Fall 2021

THE PRESENCE OF SEA LICE ON STEELHEAD TROUT AT A MARINE FARM IN NH WATERS AND THE UTILIZATION OF LUMPFISH AS A BIOLOGICAL DELOUSER

Michael Brian Doherty
University of New Hampshire, Durham

THE PRESENCE OF SEA LICE ON STEELHEAD TROUT AT A MARINE FARM IN NH WATERS AND THE UTILIZATION OF LUMPFISH AS A BIOLOGICAL DELOUSER

By

Michael Brian Doherty
Bachelor of Science, Environmental Biology
Plymouth State University, 2014

THESIS

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Master of Science
In
Biological Sciences: Marine Biology

September 2021

COMMITTEE PAGE

This thesis/dissertation was examined and approved in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences: Marine Biology by:

Thesis Director, Dr. Elizabeth A. Fairchild,
Research Associate Professor in Biological Sciences,
University of New Hampshire

Dr. Michael Chambers, Aquaculture Specialist, Associate Professor of Extension, School of Marine Science and Ocean Engineering, NH Sea Grant and UNH Cooperative Extension, University of New Hampshire

Dr. Nathan Furey, Assistant Professor in Biological Sciences,
University of New Hampshire

On July 28, 2021

Approval signatures are on file with the University of New Hampshire Graduate School

ACKNOWLEDGEMNTS

Funding and support for this project comes from the University of New Hampshire NH Agricultural Experiment Station, New Hampshire Sea Grant, United States Department of Agriculture National Institute of Food and Agriculture, and the Northeastern Regional Aquaculture Center.

Thank you to my advisor, Dr. Elizabeth Fairchild. You have been so supportive and an amazing mentor to me, your dedication to my learning is something I am truly grateful for. I thank you for bringing me into your lab, and introducing me to the amazing qualities of lumpfish, which I am convinced is rapidly becoming one of the most charismatic fishes in the Gulf of Maine. And thank you; Dr. Nathan Furey and Dr. Michael Chambers. Both of you have shared your knowledge, experience, and time and have taught me so much over the last two years. I'm fortunate to have had such accomplished and dedicated scientists as mentors during my graduate program.

Additional gratitude is deserved by my graduate and undergraduate colleagues; Mary Kate Munley, Caitlin Shanahan, Nathaniel Spada, Olivia Fitzgerald, and Shelly Lancaster. Their help, support, and friendship has been invaluable to me and key to the progress towards my own degree, I hope I can be as much of an influence in their academic careers as they have been on mine. The laborious hours in the rain, cold, or heat netting fish and sieving sea lice will not be forgotten.

The aquaculture team at NH Sea Grant deserves gratitude as well. Thank you, Arron Jones, Erich Berghahn, and Peter Roy. Help with the small cage sea lice sampling is much appreciated, as well as the sampling of steelhead at AquaFort. Not only did you provide me with a wealth of knowledge from your own experiences, but I deeply appreciate the fun and friendly work environment that existed whenever we collected sea lice. And I appreciate the small boat operations lessons I received on our trips out to AquaFort. And thank you to the Coastal Marine Lab Supervisor, Nathan Rennels. Your tireless and often stressful work allows so much valuable research to take place.

TABLE OF CONTENTS

CHAPTER PAGE

ACKNOWLEDGEMNTS iii

TABLE OF CONTENTS v

LIST OF TABLES vi

LIST OF FIGURES viii

ABSTRACT x

INTRODUCTION 1

CHAPTER 1: AN ASSESSMENT OF SEA LICE INFESTATIONS AT AN EXPERIMENTAL STEELHEAD TROUT AQUACULTURE FARM IN COASTAL NEW HAMPSHIRE 9

Introduction 9

Methods 12

Results 16

Discussion 23

Conclusion 27

CHAPTER 2: EXAMINING THE USE OF LUMPFISH AS A CLEANERFISH OF STEELHEAD TROUT IN EXPERIMENTAL AQUACULTURE PENS IN NEW HAMPSHIRE WATERS 29

Introduction 29

Methods 33

Results 40

Discussion 69

Conclusions 74

FINAL CONCLUSIONS 77

REFERENCES 80

APPENDICES 86

Appendix A 86

Appendix B. 91

Appendix C. 93

Appendix D. 100

LIST OF TABLES

TABLE PAGE

Table 1.1. Weekly sea lice assessment data for Trout Lodge fish (n=15 each week). sampled November 3, 2020, to June 11, 2021. ... 18

Table 1.2. Weekly sea lice assessment data for Riverence fish (n=15) sampled December 22, 2020 to June 4, 2021. ... 19

Table 2.1. Trial dates and trout strains used. ... 33

Table 2.2. Experimental design of the trials including initial fish stocking densities. ... 35

Table 2.3. Trial 1 initial and final trout numbers and growth information. ... 43

Table 2.4. Trial 1 mean biomass per lumpfish in each cage at the start of the trial and at the end of the trial, with specific growth rate. ... 44

Table 2.5. Trial 2 mean biomass per trout in each cage at the start of the trial and at the end of the trial, with specific growth rate. ... 45

Table 2.6. Trial 2 mean biomass per lumpfish in each cage at the start of the trial and at the end of the trial, with specific growth rate. ... 46

Table 2.7. Trial 1 lumpfish gut contents. ... 48

Table 2.8. Trial 1 trout gut contents. ... 48

Table 2.9. Trial 2 lumpfish gut contents. ... 49

Table 2.10. Trial 2 trout gut contents. ... 49

Table 2.11. Trial 1 mean lice loads on steelhead trout (n=15) and lumpfish (n=3) over the five sampling periods. ... 53

Table 2.12. All subsets regression model selection table for Trial 1 comparison of mean lice loads by fish species ... 54

Table 2.13. Parameter estimates for most parsimonious model selected for Trial 1 comparison of mean lice loads by fish species (GLM). ... 54

Table 2.14. All subsets regression model selection table for Trial 1 mean weekly lice loads of trout by hide design ... 55

Table 2.15. Parameter estimates for Trial 1 mean weekly lice loads of trout by hide design (generalized linear model, JMP). ... 56

Table 2.16. All subsets regression model selection table for Trial 1 mean weekly lice loads of trout by cleanerfish treatment ... 57

Table 2.17. Parameter estimates for Trial 1 mean weekly lice loads of trout by cleanerfish treatment (generalized linear model, JMP). ... 58

Table 2.18. All subsets regression model selection table for Trial 1 mean weekly lice loads of lumpfish by hide design ... 59

Table 2.19. Parameter estimates for Trial 1 mean weekly lice loads of lumpfish by hide design (generalized linear model, JMP). 60
Table 2.20. Trial 2 mean lice loads on steelhead trout (n=15) and lumpfish (n=3) over the five sampling periods. 61
Table 2.21. All subsets regression model selection table for Trial 2 comparison of mean lice loads by fish species 62
Table 2.22. Parameter estimates for Trial 2 comparison of mean lice loads by fish species (generalized linear model, JMP). 62
Table 2.23. All subsets regression model selection table for Trial 2 mean weekly lice loads of trout by hide design 63
Table 2.24. Parameter estimates for Trial 2 mean weekly lice loads of trout by hide design (generalized linear model, JMP). 64
Table 2.25. All subsets regression model selection table for Trial 2 mean weekly lice loads of trout by cleanerfish treatment 65
Table 2.26. Parameter estimates for Trial 2 mean weekly lice loads of trout by cleanerfish treatment (generalized linear model, JMP). 66
Table 2.27. All subsets regression model selection table for Trial 2 mean weekly lice loads of lumpfish by hide design 68
Table 2.28. Parameter estimates for Trial 2 mean weekly lice loads of lumpfish by hide design (generalized linear model, JMP). 68
Table B.1. Lice assessment data from the UNH steelhead trout farm from October 18, 2018 to December 14, 2018. 92
Table B.2. Lice assessment data 92
Table C.1. Trout stocking densities (kg/L) in each cage. 95
Table C.2. Trial experimental design. 95

LIST OF FIGURES

FIGURE PAGE

Figure 1.1. A gravid, female *Caligus elongatus* (right) alongside a gravid, female *Lepeophtheirus salmonis* (left). Photo: M. Pietrak, USDA. 14

Figure 1.2. Identifying *Caligus* lunule circled in red. Species: *Caligus curtus*. 14

Figure 1.3. Male (left) and female (right) *Caligus elongatus*. Note the difference in abdomen width relative to the cephalothorax. 15

Figure 1.4. Egg strands hanging off of an adult female *Caligus elongatus*. 15

Figure 1.5. Total mean lice loads per fish for both steelhead trout strains throughout the assessment period. 17

Figure 1.6. Mean gravid lice loads on both steelhead trout strains throughout the assessment period. 19

Figure 1.7. Lice sex ratio recorded on both steelhead trout strains over the assessment period. . 20

Figure 1.8. Lice life stage ratio recorded on both steelhead trout strains over the assessment period. 21

Figure 1.9. Mean daily temperatures at AquaFort between November 28, 2020 and May 5, 2021. 22

Figure 1.10. Relationship between mean lice loads of Trout Lodge fish and mean daily temperature of the sampling day, r^2= 0.16. 22

Figure 1.11. Relationship between mean lice loads of Riverence fish and mean daily temperature of the sampling day, r^2= 0.28. 23

Figure 2.1. Cages affixed to the platform under the Pier. 34

Figure 2.2. Two different hide designs displayed within cages. 35

Figure 2.3. Identifying *Caligus* lunule circled in red. Species: *Caligus curtus*. 37

Figure 2.4. Male (left) and female (right) *Caligus elongatus*. Note the difference in abdomen width. 38

Figure 2.5. Two egg strands (circled in red) hanging off of an adult female *Caligus elongatus*. 38

Figure 2.6. Daily mean water temperature for each of the trials. 41

Figure 2.7. Trial 1 Survival rate of trout and lumpfish throughout the five weeks in 2020. 41

Figure 2.8. Mean biomass per trout in each cage at the beginning and end of Trial 1 43

.... 44

Figure 2.9. Trial 1 mean biomass per lumpfish in each cage at the beginning and end of the trial. 44

Figure 2.10. Trial 2 mean biomass per trout in each cage at the beginning and end of the trial.. 45

Figure 2.11. Trial 2 mean biomass per lumpfish in each cage at the beginning and end of the trial. 46
Figure 2.12. Trial 1 mean weekly lice loads of all trout (orange) and all lumpfish (blue) 55
Figure 2.13. Trial 1 mean weekly lice loads of trout by hide design 56
Figure 2.14. Trial 1 overall mean lice loads of trout by hide design. 57
Figure 2.15. Trial 1 mean weekly lice loads of trout by cleanerfish treatment 58
Figure 2.16. Trial 1 overall mean lice loads of trout by cleanerfish treatment 59
Figure 2.17. Trial 1 mean weekly lice loads of lumpfish by hide design treatment 60
Figure 2.18. Trial 2 mean weekly lice loads of all trout (orange) and all lumpfish (blue), ... 63
Figure 2.19. Trial 2 mean weekly lice loads of trout by hide design 64
Figure 2.20. Trial 2 overall mean lice loads of trout by hide design 65
Figure 2.21. Trial 2 mean weekly lice loads of trout by cleanerfish treatment 67
Figure 2.22. Trial 2 overall mean lice loads of trout by cleanerfish treatment 67
Figure 2.23. Trial 2 mean weekly lice loads of lumpfish by hide design treatment 69
Figure A. 1. Male (left) and female (right) *Caligus elongatus* 87
Figure A.2. *Caligus elongatus* sea lice 87
Figure A. 3. *Lepeophtheirus salmonis*. 88
Figure B.1 Mean lice on steelhead trout between October 18, 2018 and December 14, 2018. 91
Figure B.2. Mean lice and mean gravid lice on steelhead trout between June 10, 2019 and July 16, 2019 92
Figure C.1. Cages affixed to the platform under the Pier. 94
Figure C.2. Two different hide designs tested within cages. 96
Figure C.3. Mean daily temperatures during the Kamloops preliminary caging trial. 98
Figure C.4. Survival rate of steelhead trout (blue line) and lumpfish (orange line) throughout the Kamloops preliminary caging trial. 98

ABSTRACT

Sea lice are copepodid ectoparasites that infect fish, and cost salmonid farmers millions of dollars each year in damaged product and mitigation efforts. Conventional treatments can unintentionally impact the ambient environment and lead to the lice developing resistance to the treatments. In recent years, lumpfish (*Cyclopterus lumpus*) have been utilized successfully to naturally clean sea lice from infected salmonids in European and Atlantic Canadian farms, however this technology has yet to be used in US farms. In New Hampshire coastal waters, New Hampshire Sea Grant and the University of New Hampshire operate an experimental steelhead trout (*Oncorhynchus mykiss*) farm, AquaFort. To understand seasonal occurrence of lice populations at AquaFort and which lice species are present, steelhead were subsampled weekly for sea lice during farm use throughout 2019-2021. Lice abundance, species present, sex ratio, life stage, and occurrence of gravid females were determined. Lice loads (mean lice per fish) peaked on January 19, 2020, at 3.60 lice per fish, and the dominant species observed was *Caligus elongatus* (n=930) though some individuals of *Caligus curtus* were observed (n=9). Female lice made up 74% of the lice population throughout the assessment, and adults made up 87% of all lice observed throughout the assessment. The lice loads of gravid females peaked on February 18, 2021, at 2.20 gravid lice per fish.

To understand how lumpfish could mitigate sea lice infestations, small, *in situ*-experimental cages were stocked with different treatments of steelhead trout (strain), lumpfish (presence, absence), and lumpfish hide designs (kelp, PVC panels). Water temperature, fish survival, lice loads, and lumpfish stomach contents were analyzed throughout two 5-week trials to examine lumpfish impacts on sea lice loads. In both caging trials, hide design affected mean lice loads on trout, with lower lice loads in cages containing kelp hides, and in one trial, lice loads were lower in cages containing lumpfish versus no cleanerfish, however, there was no evidence of sea lice within lumpfish stomachs at the end of each trial. Water temperature and lumpfish size differed between the two trials suggesting that cleanerfish size, hide design, and water temperature are key variables to consider for effective sea lice control. These foundational studies contribute towards developing best practices of lumpfish use for sea lice mitigation on steelhead trout, such as when to implement lumpfish to maximize their welfare and cleaning capabilities. This will ultimately lead towards the goal of increasing the sustainability and production of steelhead trout aquaculture in NH coastal waters.

INTRODUCTION

Global aquaculture

Aquaculture is a growing global industry. Currently, almost half of all seafood consumed around the world comes from aquaculture, but by 2030, aquaculture production is expected to increase by 32% from 2018, producing an estimated 109 million tons of food per year (FAO, 2020). While estimates vary, the Food and Agriculture Organization (FAO) of the United Nations states that by 2030 aquaculture will likely account for 54% of the world's seafood (including products not for human consumption) production, outproducing wild fisheries for the first time. Currently, fish supplies more than 20% of the average per capita consumption of animal protein for over 3 billion people (FAO, 2020). The importance of ensuring the sustainable growth of aquaculture as a food source is illuminated by the stagnation or decline of landings of various wild fisheries (FAO, 2020), and the ever-increasing human population, expected to reach 8.5 billion people by 2030 (UN-DESA Population Division, 2019), increasing pressure on global food resources.

Aquaculture in the U.S. and salmonids

Reflective of the global trend, aquaculture production in the United States has been increasing, yet the U.S. currently ranks only 17th in world aquaculture production (NMFS, 2021). While many U.S. coastal communities have a rich history of fishing and seafood harvest, the United States has a seafood trade deficit because about 90% of what the nation consumes is imported from foreign markets. In 2019, this seafood trade deficit totaled $16.9 billion (NMFS, 2021). The most imported finfish category in the United States is salmon, and Atlantic salmon (*Salmo salar)* represents the majority of imported salmon (NOAA Fisheries, 2019; NMFS, 2021). The native range of Atlantic salmon includes the Northeastern United States, however due to habitat disruption, pollution, and over-exploitation, wild populations are low, with many having been completely extirpated for decades (Parrish et al., 1998). As a result, in the United States, Atlantic salmon are prohibited from both commercial and recreational fishing. In addition to Atlantic salmon being the most imported finfish in the U.S., it is also the most valuable finfish grown in the U.S., with a nation-wide value of $66.5 million produced in 2018 (NMFS, 2021). The global aquaculture industry has helped meet the high consumer demand for Atlantic salmon by producing 2.4 million metric tons of Atlantic salmon in 2018 (FAO, 2020).

Farmed salmonids are the largest traded fish commodity by value (FAO, 2020), with Atlantic salmon being the greatest contributing species. Another species, rainbow trout (*Oncorhynchus mykiss*) in freshwater or steelhead trout when in saltwater, ranks 15th in finfish global production by weight, with a total of 848,000 metric tons produced globally in 2018 (FAO, 2020). *O. mykiss* has been cultured in the United States since the 1870's (Hardy, 2002). Salmonid hatcheries in the U.S. first existed to stock eggs and fry into freshwater systems across North America, and eventually provided eggs for hatcheries in other continents (NOAA Fisheries, 2021).

Sea lice in aquaculture

Disease is a major issue for all forms of agriculture, including aquaculture. One of the greatest challenges currently facing salmonid farmers, in particular, is the prevalence of parasitic sea lice. Sea lice are parasitic copepods that feed on the skin, mucus, and blood of fish. As they feed, the lice cause wounds in the fish that are open to infection, damage the muscle, and cause stress, which all lead to further health problems, a decline in growth rate, and even mortality to the host (Bjordal, 1994).

The common species of sea lice in the North Atlantic are *Lepeophtheirus salmonis* and *Caligus elongatus*. While both species infect salmonids, *L. salmonis* parasitizes salmonids more than other fish families. *C. elongatus* is more of a generalist parasite, infecting more diverse groups of fishes throughout the oceans (Costello, 2006). Because the early developmental stages of the louse are planktonic, the louse will float freely through two nauplius stages until it has developed into a copepodid (Hemmingsen et al., 2020). The copepodid is the first parasitic stage at which point it will identify and attach onto a new host and begin to parasitize it as it continues its development into a chalimus. In *C. elongatus*, there are four chalimus stages before the louse develops into an adult (Hemmingsen et al., 2020). The generation time of *C. elongatus* at 10 °C is estimated to be about 35 days (Hemmingsen et al., 2020). In areas with open water salmon production, the high density of fish can promote a rapid and costly infestation by the ectoparasite (Frazer et al., 2012). Costing hundreds of millions of dollars each year, this is the greatest cost facing salmon aquaculture (Costello, 2009).

Sea lice solutions

To combat sea lice damage to farmed fish, farmers have used a number of methods in their salmonid operations. Historically, chemotherapeutics in the form of antibiotic feeds and parasiticide baths were used, though many of these have been banned in salmon producing countries (Hemmingsen et al., 2020; Mavraganis et al., 2020). Currently, in other countries, the most common chemical treatments still in use include avermectins, benzoyl ureas, hydrogen peroxide, organophosphates, and pyrethroids (Hemmingsen et al., 2020) which range from bath treatments to in-feed treatments; all of these chemicals are prohibited in U.S. waters. Many chemotherapeutics have been found to cause damage to non-target species in the environment (Haya et al., 2005), and sea lice can eventually become resistant to the chemical treatments

(Denholm et al., 2002; Lam et al., 2020). In addition to chemotherapeutics, farms use alternative treatments to decrease the abundance of sea lice. Longer fallow periods before stocking ocean cages help keep infestations low for a time (Pike and Wadsworth, 1999). Snorkel cages are open water cages that are designed to keep salmonids below the water's surface layer, while providing access to air in the center of the cage via a "snorkel". These are effective at reducing sea lice abundance (Oppedal et al., 2017) but these cage designs are only effective if the farm has access to deep-water sites. Cages with lice skirts are widely used, with a "skirt" of copper or other materials, encircling the cage to prevent planktonic lice from entering the cage area. Additionally, some farms utilize freshwater bath treatments which effectively remove lice; however, some researchers are concerned that lice might develop a resistance to freshwater (Groner et al., 2019). Some farms utilize thermal treatments and laser treatments as well (Lekang et al., 2016). While these treatments can help mitigate parasitic infestations, they are costly. Another method being utilized by salmonid farms in Atlantic Canada and Europe is biological delousing by cleanerfish; this treatment is currently being researched in the United States.

Cleanerfish are species that exhibit mutualistic cleaning behavior and will remove parasites and pests from other organisms, and their use in salmonid aquaculture is a proven effective way to combat *L. salmonis* and *C. elongatus* (Bjordal, 1988; Deady, et al., 1995; Skiftesvik et al., 2014; Imsland et al., 2018). Wrasse species such as the goldsinny wrasse, *Ctenolabrus rupestris,* and ballan wrasse, *Labrus bergylta,* have been identified as mutualistic cleaners, and their effectiveness in salmonid aquaculture has been known for a number of decades (Bjordal, 1988). Wrasse used as cleanerfish operate best in temperate waters (Sayer and Reader, 1996) and do not feed as effectively in cold waters, however, the wrasse species commonly used by European salmon farms are not native to the western Atlantic. In addition to wrasses, another cleanerfish has been identified more recently and put to effective use in salmonid aquaculture: the lumpfish, *Cyclopterus lumpus* (Imsland, et al., 2014). Because *C. lumpus* is native to both the western and eastern North Atlantic Ocean, feeds in cold waters, and is relatively easy to hatch and rear in intensive culture (Brown, 1989), it is of great interest to the aquaculture industry in North American and Europe.

Lumpfish biology, ecology, and human use

Lumpfish are found throughout the North Atlantic, from New Jersey, U.S. to Greenland in the west and Iceland in the North. In the east, they are found from Spain to Norway (Treasurer, 2018). Lumpfish are not very strong swimmers and do not possess a swim bladder. They are equipped, however, with a set of modified pelvic fins that act as a suction disk, allowing them to attach to surfaces and conserve energy (Hvas et al., 2018). In the wild, they might attach to a rocky structure or macro algae. Juvenile lumpfish have two effective strategies for foraging (Killen et al., 2007). One strategy is to suction onto a substrate and wait for prey to come to them, which is often employed when prey is plentiful. The other strategy is to expend energy and actively swim in search of prey. In either case, suctioning to a solid surface is necessary and provides a place to rest and conserve energy for active foragers, or a place to "sit and wait" for prey.

Although adult lumpfish typically remain in pelagic waters around 50-60 meters deep (Davenport, 1985), in the spring, sexually mature lumpfish about three or four years of age or older move inshore to spawn in shallow, rocky substrate. During this time, males turn a bright orange or red and find a suitable nesting site. Females will lay up to three clutches of eggs, which males then fertilize. Females can lay anywhere from 100,000 to 400,000 eggs in a spawning season (Treasurer, 2018). Males will then protect the fertilized egg clutches until hatched, which can take 20 to 40 days depending on temperature (Treasurer, 2018). Females do not remain at the nest after spawning and eventually return offshore. It is unclear how many spawning seasons an individual female will participate in, as survival rates drop after spawning (Kasper et al., 2014; Treasurer, 2018). Throughout egg development, in addition to guarding the clutches from predators, male lumpfish will fan their fins and ventilate water through the egg clutches, keeping the eggs oxygenated and clean. When the lumpfish hatch from the eggs, they are reliant on their yolk sac for their first few days but otherwise appear more juvenile than larval. Younger fish are more likely to forage in macroalgae and eat small copepods and amphipods. As they grow, their prey becomes more diverse and larger, inclusive of small fish, ctenophores, and larger crustaceans (Davenport, 1985; Moring, 1989).

Fisheries for *C. lumpus* exist in Norway, Greenland, Iceland, and Canada (Kennedy et al., 2019), but not in the United States, even though they are commonly found in the Gulf of Maine. Traditionally the fisheries existed almost entirely for the fish's roe which is used as an alternative to sturgeon caviar. Interest in lumpfish aquaculture started due to the popularity of the fish's roe (Brown et al., 1992; Martin-Robichaud, 1992), however today, lumpfish aquaculture is propelled by the demand for cleanerfish by salmon farms, and adult fish are now harvested to supply broodstock for hatcheries. Lumpfish also are relatively easy to rear. Lumpfish hatcheries in Norway, U.K., Iceland, and Canada produced a combined yield of over 40 million fish in 2018 (Fairchild, pers. comm.), with just over 31 million lumpfish being deployed in Norwegian salmonid farms alone in 2018 (Norwegian Directorate of Fisheries, 2021). However, even with the advances in hatchery practices, more work needs to be done on captive breeding in order to reduce the recurring annual need for wild caught broodstock, including optimizing insemination techniques, disease management, and trait selection. (Powell et al., 2018).

Lumpfish as a biological delouser

C. lumpus are effective cleanerfish in salmonid aquaculture and are capable of removing up to 97% of mature female sea lice from farmed Atlantic salmon (Imsland et al., 2018). Juvenile and subadult lumpfish are the most effective delousers; the ideal size for deploying individuals in ocean cages is anywhere from 20-140g (Imsland et al., 2016; Imsland et al., 2021), though lumpfish may continue to be effective cleaners between 140 g to 250 g (Imsland et al., 2016). Based on stomach content analysis of lumpfish already deployed in salmon cages, Eliasen et al. (2018) found that larger lumpfish consumed less sea lice and more of the salmon feed. This apparent relationship between lumpfish size and sea lice consumption reinforces what Imsland et al. (2016) concluded using a large-scale farm as a testing site: smaller lumpfish are better cleaners.

To optimize delousing abilities, recommended *C. lumpus* stocking density in commercial *S. salar* cages is about 10-15% of the salmonid density (Imsland et al., 2014). Additionally, lumpfish prefer cool water (<16 °C), making them ideal cleanerfish for colder regions (Nytro et al., 2014; Mortensen et al., 2020) or for colder months when wrasse species may not be as effective. Lumpfish also require “hides” which are typically plastic plates or artificial kelp curtains suspended in the cage. The hides create suitable surface area within the ocean cages for lumpfish to attach to with their sucker and rest on when not foraging and are necessary to promote *C. lumpus* welfare (Imsland et al., 2014; Conlon, 2019). Commercial farms utilizing lumpfish often use large curtains of fake kelp, which can be costly (Conlon, 2019). Several studies have tested the effects of different types of hides (Imsland et al., 2014; Conlon, 2019), and have found that hide preference among lumpfish initially skew towards thin plastic sheeting, but after 48 hours there is no difference in hide preference (Imsland et al., 2014). Additionally, studies show that for hide coloration, lumpfish prefer black hides over white, blue, and green hides (Imsland et al., 2014). Though these studies do provide information on hide utilization, more research is needed to further explore hide designs and lumpfish behavior.

