Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon

Kaitlyn Charmley

Department of Integrative Biology, Biological Sciences, University of Guelph, Guelph, ON Canada. Faculty supervisor: Dr. Elizabeth Boulding.
For correspondence, please email: katecharmley1@gmail.com.

Abstract

Sea lice are detrimental ectoparasites that attach to Atlantic salmon causing physiological damage and costing farmers millions in inventory loss and treatments. Cleaner fishes have been introduced into sea cages to act as a biological control of sea lice which is likely a solution for Canadian salmon aquaculture industries. To improve cleaner fish foraging efficiency, this study seeks to determine the optimal habitat for cleaner fishes in Canadian aquaculture. I hypothesized that to be effective cleaner fishes, both the cunners and the lumpfish require habitats that provide them with shelter and places for rest because neither species live solely in the water column. My second hypothesis was that the cunners and the lumpfish require different habitats due to their different morphologies. Habitat comparisons were conducted with three habitats and a control in each individual fishes tank for a total of 8 cunners and 25 lumpfish. It was determined that only cunners required shelter, possibly due to the lumpfish's ability to adhere to the glass tank walls for rest. Moreover, there was no significant difference in habitat preference between the two species. However, the lumpfish were less preferential between habitat and preferred three of the four habitats equally. It should be noted that the lumpfish and the cunners utilized the same habitats in separate ways to better fit their species-specific requirements; so future research on the co-existence of the two species could lead to increased foraging efficiency through two-species cleaner fish systems.

Keywords: Atlantic salmon, sea lice, cunner, lumpfish, biological control, habitat selection

Introduction

Sea lice (*Lepeophtherirus salmonis* and *Caligus* spp.) are ectoparasitic copepods that infest Atlantic salmon (*Salmo salar*) costing farmers 6% of their yearly production value in affected countries (Costello, 2006). In 2006, the salmonid marine market was worth CAD \$10.3 billion worldwide (Costello, 2009). Canada is an important player in this market, accounting for 7% of the global salmon production in 2005 (Tacon, 2005). Economically, sea lice infestations have the largest impact of any parasite on the salmonid market (Costello, 2009). This loss in revenue is both directly from inventory loss and from the cost of treating infected salmon. There are detrimental impacts on the welfare of the salmonid impacted by these sea lice infestations, causing physiological damage, and in extreme cases, stress-induced death (Costello, 2006; Brauner et al., 2012).

Currently, the most extensively used method for managing sea lice infestations worldwide is the use of

chemotherapeutant chemicals, as it has historically been effective and relatively inexpensive (Johnson, Constible, & Richard, 1993; Treasurer, Wadsworth, & Grant, 2000; Aaen, Helensen, Bakke, Kaur, & Horsberg, 2015). However, there are some critical issues with this method; it can be harmful to other non-target marine organisms, it has negative public health perceptions, and resistance by sea lice has begun occurring in many locations (Aaen et al., 2015; Costello, 2009; Fairchild et al., 2010; Jones, Sommerville, & Wootten, 1992; Torrisen, et al., 2013; Roth, Richard, Dobson, & Rae, 1996; Treasurer et al., 2000). An additional control method currently being developed is mechanical removal of sea lice through hot water sprays, high water pressure systems and lasers (Torrisen et al., 2013).

An alternative control method is using cleaner fishes as a biological control for the sea lice, which has been used successfully in Norway since 1987 (Bjordal, 1991; Dedy,

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

Varian, & Fives, 1995). European sea lice infestations are being managed there by the lumpfish (*Cyclopterus lumpus*) and European species of wrasse (Labridae); particularly the goldsinny (*Ctenolabrus rupestris*), the corkwing (*Symphodus melops*) and the ballan wrasse (*Labrus bergylta*) (Skiftesvik et al., 2013). Cleaner fishes are raised alongside the salmon, consuming sea lice and creating a mutualistic relationship between the two species of fishes (Bjordal, 1991). Both the wrasse and the lumpfish feed opportunistically and will consume a variety of prey, depending on availability, and have been observed foraging on sea lice in previous cleaner fish research (Imsland et al. 2014).

In Canada we have had to find our own suitable native species, and the two species that will most likely be effective cleaner fishes are a Western Atlantic population of lumpfish and a Western Atlantic wrasse called cunners (*Tautogolabrus adspersus*). The cunners are a type of cleaner wrasse, however they are not closely related to any of the species used as cleaner fishes in Europe.

