship between lumpfish mean weight and rearing density for the 2019 (blue line, blue circles) and 2021 (gray line, gray triangles) cohorts reared at the UNH CML.

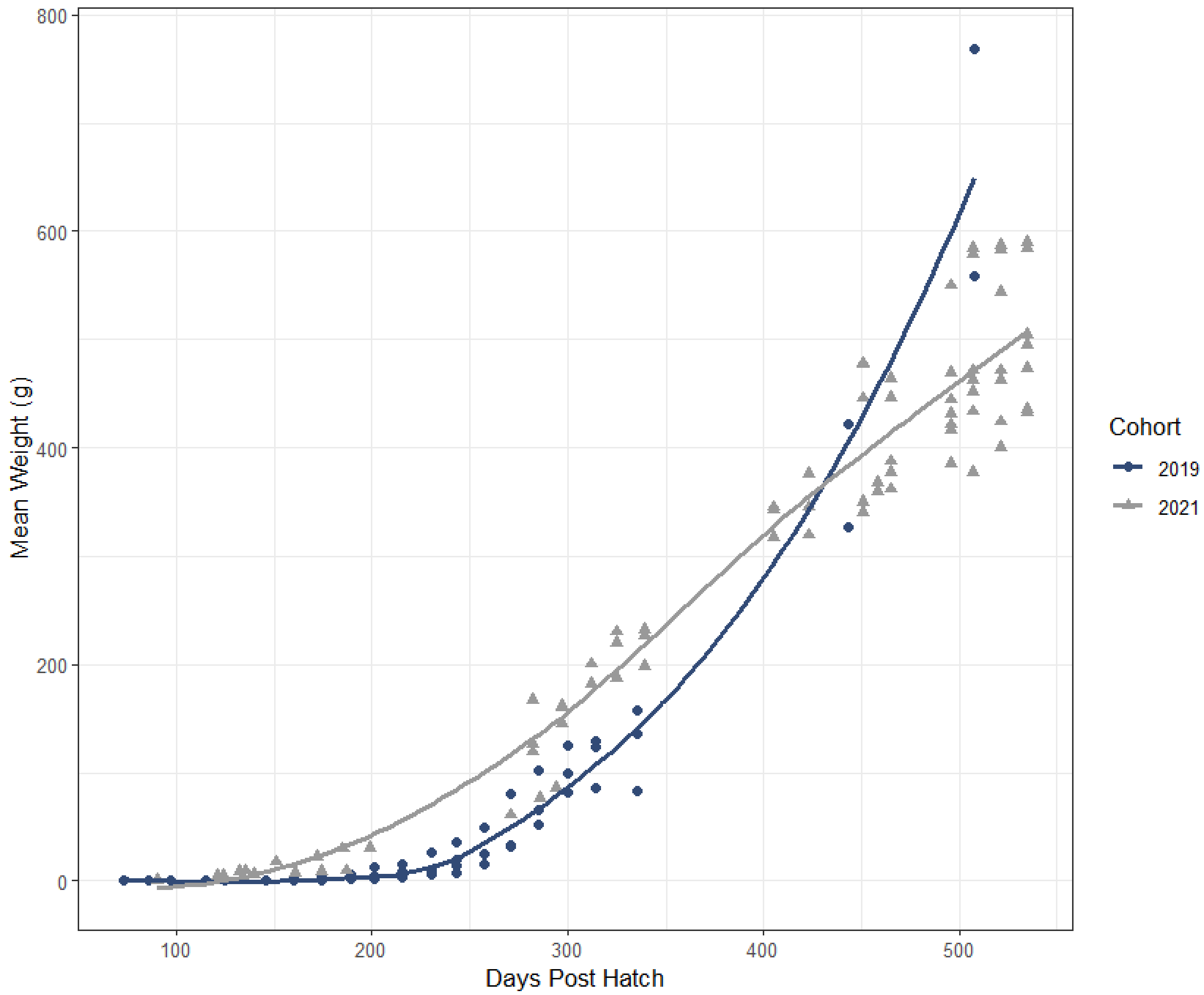

Figure A6. The relationship between lumpfish mean weight and lumpfish age (days post hatch) for the 2019 (blue line, blue circles) and 2021 (gray line, gray triangles) cohorts reared at the UNH CML. Mean weights for the 2019 cohort ranged from 0.26 g to 768.07 g. Mean weights for the 2021 cohort ranged from 0.86 g to 589.43 g. Locally weighted smoothing regression lines were fitted to the data for better trend visualization.