Justification and introduction to thesis chapters

As aquaculture grows, collecting sea lice data in areas where salmonids are currently growing in marine cages, such as New Hampshire, is important for understanding how lice infestations occur and how they can be managed locally. And, as the use of lumpfish as biological delousers of salmonids expands, more research is needed to understand delousing practices so lumpfish can be implemented in the United States, specifically in NH waters. The following chapters detail a sea lice assessment on steelhead trout and potential use of lumpfish as a delouser in NH waters. Chapter One provides a 30-week assessment of sea lice on an experimental salmonid farm in coastal NH, and identifies some characteristics of sea lice infestations on steelhead trout raised through a winter and spring productions cycle. In Chapter Two, the cleaning capabilities of lumpfish on steelhead trout are evaluated in a series of small *in situ* caging experiments in NH waters. Local sea lice monitoring, better understanding lumpfish use in steelhead trout aquaculture, and further exploration of hide designs can all help ensure sustainable aquaculture practices in the Gulf of Maine.

CHAPTER 1: AN ASSESSMENT OF SEA LICE INFESTATIONS AT AN EXPERIMENTAL STEELHEAD TROUT AQUACULTURE FARM IN COASTAL NEW HAMPSHIRE

Introduction

As demand for seafood continues to climb, and landings from wild fisheries remain steady, aquaculture continues to grow. Between 2001 and 2018, global aquaculture production grew by an average of 5.3% each year (FAO, 2020). Atlantic salmon (*Salmo salar*) by weight was the 9th top cultured finfish in the world in 2018 (FAO, 2020). Additionally, it is the most valuable marine finfish reared in the U.S, with a nation-wide value of $66.5 million produced in 2018 (National Marine Fisheries Service, 2021). Lower on the list of globally produced finfish is the steelhead trout (*Oncorhynchus mykiss*), which ranks 15th in finfish global production by weight. While *O. mykiss* has been cultured in the United States since the 1870's (Hardy, 2002), much of the past trout farming techniques were for stocking freshwater systems with rainbow trout for angling, as well as providing a valuable food fish. Like Atlantic salmon, marine culture of steelhead trout has increased with advances in technology and feed formulations (Hardy, 2002).

Parasitic sea lice are the most expensive challenge facing salmonid farmers, costing hundreds of millions of dollars to the industry each year (Costello, 2009). The parasites cause damage to product by attaching and consuming tissue of the salmonid. This can reduce the fish's growth, induce stress, and leave the fish open to secondary infection (Bjordal, 1994). Additionally, if lice loads are high enough, they can induce mortality of the host fish (Bjordal, 1994). Damage to the visible exterior of the host fish can also lead to public relations concerns and difficulties with marketing the product. can cause concerns. Sea lice hatch from egg stands, and begin their life in a planktonic stage, known as a nauplius. As the larval louse develops, it grows into a copepodid stage, and becomes parasitic. If the copepodid identifies and attaches to a host fish, it will continue its development into a chalimus louse, of which there are multiple stages. After the chalimus stage, the louse develops into a sexually mature adult (Costello, 2006).

There are numerous species of parasitic sea lice which infect finfish. The common species in the North Atlantic are *Lepeophtheirus salmonis* and *Caligus elongatus*. While both species infect salmonids, *L. salmonis* parasitizes salmonids more than other fish families. *C. elongatus* is more of a generalist parasite, infecting more diverse groups of fishes throughout the oceans (Costello, 2006), and is one of the most common species observed in Atlantic aquaculture operations (Hemmingsen et al., 2020). *Caligus clemensi* is found on a diverse number of fish species in the North Pacific. *Caligus rogercresseyi* and *Caligus teres* are both found in the South Pacific Ocean. *Caligus curtus,* often called the cod louse, is found in the North Atlantic Ocean, and infects gadids, although it has been observed on a variety of other fishes such as salmonids and elasmobranchs (Hemmingsen et al., 2020). While many species impact salmonid farms, *L. salmonis* is exceptionally damaging to the fish it parasitizes, in part due to its large size (Hemmingsen et al., 2020). Concentrations of this species are also regulated within some salmon farming regions (Abolofia et al., 2017). For example, Norway requires monitoring of sea lice infestations and requires farms to treat lice if the number of adult female *L. salmonis* reaches 0.5 lice per fish (Abolofia et al., 2017). *L. salmonis* is typically found in the North Atlantic and North Pacific oceans. In the Gulf of Maine, sea lice have been monitored in the Bay of Fundy (Hogans and Trudeau, 1989; Hemmingsen et al., 2020) and in Maine waters by Atlantic salmon farms (Cooke Aquaculture Inc., data unavailable to the public). In NH waters, only sporadic and recent (2018, 2019) sea lice sampling has occurred by UNH (Fairchild, unpublished data). Not much is known about the presence and occurrence of lice species in NH coastal waters over a full salmonid growing season. Temperature is considered a major factor in lice infestations, with cooler water contributing to slower growth in sea lice and lower infection levels (Hemmingsen et al., 2020). In addition to temperature, lice infestations are also impacted by host density. Higher host densities will lead to a higher risk of infestation (Frazer et al., 2012). Salinity plays a ole in lice infestations as well, as lice are sensitive to lower salinities (Frazer et al., 2012).

In Maine, there are currently 24 leased ocean farm sites available for marine salmonid production (MDMR 2021), half of which are unused due to historically high sea lice concentrations (J. Robinson, 2019). In New Hampshire, there are currently no active commercial sites for marine salmonid production; however there is an active lease for an experimental steelhead trout aquaculture farm off of New Castle, NH. The farm, known as "AquaFort," is located in approximately 7.6 meters of water (at low tide) and consists of two 454 m^3 cages, each cage measuring 6.1 m x 6.1 m x 12.2 m. AquaFort is operated by New Hampshire Sea Grant and designed to produce 13,608 kg of steelhead trout, 4,536 kgs of blue mussels, and spools of sugar kelp in an Integrated Multi-Trophic Aquaculture (IMTA) system (NHSG, 2021). AquaFort has been used since 2018, but succeeded smaller UNH aquaculture projects focused on steelhead trout for approximately 15 years (Chambers, pers. comm.).

Even at a small-scale, salmonid farms may face challenges with parasitic sea lice. As a result, sea lice sampling strategies for the UNH farm were developed in 2018, but due to truncated growing seasons, lice were only monitored over five weeks in 2018 and five weeks in 2019 (Appendix B). Thus, the effects of sea lice on the steelhead trout farm have never been assessed throughout an entire production run spanning multiple seasons. Additionally, lice populations in the southern Gulf of Maine are not well known. This chapter focuses on a multi-year, broad assessment of sea lice at the NH steelhead trout farm to inform farm managers and other potential finfish operations of the local lice populations both at AquaFort, but also in the larger southern Gulf of Maine region.

Methods

Sea lice were assessed on steelhead trout at AquaFort off the coast of New Castle, NH (43-04'6" N, 70-42'31" W) between November 3, 2020 and June 11, 2021.

Trout

Two strains of steelhead trout, Trout Lodge and Riverence, were tested for their abilities to quickly acclimate from freshwater to seawater. The names of each strain refer to the companies that produced the eggs. The fish were transferred from Sumner Brook Hatchery in Ossipee, NH and approximately 3,000 individuals of each strain were stocked into separate cages in the AquaFort system between October 21-23 (Trout Lodge) and October 23-27 (Riverence), 2020. Trout Lodge fish were sampled for sea lice weekly unless weather conditions prevented access to the farm site, whereas Riverence fish were only sampled monthly due to the increased effort required to capture them (see below for further details).

Temperature

Temperature was collected every 30 minutes between November 28, 2020 and May 5, 2021 with a HOBO data logger (Onset Computer Corp.) positioned approximately one meter below the surface within the AquaFort cage. Daily temperature means were calculated and used for data analysis.

Sea lice collection

Lice were sampled from Trout Lodge fish weekly with the actual sampling day dependent on tide, ocean conditions, and steelhead feeding schedules. The UNH R/V *Red Cloud* was loaded with an Xactic™ (Ontario, Canada) filled with freshwater treated for chlorine and heavy metal. After leaving the UNH pier, the vessel was tied to AquaFort. A long dipnet was then used to collect a total of fifteen steelhead from the net pen. Each fish was placed in an individual 19-L bucket half filled with the treated freshwater for approximately two minutes. After two-minute exposure to freshwater, sea lice infecting the fish will release and fall off into the bucket. After that time period, the fish were gently placed back into the net pen. After each fish was returned to the net pen, the contents of each freshwater bath were poured through the 180-µm sieve. The contents of the sieve were then poured into a 473-mL plastic storage container, and stored for transfer back to CML for analysis. The same sea lice sampling protocol was used to sample the Riverence fish monthly with the exception that sometimes a seine net was needed to collect the fish in addition to the dipnet because the fish occupied deeper zones within the net pen.

Sea lice assessment

At the Coastal Marine Laboratory (CML), the contents from each fish sampling were examined the same day under an Olympus SZ61 stereomicroscope at 6.7x magnification and the number, sex, species, and estimated life stage of all sea lice found were recorded. Sea lice were differentiated between *Caligus spp.* and *Lepeophtheirus salmonis* by the presence of lunules (Figures 1.1, 1.2). Sex was identified (Figure 1.3) in adult and young adults by observing the relative width of the abdomen as male lice have a relatively thinner posterior region than females

(Hemmingsen, et al. 2020). Gravid females also were noted and counted (Figure 1.4). Lastly, life history stage was estimated and recorded based on louse body shape and size (Hemmingsen et al. 2020).

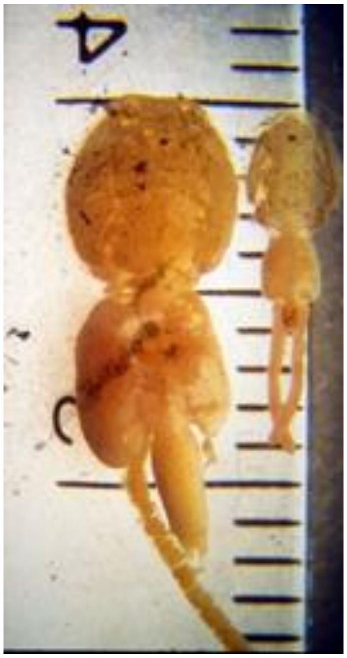

Figure 1.1. A gravid, female *Caligus elongatus* (right) alongside a gravid, female *Lepeophtheirus salmonis* (left). Photo: M. Pietrak, USDA.

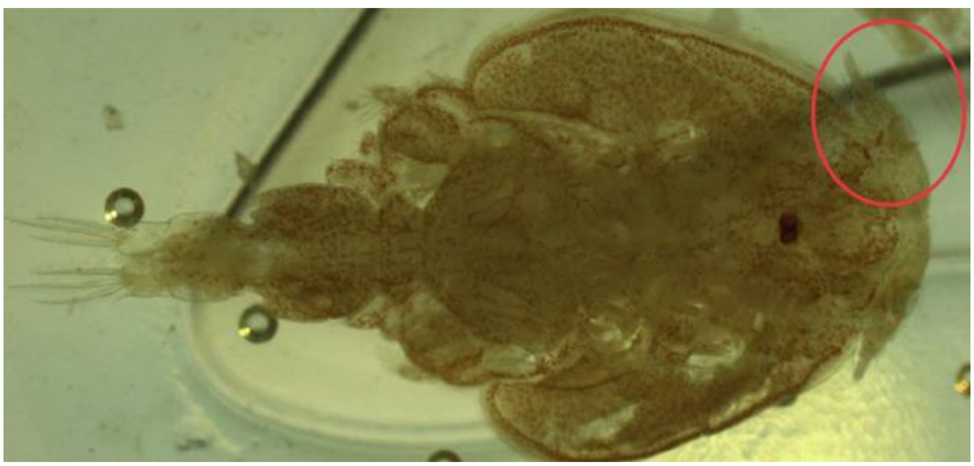

Figure 1.2. Identifying *Caligus* lunule circled in red. Species: *Caligus curtus*.

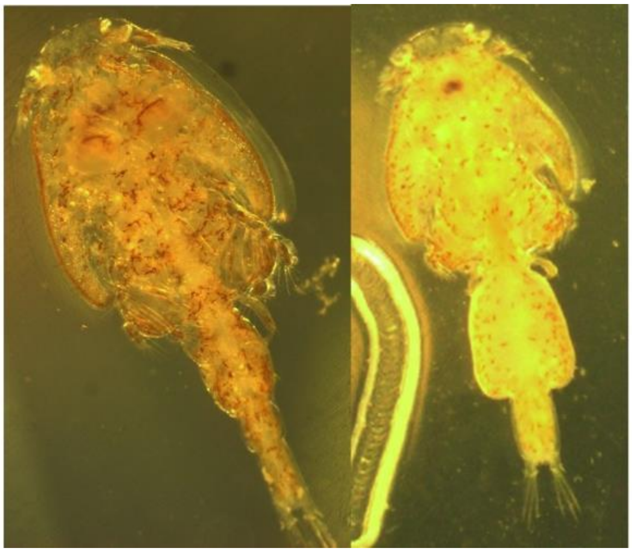

Figure 1.3. Male (left) and female (right) *Caligus elongatus*. Note the difference in abdomen width relative to the cephalothorax.

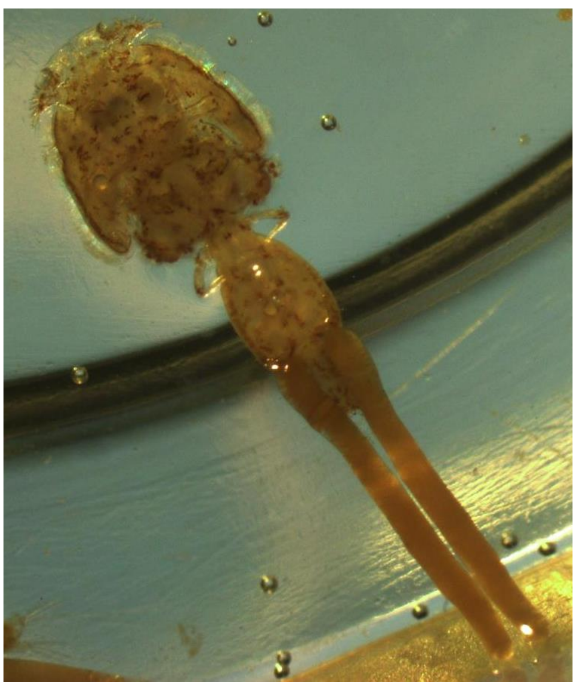

Figure 1.4. Egg strands hanging off of an adult female *Caligus elongatus*.

Statistical analysis

Data were analyzed using JMP Pro 15. Mean lice loads were calculated as the mean number of lice per steelhead trout for each sampling of 15 fish. Lice loads on steelhead strains were compared using student's t-test. An alpha value of 0.05 was used to indicate significance. A linear regression model was used to examine correlations between mean daily temperatures and mean lice loads.

Results

Mean lice load

Weekly (30 sampling dates) lice assessments of Trout Lodge fish ranged from 0.13 to 3.60 lice per fish. There was an increase of lice loads from the beginning of the assessment (11/03/2020) to the peak of 3.60 lice per fish on January 19 (Figure 1.5, Table 1.1). Lice loads decreased after the peak until April 28, 2021, when lice loads started to increase again. Monthly (6 sampling dates) assessments on Riverence fish ranged from 0.47 to 1.93 lice per fish. The mean lice loads on Riverence fish increased from December 2020 to February 24, 2021, when it peaked at 1.90 mean lice per fish. Following this peak, mean lice loads declined to April 28, and there was an increased mean lice load on the final sampling date of June 11 (Figure 1.5, Table 1.2).

Gravid females

Mean lice loads of gravid females followed similar trends as the total mean lice loads per steelhead trout throughout the assessment. The mean lice load of gravid females on Trout Lodge fish ranged from 0.00 to 2.20 gravid female lice per fish (Figure 1.6, Table 1.1). The mean lice load of gravid females on Riverence fish ranged from 0.27 to 0.80 gravid female lice per fish (Figure 1.6, Table 1.2).

Strain Comparison

Of the six sampling days when both Trout Lodge and Riverence fish were sampled for lice, Trout Lodge fish regularly had more sea lice present than Riverence fish (Figure 1.5), with a mean lice load of 1.85 lice per Trout Lodge fish over all sampling dates, compared to a mean lice load of 1.16 lice per Riverence fish over all sampling dates. However, overall there were no statistically significant differences between the mean lice loads on each steelhead strain (paired t-test, $t(10)= 1.89$, $p=0.08$).

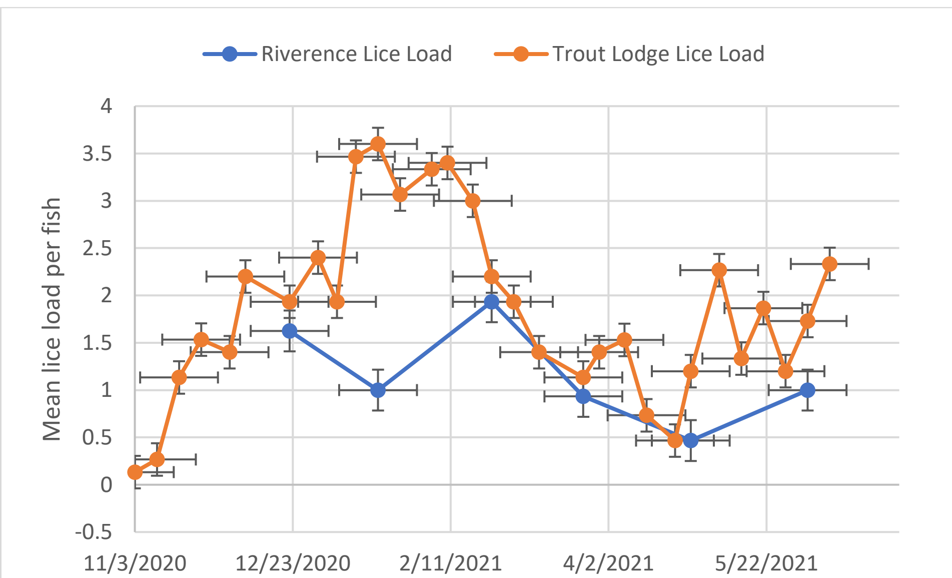

Figure 1.5. Total mean lice loads per fish for both steelhead trout strains throughout the assessment period. Error bars represent standard error.

Table 1.1. Weekly sea lice assessment data for Trout Lodge fish (n=15 each week). sampled November 3, 2020, to June 11, 2021.

Week	Date	Total Lice	Mean Lice Load	Mean Gravid Lice Load
1	11/3/2020	2	0.13	0.13
2	11/10/2020	4	0.27	0.00
3	11/17/2020	17	1.13	0.27
4	11/24/2020	23	1.53	0.47
5	12/3/2020	21	1.40	0.40
6	12/8/2020	33	2.20	0.73
7	12/22/2020	29	1.93	1.07
8	12/31/2020	36	2.40	1.40
9	1/6/2021	29	1.93	0.93
10	1/12/2021	52	3.47	1.40
11	1/19/2021	54	3.60	1.53
12	1/26/2021	46	3.07	1.67
13	2/5/2021	50	3.33	1.60
14	2/10/2021	51	3.40	1.87
15	2/18/2021	45	3.00	2.20
16	2/24/2021	33	2.20	1.53
17	3/3/2021	29	1.93	1.00
18	3/11/2021	21	1.40	0.87
19	3/25/2021	17	1.13	0.93
20	3/30/2021	21	1.40	1.00
21	4/7/2021	23	1.53	1.07
22	4/14/2021	11	0.73	0.53
23	4/23/2021	7	0.47	0.33
24	4/28/2021	18	1.20	0.47
25	5/7/2021	34	2.27	1.13
26	5/14/2021	20	1.33	0.80
27	5/21/2021	28	1.87	1.33
28	5/28/2021	18	1.20	1.07
29	6/4/2021	26	1.73	1.33
30	6/11/2021	35	2.33	0.50

Table 1.2. Weekly sea lice assessment data for Riverence fish (n=15) sampled December 22, 2020 to June 4, 2021.

Week	Date	Total Lice	Mean Lice Load	Mean Gravid Lice Load
1	12/22/2020	26	1.63	0.80
2	1/19/2021	15	1.00	0.27
3	2/24/2021	29	1.93	0.80
4	3/25/2021	14	0.93	0.47
5	4/28/2021	7	0.47	0.27
6	6/4/2021	15	1.00	0.47

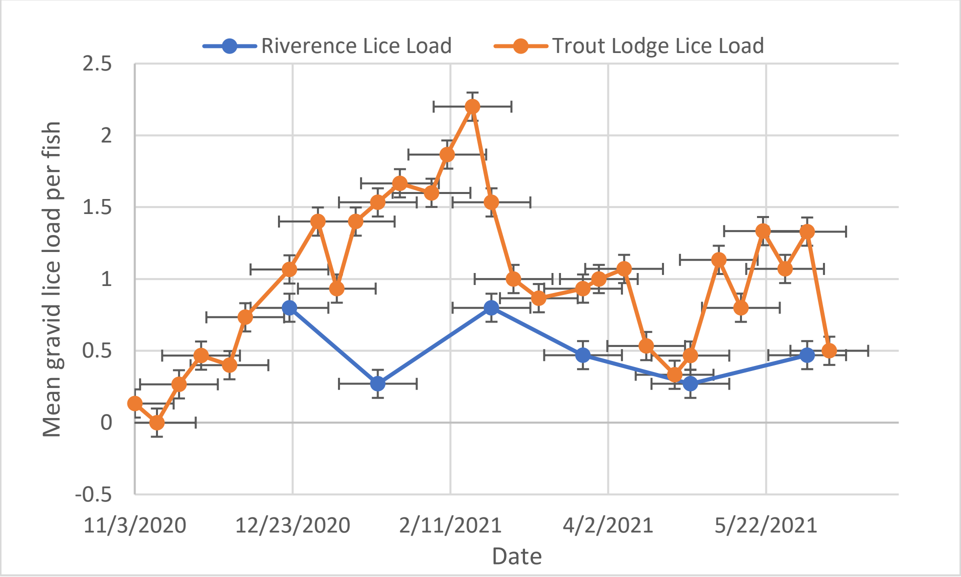

Figure 1.6. Mean gravid lice loads on both steelhead trout strains throughout the assessment period. Error bars represent standard error.

Lice species, sex, and life stage

The primary lice species observed throughout this assessment among both steelhead trout strains was *C. elongatus*. *L. salmonis* individuals were not observed on either of the steelhead strains. On the Trout Lodge fish, 99% of lice recorded (n=824) were *C. elongatus*, and 1% (n=9) were *C. curtus*. On Riverence fish, 100% of the lice recorded were *C. elongatus* (n=106). Throughout the assessment, female lice were more prevalent than male lice on fish. Of adult sea lice on Trout Lodge fish, 73% were female and 27% were male (Figure 1.7). Similarly, of adult sea lice on Riverence fish, 74% were female and 26% male (Figure 1.7). Adult lice made up the majority of lice observed. Of all sea lice observed on Trout Lodge fish, 88% were adults and 12% were pre-adult stages (Figure 1.8). Of all sea lice observed on Riverence fish, 85% were adults and 15% were pre-adult stages (Figure 1.8).

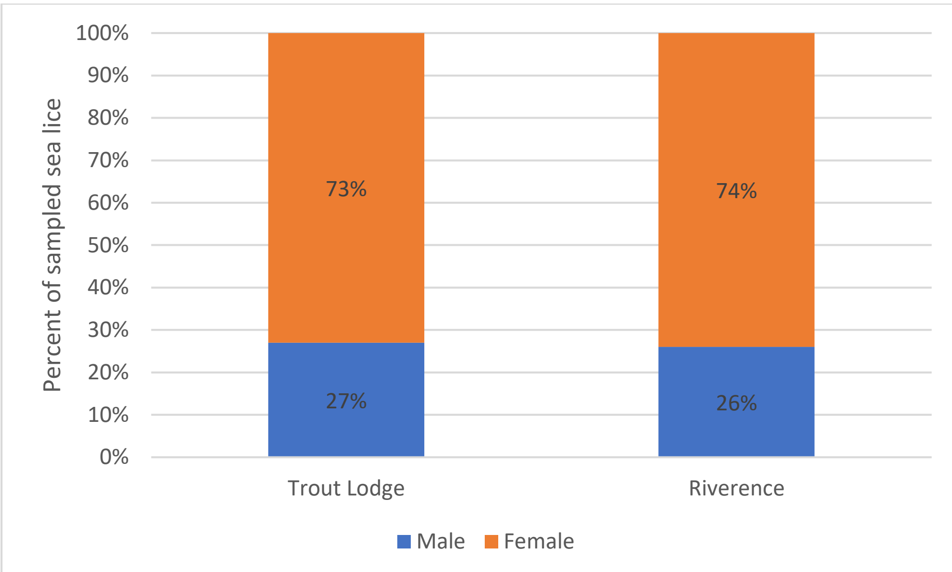

Figure 1.7. Lice sex ratio recorded on both steelhead trout strains over the assessment period.