These species are new to aquaculture and consequently, not much is presently known regarding their biological requirements. A few locations on the East Coast of Canada have successfully spawned and raised these species (Boyce et al., 2018). An early study found that the cleaner fishes are not foraging sea lice as productively as their European counterparts (MacKinnon, 1995). I speculated that the reason for this inefficient foraging is due to the unsuitable tank habitats.

Habitat selection is defined as behavioural responses that result in uneven use of different habitats to increase survival and fitness of that individual (Rice & Owsley, 2005). Quality habitats can provide different benefits for organisms, for example predator avoidance, provision of food, and protection from abiotic changes (Rice & Owsley, 2005). A study conducted by Mittelbach (1981) found that the bluegills (*Lepomis macrochirus*) switched their habitat over time to coincide with prey abundances changes, choosing a habitat that will increase their foraging efficiency. This suggests that other species of fish often select habitats that will maximize species specific foraging returns. Thus, in aquaculture a possible mechanism to increase foraging efficiency is to provide the species with its optimal habitat.

In their natural habitat, juvenile cunners have refuges that they prefer to reside in, foraging only to limited areas surrounding the refuge due to the risk of predation (Bradbury, Green, & Bruce-Lockhart, 1995; Tupper & Juanes, 2017). Lumpfish prefer to 'sit-and-wait' for prey when prey is abundant by attaching to a substrate with their ventral sucker to save energy (Killen, Brown, & Gamperl, 2007). Consequently, it is possible that the explanation for the reduced foraging efficiency is because the empty tanks do not provide the cunners and the lumpfish with the shelter they require for these activities. It is unknown if providing the cunners and the lumpfish with shelters in aquaculture tanks

will increase their foraging efficiency and if so, what types of shelters will promote this.

My goal was to determine the optimal habitat for cleaner fishes in Canadian aquaculture. Firstly, I hypothesized that for the cunners and the lumpfish to be effective cleaner fish, they would require habitats that provide them with shelter and places for rest because neither species lives solely in the water column due to species-specific biological traits. The cunners require these shelters because they are torpid at night and the lumpfish require these shelters because they lack a swim bladder, thus both species need a place that can act as refuge during this time (Olla, Bejda, & Martin, 1975; Davenport & Kjorsvik, 1986). I predicted that the cunners and the lumpfish would prefer to reside in one of the provided shelters available to them as refuge as opposed to the empty habitat. My second hypothesis was that cunners and lumpfish require different habitats due to their different morphologies; cunners are slender fish and thus prefer small locations (MacKinnon, 1995), whereas lumpfish are nearly spherical and possess ventral suction discs that allow them to adhere to various surfaces (Fisheries and Oceans Canada, 2011). For this hypothesis, I predicted that (1) the preferred habitat would be different for the cunners and the lumpfish and more specifically that (2) the cunners will show preference for the artificial kelp due to its many locations to hide in and the lumpfish will show preference for the plastic pipe because its smooth surface will allow them to properly adhere to it.

To test these predictions, I conducted habitat comparisons between three different shelter types and one control to determine the preferences between the shelters.

Methods

Study Species

Cunners and lumpfish are both diurnal marine fish that live in eastern Canada (MUN, 2015). Cunners can reach to a maximum size of 43cm (MUN, 2015) and lumpfish can reach up to a maximum size of 61cm (Nytrø et al., 2014). Cunners become torpid at night and when the temperature drops below 6°C—a temperature that lumpfish are able to continue to thrive below (Sayer & Davenport, 1996; Nytrø et al., 2014). Prior to receiving the fishes, the cunners were raised in communal tanks provided with plant-like hides and the lumpfish were raised in communal tanks with provided shelves hanging in the middle of the water column. One day prior to the commencement of the study, the cunners had a mean weight of 6.60g (s=3.20g) and the lumpfish had a mean weight of 18.09g (s=4.66g).

Study Site

I conducted my research in the Hagen Aqualab at the University of Guelph as an undergraduate research project under Dr. Elizabeth Boulding, who had obtained an animal

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

utilization protocol (AUP: #3962) for all fishes utilized in this study. I used 8 juvenile cunners and 25 juvenile lumpfish, each in an individual tank with dimensions 40cm L x 20cm W x 25cm H. Each individual tank was labeled to differentiate between species, as well as individual fishes. Each tank was marked at halfway-points along the length and width to divide each tank into four equal parts.