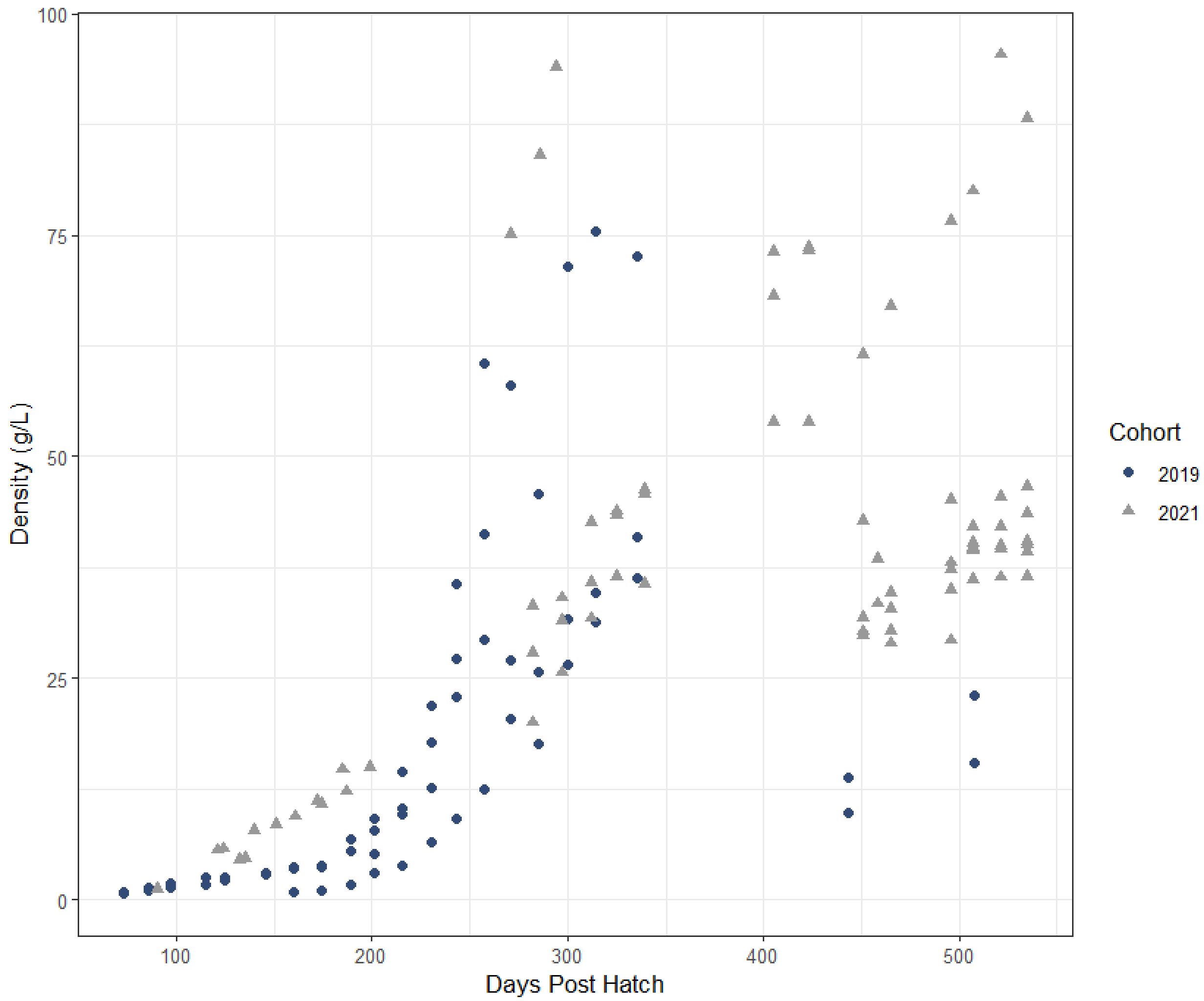

Figure A7. The relationship between lumpfish rearing density and lumpfish age (days post hatch) for the 2019 (blue circles) and 2021 (gray triangles) cohorts reared at the UNH CML. Rearing density for the 2019 cohort ranged from 0.64 g/L to 75.46 g/L. Rearing density for the 2021 cohort ranged from 1.13 g/L to 95.46 g/L.

Appendix B

University *of* New Hampshire

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26-Mar-2021

Fairchild, Elizabeth A
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IACUC #: 210307
Project: Advancing Lumpfish Aquaculture and Their Use As Biological Delousers in Salmonid Ocean Farms
Approval Date: 25-Mar-2021

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category D in Section V of the Application for Review of Vertebrate Animal Use in Research or Instruction - *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 Susan Jalbert at 862-3536.

For the IACUC,

Julie Simpson, Ph.D.
Director

cc: File