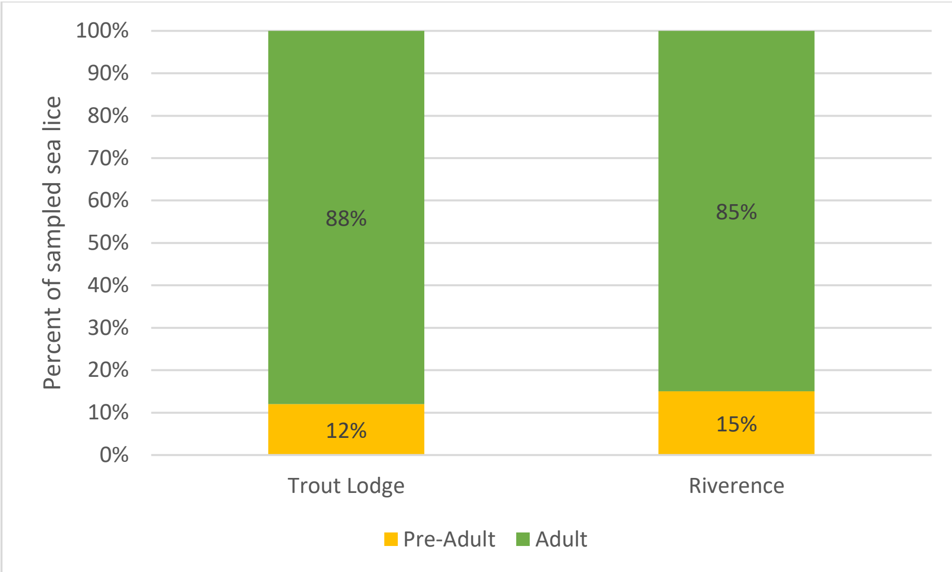

Figure 1.8. Lice life stage ratio recorded on both steelhead trout strains over the assessment period.

Temperature

Mean daily temperatures at AquaFort ranged from 2.8 °C to 8.8 °C between November 28, 2020, and May 5, 2021 (Figure 1.9). There was a significant correlation between an increased temperature and a decreased lice load during this time period for Trout Lodge fish ($p=0.04$, Figure 1.10), but not for Riverence fish ($p=0.28$, Figure 1.11).

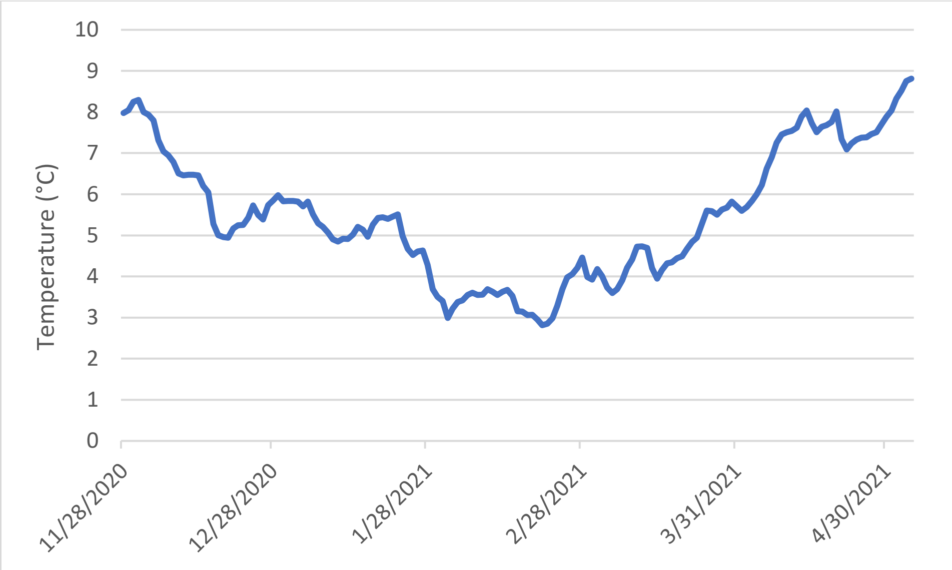

Figure 1.9. Mean daily temperatures at AquaFort between November 28, 2020 and May 5, 2021.

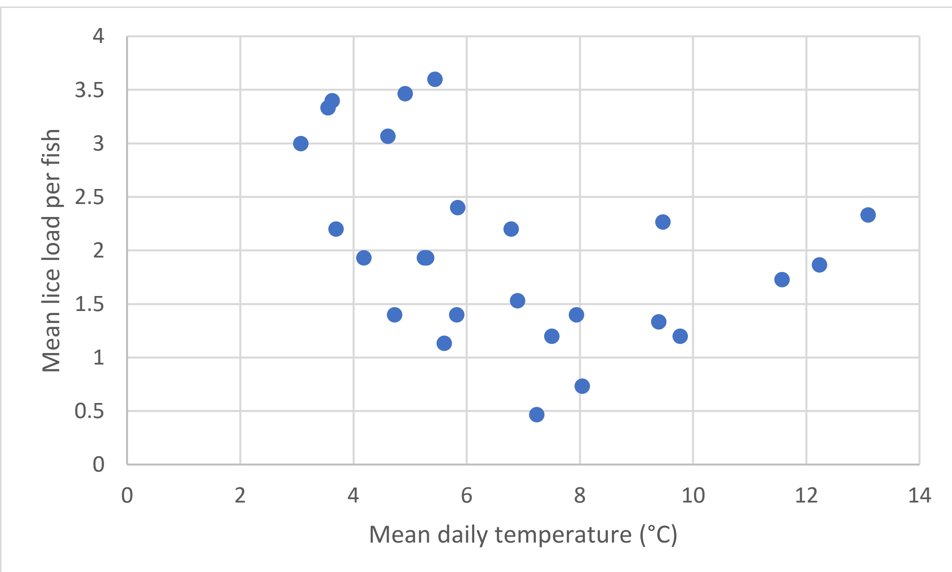

Figure 1.10. Relationship between mean lice loads of Trout Lodge fish and mean daily temperature of the sampling day, r^2= 0.16.

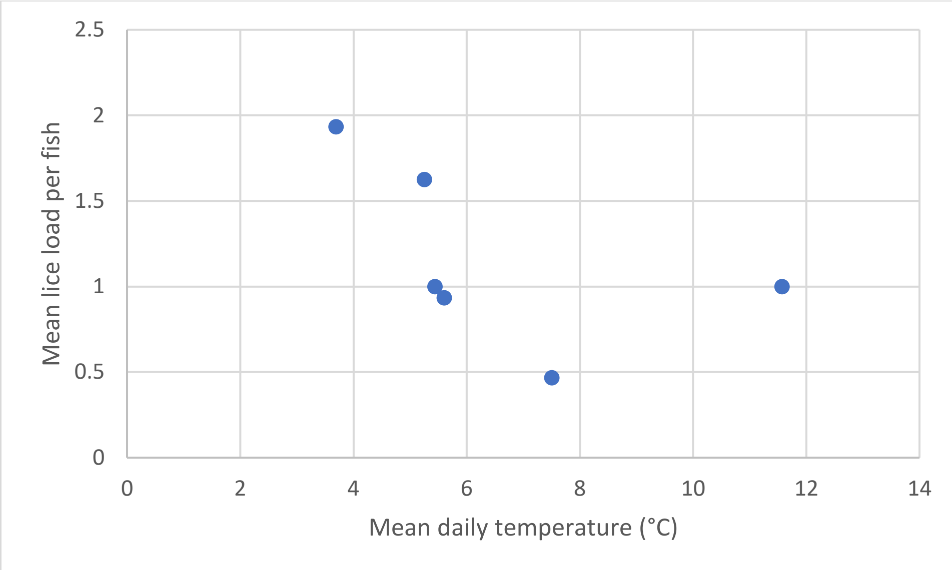

Figure 1.11. Relationship between mean lice loads of Riverence fish and mean daily temperature of the sampling day, r^2= 0.28.

Discussion

Temperature and mean lice loads

Sea lice loads fluctuated at the New Hampshire steelhead trout farm AquaFort throughout the production run in the absence of lice treatment (Figure 1.5). Both Trout Lodge and Riverence steelhead strains were infected primarily by female, adult *Caligus elongatus,* and to a lesser extent, male or subadult *C. elongatus.* Of lice observed in this assessment, only 12%-15% were either copepodid or chalimus stage lice (Figure 1.8). The majority of lice observed were adult stage lice.

Sea lice infestations are influenced by a variety of factors including salinity, depth, host density, host species, and water temperature. For example, *L. salmonis* is less likely to survive and infect Atlantic salmon if salinity is below 29 ppt (Bricknell et al., 2006). Fish species might also impact how a louse is able to attach or develop on its host. For example, in a lab study, lice development and retention were lower on Chinook salmon than on Atlantic salmon (Bui et al., 2018). Though this assessment focused only on a single species, it does raise questions about comparisons between two genetic strains of steelhead trout in the context of sea lice loads. In this assessment, comparisons between strains are difficult to make because of the small number of Riverence sampling dates in relation to Trout Lodge sampling dates. Lastly, water temperature has a major influence on sea lice infestations (Hemmingsen et al., 2020). The optimal water temperature of *C. elongatus*, the predominant louse species observed in this assessment, is 14 °C (Hogans and Trudeau, 1989; Hemmingsen et al., 2020). Lower water temperatures will prolong the early life stages of the louse. In this assessment, lice loads increased as temperatures decreased (Figure 1.10, Figure 1.11). While unexpected, this pattern may be attributed to when the steelhead trout production run began. Fish were stocked in mid-autumn, and grow-out took place over the winter. As temperatures dropped after stocking, lice loads increased independently of temperature because of the sudden presence of a concentration of approximately 6,000 host fish. Maximum lice loads were observed in January and February, then began to decline, even as water temperatures began to warm. In April, lice loads began to increase, and were highest May-June with the maximum recorded levels in early June 11 for both steelhead strains. Mean daily water temperatures recorded ranged from 2.8 °C to 8.8 °C between December 3, 2020 and June 11, 2021. These temperatures remained lower than the ideal temperature of 14 °C laid out by Hogans and Trudeau (1989), which indicates that host density was more of a factor in *C. elongatus* infestations than temperature throughout the increasing lice loads between November, 2020 and January, 2021.

Hogans and Trudeau (1989) found that *C. elongatus* loads on Atlantic salmon in the Bay of Fundy were at their peak in the month of October (1988), reaching as high as 47 lice per fish, but with a mean of 18 lice per fish (Hogans and Trudeau, 1989; Saksida et al., 2015). The steelhead trout examined in this assessment were stocked in October of 2020, and sampling did not occur until November 3, 2020, which yielded the smallest lice loads (0.13 lice per fish) seen throughout the season. AquaFort holds a smaller population of salmonids compared to the commercial farms examined by Hogans and Trudeau (1989), and therefore smaller lice loads should be expected based on host density. However, considering temperature, an October stocking period can be expected provide temperature regulation to maximum lice load intensity, as the peak (3.6 lice per fish) observed in the AquaFort assessment took place in January, 2021, when ocean temperatures are consistently cool (less than 6 °C). This indicates that an October stocking period, and winter production run can contribute to lower lice load intensities.

Norway regulates sea lice infestations in salmonid farms with treatment occurring when lice loads exceed 0.5 adult female lice per fish, but only *L. salmonis* and not *C. elongatus*. However, if the 0.5 adult female lice per fish threshold applied to *C. elongatus* in NH, treatment would have been required for 23 out of the 30 weeks that Trout Lodge fish were sampled between November 3, 2020 and June 11, 2021 (Table 1.1). This species is not regulated as such however, due to the relatively less substantial damage done to infected salmonid hosts compared to damage done by *L. salmonis.* Though this lice assessment does indicate lice loads are lower than past studies (Hogans and Trudeau, 1989) if salmonids are grown through a winter production run, sea lice preventative strategies or treatments may still be necessary.

While there were no significant differences in lice loads between the two strains of steelhead trout sampled throughout the assessment period (Figure 1.5), it is possible that strains of steelhead trout could be selected for traits that promote resistance to sea lice. An examination of Atlantic salmon resistance to sea lice identified three quantitative trait loci that influenced resistance to the lice species *C. rogercresseyi* (Robledo et al., 2019). While the two strains of steelhead being grown in AquaFort were being compared by NH Sea Grant to compare successful acclimation to salt water, it is difficult to compare lice loads because of the lower number of sampling dates conducted for the Riverence strain, versus the Trout Lodge strain. Further exploration of these genetic effects should be completed in steelhead trout, and the information could be used to develop more lice-resistant strains of salmonids.

Species observed

Sea lice observed on cage farmed steelhead trout in coastal New Hampshire waters was dominated by *Caligus elongatus*, much like previous findings in the Bay of Fundy (Hogans and Trudeau, 1989), in which *C. elongatus* made up 91% of the sea lice observed. Similar to sea lice species composition in the Bay of Fundy where *C. curtus* were present but had a negligible presence (0.07%; Hogans and Trudeau, 1989), 1% of lice recorded (n=9 lice) at AquaFort were *C. curtus*. However this assessment differs from that of Hogans and Trudeau (1989) in that *L. salmonis* were not present in NH whereas they made up 8% of lice observed in the Bay of Fundy. Although *L. salmonis* was not observed throughout this assessment, the species is present in the Gulf of Maine, infects farmed salmon in Maine and Canada, and parasitizes wild fishes like the three-spined stickleback (*Gasterosteus aculeatus*) in the region (Pietrak et al., 2019). Sampling and analyzing sea lice should be a regular process in any salmonid aquaculture site to better understand environmental risks associated with lice and to guide treatment of lice infestations.

Conclusion

Our assessment revealed that in NH waters, *C. elongatus* is the dominant sea lice species infecting farmed steelhead trout, and to a much lesser degree, *C. curtus*. To date, *L. salmonis* has not been observed in NH waters, though this species is present and has been recorded in the Gulf of Maine. This assessment provides a broad, but important look into sea lice populations and infestations on steelhead trout in NH waters over the course of 30 weeks. Lice loads should continue to be monitored in any open-water marine finfish farming operation in NH waters. Collecting these data allows for responsible decision-making regarding fish stocking strategies and sea lice treatment options. For example, because the maximum lice load observed in this assessment was far below previous maximum observations in the Gulf of Maine (Hogans and Trudeau, 1989) and was recorded in January, it might be inferred that stocking steelhead trout in mid-fall and growing them out through a winter and spring season for an early summer harvest holds potential to avoid larger sea lice infestations. If lice loads begin to increase in warmer water, as seen between April and June in this assessment, treatment options can be planned and employed in early spring before lice infestations intensify. Continued monitoring of lice loads can also contribute to a better understanding of lice populations in the Gulf of Maine and can help manage risk associated with finfish aquaculture and sea lice in any future operations.

Future sampling efforts should continue as detailed above, however; valuable information could be gained by expanding the sampling strategy. Incorporating plankton tows around the farm site to sample for planktonic nauplii and copepodid stages of sea lice would provide additional information about the dynamics of sea lice infestations within an active farm site. Similar studies conducted in Scotland (McKibben and Hay, 2004) and in the Bay of Fundy, Canada (Nelson et al., 2018) to record larval *L. salmonis* around farm sites can help shape future studies looking at planktonic *C. elongatus* in NH waters. These data could provide valuable baseline records for lice treatment options that might target planktonic lice not only entering the farm, but hatching from within the farm (Figure 1.6). All new and continued monitoring of sea lice in NH waters would help inform farmers and managers of how best to mitigate sea lice at all life stages. Promoting sustainable aquaculture practices through minimizing sea lice infestations are an important step towards a healthy, sustainably growing aquaculture industry.

CHAPTER 2: EXAMINING THE USE OF LUMPFISH AS A CLEANERFISH OF STEELHEAD TROUT IN EXPERIMENTAL AQUACULTURE PENS IN NEW HAMPSHIRE WATERS

Introduction

Aquaculture is growing as an industry and a major food source globally. Between 2001 and 2018, global aquaculture production grew by an average of 5.3% each year (FAO, 2020) while landings from wild fisheries have remained largely steady. United States aquaculture production, including both freshwater and marine products, was valued at $1.5 billion in 2018, an increase of 1.8% from 2017, according to the most recent survey statistics (NMFS, 2021). Marine aquaculture production was valued at $430 million, 41% of which originates from the U.S Atlantic coast. Atlantic salmon (*Salmo salar*) is the most valuable marine finfish grown in the U.S, with a nation-wide value of $66.5 million produced in 2018 (NMFS, 2021). U.S Atlantic salmon exports in 2018 were valued at $64.4 million, while the country imported $1.57 billion the same year (NMFS, 2021). This deficit contributes to the total seafood trade deficit of $16.8 billion in the U.S. While the U.S contributes about 4% of the world's seafood exports, it is the top seafood importer, responsible for 14% of the world's seafood imports (FAO, 2020). And though the domestic aquaculture industry has grown substantially in recent years, the U.S still only ranks 17th on the list of top aquaculture-producing countries (FAO, 2020; NMFS, 2021). Because of the high demand for, low supply of, and high value of salmonids like Atlantic salmon, there is potential for further growth of domestic salmonid production.

One of the greatest challenges facing salmonid farmers is the prevalence of parasitic sea lice. Sea lice are parasitic copepods that feed on the skin, mucus, and blood of the host fish. As they feed, the lice cause wounds in the fish that are open to infection, damage the muscle, and cause stress, which all lead to more health problems, a decline in growth rate, and even mortality to the host (Bjordal, 1994). The common sea lice species in the North Atlantic Ocean are *Lepeophtheirus salmonis* and *Caligus elongatus*. While both species infect salmonids, *L. salmonis* parasitizes salmonids more than other fish families, whereas *C. elongatus* is more of a generalist parasite, infecting more diverse groups of fishes throughout the oceans (Costello, 2006). These ectoparasites cost the global salmonid aquaculture industry hundreds of millions of dollars each year due to downgraded product quality and market price, and because mitigating sea lice infestations at the farm is so costly (Costello, 2009).

Farmers use a number of methods to prevent occurrence and severity of sea lice infestations in salmonid cage farms. In the past, chemotherapeutics in the form of parasiticide baths or in-feed treatments were most commonly used. The use of chemotherapeutics as lice treatments have led to resistance among sea lice (Denholm et al., 2002) as well concerns with parasiticides damaging non-target wild crustaceans (Haya et al., 2005). With these unintended effects of chemotherapeutics, most countries, including the US, have banned their use and alternative treatments have been employed to combat sea lice infestations. One example is using long fallow periods prior to stocking ocean cages. This strategy can help keep infestations low for a period (Pike and Wadsworth, 1999), though it contributes to lost revenue for the farms, and the benefits are temporary. Variations in cage designs have been explored to prevent sea lice from attaching to hosts. Cages with lice skirts are widely used, with a “skirt” of copper or other material encircling the cage to prevent planktonic lice from entering the cage area. Another example of cage designs includes snorkel cages. These are designed to keep salmon in deeper water and are effective at reducing sea lice abundance (Oppedal et al., 2017). Both of these designs act by separating the hosts from planktonic lice that are typically found in the upper layer of the water column. These methods can be costly, and if site conditions are not ideal for the barrier design, low dissolved oxygen within the cage barrier can become an issue (Barrett et al., 2020). Additionally, some farms utilize freshwater and thermal bath treatments which effectively remove lice. Freshwater treats fish infected with sea lice; however, some researchers are concerned that lice might develop a resistance to freshwater (Groner et al., 2019). Thermal baths are effective at removing lice, but cost and stress on the fish act as trade-offs for this method. Another strategy for dealing with sea lice is selecting for lice-resistance among salmonid strains (Barrett et al., 2020). This selective breeding method is being explored, but can be costly and time-consuming.

Though strategies for sea lice prevention and treatment are plentiful, many are expensive, or not yet developed in U.S marine aquaculture operations. However, another method being utilized by salmonid farms in Atlantic Canada and Europe is biological delousing by cleanerfish. Cleanerfish are simply a species of fish that will remove parasites off of another fish. This treatment is currently being researched in the United States, focused on lumpfish (*Cyclopterus lumpus)*. Lumpfish are native to the Gulf of Maine, and are a proven cleanerfish in salmonid farming operations (Imsland et al., 2018). When stocked into salmon farms, properly sized lumpfish are capable of removing up to 97% of mature female sea lice from the cages (Imsland et al., 2018) without negatively impacting the salmon (Imsland et al., 2014a). Based on European case studies integrating cleanerfish into Atlantic salmon farms, lumpfish should be utilized:

1. when 20-140g as juvenile and subadult lumpfish are the most effective delousers (Imsland et al., 2016; Eliasen et al., 2018; Imsland et al., 2021);
2. in colder regions or colder months (Mortensen et al., 2020) since lumpfish prefer water below 16 °C;

3. at about 10-15% of the salmonid density (Imsland et al., 2014b); and
4. include “hides” or lumpfish habitat to promote *C. lumpus* welfare (Imsland et al., 2014c; Conlon, 2019). Typically hides are plastic plates or artificial kelp curtains suspended in the cage to create suitable surface area for lumpfish to attach to with their sucker and rest on when not foraging.

Although Atlantic salmon are not reared in ocean cages in New Hampshire waters, another salmonid, steelhead trout (*Oncorhynchus mykiss*), have been raised in experimental cage systems by the University of New Hampshire (UNH) since 2013. Steelhead trout are advantageous to raise in ocean cages because they adapt to saltwater quickly, have fast growth rates, and can tolerate a wide range of temperatures (Chambers, 2013). Additionally, steelhead trout is in high demand, and marketed at $14.99 per pound (7/9/21; Seaport Fish Market, Rye, NH) or higher. There is great potential to commercialize and scale up steelhead trout production, boosting the economy of the coastal NH community, and relieving pressure from some overfished species by diversifying the domestic local seafood available. However, like Atlantic salmon raised in open ocean aquaculture cages, steelhead trout are affected by sea lice infestations and the use of cleanerfish to control the parasites could be beneficial and help grow the industry. Because all of the major research on utilizing lumpfish as cleanerfish has focused on Atlantic salmon, little is known about the expected interactions between steelhead trout and lumpfish. Therefore, there is a need to assess the cleaning behavior of lumpfish with steelhead trout as well as examining the use of hides in the sea cages and if they impact cleaning behavior or fish welfare. This chapter focuses on two trials of small cage experiments, testing the cleaning behaviors of lumpfish with steelhead trout, and the impact hide designs might have on lumpfish cleaning behavior.

Methods

Two cage trials evaluating the effectiveness of lumpfish as cleanerfish on two different strains of steelhead trout were conducted at the UNH Judd Gregg Marine Research Complex Pier in New Castle, NH (Table 2.1). Strains were selected because of the size and availability of fish for each of the trial periods. Both trials occurred over five weeks; the first trial took place from October 14 to November 18, 2020, and the second trial took place from November 23 to December 28, 2020.

Table 2.1. Trial dates and steelhead trout strains used.

Trial	Start Date	End Date	Number Sampling Days	Trout Strain	Cargill EWOS® feed size
1	10/14/2020	11/18/2020	5	Trout Lodge	8.0 mm
2	11/23/2020	12/28/2020	5	Riverence	8.0 mm

Fish source

Steelhead trout were acquired from Sumner Brook Fish Farm in Ossipee, NH and trucked to the Coastal Marine Laboratory (CML) in New Castle, NH a minimum of three weeks prior to the onset of the caging studies. Upon arrival to the CML, steelhead were transferred via dipnet from the truck to a 1.8-m diameter round acclimation tank supplied with flow-through, ambient sea water, oxygen, and air. Steelhead were handfed Cargill EWOS® 8.0 mm dry pellets twice daily until caging studies began. Different steelhead trout strains (Riverence, Trout Lodge) were kept in separate acclimation tanks. Existing, cultured lumpfish, reared and housed at the CML, were used in the caging trials.

Cages

Six, small (785 L, 1-m diameter x 1-m depth), cylindrical cages constructed with an HDPE plastic frame and 5 cm mesh netting were used as experimental units to evaluate the cleanerfish ability of lumpfish with steelhead trout. Cages were weighted at the bottom and lined with buoys along the top. The lids were hinged to allow easy access into the cage. Lids were secured with twist-ties to prevent fish egress or entry of predators. The cages were suspended in the water and secured in a bay under the UNH Judd Gregg Marine Research Complex Pier (Figure 2.1).

Figure 2.1. Cages affixed to the platform under the Pier.

Cage densities

At the beginning of each trial, each cage was stocked with 15 steelhead trout. The approximate density of 0.004 to 0.005 kg/L was chosen (Table 2.2) so that fish density would be high enough to promote schooling behavior yet not exceed the carrying capacity of the cages. Four of these six cages also were stocked with three lumpfish each. Although stocking lumpfish at 15% density in relation to the salmonid density is an effective approach to elicit cleanerfish behavior (Imsland et al., 2014b), Lumpfish were stocked at 20% density to offset any lumpfish mortality. The remaining two cages served as controls and were void of lumpfish (Table 2.2).

Table 2.2. Experimental design of the trials including initial fish stocking densities.

Cage #	Trout	Lumpfish	Hide Design	Trout Lodge Stocking Density (kg/L)	Riverence Stocking Density (kg/L)
1	15	3	Kelp	0.00420	0.00499
2	15	3	PVC	0.00510	0.00487
3	15	0	None	0.00434	0.00454
4	15	3	PVC	0.00440	0.00478
5	15	0	None	0.00436	0.00510
6	15	3	Kelp	0.00427	0.00532

Hides

In the four cages containing lumpfish, two designs of lumpfish hides were evaluated: fake kelp and PVC panels (Table 2.2). The fake kelp hide design was a 10 mm polyester rope with strips of full weight black plastic sheeting (80 cm X 5.5 cm) woven through to mimic macroalgae. The PVC hide design was made of a 76 cm length of 10-cm diameter PVC pipe cut down the middle and bolted together to form a “w” shaped panel. Both hide designs were securely tied to the cages at the top and bottom to minimize the pull of the current (Figure 2.2). Cages were randomly affixed to the floating platform under the Pier.

Figure 2.2. Two different hide designs displayed within cages.

Stocking cages

For each trial, steelhead trout were loaded into an oxygen supplied Xactic™ filled with seawater and trucked from the CML to the Pier. From there, batch weights (kg) were taken of 15 steelhead per batch in tared buckets with sea water. The bucket then was lowered to the float and the fish were emptied into a single cage. This process was repeated for all six cages. Lumpfish, weighed individually in the CML, were transported to the Pier in four 19-L buckets, and released into the four respective cages.

Trial protocols

Fish were fed twice daily at low tide. Steelhead trout were fed Cargill EWOS® 8.0 mm diet at 2%-2.5% body weight/day, slightly higher than the recommended 1.5% body weight/day (M. Chambers, pers. comm.), to compensate for currents and lost pellets. Lumpfish were fed 4.0 mm Skretting (Nutreco) Clean Assist diet at 2% body weight/day. Water temperature was recorded every 2 hours with HOBO data loggers (Onset Computer Corp.) positioned approximately 1 m deep within the cages. Because the Pier overhead shadowed a portion of the bay, one data logger was secured on the inside of the back-left cage, while the other was secured on the inside of the front-right cage, to account for any parameter differences.