Study Conditions

The juvenile lumpfish and cunners were received on the same day from Memorial University and Cooke Aquaculture Inc., respectively. After the fishes arrived, I allowed them to acclimate to their empty tanks for one week before starting any trials. Conditions for the fishes remained constant throughout the entirety of the experiment. The study area followed a 12L:12D photoperiod based on the Atlantic Daylight Time zone and there was a recirculating saltwater system with a constant temperature of 11°C and a salinity of 31 g/L.

Shelter Specifications

The three shelters I studied were: artificial grass turf with a 5cm³ weighted rock placed in the middle of it (“Grass”), black plastic pipe with a diameter of 9cm and a length of 10cm (“Pipe”), and pieces of cut reinforced polyethylene tarp held together at the base to resemble kelp (“Plant”). Examples of the three materials I used for these shelters can be found in **Figure 1**. I also had an empty habitat as a control. I defined habitat complexity as the amount of separate locations in that shelter there was for the fish to reside. Thus, the “Grass” shelter was the least complex, followed by the “Pipe” shelter, and the “Plant” shelter was the most complex. Each shelter was a maximum of 20cm length and 10cm width to fit in one quarter of the tank.

Habitat Comparisons

After the fishes acclimated to their empty tanks, I conducted habitat comparisons. I compared the three shelters and the control against each other, each habitat taking up one quarter of the tank. I studied combinations of the four shelters in all 33 tanks simultaneously to obtain 25 independent observations for the lumpfish and eight independent observations for the cunners each day. The quarter of the tank that each habitat occupied was randomly selected for each individual tank. I put the shelters in the tanks in the morning of day 1 and let them acclimate throughout the day with the shelters in their tanks. For the subsequent seven days, I recorded which habitat each fish was residing in one hour before the photoperiod lights turned off in the evening. In the occurrence that a fish was residing directly on top of a dividing line in the tank, the habitat that the head of the fish was residing in was counted as the fish being present there.

Statistical Analyses

To analyse the habitat preferences within the species I conducted two Kruskal-Wallis tests, one for cunners and one for lumpfish, followed by two Dunn’s post hoc tests. Before conducting these tests, I summed the number of fish in each habitat on each of the seven days for the cunners (n=8) and lumpfish (n=25) separately to obtain seven replicates for each species/shelter combination.

To analyse the habitat preferences between the two species, I conducted two t-tests on the mean proportions of fish residing in the plant shelter and in the pipe shelter between the two species. Prior to this, using the independent observations for each species, I found the proportion of cunners and the proportion of lumpfish in the four habitats for all seven days. I conducted an arcsine square root transformation on the proportions of fish in each habitat to correct for the data not being normally distributed and to correct for the unequal variances in the populations.

Results

Within Species

The within species non-parametric independent samples Kruskal-Wallis test for the cunners found that there was strong evidence ($p < 0.001$) that the distribution of the number of fish was not the same across all habitats ($N=28$, $t=21.244$, $df=2$) (**Figure 2**). Dunn’s pairwise tests between the four habitats found that there was strong evidence ($p < 0.001$) that there was preference by the cunners for the “Plant” habitat over the control habitat ($t=-19.857$). All p-values were corrected with the Bonferroni adjustment for multiple individual comparisons. The median number of cunners that selected the plant was 5.0 compared to 0.0 for the control. The median number of cunners in the pipe habitat and the grass habitat were 2.0 and 1.0, respectively. However, all other pairwise comparisons showed no significant difference in preference.

The within species non-parametric independent samples Kruskal-Wallis test for the lumpfish found strong evidence ($p < 0.001$) that the distribution of the number of fish in the habitats was not the same ($N=28$, $t=17.902$, $df=2$) (**Figure 3**). I then conducted Dunn’s pairwise test between the four habitats and found that there was significant preference by the lumpfish against the “Grass” habitat for the “Pipe” habitat evidence ($p < 0.001$), the “Plant” habitat ($p=0.043$) and the control habitat ($p=0.028$). All p-values were corrected with the Bonferroni adjustment for multiple individual comparisons. The median number of lumpfish that selected the pipe was 9.0, the median number that selected the control was 8.0, the median number for the plant was 6.0 and for the grass the median number of lumpfish that selected it was 1.0. All other pairwise comparisons between habitats

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

showed no significant difference in preference by the lumpfish.

Between Species

I conducted two separate t-tests to analyse the difference in proportion of the two species that preferred the “Plant” and that preferred the “Pipe.” Since the arcsine square root transformation was conducted prior, the Levene’s test for both the “Plant” and the “Pipe” were not significant and thus equal variances was assumed for both t-tests. The independent-samples t-test for the plant found there was a significantly higher proportion of cunners ($M=0.864$, $SD=0.189$) than lumpfish ($M=0.561$, $SD=0.926$) that selected it; $t(12)=3.793$, $p=0.003$ (**Figure 4**).