Sea lice sampling

All fish were assessed for sea lice weekly. For each cage, every fish (including lumpfish when applicable) was removed with a dipnet, then immersed in an individual freshwater bath (19-L bucket filled halfway with freshwater) for 90-120 seconds to remove sea lice. Then the fish were transferred to a sea water holding tank until all fish had been processed from the cage. After all fish from a given cage were assessed, they were returned to their cage. Each freshwater bath sample was poured through a 180-µm sieve, and the collected contents within the sieve were washed with freshwater into a small (473-946 mL) plastic container. This process then was repeated for each of the remaining cages. The containers of the sieved contents of the freshwater baths were then brought back to the CML and processed either immediately or refrigerated and processed within 36 hours. Any dead fish were immediately removed from cages, sampled for lice, weighed, and stomach contents assessed.

Sea lice assessment

The contents from each sea lice sampling were examined under an Olympus SZ61 stereomicroscope at 6.7x magnification and the number, sex, species, and estimated life stage of all sea lice found were recorded. Sea lice were differentiated between *Caligus sp.* and *Lepeophtheirus salmonis* by the presence of lunules (Figure 2.3; González and Carvajal, 2003; Hemmingsen et al., 2020; Appendix A). Sex was identified (Figure 2.4) in adult and young adults by observing the relative width of the abdomen as male lice have a relatively thinner posterior region than females (Hemmingsen, et al. 2020). Gravid females also were noted and counted (Figure 2.5). Lastly, life history stage was estimated and recorded based on louse body shape and size (González and Carvajal, 2003; Hemmingsen et al., 2020).

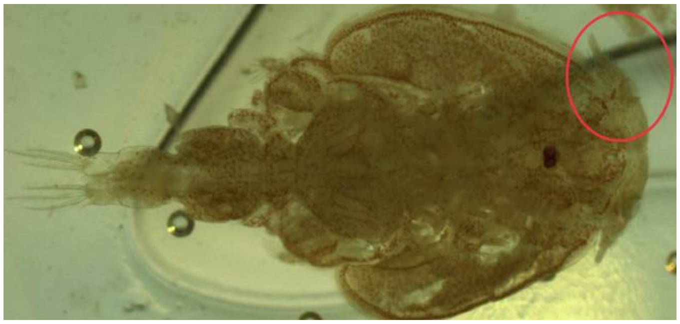

Figure 2.3. Identifying *Caligus* lunule circled in red. Species: *Caligus curtus*.

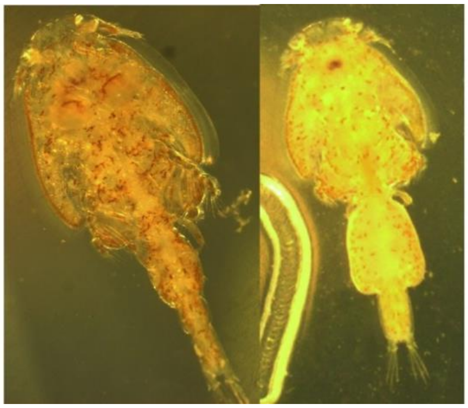

Figure 2.4. Male (left) and female (right) *Caligus elongatus*. Note the difference in abdomen width.

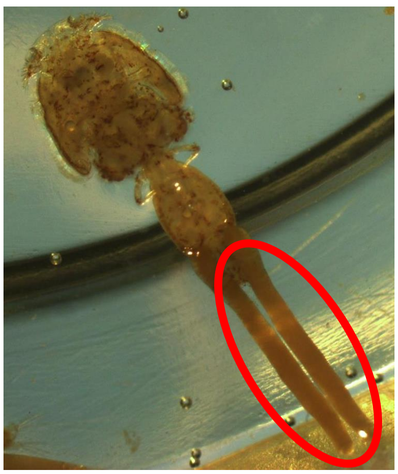

Figure 2.5. Two egg strands (circled in red) hanging off of an adult female *Caligus elongatus*.

End of trial

After the final sampling period, steelhead trout were batch-weighed, and then both steelhead and lumpfish euthanized with an overdose of tricaine methanesulfonate (MS-222). All lumpfish were dissected, in each trial, while only a subsample of two steelhead trout per cage were dissected in Trial 1, and three steelhead trout per cage in Trial 2. The fish were dissected either immediately after being euthanized, or frozen and dissected approximately one week later. The digestive tract was weighed, and the contents of the stomach identified, and stomach fullness (%) quantified based on a visual assessment.

Statistical analysis

Data were analyzed using JMP Pro 15. Fish mortality was tested against hide design using an analysis of variance (ANOVA) to compare the number of fish mortalities between the three hide groups (kelp, PVC, and control). An alpha value of 0.05 was used to indicate significance. Specific growth rate was calculated using the equation (Hopkins, 1992):

$$((\ln(\text{final weight}) - \ln(\text{initial weight})) / \text{time} * 100$$

A one-way ANOVA tested if the specific growth rate differed between hide groups. An alpha value of 0.05 was used to indicate significance. An analysis of gut contents included a calculation of the gastro-somatic index (GSI) of the fish. GSI was calculated using the equation:

$$(\text{gut weight} / \text{total weight}) * 100$$

Mean lice loads were calculated as the number of lice per fish. Lice load analyses were completed using a Generalized Linear Model (GLM) with normal distribution. Model selection using an all-subsets regression was conducted. The most parsimonious model was identified and selected as the model with the lowest corrected Akaike information criterion (AICc) value. Once the most parsimonious model was selected, results of F-tests were used on the individual variables to determine significance (alpha= 0.5). Lice loads were tested with the GLM to determine effects of sample week, hide design, cleanerfish treatment, and interactions. The GLM was also used to determine whether lice loads differed between steelhead trout and lumpfish.

Results

Water temperature and fish survival

Mean daily water temperature during Trial 1 between October 14 and November 18, 2020 fluctuated between 8.7 °C and 13.0 °C with a five week mean (± 1 s.d.) of 11.2 ± 1.4 °C (Figure 2.6). Mean daily water temperature during Trial 2 between November 23 and December 28, 2020 fluctuated between 3.3 °C and 11.9 °C with a five week mean of 6.1 ± 1.8 °C (Figure 2.6). Overall steelhead trout survival rate in Trial 1 was 94%, with a total of five mortalities, not including an additional escapee. Steelhead mortalities in Trial 1 were observed on October 16, November 4, November 8, November 9, and November 16. Overall lumpfish survival rate in Trial 1 was 67% with a total of four mortalities (Figure 2.7). Lumpfish mortalities were observed on October 28, November 4, November 9, and November 13. Neither lumpfish survival (one-way ANOVA, $p > 0.30$) nor steelhead trout survival (one-way ANOVA, $p = 0.54$) were significantly affected by hide design in Trial 1. Steelhead trout survival rate in Trial 2 was 99%, with a single mortality observed in an experimental kelp hide cage (Cage 1) on the final day of the trial. Lumpfish survival rate in Trial 2 was 100%.

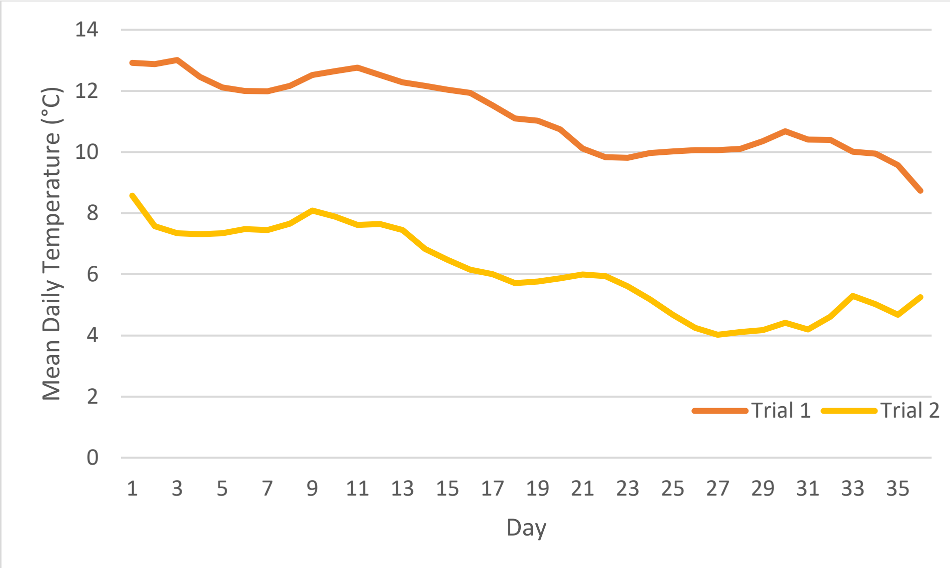

Figure 2.6. Daily mean water temperature for each of the trials.

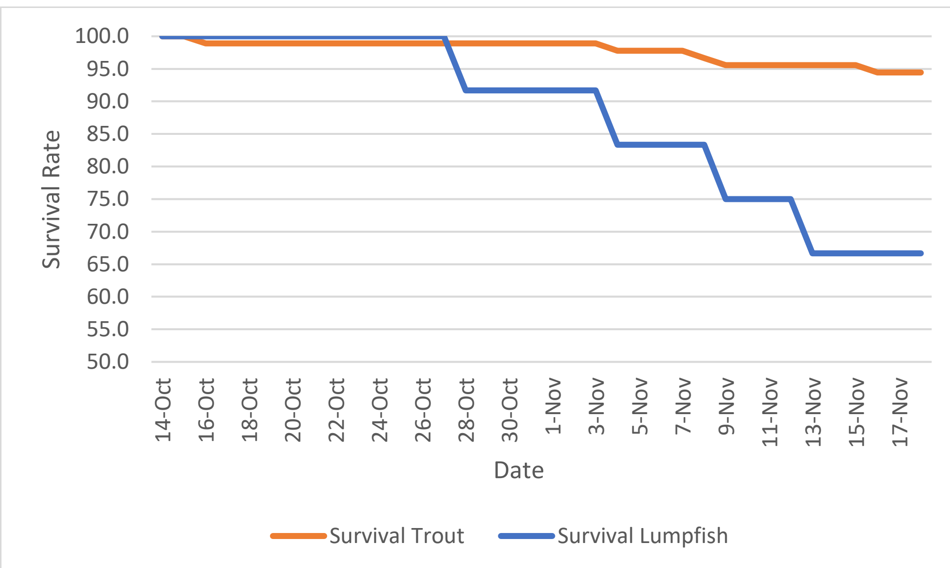

Figure 2.7. Trial 1 Survival rate of steelhead trout and lumpfish throughout the five weeks in 2020.

Fish growth

In Trial 1, mean biomass per steelhead trout increased in each cage (Figure 2.8). Total mean biomass per steelhead trout among all cages at the start and end of Trial 1 was 232.6 g and

316.2 g, respectively. The specific growth rate of Trial 1 steelhead trout ranged from 0.45 to 1.18, with a mean of 0.88 (Table 2.3). Mean biomass per lumpfish increased in two of the four cages containing lumpfish (Figure 2.9). Total mean biomass per lumpfish among all cages at the start and end of Trial 1 was 155.1 g and 177.4 g, respectively. The specific growth rate of lumpfish in Trial 1 ranged from -0.33 to 0.98 with a mean value of 0.39 (Table 2.4). Neither steelhead trout specific growth rate (one-way ANOVA, p=0.11) nor lumpfish specific growth rate (one-way ANOVA, p=0.97) was significantly affected by hide design in Trial 1.

In Trial 2, mean biomass per steelhead trout increased in each cage (Figure 2.10). Total mean biomass per steelhead trout among all cages at the start and end of Trial 2 was 258.1 g and 294.9 g, respectively. The specific growth rate of Trial 2 steelhead trout ranged from 0.34 to 0.52 (Table 2.5). Mean biomass per lumpfish increased in two of the four cages containing lumpfish (Figure 2.11). Total mean biomass per lumpfish among all cages at the start and end of Trial 2 was 301.5 g and 307.2 g, respectively. The specific growth rate of lumpfish in Trial 2 ranged from -0.08 to 0.18 (Table 2.6). Neither steelhead trout specific growth rate (one-way ANOVA, p=0.89) nor lumpfish specific growth rate (one-way ANOVA, p=1.00) was significantly affected by hide design in Trial 2.

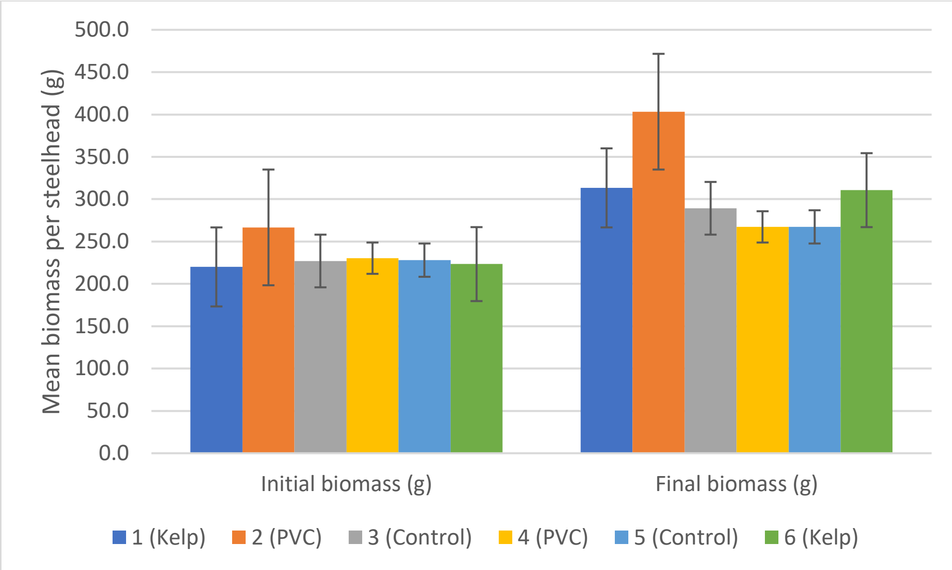

Figure 2.8. Mean biomass per steelhead trout in each cage at the beginning and end of Trial 1. Legend numbers refer to individually numbered cages, and hide design of the corresponding cage is described in parentheses. Error bars represent standard error.

Table 2.3. Trial 1 initial and final steelhead trout numbers and growth information.

	Initial		Final		
Cage	Number of Trout	Mean Biomass per Fish (g)	Number of Trout	Mean Biomass per Fish (g)	Specific Growth Rate
1 (Kelp)	15	220.0	15	313.3	1.01
2 (PVC)	15	266.7	15	403.3	1.18
3 (Control)	15	227.0	13	289.2	0.69
4 (PVC)	15	230.3	11	313.3	0.88
5 (Control)	15	228.0	15	267.3	0.45
6 (Kelp)	15	223.3	15	310.7	0.94
Totals/Means	**90**	**232.6**	**84**	**316.2**	**0.88**

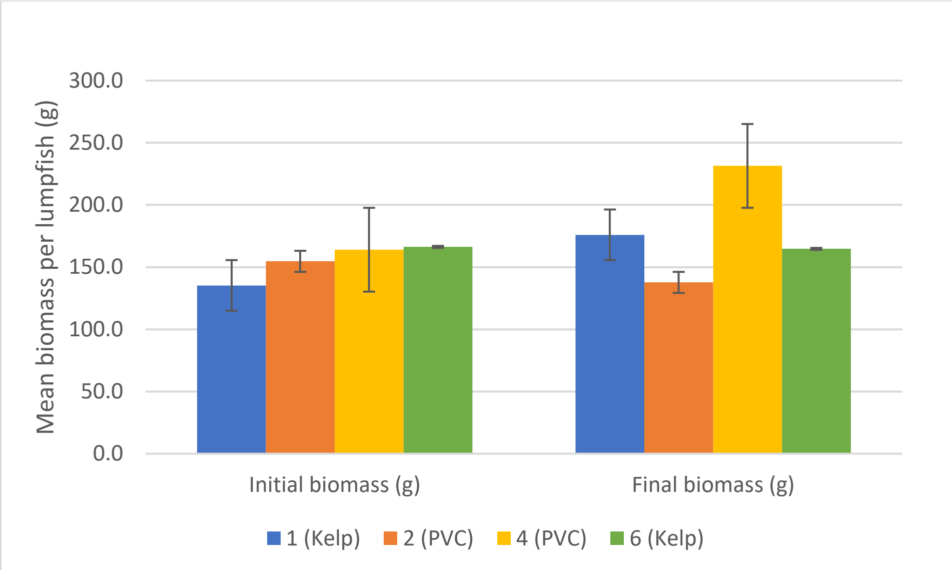

Figure 2.9. Trial 1 mean biomass per lumpfish in each cage at the beginning and end of the trial. Legend numbers refer to individually numbered cages, and hide design of the corresponding cage is described in parentheses. Error bars represent standard error.

Table 2.4. Trial 1 mean biomass per lumpfish in each cage at the start of the trial and at the end of the trial, with specific growth rate.

	Initial		Final		
Cage	Number of Lumpfish	Mean Biomass per Fish (g)	Number of Lumpfish	Mean Biomass per Fish (g)	Specific Growth Rate
1 (Kelp)	3	135.3	2	176.0	0.75
2 (PVC)	3	154.7	1	137.7	-0.33
3 (Control)	0	0.0	0	0.0	Not Applicable
4 (PVC)	3	164.0	2	231.4	0.98
5 (Control)	0	0.0	0	0.0	Not Applicable
6 (Kelp)	3	166.3	3	164.7	-0.03
Totals/Means	**12**	**155.1**	**8**	**177.4**	**0.39**

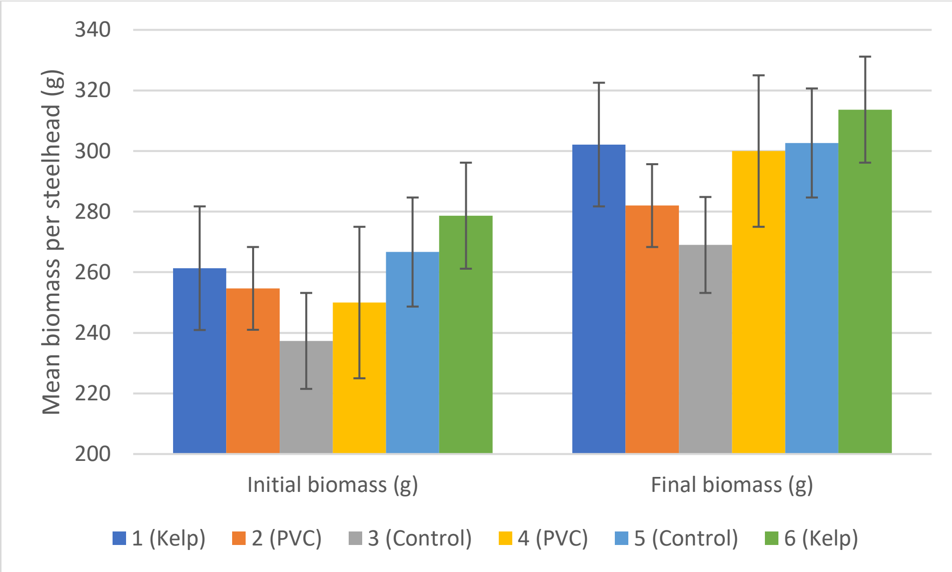

Figure 2.10. Trial 2 mean biomass per steelhead trout in each cage at the beginning and end of the trial. Legend numbers refer to individually numbered cages, and hide design of the corresponding cage is described in parentheses. Error bars represent standard error.

Table 2.5. Trial 2 mean biomass per steelhead trout in each cage at the start of the trial and at the end of the trial, with specific growth rate.

	Initial		Final		
Cage	Number of Trout	Mean Biomass per Fish (g)	Number of Trout	Mean Biomass per Fish (g)	Specific Growth Rate
1 (Kelp)	15	261.3	14	302.1	0.41
2 (PVC)	15	254.7	15	282.0	0.29
3 (Control)	15	237.3	15	269.0	0.36
4 (PVC)	15	250.0	15	300.0	0.52
5 (Control)	15	266.7	15	302.7	0.36
6 (Kelp)	15	278.7	15	313.7	0.34
Totals/Means	**90**	**258.1**	**89**	**294.9**	**0.38**

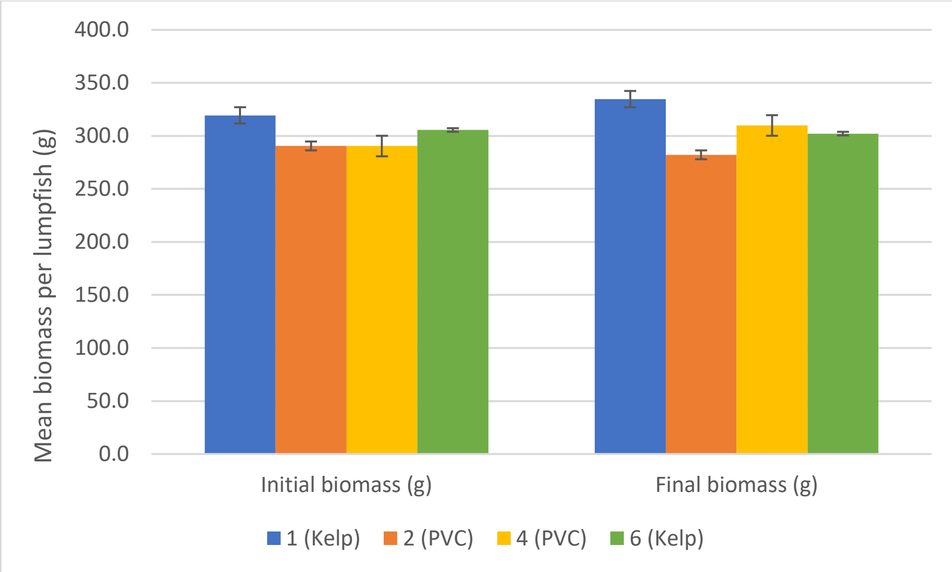

Figure 2.11. Trial 2 mean biomass per lumpfish in each cage at the beginning and end of the trial. Legend numbers refer to individually numbered cages, and hide design of the corresponding cage is described in parentheses. Error bars represent standard error.

Table 2.6. Trial 2 mean biomass per lumpfish in each cage at the start of the trial and at the end of the trial, with specific growth rate.

	Initial		Final		
Cage	Number of Lumpfish	Mean Biomass per Fish (g)	Number of Lumpfish	Mean Biomass per Fish (g)	Specific Growth Rate
1 (Kelp)	3	319.4	3	334.7	0.13
2 (PVC)	3	290.5	3	282.2	-0.08
3 (Control)	0	0.0	0	0.0	Not Applicable
4 (PVC)	3	290.4	3	309.8	0.18
5 (Control)	0	0.0	0	0.0	Not Applicable
6 (Kelp)	3	305.6	3	302.2	-0.03
Totals/Means	**12**	**301.5**	**12**	**307.2**	**0.05**

Gut contents and analysis

Trial 1: Of the twelve lumpfish dissected, 67% had stomachs that were at least half full, including 25% that had entirely full stomachs. Additionally, all lumpfish had pellets or traces of pellets in their stomachs, with 25% of lumpfish having clearly consumed steelhead trout pellets (Table 2.7). One lumpfish stomach contained Styrofoam debris. Sea lice were not present in any of the lumpfish stomachs in Trial 1. The gastro-somatic index (GSI) of the lumpfish ranged from 4.27 to 12.37 with a mean value (± 1 s.d.) of 8.84 ± 5.92. Of the eleven steelhead trout dissected, 64% had stomachs that were at least half full, including 27% with entirely full stomachs. Vegetation was found in 36% of the steelhead trout (Table 2.8). One steelhead trout stomach contained an unidentified bivalve shell fragment. Steelhead trout GSI in Trial 1 ranged from 4.64 to 13.0, with a mean value of 8.67 ± 7.03.

Trial 2: Of the twelve lumpfish dissected, 75% had stomachs that were at least half full, including 66.7% with entirely full stomachs. Additionally, 83% of lumpfish had pellets or traces of pellets in their stomachs (Table 2.9). One lumpfish stomach contained a clear, unidentified, jelly-like substance. Sea lice were not present in any of the lumpfish stomachs in Trial 2. Lumpfish GSI ranged from 4.82 to 13.05, with a mean value of 8.90 ± 10.69. Of the eighteen steelhead trout dissected, 73% had stomachs that were at least half full, including 19% with entirely full stomachs. Pellets were found in in the digestive tracts of 87% of the steelhead trout and vegetation in 47% (Table 2.10). Steelhead trout GSI ranged from 5.36 to 15.54, with a mean value of 10.17 ± 5.23.

Table 2.7. Trial 1 lumpfish gut contents.

Cage #	Fish Weight (g)	Fish Length (cm)	Gut Weight (g)	Gut Contents	Presence of Lice	% Fullness	GSI
1	143.2	n/a	6.12	small amount of pellets	no	20	4.27
1	205.62	16.5	21.56	full of pellets	no	100	10.49
1	146.38	15.0	15.97	full of steelhead trout pellets	no	100	10.91
2	203.6	n/a	10.71	pellets, steelhead trout pellets	no	75	5.26
2	127.72	n/a	10.12	pellets	no	50	7.92
2	137.74	15.5	13.47	some evidence of pellets	no	5	9.78
4	189.1	15.6	13.1	pellets, additional steelhead trout pellets	no	50	6.93
4	198.6	16.5	18.42	pellets	no	75	9.27
4	264.15	18.5	29.66	pellets, Styrofoam	no	100	11.23
6	161.13	16	19.93	pellets	no	60	12.37
6	159.7	17.5	16.99	pellets	no	10	10.64
6	173.21	16.5	12.08	some evidence of pellets	no	20	6.97

Table 2.8. Trial 1steelhead trout gut contents.

Cage #	Fish Weight (g)	Fish Length (cm)	Gut Weight (g)	Gut Contents	% Fullness	GSI
1	243.6	28	20.37	pellets	50	8.36
1	142.5	25.5	n/a	pellets	100	n/a
2	354.8	30	46.12	pellets	30	13.00
3	164.6	26	20.35	pellets	100	12.36
3	148.6	26.5	12.41	pellets, vegetation	25	8.35
4	116.3	24	6.49	pellets	10	5.58
4	165	27	11.18	half pellets, half vegetation	50	6.78
5	177.7	27.5	8.25	non identifiable	20	4.64
5	192.6	27	18.01	pellets	50	9.35
6	148.2	27	14.27	pellets, vegetation	100	9.63
6	143.1	27	n/a	pellets, vegetation, shell fragment	50	n/a

Table 2.9. Trial 2 lumpfish gut contents.

Cage #	Fish Weight (g)	Fish Length (cm)	Gut Weight (g)	Gut Contents	Presence of Lice	% Fullness	GSI
1	280.3	20	18.8	pellets	no	90	6.71
1	422.7	21.6	43.2	pellets	no	100	10.22
1	301.2	20.0	20.4	pellets	no	90	6.77
2	243.7	18.5	26.5	pellets	no	80	10.87
2	260.8	18.9	24.9	pellets	no	20	9.55
2	342	20.6	16.5	liquid/bile	no	10	4.82
4	429.4	22.5	44.1	pellets	no	100	10.27
4	319.3	20	20.3	clear jelly	no	30	6.36
4	180.9	16.6	23.6	pellets	no	80	13.05
6	288.6	19.6	32.1	pellets	no	100	11.12
6	290.8	19.5	24.5	pellets	no	90	8.43
6	327.1	20	28.3	pellets	no	100	8.65

Table 2.10. Trial 2 steelhead trout gut contents.

Cage #	Fish Weight (g)	Fish Length (cm)	Gut Weight (g)	Gut Contents	% Fullness	GSI
1	298.1	30.2	37.6	pellets	70	12.61
1	183.7	26.1	18.9	pellets and vegetation	90	10.29
1	243.3	26.8	37.8	pellets	100	15.54
2	326	30.2	41.8	pellets	90	12.82
2	390.4	31	48	pellets	30	12.30
2	314.2	30.4	32.6	pellets	50	10.38
3	142.5	26.5	8.2	vegetation	20	5.75
3	144.6	25.3	7.9	pellets and vegetation	40	5.46
3	162.3	25.4	8.7	vegetation	25	5.36
4	318.3	29.7	47.2	pellets	80	14.83
4	270.1	29.7	33	pellets	100	12.22
4	180	27.5	14.9	vegetation	60	8.28
5	130.1	22.9	16	pellets	70	12.30
5	448.5	33.1	59.3	pellets	100	13.22
5	137.8	24.8	10.4	possible digested pellets	5	7.55
6	300.7	31	34.5	pellets	60	11.47
6	170.5	27	10.1	vegetation	20	5.92
6	132.1	24.9	9	vegetation	10	6.81

Mean sea lice loads

The overall mean lice load (the mean number of lice per fish per sampling period) on steelhead trout between all cages in Trial 1 ranged from 0.07 to 0.70 lice per fish (Table 2.11). The overall mean lice load on lumpfish between all cages in Trial 1 ranged from 0.33 to 2.04 lice per fish (Table 2.11). For the comparison of mean lice loads between lumpfish and steelhead in Trial 1, the most parsimonious model retained the species, week, and species by week interaction variables (Table 2.12). The GLM F-tests for each of the retained variables revealed lice loads were not significantly different between lumpfish and steelhead trout ($p= 0.58$) throughout Trial 1. There was, however, a strong weekly effect ($p< 0.001$) on lice loads, as well as a strong interaction ($p=0.01$) between fish species and week (GLM, AICc=140.21, Table 2.12, Table 2.13, Figure 2.12).

For the comparison of mean lice loads on steelhead between hide design treatments in Trial 1, the most parsimonious model retained the hide design and week variables, and did not retain the interaction variable between hide design and week (Table 2.14). The GLM F-tests of these retained variables revealed that steelhead lice loads were significantly affected by hide design ($p=0.01$), as well as week ($p<0.001$). In Trial 1, lice loads were 40% lower in kelp hide cages than in PVC hide cages, and 46% lower than control (no lumpfish, no hide) cages (GLM, AICc= -1.85, Table 2.14, Table 2.15, Figure 2.13, Figure 2.14).

For the comparison of mean lice loads on steelhead between cages with and without the lumpfish treatment in Trial 1, the most parsimonious model retained the lumpfish and week variables. The interaction variable between lumpfish and week was not retained (Table 2.16). The results of the GLM F-tests of the retained variables revealed that steelhead lice loads were significantly higher in control cages than they were in cages with lumpfish ($p=0.04$) in Trial 1.

There was also a strong weekly effect ($p<0.001$) on mean lice loads of steelhead (GLM, AICc= 0.0094, Table 2.16, Table 2.17, Figure 2.15, Figure 2.16). Lice loads on steelhead in cages with lumpfish were 37% lower than in control cages in Trial 1. For the comparison of mean lice loads on lumpfish between hide design treatments, the most parsimonious model retained the week variable (Table 2.18). The hide design and interaction between week and hide design variables were not retained. The results of F-tests in Trial 1 show significant effects by sample week ($p=0.02$) on lumpfish mean lice loads (GLM, AICc= 80.48, Table 2.18, Table 2.19, Figure 2.17),

The overall mean lice load on steelhead trout between all cages in Trial 2 ranged from 0.01 to 0.08 lice per fish (Table 2.20). The overall mean lice load on lumpfish between all cages in Trial 2 ranged from 0.08 to 0.17 lice per fish (Table 2.20). For the comparison of mean lice loads between lumpfish and steelhead in Trial 2, the most parsimonious model retained the species variable (Table 2.21). The model did not retain the week or interaction between species and week variables. The results of the GLM F-tests on the species variable revealed that lice loads were not significantly different between lumpfish and steelhead trout ($p=0.52$) in Trial 2 (GLM, AICc=-64.65, Table 2.21, Table 2.22, Figure 2.18).

For the comparison of mean lice loads on steelhead by hide design in Trial 2, the most parsimonious model retained the hide design variable (Table 2.23). The model did not retain the week and interaction between hide design and week variables. The GLM F-test results revealed steelhead lice loads were significantly affected by hide design in Trial 2 ($p=0.02$). In Trial 2, lice loads were lower in both kelp and PVC hide designs than in the control cages; there were no differences in lice loads between the two hide treatments. (GLM, AICc= -76.78, Table 2.23, Table 2.24, Figure 2.19, Figure 2.20). The mean lice loads in cages with kelp hide designs were approximately 85% lower than those in control cages, while the lice loads in cages with PVC hide designs were approximately 61% lower than those in control cages in Trial 2 (Figure 2.20).

For the comparison of mean lice loads on steelhead between cages with and without the lumpfish treatment Trial 2, the most parsimonious model retained the lumpfish, week, and interaction between lumpfish and week variables (Table 2.25). The GLM F-tests of the variables revealed steelhead lice loads did not significantly differ between control cages and cages with lumpfish ($p=0.25$) in Trial 2. There was, however, a strong effect on lice loads by week ($p<0.001$), and a strong interaction term ($p<0.001$) between cleanerfish treatment and week in Trial 2 (GLM, AICc= -83.29, Table 2.25, Table 2.26, Figure 2.21, Figure 2.22).

For the comparison of mean lice loads on lumpfish between hide design treatments in Trial 2, the most parsimonious model retained the hide design variable (Table 2.27). The model did not retain the week or interaction between hide design and week variables. The GLM F-test results reveal that mean lice loads on lumpfish were not affected by hide design ($p=0.38$) in Trial 2 (GLM, AICc=-7.41, Table 2.27, Table 2.28, Figure 2.23).

Table 2.11. Trial 1 mean lice loads on steelhead trout (n=15) and lumpfish (n=3) over the five sampling periods.

Date	Week	Hide (Cage #)	Total Lice on Trout	Mean Lice Load Trout	Total Lice on Lumpfish	Mean Lice Load Lumpfish
10/21/2020	1	kelp (1)	0	0.00	2	0.67
	1	kelp (6)	0	0.00	0	0.00
	1	PVC (2)	2	0.13	1	0.33
	1	PVC (4)	3	0.20	1	0.33
	1	Control (3)	1	0.07		
	1	Control (5)	0	0.00		
10/28/2020	2	kelp (1)	1	0.07	0	0.00
	2	kelp (6)	5	0.33	3	1.00
	2	PVC (2)	5	0.33	4	1.33
	2	PVC (4)	7	0.47	5	1.67
	2	Control (3)	4	0.31		
	2	Control (5)	7	0.47		
11/4/2020	3	kelp (1)	6	0.40	18	6.00
	3	kelp (6)	3	0.20	8	2.67
	3	PVC (2)	17	1.13	9	3.00
	3	PVC (4)	8	0.53	5	1.67
	3	Control (3)	12	0.92		
	3	Control (5)	15	1.00		
11/11/2020	4	kelp (1)	9	0.60	3	1.00
	4	kelp (6)	11	0.73	13	4.33
	4	PVC (2)	11	0.73	7	2.33
	4	PVC (4)	6	0.40	1	0.50
	4	Control (3)	7	0.54		
	4	Control (5)	12	0.80		
11/18/2020	5	kelp (1)	4	0.27	2	1.00
	5	kelp (6)	1	0.07	2	0.67
	5	PVC (2)	4	0.27	6	3.00
	5	PVC (4)	3	0.25	0	0.00
	5	Control (3)	7	0.54		
	5	Control (5)	6	0.40		
		TOTALS	177	0.40	90	1.61

Table 2.12. All subsets regression model selection table for Trial 1 comparison of mean lice loads by fish species, where models (defined by the variables retained) are ranked by lowest to highest AICc. ΔAICc represents how close to the top model a given model is, and results of F-tests for retained variables are given as p-values for each model. An "x" denotes a variable that was not retained for a given model.

Trial 1 Lice Load Comparison Between Steelhead and Lumpfish					
Model	AICc	ΔAICc	P-value Treatment	P-value Week	P-value Interaction
species + week + species*week	140.2117	0	0.57528	0.00003	0.01117
species + week	140.952	0.7403	0.00001	0.0014	x
species	148.5284	8.3167	0.0002	x	x
species*week	152.5923	12.3806	x	x	0.0015
week	157.4006	17.1889	x	0.0127	x

Table 2.13. Parameter estimates for most parsimonious GLM selected for Trial 1 comparison of mean lice loads by fish species, where Term defines which variable is being tested (sample weeks: 1-5, species: lumpfish and steelhead). The Estimate defines the estimate value for a given term. L-R Chisquare is the likelihood ratio Chi Square for a given term. Prob> Chisq is the p value for a given term's estimate. The Lower CL defines the lower limit of the 95% confidence interval for a given term's estimate, and Upper CL defines the upper limit of the 95% confidence interval for a given term's estimate.

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
intercept	0.200	0.238	0.704	0.402	-0.275	0.675
sample week[2-1]	0.465	0.336	1.876	0.171	-0.207	1.136
sample week[3-2]	1.351	0.336	14.012	0.000	0.680	2.023
sample week[4-3]	-0.678	0.336	3.913	0.048	-1.349	-0.006
sample week[5-4]	-0.606	0.336	3.147	0.076	-1.277	0.066
species[lumpfish]	0.133	0.238	0.314	0.575	-0.341	0.608
sample week[2-1]*species[lumpfish]	0.202	0.336	0.361	0.548	-0.469	0.874
sample week[3-2]*species[lumpfish]	0.982	0.336	7.886	0.005	0.311	1.653
sample week[4-3]*species[lumpfish]	-0.614	0.336	3.230	0.072	-1.285	0.058
sample week[5-4]*species[lumpfish]	-0.269	0.336	0.639	0.424	-0.941	0.402

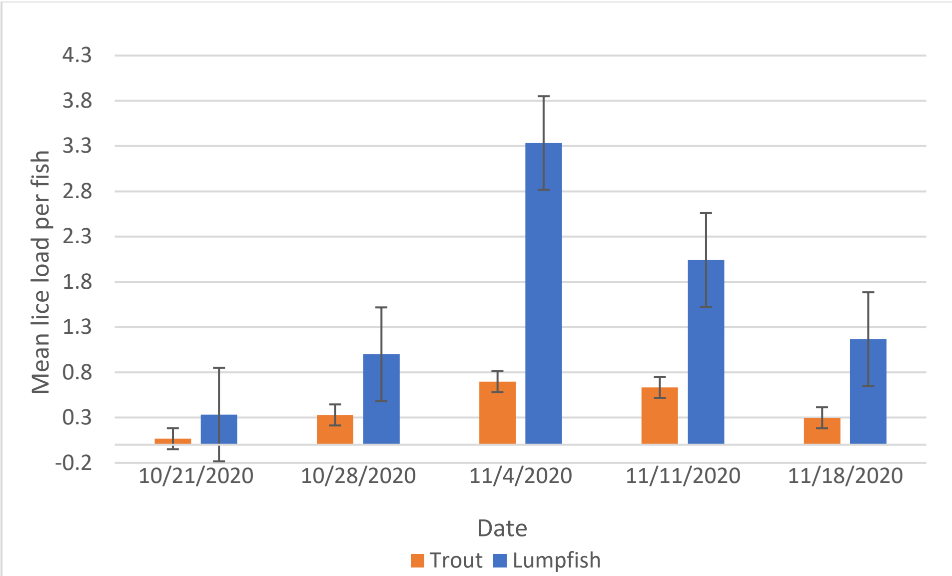

Figure 2.12. Trial 1 mean weekly lice loads of all steelhead trout (orange) and all lumpfish (blue), where lice load is the mean number of lice per fish. Error bars represent standard error.

Table 2.14. All subsets regression model selection table for Trial 1 mean weekly lice loads of steelhead trout by hide design, where models (defined by the variables retained) are ranked by lowest to highest AICc. ΔAICc represents how close to the top model a given model is, and results of F-tests for retained variables are given as p-values for each model. An "x" denotes a variable that was not retained for a given model.

Trial 1 Steelhead Lice Load by Hide Design					
Model	AICc	ΔAICc	P-value Treatment	P-value Week	P-value Interaction
hide + week	-1.8508	0	0.00668	<0.0001	x
week	0.9615	2.8123	x	<0.0001	x
hide	18.8659	20.7167	0.1648	x	x
hide + week + hide*week	28.917	30.7678	0.3188	<0.0001	0.00953
hide*week	36.145	37.9958	x	x	0.4041

Table 2.15. Parameter estimates for most parsimonious GLM selected for Trial 1 mean weekly lice loads of steelhead trout by hide design, where Term defines which variable is being tested (sample weeks: 1-5, species: lumpfish and steelhead). The Estimate defines the estimate value for a given term. L-R Chisquare is the likelihood ratio Chi Square for a given term. Prob> Chisq is the p value for a given term's estimate. The Lower CL defines the lower limit of the 95% confidence interval for a given term's estimate, and Upper CL defines the upper limit of the 95% confidence interval for a given term's estimate.

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
intercept	0.067	0.065	1.020	0.313	-0.066	0.199
hide[PVC]	0.040	0.041	0.908	0.341	-0.044	0.124
hide[kelp]	-0.139	0.041	9.527	0.002	-0.222	-0.055
week[2-1]	0.262	0.093	7.122	0.008	0.075	0.450
week[3-2]	0.369	0.093	12.769	0.000	0.182	0.557
week[4-3]	-0.064	0.093	0.476	0.490	-0.251	0.123
week[5-4]	-0.336	0.093	10.932	0.001	-0.523	-0.149

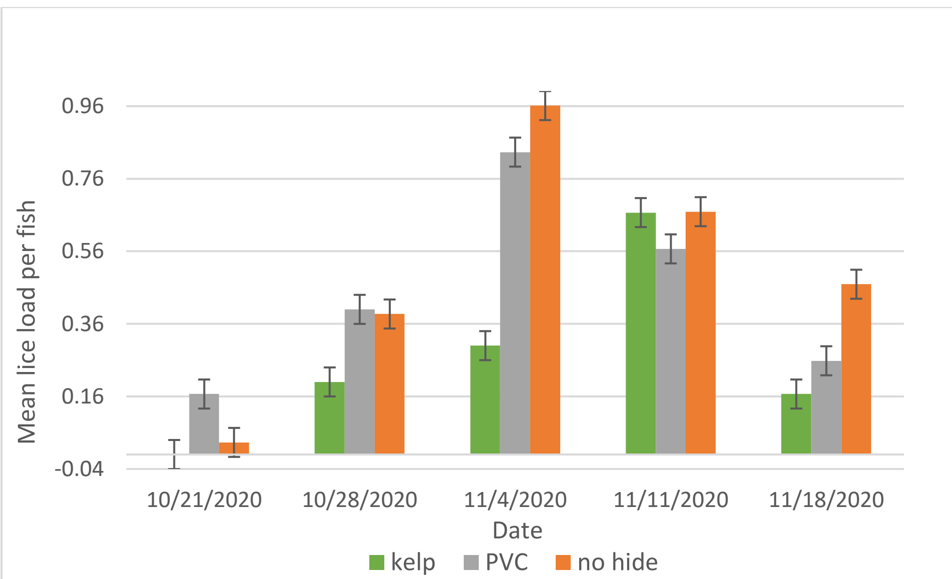

Figure 2.13. Trial 1 mean weekly lice loads of steelhead trout by hide design, where lice load is the mean number of lice per fish. Error bars represent standard error.

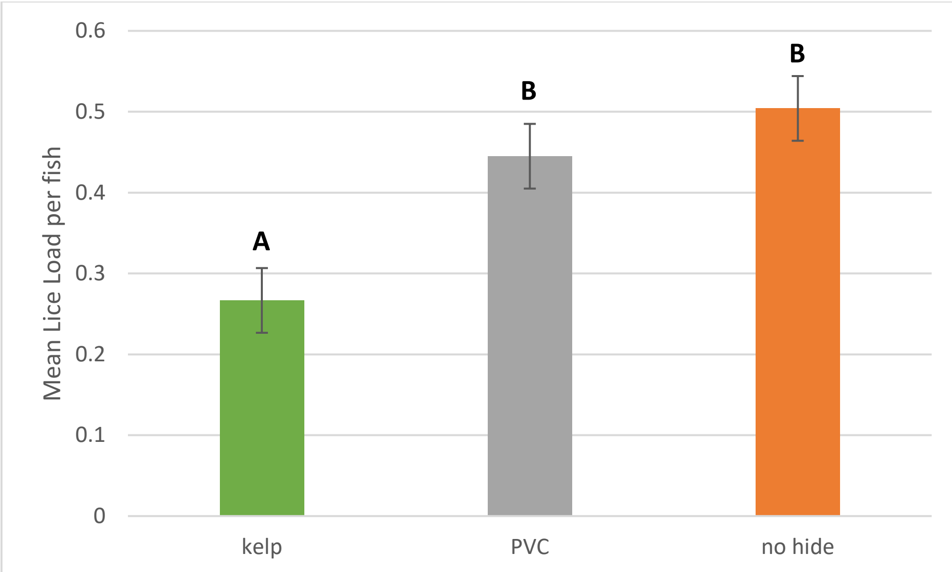

Figure 2.14. Trial 1 overall mean lice loads of steelhead trout by hide design, where lice load is the mean number of lice per fish. Unique letters signify statistical differences between the treatments based on results of the GLM F-test ($p<0.05$). Error bars represent standard error.

Table 2.16. All subsets regression model selection table for Trial 1 mean weekly lice loads of steelhead trout by cleanerfish treatment, where models (defined by the variables retained) are ranked by lowest to highest AICc. ΔAICc represents how close to the top model a given model is, and results of F-tests for retained variables are given as p-values for each model. An “x” denotes a variable that was not retained for a given model.

Trial 1 Steelhead Lice Load by Cleaner Treatment					
Model	AICc	ΔAICc	P-value Treatment	P-value Week	P-value Interaction
lumpfish + week	0.0094	0	0.03613	<0.0001	x
week	0.9615	0.9521	x	<0.0001	x
lumpfish + week + lumpfish*week	11.671	11.6616	0.71771	<0.0001	0.20565
lumpfish	18.1197	18.1103	0.1955	x	x
lumpfish*week	25.9618	25.9524	x	x	0.6334

Table 2.17. Parameter estimates for most parsimonious GLM selected for Trial 1 mean weekly lice loads of steelhead trout by cleanerfish treatment, where Term defines which variable is being tested (sample weeks: 1-5, species: lumpfish and steelhead). The Estimate defines the estimate value for a given term. L-R Chisquare is the likelihood ratio Chi Square for a given term. Prob> Chisq is the p value for a given term's estimate. The Lower CL defines the lower limit of the 95% confidence interval for a given term's estimate, and Upper CL defines the upper limit of the 95% confidence interval for a given term's estimate.

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
intercept	0.091	0.073	1.537	0.215	-0.056	0.239
week[2-1]	0.262	0.102	6.018	0.014	0.057	0.468
week[3-2]	0.369	0.102	10.936	0.001	0.163	0.575
week[4-3]	-0.064	0.102	0.395	0.530	-0.270	0.142
week[5-4]	-0.336	0.102	9.323	0.002	-0.542	-0.130
cleaning treament[control]	0.074	0.034	4.391	0.036	0.005	0.143

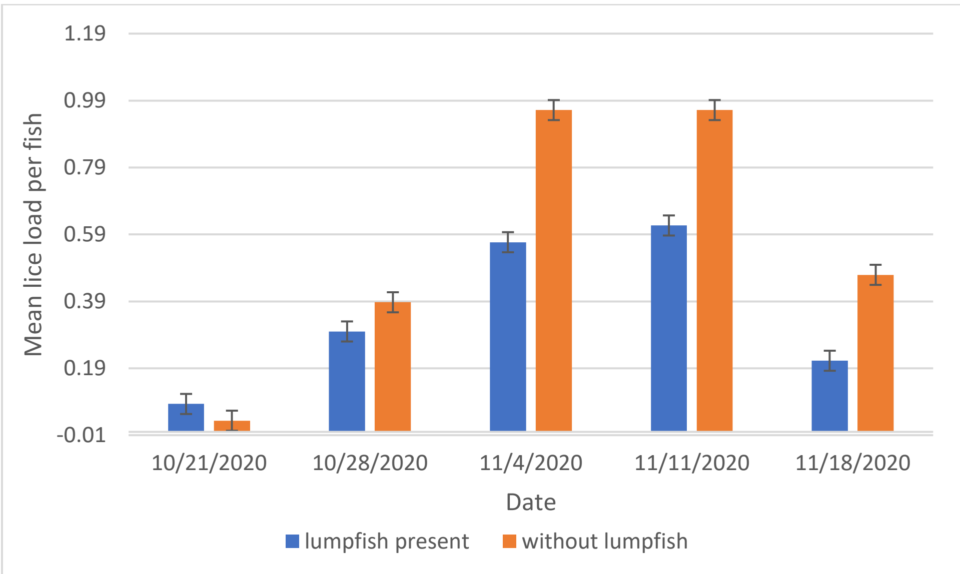

Figure 2.15. Trial 1 mean weekly lice loads of steelhead trout by cleanerfish treatment, where lice load is the mean number of lice per fish. Error bars represent standard error.

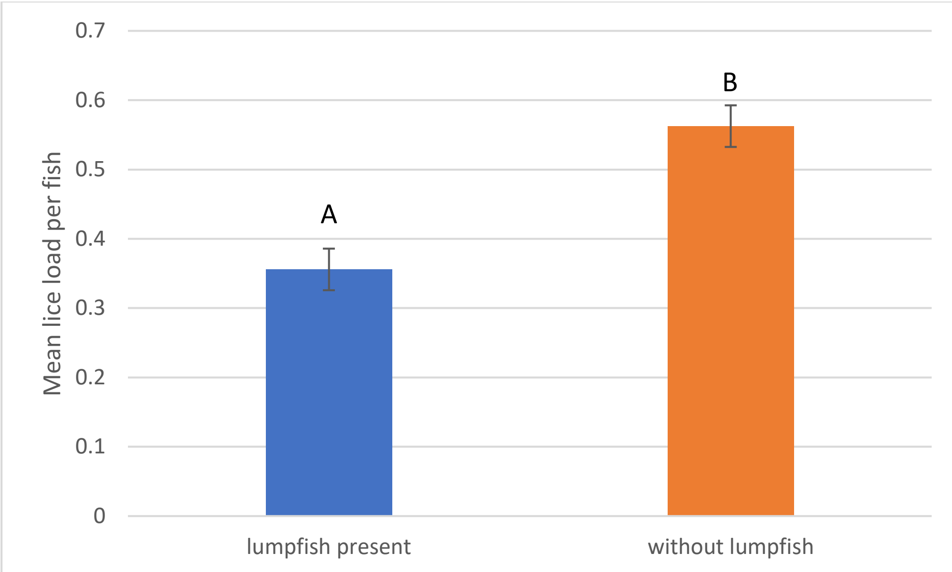

Figure 2.16. Trial 1 overall mean lice loads of steelhead trout by cleanerfish treatment, where lice load is the mean number of lice per fish. Unique letters signify statistical differences between the treatments based on results of the GLM F-test ($p<0.05$). Error bars represent standard error.

Table 2.18. All subsets regression model selection table for Trial 1 mean weekly lice loads of lumpfish by hide design, where models (defined by the variables retained) are ranked by lowest to highest AICc. ΔAICc represents how close to the top model a given model is, and results of F-tests for retained variables are given as p-values for each model. An "x" denotes a variable that was not retained for a given model.

Trial 1 Lumpfish Lice Load by Hide Design					
Model	AICc	ΔAICc	P-value Treatment	P-value Week	P-value Interaction
week	80.4824	0	x	0.0173	x
hide	81.3092	0.8268	0.6447	x	x
hide + week	84.965	4.4826	0.53275	0.01608	x
hide*week	89.2852	8.8028	x	x	0.5252
hide + week + hide*week	110.7463	30.2639	1	0.00454	0.20787

Table 2.19. Parameter estimates for most parsimonious GLM selected for Trial 1 mean weekly lice loads of lumpfish by hide design, where Term defines which variable is being tested (sample weeks: 1-5, species: lumpfish and steelhead). The Estimate defines the estimate value for a given term. L-R Chisquare is the likelihood ratio Chi Square for a given term. Prob> Chisq is the p value for a given term's estimate. The Lower CL defines the lower limit of the 95% confidence interval for a given term's estimate, and Upper CL defines the upper limit of the 95% confidence interval for a given term's estimate.

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
intercept	0.333	0.570	0.339	0.561	-0.840	1.507
week[2-1]	0.667	0.807	0.672	0.412	-0.993	2.327
week[3-2]	2.333	0.807	6.992	0.008	0.673	3.993
week[4-3]	-1.292	0.807	2.413	0.120	-2.952	0.368
week[5-4]	-0.875	0.807	1.144	0.285	-2.535	0.785

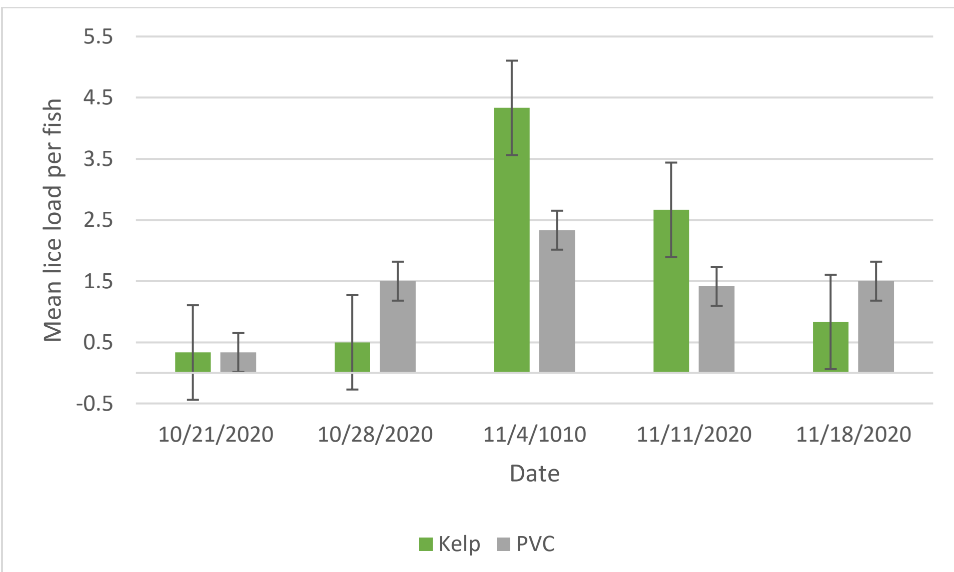

Figure 2.17. Trial 1 mean weekly lice loads of lumpfish by hide design treatment, where lice load is the mean number of lice per fish. Error bars represent standard error.

Table 2.20. Trial 2 mean lice loads on steelhead trout (n=15) and lumpfish (n=3) over the five sampling periods.

Date	**Week**	**Hide (Cage #)**	**Total Lice on Trout**	**Mean Lice Load Trout**	**Total Lice on Lumpfish**	**Mean Lice Load Lumpfish**
11/30/2020	1	kelp (1)	0	0.00	0	0.00
	1	kelp (6)	1	0.07	0	0.00
	1	PVC (2)	0	0.00	2	0.67
	1	PVC (4)	1	0.07	0	0.00
	1	Control (3)	1	0.07		
	1	Control (5)	1	0.07		
12/7/2020	2	kelp (1)	0	0.00	0	0.00
	2	kelp (6)	1	0.07	0	0.00
	2	PVC (2)	2	0.13	0	0.00
	2	PVC (4)	1	0.07	0	0.00
	2	Control (3)	0	0.00		
	2	Control (5)	1	0.07		
12/14/2020	3	kelp (1)	0	0.00	0	0.00
	3	kelp (6)	0	0.00	1	0.33
	3	PVC (2)	0	0.00	0	0.00
	3	PVC (4)	1	0.07	0	0.00
	3	Control (3)	4	0.27		
	3	Control (5)	3	0.20		
12/21/2020	4	kelp (1)	0	0.00	0	0.00
	4	kelp (6)	0	0.00	0	0.00
	4	PVC (2)	0	0.00	0	0.00
	4	PVC (4)	0	0.00	0	0.00
	4	Control (3)	1	0.07		
	4	Control (5)	0	0.00		
12/28/2020	5	kelp (1)	0	0.00	0	0.00
	5	kelp (6)	0	0.00	0	0.00
	5	PVC (2)	0	0.00	0	0.00
	5	PVC (4)	0	0.00	1	0.33
	5	Control (3)	0	0.00		
	5	Control (5)	2	0.13		
		TOTALS	20	0.06	4	0.07

Table 2.21. All subsets regression model selection table for Trial 2 comparison of mean lice loads by fish species, where models (defined by the variables retained) are ranked by lowest to highest AICc. ΔAICc represents how close to the top model a given model is, and results of F-tests for retained variables are given as p-values for each model. An "x" denotes a variable that was not retained for a given model.

Trial 2 Lice Load Comparison Between Steelhead and Lumpfish					
Model	AICc	ΔAICc	P-value Treatment	P-value Week	P-value Interaction
species	-64.6503	0	0.5177	x	x
week	-60.6982	3.9521	x	0.42	x
species*week	-57.9073	6.743	x	x	0.8931
species + week	-58.4375	6.2128	0.50115	0.41527	x
species + week + species*week	-49.871	14.7793	0.08982	0.28125	0.44607

Table 2.22. Parameter estimates for most parsimonious GLM selected for Trial 2 comparison of mean lice loads by fish species, where Term defines which variable is being tested (sample weeks: 1-5, species: lumpfish and steelhead). The Estimate defines the estimate value for a given term. L-R Chisquare is the likelihood ratio Chi Square for a given term. Prob> Chisq is the p value for a given term's estimate. The Lower CL defines the lower limit of the 95% confidence interval for a given term's estimate, and Upper CL defines the upper limit of the 95% confidence interval for a given term's estimate.

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
intercept	0.056	0.017	9.534	0.002	0.021	0.090
species[lumpfish]	0.011	0.017	0.418	0.518	-0.023	0.045

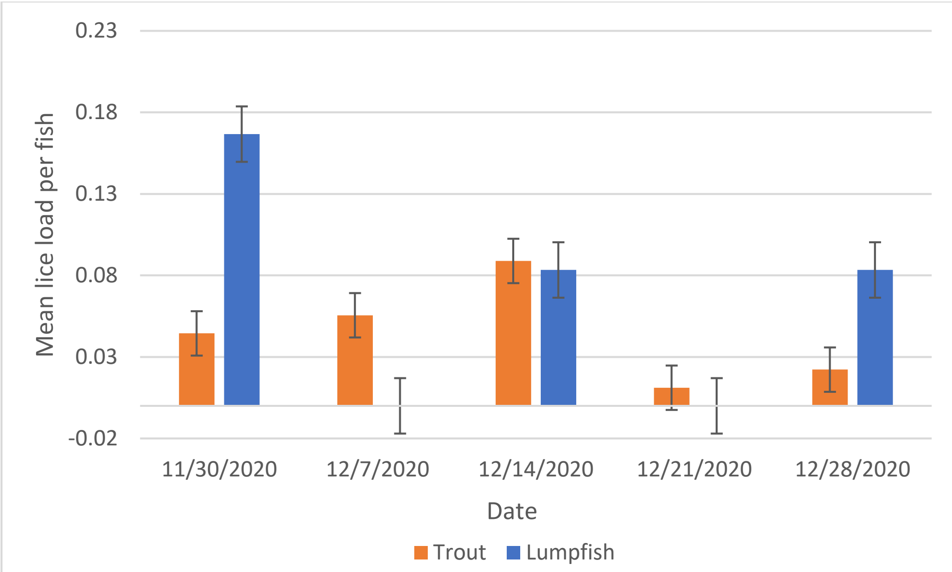

Figure 2.18. Trial 2 mean weekly lice loads of all steelhead trout (orange) and all lumpfish (blue), where lice load is the mean number of lice per fish. Error bars represent standard error.

Table 2.23. All subsets regression model selection table for Trial 2 mean weekly lice loads of steelhead trout by hide design, where models (defined by the variables retained) are ranked by lowest to highest AICc. ΔAICc represents how close to the top model a given model is, and results of F-tests for retained variables are given as p-values for each model. An “x” denotes a variable that was not retained for a given model.

Trial 2 Steelhead Lice Load by Hide Design					
Model	AICc	ΔAICc	P-value Treatment	P-value Week	P-value Interaction
hide	-76.7799	0	0.0216	x	x
hide*week	-74.912	1.8679	x	x	0.0005
hide + week	-71.1782	5.6017	0.00833	0.10505	x
week	-68.8084	7.9715	x	0.2186	x
hide + week + hide*week	-51.7837	24.9962	0.44441	0.00114	0.00011

Table 2.24. Parameter estimates for most parsimonious GLM selected for Trial 2 mean weekly lice loads of steelhead trout by hide design, where Term defines which variable is being tested (sample weeks: 1-5, species: lumpfish and steelhead). The Estimate defines the estimate value for a given term. L-R Chisquare is the likelihood ratio Chi Square for a given term. Prob> Chisq is the p value for a given term's estimate. The Lower CL defines the lower limit of the 95% confidence interval for a given term's estimate, and Upper CL defines the upper limit of the 95% confidence interval for a given term's estimate.

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
intercept	0.044	0.010	14.111	0.000	0.023	0.066
hide[control]	0.042	0.015	7.195	0.007	0.012	0.072
hide[PVC]	-0.011	0.015	0.558	0.455	-0.041	0.019

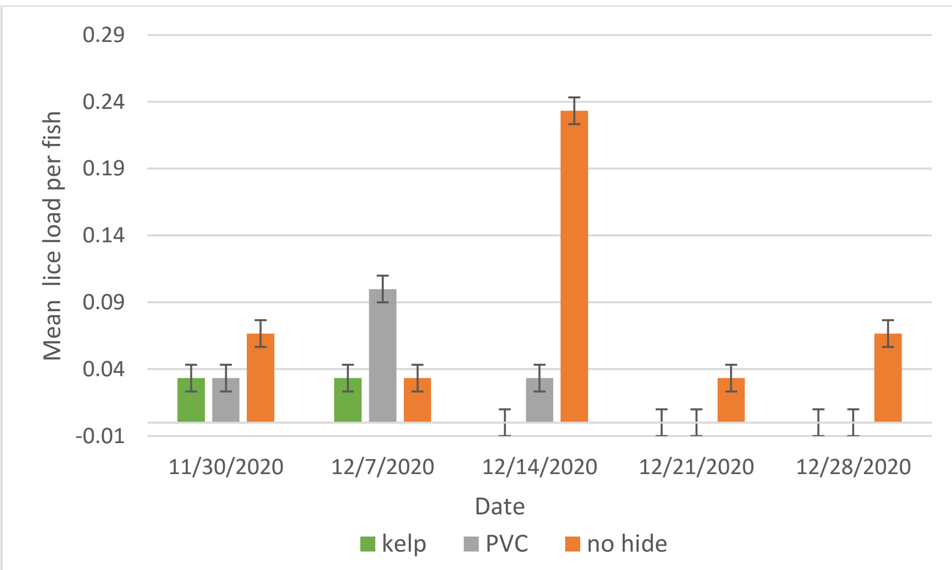

Figure 2.19. Trial 2 mean weekly lice loads of steelhead trout by hide design, where lice load is the mean number of lice per fish. Error bars represent standard error.

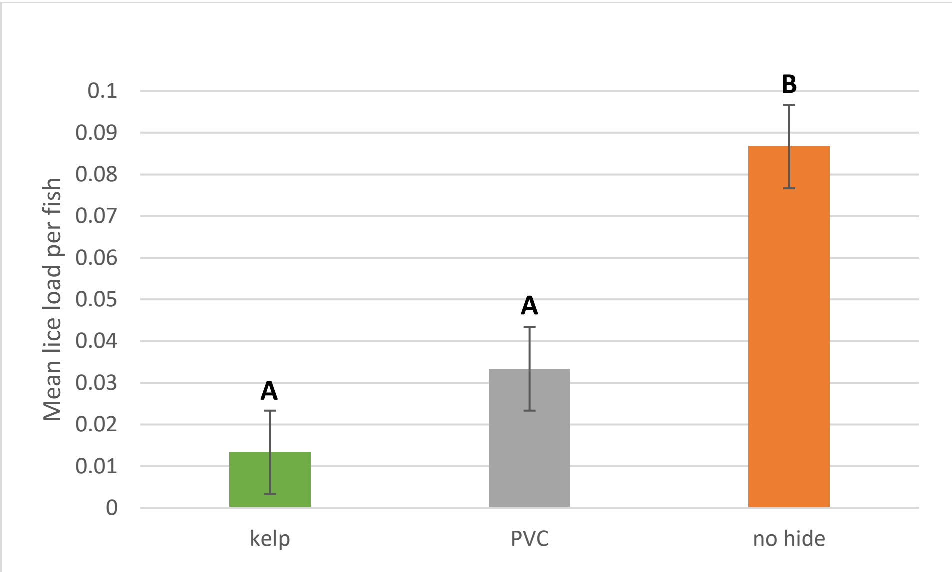

Figure 2.20. Trial 2 overall mean lice loads of steelhead trout by hide design, where lice load is the mean number of lice per fish. Unique letters signify statistical differences between the treatments based on results of the GLM F-test ($p<0.05$). Error bars represent standard error.

Table 2.25. All subsets regression model selection table for Trial 2 mean weekly lice loads of steelhead trout by cleanerfish treatment, where models (defined by the variables retained) are ranked by lowest to highest AICc. ΔAICc represents how close to the top model a given model is, and results of F-tests for retained variables are given as p-values for each model. An “x” denotes a variable that was not retained for a given model.

Trial 2 Steelhead Lice Load by Cleaner Treatment					
Model	AICc	ΔAICc	P-value Treatment	P-value Week	P-value Interaction
lumpfish + week + lumpfish*week	-83.2859	0	0.24553	0.00014	0.00002
lumpfish*week	-80.6291	2.6568	x	x	0.0015
lumpfish	-78.8547	4.4312	0.0079	x	x
lumpfish + week	-74.1696	9.1163	0.00301	0.11247	x
week	-68.8084	14.4775	x	0.2186	x

Table 2.26. Parameter estimates for most parsimonious GLM selected for Trial 2 mean weekly lice loads of steelhead trout by cleanerfish treatment, where Term defines which variable is being tested (sample weeks: 1-5, species: lumpfish and steelhead). The Estimate defines the estimate value for a given term. L-R Chisquare is the likelihood ratio Chi Square for a given term. Prob> Chisq is the p value for a given term's estimate. The Lower CL defines the lower limit of the 95% confidence interval for a given term's estimate, and Upper CL defines the upper limit of the 95% confidence interval for a given term's estimate.

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
intercept	0.050	0.014	10.388	0.001	0.021	0.079
treatment[lumpfish]	-0.017	0.014	1.349	0.246	-0.045	0.012
sampling week[2-1]	0.000	0.020	0.000	1.000	-0.041	0.041
sampling week[3-2]	0.075	0.020	11.466	0.001	0.034	0.116
sampling week[4-3]	-0.108	0.020	20.360	<.0001	-0.149	-0.068
sampling week[5-4]	0.017	0.020	0.682	0.409	-0.024	0.057
sampling week[2-1]*treatment[lumpfish]	0.033	0.020	2.639	0.104	-0.007	0.074
sampling week[3-2]*treatment[lumpfish]	-0.125	0.020	24.897	<.0001	-0.166	-0.084
sampling week[4-3]*treatment[lumpfish]	0.092	0.020	15.838	<.0001	0.051	0.132
sampling week[5-4]*treatment[lumpfish]	-0.017	0.020	0.682	0.409	-0.057	0.024

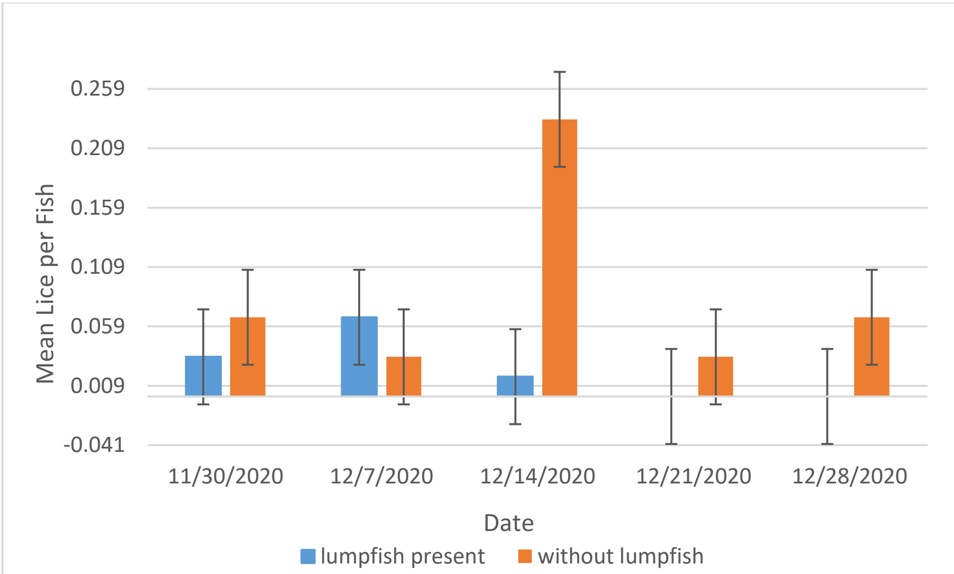

Figure 2.21. Trial 2 mean weekly lice loads of steelhead trout by cleanerfish treatment, where lice load is the mean number of lice per fish. Error bars represent standard error.

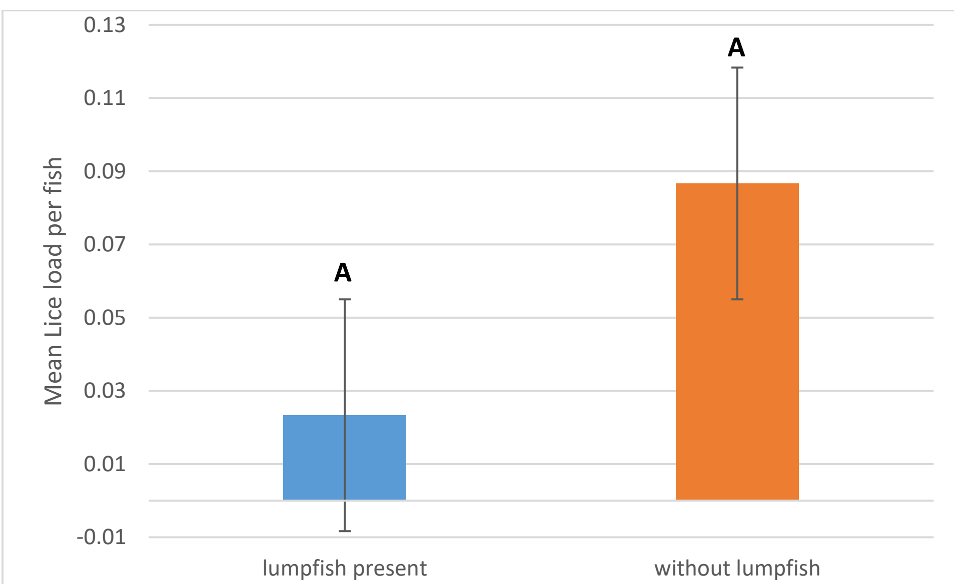

Figure 2.22. Trial 2 overall mean lice loads of steelhead trout by cleanerfish treatment, where lice load is the mean number of lice per fish. Unique letters signify statistical differences between the treatments based on results of the GLM F-test ($p<0.05$). Error bars represent standard error.

Table 2.27. All subsets regression model selection table for Trial 2 mean weekly lice loads of lumpfish by hide design, where models (defined by the variables retained) are ranked by lowest to highest AICc. ΔAICc represents how close to the top model a given model is, and results of F-tests for retained variables are given as p-values for each model. An "x" denotes a variable that was not retained for a given model.

Trial 2 Lumpfish Lice Load by Hide Design					
Model	AICc	ΔAICc	P-value Treatment	P-value Week	P-value Interaction
hide	-7.4128	0	0.3758	x	x
week	1.4415	8.8543	x	0.5761	x
hide*week	2.3112	9.724	x	x	0.7317
hide + week	5.404	12.8168	0.34031	0.55508	x
hide + week + hide*week	29.8707	37.2835	0.01645	0.38033	0.12569

Table 2.28. Parameter estimates for most parsimonious GLM selected for Trial 2 mean weekly lice loads of lumpfish by hide design, where Term defines which variable is being tested (sample weeks: 1-5, species: lumpfish and steelhead). The Estimate defines the estimate value for a given term. L-R Chisquare is the likelihood ratio Chi Square for a given term. Prob> Chisq is the p value for a given term's estimate. The Lower CL defines the lower limit of the 95% confidence interval for a given term's estimate, and Upper CL defines the upper limit of the 95% confidence interval for a given term's estimate.

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
intercept	0.067	0.037	2.968	0.085	-0.010	0.143
hide[kelp]	-0.033	0.037	0.784	0.376	-0.110	0.043

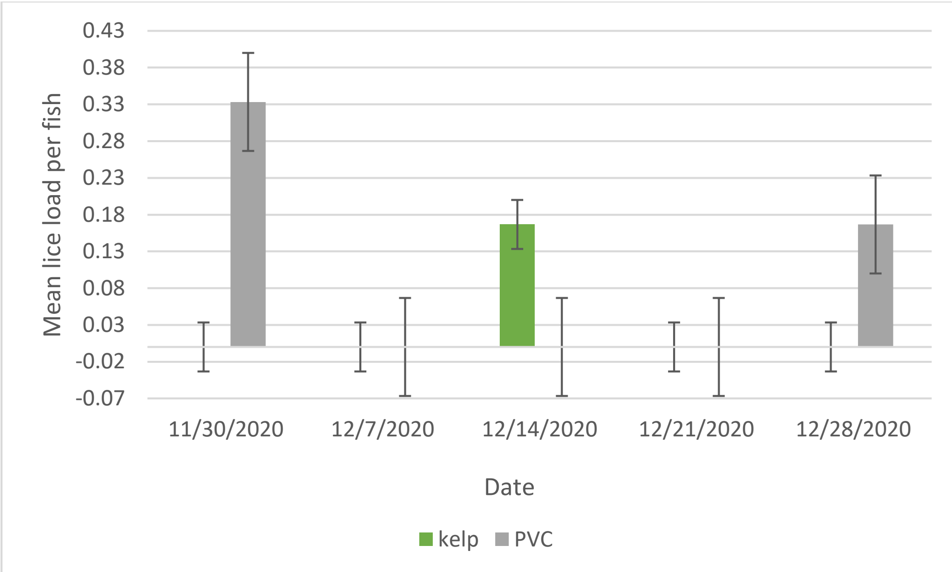

Figure 2.23. Trial 2 mean weekly lice loads of lumpfish by hide design treatment, where lice load is the mean number of lice per fish. Error bars represent standard error.

Discussion

Fish welfare

Trout survival was high (94-99%) in both Trial 1 (Figure 2.7) and Trial 2. Lumpfish survival was high (100%) in Trial 2, but lower in Trial 1 (67%; Figure 2.7). It is important to consider that temperature does have an effect on both steelhead trout and lumpfish wellbeing. The overall mean temperature of Trial 1 (October 14 - November 18) was 11.2 ± 1.4 °C. The Overall mean temperature of Trial 2 (November 23- December 28) was much lower, at 6.1 ± 1.8 °C. This difference in temperature is not unexpected for these times of the year, and could play a role in fish survival. Steelhead trout can survive in a wide range of temperatures, from 0 °C up to 27 °C (FAO, 2005), however specific thermal tolerances can be strain dependent, and aquaculturists might select for higher temperature tolerant traits to produce more tolerant strains depending on environmental rearing conditions. For example, Hartman and Porto (2014) reviewed the critical thermal maxima (CTM) for three steelhead strains above optimal temperatures, including Kamloops by Trout Lodge. All three strains had a CTM greater than 31.0 °C, though Kamloops had the lowest CTM at 31.1 °C. While this particular strain by Trout Lodge may be different from the Trout Lodge strain used in this study, it does highlight the fact that tolerance to environmental conditions are often strain dependent. Lumpfish typically have even lower tolerances to temperature, but thermal optima shift as the fish grow. Lumpfish between 20 g and 40 g thrive in 16 °C water, whereas as the fish grow, their thermal optima decrease. Lumpfish that are 100 g to 110 g grow optimally at 13 °C and fish that are 120 g to 200 g grow optimally at 8.9 °C (Nytro et al., 2014). Another study that examined thermal performance among other physiological responses found that larger lumpfish (300 g) exposed to 18 °C suffered from low survival compared to other large (300 g), and small (75 g) lumpfish kept at 15 °C, 9 °C, and 3 °C (Hvas et al., 2018). The lumpfish used in Trial 1 had an initial mean weight of 155.1 g, while the five-week mean temperature was 11.2 ± 1.4 °C (Figure 2.6). While the mean temperature through Trial 1 was not as high as mortality-inducing temperatures described by Hvas et al. (2018), it is higher than the optimal temperature described by Nytro et al. (2014), and could have affected lumpfish wellbeing. Further, the reproductive abilities of mature adult lumpfish (165 g to 448 g) can be compromised when water temperature exceeds 14 °C, and lower temperatures are advised for maintaining adult broodstock (Pountney et al., 2020). The natural behavior of lumpfish validates these thermal preference studies, as small (10 g to 200 g), young fish often spend time in nearshore and intertidal waters, but will migrate to cooler offshore waters as they grow (Powell et al., 2018), something that the caged fish were prevented from doing.

Fish growth and diet

Steelhead trout grew in every cage during both trials (Figure 2.8, Figure 2.10, Table 2.3, Table 2.5) and 79% of all (Trial 1 and Trial 2) dissected steelhead had pellets in their digestive tract (Table 2.8, Table 2.10). Mean specific growth rate was higher during Trial 1 than the cooler weeks of Trial 2. Lumpfish did not impede steelhead growth, corroborating past studies that show small (less than 360 g) lumpfish do not inhibit the growth rate of farmed Atlantic salmon (Imsland et al., 2014a). When lumpfish of two different size classes (54 g and 360 g) were stocked into salmon cages, the smaller lumpfish did not negatively affect the growth of the salmon, but the larger lumpfish did inhibit salmonid growth (Imsland et al., 2014a).

Although 92% of the lumpfish between both trials had pellets in their digestive tract at the time of dissection (Table 2.7, Table 2.9), not all lumpfish grew during the trials (Figure 2.9, Figure 2.11, Table 2.4, Table 2.6). Because the mean lumpfish weights were averaged by cage, and individual lumpfish were not tagged during the trials, individual growth rates and stomach contents cannot be linked.

Lice loads and lumpfish diet

Lice loads were lower in cages containing lumpfish than in those without lumpfish in Trial 1 but not in Trial 2. In addition to lower lice loads being observed across all cages in Trial 2, this difference may also have been cleanerfish size related. Trial 1 fish (155.1 g) were smaller than Trial 2 fish (301.5g). Lumpfish are most effective cleaners when they are small. The optimal cleaning size for lumpfish is 40 g to 140 g (Imsland et al., 2021). Imsland et al. (2014a) compared 54 g lumpfish to 360 g lumpfish stocked with Atlantic salmon and found that the larger lumpfish competed more with salmon for feed pellets and were not as effective at removing sea lice as the smaller lumpfish. While the larger lumpfish did reduce lice infestations compared to control cages, the smaller size class reduced infestations more consistently, and to a greater extent (Imsland et al., 2014a). Additionally, the larger size lumpfish impeded the growth of Atlantic salmon. Lumpfish size, however, is not the only determinant of cleanerfish efficacy as differences between lumpfish families also exist (Imsland et al. 2021). For example, in a study by Imsland et al. (2021), two lumpfish families with the highest sea lice consumption levels occurred in 40 g to 79 g fish, while other families had the highest sea lice consumption in 40 g to 179 g fish. Overall, the amount of sea lice consumed by lumpfish decreased with increasing size classes tested among all families.

Lumpfish are naturally opportunistic feeders. Younger lumpfish are more likely to forage amongst macroalgae and will graze on smaller crustaceans such as amphipods and copepods (Powell et al., 2018). As they grow larger, lumpfish will forage for ctenophores and larger crustaceans. None of the lumpfish dissected had evidence of sea lice or any other crustacean in their digestive tracts, but it is possible that sea lice had been consumed and fully digested prior to gut analysis. The lumpfish used in this study were large enough to forage for larger crustaceans and even ctenophores, and might not have been targeting crustaceans as small as sea lice.

While larger lumpfish will still graze on sea lice (Imsland et al., 2016; Eliasen et al., 2018), they are more likely than smaller lumpfish to compete with salmon for pellets and will not graze on lice as much as their smaller counterparts. Imsland et al. (2016) tested lumpfish of three size classes (small: 22.6 ± 0.7 g, medium: 77.4 ± 3.6 g, and large: 113.5 ± 2.1 g), and competition with Atlantic salmon for food was significantly lower in the small size class than in the medium or large classes. Eliasen et al. (2018) tested five size classes of lumpfish (>50 g, 50-

99 g, 100-149 g, 150-199 g, >200 g) in salmon farms in the Faroe Islands. Lumpfish of increasing size classes consumed decreasing numbers of sea lice. Alternatively, lumpfish of increasing size classes had increasing rates of salmon feed in their stomachs. In these trials, the lumpfish used were either on the upper size limits of appropriate cleanerfish size (Trial 1 lumpfish mean weights ranging from 135.3 g to 166.3 g per cage) or larger than the recommended cleanerfish size (Trial 2 lumpfish: 290.4 g to 319.4 g per cage). Like studies by Imsland et al. (2016) and Eliasen et al. (2018), there was some degree of interspecific food competition observed. In Trial 1, 25% of lumpfish clearly had steelhead trout pellets in their stomachs.

The development of *Caligus elongatus*, the only louse species observed in these trials, is affected by temperature (Hemmingsen et al., 2020). According to Hemmingsen et al.'s (2020) review, the optimal water temperature of *C. elongatus* is 14 °C with lower water temperatures prolonging the early, non-parasitic life stages of the louse. In Trial 1, there was a total mean lice load of 0.40 lice per steelhead trout over all five weeks (Table 2.11), with a total of 177 lice collected throughout the trial. In Trial 2, there was a total mean lice load of 0.06 lice per steelhead trout over all five weeks (Table 2.20), with a total of 20 lice collected throughout the trial. The total mean temperature throughout Trial 1 was 11.2 ± 1.4 °C, while the total mean temperature throughout Trial 2 was 6.1 ± 1.8 °C (Figure 2.6). The lower number of lice observed in Trial 2 compared to Trial 1 was likely a result of lower mean temperatures during the trial.

Hide design

Researchers and farmers agree that replicating shelter (hides) for lumpfish used in aquaculture pens is a best practice (Imsland et al., 2015) for lumpfish welfare. Several studies have tested the effects of different types of hides with cleanerfish in salmon farms (Imsland et al., 2014c; Conlon, 2019). In one study, lumpfish preferred to adhere onto plastic panels or plastic tubes, and avoided car tires, concrete tubes, and stone (Imsland et al., 2014c). Commercial farms often use large curtains of fake kelp, which can be costly (Conlon, 2019). Conlon (2019) explored different and more cost-effective lumpfish hide options including recycled materials from commercial aquaculture operations, and found that of the designs tested, lumpfish preferred flat plastic sheeting. Color preference was also evaluated; lumpfish preferred black hides over blue, white, and green colored hides (Conlon, 2019). In Trial 1, lice loads on steelhead trout were significantly different between hide treatments. In this study, lumpfish behavior in relation to the different hides was not quantified, but rather sea lice load, and indirectly cleanerfish efficacy, were evaluated between hide types. Based on personal observations, lumpfish used PVC hides for resting more so than kelp hides, but those observations were not quantified and recording the hide use was not a part of the protocol. However, sea lice load of steelhead varied depending on the type of hide used. In Trial 1, the cages with kelp hides had significantly lower lice loads on steelhead than both the control cages and the cages with PVC hides (Table 2.14, Table 2.15, Figure 2.13, Figure 2.14). Over the entire trial, lice loads in cages with PVC hides were 67% higher than in cages with kelp hides, and lice loads in control cages were 89% higher than in cages with kelp hides (Table 2.15). In Trial 2, there was no difference in lice loads between cages with different hide designs, though all cages with hides in Trial 2 had significantly lower lice loads than the control cages (Table 2.23, Table 2.24, Figure 2.19, Figure 2.20).

Conclusions

In this study, conducted in a small, experimental system, lice loads on steelhead trout were reduced by the presence of lumpfish and by hide design used in the cages, with lower lice loads observed on steelhead trout in cages utilizing a fake kelp hide design. Going forward, the next logical step would be to employ what has been learned from this study and validate the beneficial use of lumpfish in a larger steelhead trout aquaculture operation, such as at the NH Sea Grant managed AquaFort farm. Using a larger pen with greater fish biomass would allow researchers to sample sea lice on a subsample of steelhead trout rather than handling every individual fish each week, which not only would better mimic commercial operations, but reduced fish handling could result in different lice loads as well as improve fish welfare. In any future study, lumpfish used should be smaller than 140 g to ensure optimal cleanerfish size potential. Additionally, evaluating lumpfish use throughout an entire steelhead trout production run would be beneficial. Future hide design studies should incorporate a quantitative method to record total time of hide use by lumpfish as well as include video monitoring to see how hides affect steelhead trout behavior. Utilizing acoustic telemetry transmitters to track behaviors within the cage can be useful as well. Ward et al. (2012) used acoustic telemetry to explore swimming behaviors of farmed cod in response to different stocking densities. The authors were able to identify different swimming behaviors and cage utilization at different stocking densities. Using this technology and similar methodology to examine lumpfish behaviors around hide usage, and interactions between lumpfish and steelhead trout would further illuminate the use of lumpfish and their behavior in steelhead trout farms, and allow greater insight into impacts on fish welfare when the two species interact.

The ever-expanding aquaculture industry, especially high-value products such as salmonids, will continue to be an integral part of seafood production and global food security. Therefore, it is important to invest energy and resources into developing and utilizing the best available sustainable practices. Industry challenges should be addressed with solutions that can benefit producers and communities, while maintaining environmental health. This research contributes to these solutions by exploring the use of lumpfish as cleanerfish of steelhead trout, a salmonid of interest for commercial production in NH coastal waters.

FINAL CONCLUSIONS

The goals of this thesis were to 1) document the presence of sea lice species and provide an assessment of sea lice infestations over a winter-spring production run of steelhead trout in NH waters and 2) examine the use of lumpfish as a biological delouser of steelhead trout in an experimental setting. These goals were addressed through a 30-week evaluation of sea lice at an active experimental aquaculture farm and through a small cage experiment examining the effects lumpfish and hide designs on sea lice infestations of steelhead trout.

In NH coastal waters, *Caligus elongatus* is the dominant louse species parasitizing farmed salmonids. *Caligus curtus* occurs to a much lesser extent (1%) and there is no evidence of *Lepeophtheirus salmonis* on steelhead trout farmed in NH coastal waters. Maximum lice loads observed during this November 2020 to June 2021 assessment were 3.6 lice per fish in January, far lower than similar sea lice assessments where salmonids were reared throughout the summer months (18 lice per fish) in the Bay of Fundy 32 years ago (Hogans and Trudeau 1989). Though the AquaFort farm site has a smaller production yield and host density than a commercial site, it is known that cooler waters have an impact on *C. elongatus* development, and a winter production run of steelhead can contribute to a reduction of maximum lice load intensity.

The presence of lumpfish in an experimental steelhead trout pen can influence lice loads. In small *in situ* cages, lice loads were lower on steelhead trout when 1) lumpfish were present and 2) kelp hides were used (versus PVC hides) during October to November (Trial 1). Though these finding were not repeated November through December (Trial 2), average lice loads in all cages were much lower than in the fall and cooler temperatures likely contributed to this decrease. Additionally, the lumpfish used in Trial 2 were, on average, larger than the recommended cleanerfish size for Atlantic salmon (Imsland et al., 2021), and, thus, may not have been as effective biological delousers as the smaller lumpfish used in Trial 1.

Comparing lice loads at the AquaFort production site and the small cage experiments at the UNH Pier, there is a clear difference. Within the small cage experiments, lice loads reached a peak of 1.13 lice per fish, during Trial 1 on November 4. The mean temperature within the small cages when the peak was recorded was 9.8 °C. The maximum lice load observed at AquaFort was more than three times that seen during the small cage experiment, at 3.6 lice per fish. This was recorded on January 19, 2021, while the mean daily temperature was 5.4 °C, indicating that the higher host density at AquaFort played a larger role in promoting sea lice infestations than the warmer temperatures observed a the UNH Pier earlier.

Going forward, it is important to consider the impacts of climate change on the marine environment, especially if investment in sustainable aquaculture continues in the Gulf of Maine watershed. The Gulf of Maine is warming faster than 99% of the world's oceans, and between 1982 and 2012, average temperature in the Gulf of Maine rose 0.03 °C per year (Pershing et al., 2015). As water temperatures warm, salmonid health, lumpfish health (see Appendix C), and sea lice infestations will be impacted. If temperatures regularly exceed 18 °C during a steelhead production run while lumpfish are present, it will lead to higher lumpfish mortality and poor fish health (Hvas et al., 2018). Higher temperatures also mean faster development of sea lice and shorter generation times, leading to higher lice loads (Hemmingsen et al., 2020). New solutions will be needed to help the aquaculture industry keep pace with changing environmental conditions while ensuring sustainable production.

The next steps in this line of research are to continue and expand sea lice sampling of steelhead trout during future production runs. By maintaining regular lice load assessments, farmers can ensure they make sea lice management decisions (i.e., when to stock fish, when and how to treat fish for lice, when to harvest fish) with the best information possible. Supplementing regular lice assessments on-site, as defined by this thesis, with plankton tows in the areas surrounding the farm also will yield more data regarding sea lice movement and sea lice settlement within an aquaculture setting. Additionally, utilizing lumpfish as a cleanerfish in a larger operation (i.e., experimental farm) will help inform best practices in improving fish welfare. Future studies utilizing lumpfish as biological delousers should consider lumpfish size and hide design. Another priority for this research is exploring how lumpfish interact with steelhead trout utilizing acoustic telemetry to study cage utilization and interactions. This future work can inform decisions regarding fish welfare.

Sustainable practices in aquaculture are an important investment as the industry continues to grow throughout the world and in the Gulf of Maine community. This thesis contributes to aquaculture sustainability by exploring the use of lumpfish, a cleanerfish species native to the Gulf of Maine, in cage production of steelhead trout, a salmonid with commercial potential.

REFERENCES

Abolofia, J., Asche, F., Wilen, J. E. (2017). The cost of lice: quantifying the impacts of parasitic sea lice on farmed salmon. Marine Resource Economics. 32(3), 329-349.

Barrett, L.T., Oppedal, F., Robinson, N., Dempster, T. (2020). Prevention not cure: A review of methods to avoid sea lice infestations in salmon aquaculture. Reviews in Aquaculture. 12(4), 2527-2543.

Bjordal, Å. (1988). Cleaning symbiosis between wrasse (*Labridae*) and lice infested salmon (*Salmo salar*) in mariculture. International Council for the Exploration of the Sea, Mariculture Committee.

Bjordal, Å. (1994). Salmon lice-problems and solutions. Fisken og Havet (Norway). no. 13. (NLH accession no. FIS).

Bricknell, I. R., Dalesman, S. J., O’Shea, B., Pert, C. C., Luntz, A. J. M. (2006). Effect of environmental salinity on sea lice *Lepeophtheirus salmonis* settlement success. Diseases of Aquatic Organisms. 71(3), 201-212.

Brown, J. A., Somerton, D. C., Methven, D. A., Watkins, J. R. (1992). Recent advances in lumpfish *Cyclopterus lumpus* and ocean pout *Macrozoarces americanus* larviculture. Journal of the World Aquaculture Society. 3, 1749-7345.

Bui, S., Halttunen, E., Mohn, A. M., Vågseth, T., Oppedal, F. (2018). Salmon lice evasion, susceptibility, retention, and development differ amongst host salmonid species. ICES Journal of Marine Science. 75(3), 1071-1079.

Chambers, M. D. (2013). Research and development of steelhead trout *Oncorhynchus mykiss* aquaculture in sea cages (Dissertation). University of New Hampshire, Durham, NH. 140 pp.

Conlon, H. (2019). Lumpfish habitat development for use in salmon farming (Master’s Thesis). University of Akureyri Faculty of Business and Science University Centre of the Westfjords, Ísafjörður, Iceland. 62 pp.

Costello, M. J. (2006). Ecology of sea lice parasitic on farmed and wild fish. Trends in Parasitology. 22(11), 475-483.

Costello, M. J. (2009). The global economic cost of sea lice to the salmonid farming industry. Journal of Fish Diseases. 32, 115-118.

Davenport, J. (1985). Synopsis of biological data on the lumpsucker *Cyclopterus lumpus* (Linnaeus, 1758). FAO Fisheries Synopsis No. 147.

Deady, S., Varian, S. J. A., Fives, J. M. (1995). The use of cleaner-fish to control sea lice on two Irish salmon (*Salmo salar*) farms with particular reference to wrasse behaviour in salmon cages. Aquaculture. 131, 73-90.

Denholm, I., Devine, G. J., Horsberg, T. E., Sevatdal, S., Fallang, A., Nolan, D. v., Powell, R. (2002). Analysis and management of resistance to chemotherapeutants in salmon lice, *Lepeophtheirus salmonis* (Copepoda: Caligidae). Pest Management Science. 58, 528-536.

Eliasen, K., Danielsen, E., Johannesen, Á., Joensen, L. L., Patursson, E. J. (2018). The cleaning efficacy of lumpfish (*Cyclopterus lumpus* L.) in Faroese salmon (*Salmo salar* L.) farming pens in relation to lumpfish size and seasonality. Aquaculture. 488, 61-65.

FAO (Food and Agriculture Organization). (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.

FAO (Food and Agriculture Organization). (2021). Cultured aquatic species information programme. *Oncorhynchus mykiss*. Cultured Aquatic Species Information Programme. Text by Cowx, I. G. In: FAO Fisheries Division [online]. Rome. Updated.

Frazer, L. N., Morton, A., Krkošek, M. (2012). Critical thresholds in sea lice epidemics: evidence, sensitivity and subcritical estimation. Proceedings of the Royal Society B: Biological Sciences. 279(1735), 1950-1958.

Groner, M.L., Laurin, E., Stormoen, M., Sanchez, J., Fast, M.D., Revie, C.W. (2019). Evaluating the potential for sea lice to evolve freshwater tolerance as a consequence of freshwater treatments in salmon aquaculture. Aquaculture Environment Interactions. 11, 507-519.

Hardy, R. W. (2002). Rainbow Trout, *Oncorhynchus mykiss*. In: Nutrient Requirements and Feeding of Finfish for Aquaculture. Webster, C.D., Lim, C. 184-202.

Hartman, K. J., Porto, M. A. (2014). Thermal performance of three rainbow trout strains at above-optimal temperatures. Transactions of the American Fisheries Society. 143(6), 1445-1454.

Haya, K., Burridge, L. E., Davies, I. M., Ervik, A. (2005). A review and assessment of environmental risk of chemicals used for the treatment of sea lice infestations of cultured salmon. Environmental Effects of Marine Finfish Aquaculture. 5, 305-340.

Hemmingsen, W., MacKenzie, K., Sagerup, K., Remen, M., Bloch-Hansen, K., Imsland, A.K.D. (2020). *Caligus elongatus* and other sea lice of the genus *Caligus* as parasites of farmed salmonids: A review. Aquaculture. 522, 735160.

Hogans, W. E., Trudeau, D. J. (1989). Preliminary studies on the biology of sea lice, *Caligus elongatus*, *Caligus curtus* and *Lepeophtheirus salmonis* (*Copepoda: Caligoida*) parasitic on cage-cultured salmonids in the lower Bay of Fundy. Canadian Technical Report of Fisheries and Aquatic Sciences. 1715, iv-14.

Hopkins, K. (1992). Reporting fish growth: A review of the basics. Journal of the World Aquaculture Society. 23(3), 173-179.

Hvas, M., Folkedal, O., Imsland, A., Oppedal, F. (2018). Metabolic rates, swimming capabilities, thermal niche and stress response of the lumpfish, *Cyclopterus lumpus*. Biology Open. 7(9), bio036079.

Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Nytrø, A. V., Foss, A., Elvegård, T. A. (2014a). Assessment of growth and sea lice infection levels in Atlantic salmon stocked in small-scale cages with lumpfish. Aquaculture. 433, 137-142.

Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Foss, A., Vikingstad, E., Elvegård, T. A. (2014b). The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.). Aquaculture. 424, 18-23.

Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Elvegård, T. A., Urskog, T. C., Mikalsen, B. (2014c). Assessment of artificial substrates for lumpfish: effect of material thickness and water current speed. Aquaculture International. 26(6), 1469-1479.

Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Nytrø, A. V., Foss, A., Elvegård, T. A. (2015). Assessment of suitable substrates for lumpfish in sea pens. Aquaculture International. 23(2), 639-645.

Imsland, A. K., Reynolds, P., Nytrø, A. v., Eliassen, G., Hangstad, T. A., Jónsdóttir, Ó. D. B., Jonassen, T. M. (2016). Effects of lumpfish size on foraging behaviour and co-existence with sea lice infected Atlantic salmon in sea cages. Aquaculture. 465, 19-27.

Imsland, A. K. D., Hanssen, A., Nytrø, A. V., Reynolds, P., Jonassen, T. M., Hangstad, T. A., Mikalsen, B. (2018). It works! Lumpfish can significantly lower sea lice infestation in large-scale salmon farming. Biology Open. 7(9), bio036301.

Imsland, A. K. D., Reynolds, P., Hangstad, T. A., Kapari, L., Maduna, S. N., Hagen, S. B., Lindberg, K. S. (2021). Quantification of grazing efficacy, growth and health score of different lumpfish (*Cyclopterus lumpus* L.) families: Possible size and gender effects. Aquaculture. 530, 735925.

Kasper, J. M., Bertelsen, B., Ólafsson, H. G., Holst, J. C., Sturlaugsson, J., Jónsson, S. P. (2014). Observations of growth and postspawning survival of lumpfish *Cyclopterus lumpus* from mark-recapture studies. Journal of Fish Biology. 84, 1958-1963.

Kennedy, J., Durif, C. M. F., Florin, A. B., Fréchet, A., Gauthier, J., Hüssy, K., Hedeholm, R. B. (2019). A brief history of lumpfishing, assessment, and management across the North Atlantic. ICES Journal of Marine Science. 76(1), 181-191.

Killen, S. S., Brown, J. A., Gamperl, A. K. (2007). The effect of prey density on foraging mode selection in juvenile lumpfish: balancing food intake with the metabolic cost of foraging. Journal of Animal Ecology. 76(4), 814-825.

Lam, C. T., Rosanowski, S. M., Walker, M., St-Hilaire, S. (2020). Sea lice exposure to non-lethal levels of emamectin benzoate after treatments: a potential risk factor for drug resistance. Scientific Reports. 10(1), 1-8.

Lekang, O.I., Salas-Bringas, C. & Bostock, J.C. (2016). Challenges and emerging technical solutions in on-growing salmon farming. Aquaculture International. 24, 757–766.

Martin-Robichaud, D. (1991). Culture of lumpfish (*Cyclopterus lumpus*) for roe production. Bulletin of the Aquaculture Association of Canada. 91(3), 83-85.

Mavraganis, T., Constantina, C., Kolygas, M., Vidalis, K., Nathanailides, C. (2020). Environmental issues of aquaculture development. Egyptian Journal of Aquatic Biology and Fisheries. 24(2), 441-450.

McKibben, M. A., Hay, D. W. (2004). Distributions of planktonic sea lice larvae *Lepeophtheirus salmonis* in the inter-tidal zone in Loch Torridon, Western Scotland in relation to salmon farm production cycles. Aquaculture Research. 35(8), 742-750.

MDMR (Maine Department of Marine Resources). (2021). https://www.maine.gov/dmr/aquaculture/leases/decisions/table.html. Accessed July 12, 2021.

Moring, J. R. (1989). Food habits and algal associations of juvenile lumpfish, *Cyclopterus lumpus* L., in intertidal waters. Fishery Bulletin. 87, 233-237.

Mortensen, A., Johansen, R. B., Hansen, Ø. J., Puvanendran, V. (2020). Temperature preference of juvenile lumpfish (*Cyclopterus lumpus*) originating from the southern and northern parts of Norway. Journal of Thermal Biology. 89, 102562.

Nelson, E. J., Robinson, S. M. C., Feindel, N., Sterling, A., Byrne, A., Pee Ang, K. (2018). Horizontal and vertical distribution of sea lice larvae (*Lepeophtheirus salmonis*) in and around salmon farms in the Bay of Fundy, Canada. Journal of Fish Disease. 41(6), 885-899.

NHSG (New Hampshire Sea Grant). (2021). https://seagrant.unh.edu/aquafort. Accessed July 12, 2021.

NMFS (National Marine Fisheries Service) (2021) Fisheries of the United States, 2019. U.S. Department of Commerce, NOAA Current Fishery Statistics No. 2019 Available at: https://media.fisheries.noaa.gov/2021-05/FUS2019-FINAL-webready-2.3.pdf?null=.

NOAA (National Oceanic and Atmospheric Administration) Fisheries. (2019). Imports and Exports of Fishery Products Annual Summary, 2018. NOAA Fisheries. NO. 2018-2. https://www.st.nmfs.noaa.gov/Assets/commercial/trade/Trade2018.pdf.

NOAA (National Oceanic and Atmospheric Administration) Fisheries. (2021). Baird Station: The First National Fish Hatchery. https://www.fisheries.noaa.gov/feature-story/baird-station-first-national-fish-hatchery. Accessed July 12, 2021.

Norwegian Directorate of Fisheries. (2021). Total number of cleanerfish put into cages with Atlantic salmon and rainbow trout (Wild catch and farmed cleanerfish). https://www.fiskeridir.no/English/Aquaculture/Statistics/Cleanerfish-Lumpfish-and-Wrasse. Accessed August 6, 2021.

Oppedal, F., Samsing, F., Dempster, T., Wright, D. W., Bui, S., Stien, L. H. (2017). Sea lice infestation levels decrease with deeper 'snorkel' barriers in Atlantic salmon sea-cages. Pest Management Science. 73(9), 1935-1943.

Parrish, D. L., Behnke, R. J., Gephard, S. R., McCormick, S. D., Reeves, G. H. (1998). Why aren't there more Atlantic salmon (*Salmo salar*)? Canadian Journal of Fisheries and Aquatic Sciences. 55, 281-287.

Pershing, A.J., Alexander, M.A., Hernandez, C.M., Kerr, L.A., Le Bris, A., Mills, K. E., Nye, J. A., Record, N. R., Scannell, H. A., Scott, J. D., Sherwood, G. D., Thomas, A.C. (2015). Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. Science. 350 (6262), 809-812.

Piasecki, W., MacKinnon, B. M. (1995). Life cycle of a sea louse, *Caligus elongatus* von Nordmann, 1832 (*Copepoda, Siphonostomatoida, Caligidae*). Canadian Journal of Zoology. 73(1), 74-82.

Pietrak, M., Jensen, A., Barbin Zydlewski, G., Bricknell, I. (2019). The three-spined stickleback, *Gasterosteus aculeatus* Linnaeus 1758, plays a minor role as a host of *Lepeophtheirus salmonis* (Krøyer 1837) in the Gulf of Maine. Journal of Fish Diseases. 42(7), 985-989.

Pike, A. W., Wadsworth, S. L. (1999). Sea lice on salmonids: Their biology and control. Advances in Parasitology. 44, 233-237.

Powell, A., Treasurer, J. W., Pooley, C. L., Keay, A. J., Lloyd, R., Imsland, A. K., Garcia de Leaniz, C. (2018). Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. Reviews in Aquaculture. 10(3), 683-702.

Powell, A., Pooley, C., Scolamacchia, M., Garcia de Leaniz, C. (2018). Review of lumpfish biology. In: Cleaner fish biology and aquaculture applications. (J. W. Treasurer, ed.), 5M Publishing Ltd., Sheffield, UK, pp. 90-113.

Pountney, S. M., Lein, I., Migaud, H., Davie, A. (2020). High temperature is detrimental to captive lumpfish (*Cyclopterus lumpus*, L) reproductive performance. Aquaculture. 522, 735121.

Robinson, J. (2019). Compliance Officer for Cooke Aquaculture USA Inc., personal communication to E. Fairchild on 3/26/19.

Robledo, D., Gutiérrez, A. P., Barría, A., Lhorente, J. P., Houston, R. D., Yáñez, J. M. (2019). Discovery and functional annotation of quantitative trait loci affecting resistance to sea lice in Atlantic salmon. Frontiers in Genetics. 10, 56.

Saksida, S. M., Bricknell, I., Robinson, S., Jones, S. (2015). Population ecology and epidemiology of sea lice in Canadian waters. Maine Sea Grant Publications. 75. https://digitalcommons.library.umaine.edu/seagrant_pub/75

Sayer, M. D. J., Reader, J. P. (1996). Exposure of goldsinny, rock cook and corkwing wrasse to low temperature and low salinity: Survival, blood physiology and seasonal variation. Journal of Fish Biology. 49, 41-63.

Skiftesvik, A. B., Blom, G., Agnalt, A. L., Durif, C. M. F., Browman, H. I., Bjelland, R. M., Mortensen, S. (2014). Wrasse (Labridae) as cleaner fish in salmonid aquaculture - The Hardangerfjord as a case study. Marine Biology Research. 10(3), 289-300.

UN-DESA (United Nations, Department of Economic and Social Affairs) Population Division (2019). Probabilistic Population Projections Rev. 1 based on the World Population Prospects 2019 Rev. 1: http://population.un.org/wpp/.

Ward, D., Føre, M., Howell, W. H., Watson, W. (2012). The influence of stocking density on the swimming behavior of adult Atlantic cod, *Gadus morhua*, in a near shore net pen. Journal of the World Aquaculture Society. 43(5), 621-634.

APPENDICES

Appendix A. A general guide to identifying two common types of sea lice, *Lepeophtheirus salmonis* and *Caligus elongatus*

A sea lice identification guide was created by former UNH undergraduate student Nathaniel Kinsman as an independent investigation (MEFB 775) during spring semester 2019 under the mentorship of Dr. Elizabeth Fairchild. Dr. Michael Pietrak of the USDA ARS NCWMAC (Franklin, ME) contributed to this project.

Figure A. 1. Male (left) and female (right) *Caligus elongatus.* An easy and consistent way to differentiate sex in *C. elongatus* is by looking at the genital section or what could be called an abdomen. Notice that the male is thin, long, and has sharp turns to the curvature. The female is squarer shaped, with large round corners. This method of identification also works on *L. salmonis*, as adult males and females of both species share these structures.

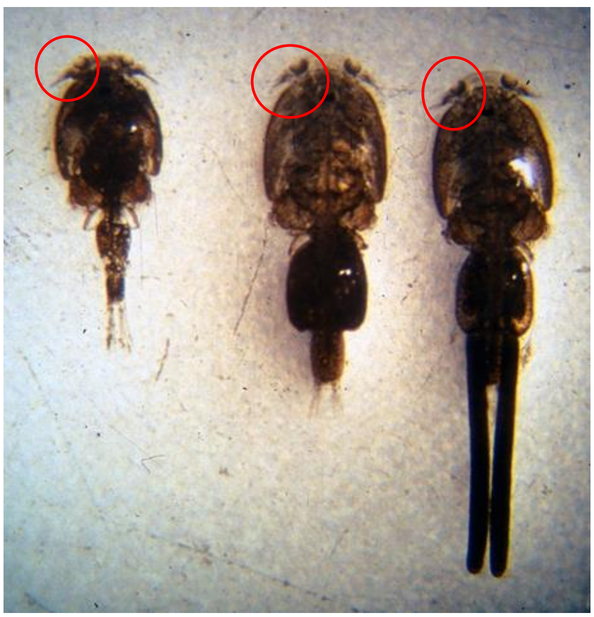

Figure A.2. *Caligus elongatus* sea lice. The far left is an adult male, the middle is an adult female, and the far right is a gravid adult female with egg sacs attached to its abdomen. The red circles denote a barb like structure on the top of the louse's head. The spherical depression at the base of the barb is known as a lunule. This lunule is more pronounced in *C. elongatus* than in *L. salmonis* (see Figure A.3), making this a good way to differentiate between the two species.

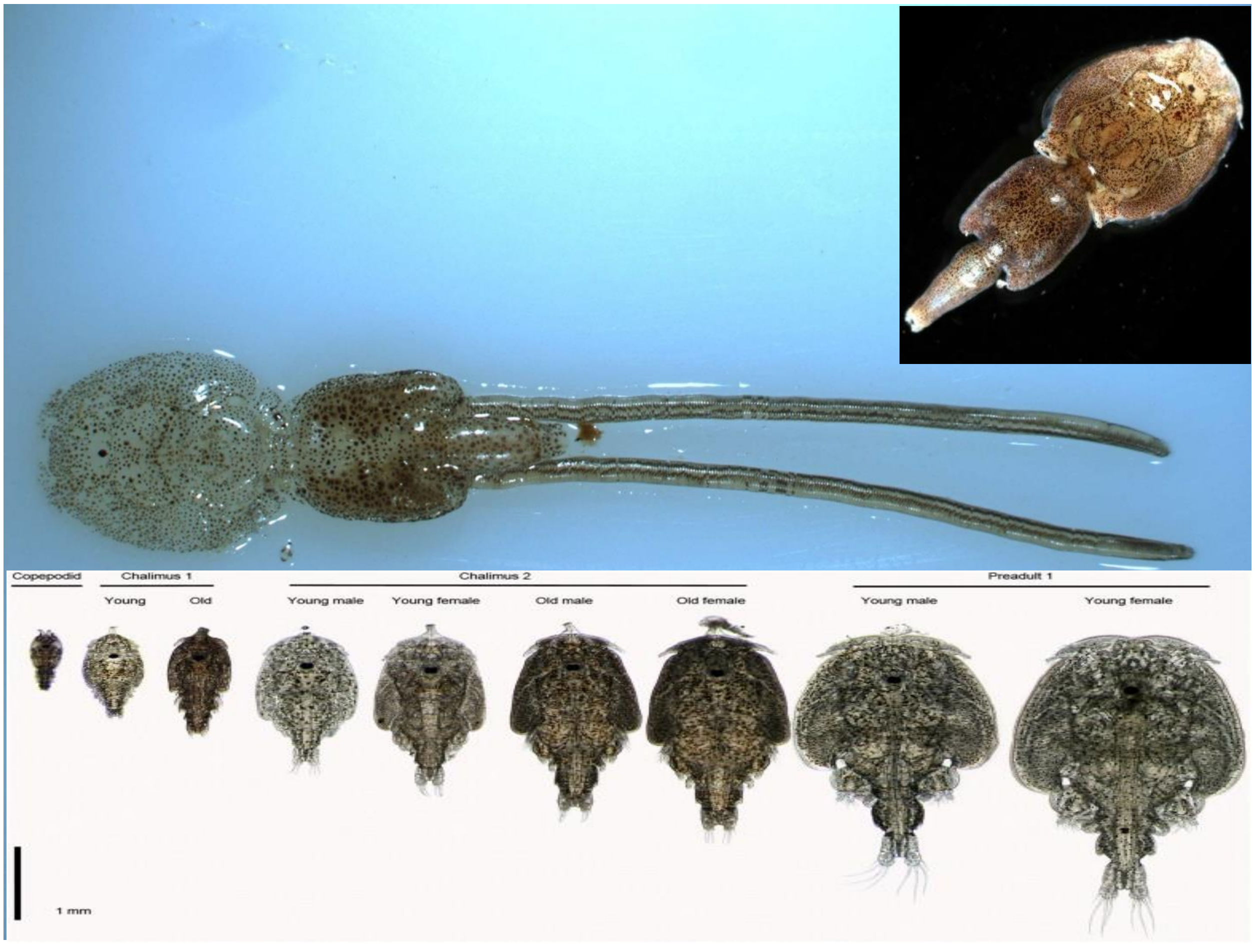

Figure A. 3. *Lepeophtheirus salmonis*. Top photo: Example of a female *L. salmonis* with egg sacs (the long tendrils) attached. Bottom photo: *L. salmonis* life cycle. Starting as free-swimming plankton, the lice moult multiple times until the adult parasitic stage. Generally, only the adult stages are noticeable with the naked eye. Sub-adult stages may look like small black dots or marks on the host fish and are hard to see without the use of a microscope. *L. salmonis* also have a small lunule (barb) like *C. elongatus*, however, it is much smaller and less pronounced.

Photo credits:

Pictures provided by Dr. Michael Pietrak (USDA ARS NCWMAC) and Foras na Mara Marine Institute (Galway, Ireland; https://www.marine.ie/Home/home).

General morphology:

Generally, *Lepeophtheirus salmonis* tends to be bigger (roughly twice the size) than most *Caligus spp*. A sea lice body consists of four regions: the cephalothorax, the fourth segment (leg-bearing portion), genital complex, and abdomen. The cephalothorax is like a broad shield or barrier that contains all the other body segments until the third leg sections; it also is what allows the lice to attach to fish (Pike and Wadsworth, 1999). In addition, lice possess a modified oral appendage to assist with holding itself to the fish. In all cases, females always are significantly larger than males, with large genital regions. Females may also have long egg sacs which are roughly the same length as the female's body. Females can potentially produce 6 to 11 pairs of these egg strings across their 7-month lifespan (Johnson and Albright, 1991).

Life stages and development:

L. salmonis has both free-swimming and parasitic life stages, all separated by moults. The period of development from egg to adult takes between 17 to 72 days (roughly) depending on temperature. The eggs hatch into nauplii, which molt to a second planktonic stage. Both these stages are non-feeding and non-parasitic, instead depending on the egg yolk for energy (Johannessen, 1977). The next stage is the copepodid stage, the first parasitic stage where the lice seek a host using primarily chemical cues. Other variables such as light, water salinity, and current also play a large roll in lice finding a host. Lice prefer to attach to fish in areas with little hydrodynamic disturbance (weak or no current). Once attached, lice feed on their host for the remainder of their development, detaching only in some cases to find a new host.

References:

Johannessen, A. (1977). Early stages of *Lepeophtheirus salmonis* (*Copepoda, Caligidae*) Sarsia 63:169-176. doi:10.1080/00364827.1978.10411336

Johnson, S. C., Albright, L. J. (1991). Development, growth, and survival of *Lepeophtheirus-Salmonis* (Copepoda, Caligidae) under laboratory conditions. J Mar Biol Assoc UK 71:425-436. doi:Doi 10.1017/S0025315400051687

Pike A, W., Wadsworth, S, L, (1999). Sea lice on salmonids: Their biology and control. In: Baker J. R., Muller, R., Rollinson, D. (eds) Advances in Parasitology 44: 233-337.

Appendix B. Sea lice sampling data from 2018 and 2019 steelhead trout production runs at the UNH steelhead trout farm

Sea lice sampling at the UNH steelhead trout farm occurred briefly during the 2018 and 2019 production runs by students in the Fairchild Lab. Here those data are reported for comparison to the lice assessment in this thesis (Chapter 1).

During the 2018 steelhead trout season, lice loads were sampled three times between October 18, 2018 and December 14, 2018 and ranged from 1.70 and 3.00 lice per fish (Figure B.1, Table B.1). During the 2019 steelhead trout season, lice loads were sampled seven times between June 10, 2019 and July 17, 2019, starting at time 0 when the fish were transferred from freshwater into the saltwater farm. Lice loads in summer 2019 ranged from 0.0 (at stocking), but after one week, increased steadily to 0.40 to 0.47 lice per fish in the final weeks before the steelhead trout were harvested (Figure B.2, Table B.2). Only *Caligus elongatus* were collected during these sampling periods.

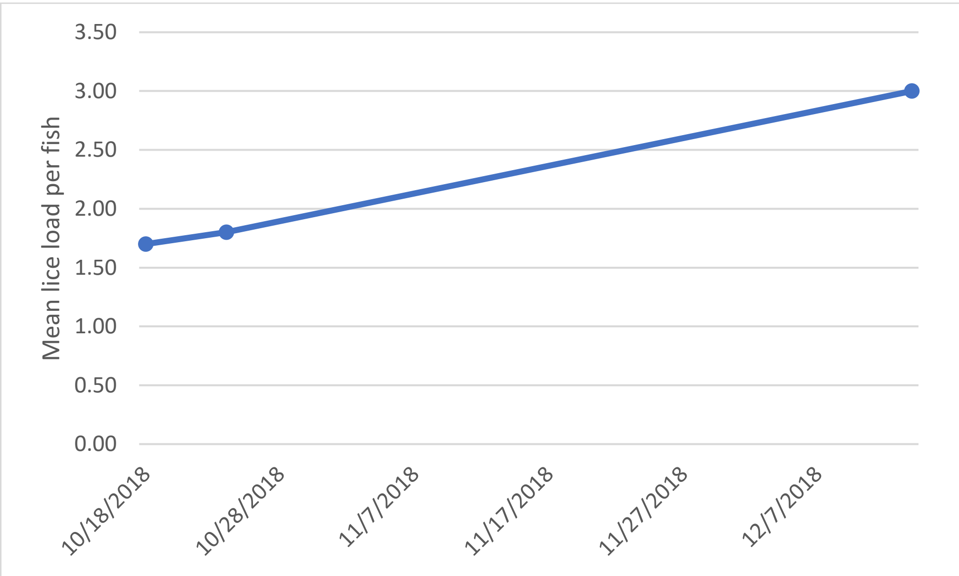

Figure B.1 Mean lice on steelhead trout between October 18, 2018 and December 14, 2018.

Table B.1. Lice assessment data from the UNH steelhead trout farm from October 18, 2018 to December 14, 2018.

Date	Week	Total Lice	Mean Lice per Trout	Total Female Lice
10/18/2018	1	17	1.70	9
10/24/2018	2	18	1.80	10
12/14/2018	3	30	3.00	20

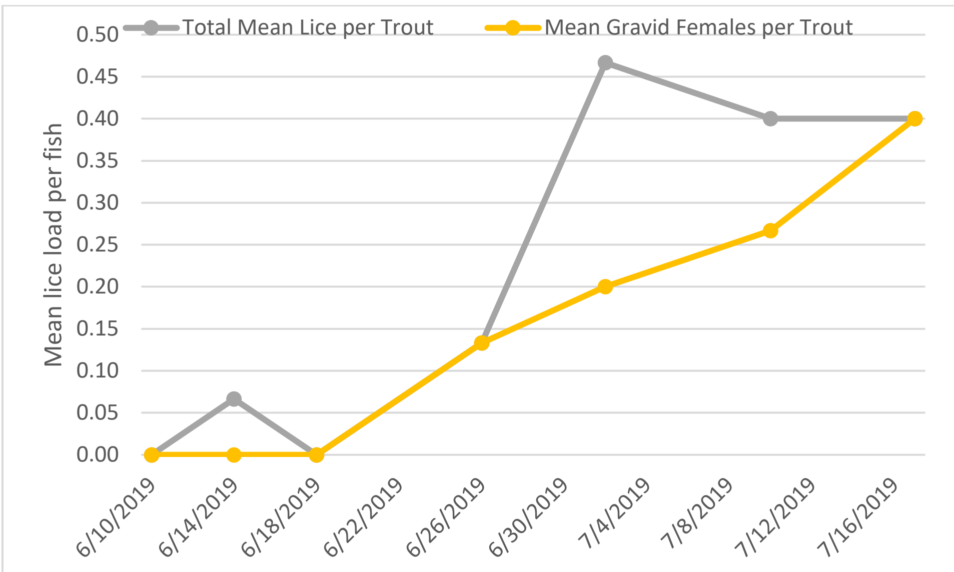

Figure B.2. Mean lice and mean gravid lice on steelhead trout between June 10, 2019 and July 16, 2019.

Table B.2. Lice assessment data from the UNH steelhead trout farm from June 10, 2019 to July 16, 2019. Week 0 refers to when the fish were transferred from freshwater and stocked into the marine farm.

Date	Week	Total Lice	Mean Lice per Trout	Mean Gravid Female Lice per Trout	Total Male Lice	Total Female Lice
6/10/2019	0	0	0.00	0.00	0	0
6/14/2019	1	1	0.07	0.00	0	1
6/18/2019	2	0	0.00	0.00	0	0
6/26/2019	3	2	0.13	0.13	0	1
7/2/2019	4	7	0.47	0.20	1	6
7/10/2019	5	6	0.40	0.27	1	5
7/17/2019	6	6	0.40	0.4	0	6

Appendix C. Preliminary lumpfish-steelhead trout caging trial to develop sampling protocols

To develop sampling protocols for evaluating the effectiveness of lumpfish as cleanerfish of steelhead trout, a three-week trial was conducted at the UNH Judd Gregg Marine Research Complex Pier in New Castle, NH from July 3 to July 24, 2020.

Fish source

Steelhead trout, *Oncorhynchus mykiss* (Kamloops strain), were acquired from Sumner Brook Fish Farm in Ossipee, NH and trucked to the Coastal Marine Laboratory (CML) in New Castle June 12, 2020, prior to the onset of the caging study. Upon arrival to the CML, steelhead trout were transferred via dipnet from the truck to a 1.8 m diameter round acclimation tank supplied with flow-through, ambient sea water, oxygen, and air. Steelhead trout were handfed Cargill EWOS® 5.0 mm dry pellets twice daily until caging studies began. Existing, cultured lumpfish, *Cyclopterus lumpus,* reared and housed at the CML, were used in the caging trials.

Cages

Six, small (785 L, 1 m diameter x 1 m depth), cylindrical cages constructed with an HDPE plastic frame and 5 cm mesh netting were used as experimental units to evaluate the cleanerfish ability of lumpfish with steelhead trout. Cages were weighted at the bottom and lined with buoys along the top. The lids were hinged to allow easy access into the cage. Lids were secured with twist-ties to prevent fish egress or entry of predators. The cages were suspended in the water and secured in a bay under the Pier (Figure C.1).

Figure C.1. Cages affixed to the platform under the Pier.

Fish

Each cage was stocked with 15 steelhead trout yielding an initial density of approximately 0.004 kg/L (Table C.1). This stocking density was chosen so that fish density would be high enough to promote schooling behavior yet not exceed the carrying capacity of the cages. Four of these six cages also were stocked with three lumpfish each. Although stocking lumpfish at 15% density in relation to the salmonid density is an effective approach to elicit cleanerfish behavior (Imsland et al., 2014), cages were stocked at 20% density to offset any potential issues with lumpfish mortality. The remaining two cages served as controls and were void of lumpfish (Table C.2).

Table C.1. Steelhead stocking densities (kg/L) in each cage.

Cage	Trout Density (kg/L)
1	0.0044
2	0.0039
3	0.0045
4	0.0041
5	0.0041
6	0.0039

Table C.2. Trial experimental design.

Cage	Trout	Lumpfish	Hide Design Treatment
1	15	3	Fake Kelp
2	15	3	PVC Panels
3	15	0	None
4	15	3	PVC Panels
5	15	0	None
6	15	3	Fake Kelp

Hides

In the four cages containing lumpfish, two kinds of lumpfish hides were evaluated: fake kelp and PVC panels (Table C.2; Figure C.2). The fake kelp hide consisted of a 10 mm polyester rope with strips of black plastic sheeting (80 cm x 5.5 cm) woven through the rope to mimic macroalgae. The PVC hide was a 76 cm length of 10 cm diameter PVC pipe cut down the middle and bolted together to form a “w” shaped panel. Both hide designs were securely tied to the cages at the top and bottom to minimize the pull of the current and cages were randomly affixed to the floating platform under the Pier.

Figure C.2. Two different hide designs tested within cages.

Stocking cages

Trout were loaded into an oxygen supplied Xactic™ filled with seawater and trucked from the CML to the Pier. From there, batch weights (kg) were taken of 15 steelhead trout per batch in tared 19 L buckets filled with sea water. The bucket then was lowered to the float and the fish were emptied into a single cage. This process was repeated for all six cages. Lumpfish, weighed individually in the CML, were transported to the Pier in four 19 L buckets, and released into the four respective cages.

Trial protocols

Fish were fed twice daily at low tide. Steelhead trout were fed Cargill EWOS® 5.0 mm diet at 1.5% body weight per day, a recommended amount (A. Jones, pers. comm.). Lumpfish were fed 4.0 mm Skretting (Nutreco) Clean Assist diet at 2% body weight per day. Water temperature and light intensity were recorded every 0.5 hours with HOBO data loggers (Onset Computer Corp.). Because the Pier overhead shadowed a portion of the bay where the cages were, one data logger was secured on the inside of the back-left cage, while the other was secured on the inside of the front-right cage to account for any parameter differences.

Sea lice sampling

All fish were assessed for sea lice weekly. For each cage, every fish (including lumpfish when applicable) was removed with a dipnet, immersed in an individual freshwater bath (19 L bucket filled halfway with freshwater) for 90-120 seconds, then transferred to a sea water holding tank until all fish had been processed from the cage. After all fish from a given cage were assessed, they were returned to their cage. Each freshwater bath sample was poured through a 180 μm sieve, and the collected contents within the sieve were washed with freshwater into a small (473-946 ml) plastic storage container. This process then was repeated for each of the six cages. The storage containers containing the sieved contents of the freshwater baths were then brought back to the CML and processed either immediately or refrigerated and processed within 36 hours. Any deceased fish were removed without sampling for sea lice, weighed, and disposed.

Preliminary results: mortality, temperature

Mean daily water temperature throughout the 21 days fluctuated between 16.0° C and 19.5° C (Figure C.3). The highest temperature recorded during this time period was 20.2° C, and the lowest temperature recorded was 13.0° C. The survival rate of steelhead trout over the entire trial was 18.9%. The survival rate of lumpfish over the entire trial was 41.7% (Figure C.4). Due to the low survival of both fish species after only three weeks, this trial was terminated, and knowledge gained was applied to Trial 1 and Trial 2 sampling methods.

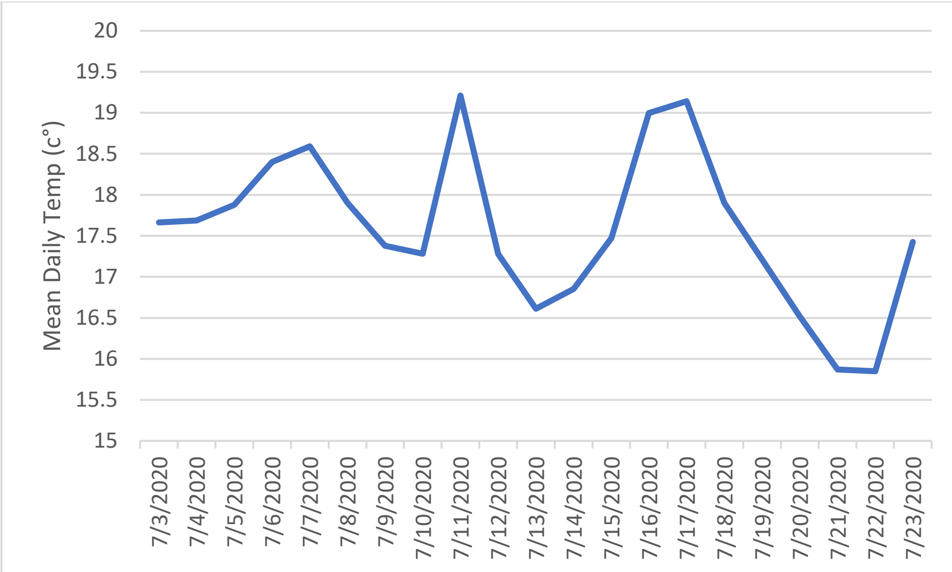

Figure C.3. Mean daily temperatures during the Kamloops preliminary caging trial.

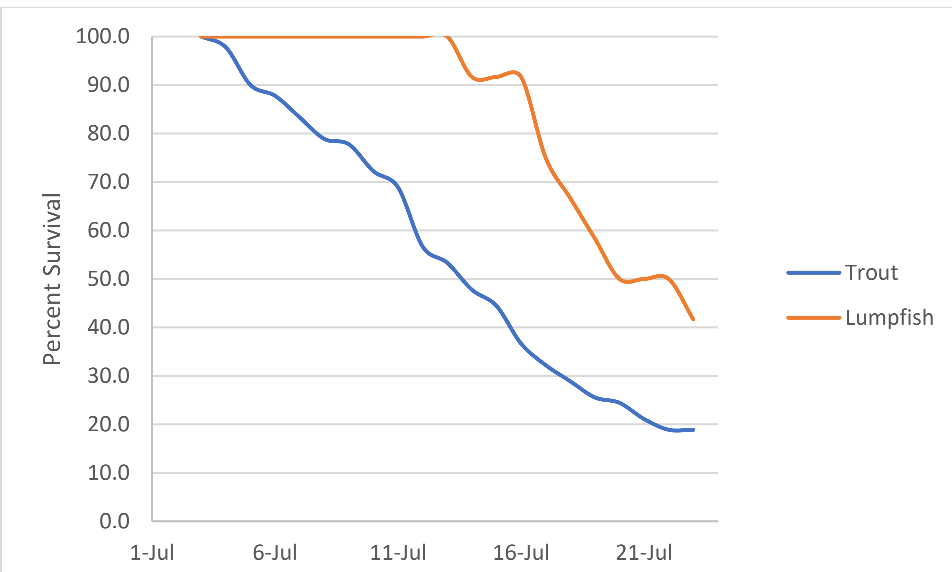

Figure C.4. Survival rate of steelhead trout (blue line) and lumpfish (orange line) throughout the Kamloops preliminary caging trial.

Preliminary trial outcome:

Based on this preliminary caging trial, the following changes were made to the sampling protocol:

1) Any dead fish must be removed from the cages and immediately sampled for sea lice.
2) Stomach content analysis of a subsample of steelhead trout will be examined at the end of each trial.
3) The effects of temperature and steelhead trout strain should be taken into consideration in future caging studies.

Following this preliminary trial, the two experimental trials were conducted during cooler seasons (see Chapter 2).

Appendix D.

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

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