There was a trend for a higher proportion of the lumpfish to select the “Pipe”. The independent-samples t-test for the pipe found that there was a borderline significant difference between the proportion of cunners ($M=0.440$, $SD=0.240$) and the proportion of the lumpfish (0.648 , $SD=0.092$) that selected it; $t(12)=-2.137$, $p=0.054$ (**Figure 5**).

Discussion

The first hypothesis regarding both species’ requirement for shelter in their habitat was supported for the cunners. This is because my prediction that they will prefer to reside in one of the shelters as opposed to the empty habitat was found to be correct. The cunners showed significant preference for the plant over the control in the Kruskal-Wallis test. The prediction was not supported with regards to the lumpfish because the Kruskal-Wallis test did not show a significant preference for any of the habitats with shelters over the control. Thus, I can state with confidence that cunners require shelter in their habitat to act as refuge whereas lumpfish require hard surfaces to attach to.

My hypothesis that the two cleaner fish species have different habitat requirements due to their different morphologies cannot be supported. The prediction that cunners would show preference for the plant shelter and the lumpfish would show preference for the pipe shelter was supported by the two Kruskal-Wallis tests. However, since the lumpfish preferred the pipe habitat, plant habitat and control habitat with no significant difference between the three, the prediction regarding the two species having different habitat preferences cannot be supported as both species showed preference for the plant habitat.

The lumpfish were found to be less particular about the habitat they selected. This is seen by the lumpfish preferring the pipe, control and plant with no significant difference between the three in the Kruskal-Wallis test. The t-test between the two species for the plant also supports this because despite the cunners and the lumpfish both showing preference for the plant, there is a significantly higher proportion of cunners that selected the plant, showing that the preference of habitats is more distributed for the lumpfish. The

lumpfish showing no significant preference for any one habitat provided could be due to them not having specific habitat requirements, all the provided habitats being adequate or possibly none of the provided habitats being optimal for their requirements.

Previous work by Tupper and Juanes (2017) tested the effects of habitat type on the time and energy spent foraging by juvenile cunners in the natural environment off the east coast of Canada. Contrary to our results and to their prediction, they found that less complex environments coincided with more time and energy spent on foraging. They defined habitat complexity as substrate rugosity and did not consider habitats such as macroalgae and seagrass. The possible explanation that they had for these unexpected results was that the difference was a response to food availability, as complex habitats have a high amount food available to the cunners and thus they did not need to leave the habitat to forage. These results give importance to our study because in aquaculture, available food is not provided by complex shelters in the same way that it supports life in the wild. Therefore, although in the wild, cunners’ foraging is maximized in less complex habitats, this is not necessarily the habitat that will maximize it in aquaculture.

Studies have found that lumpfish spend the majority of their time, other than feeding-related activities, resting in shelters (Imsland et al., 2014; Imsland et al., 2015). Imsland et al. (2014) observed the behaviour of lumpfish with and without Atlantic salmon in the same sea pen and found that in both instances, when the fish were not foraging for food, they were usually found resting on the floating seaweed provided for them. The study by Imsland et al. (2015) took this study one step further and explored the different types of substrates lumpfish preferred in sea cages. They found that lumpfish had preference for artificial substrates that are smooth and plastic as opposed to natural substrates like rocks and kelp. Contrary to this, the current study showed lumpfish having no preference against the control which goes against these studies’ findings that the lumpfish need a shelter for rest when they are not feeding. A possible explanation is that since this study was conducted in glass aquaria whereas the previous research was all conducted in sea cages, the bare glass “control” could be similar to the artificial smooth plastic provided in the previous studies and adequate for rest. The studies conducted in sea cages had no other areas for the lumpfish to rest other than the provided shelters.

No previous research has compared the habitat preferences of cunners against lumpfish. One finding of this study was that the two species had no significant difference in habitat preferences, despite their different morphologies. The fish were instead observed using the same habitats in different ways to suit their biological needs (**Figure 6**). The cunners were found burrowing below the grass and pipe and in the plant, whereas, the lumpfish were found attached to the top of these shelters with their ventral sucker. These shelters were found to therefore provide different niches for the species.

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

Since the two species would likely not compete for shelter, future studies should analyse the co-existence of cunners and lumpfish for the possibility of two-species cleaner fish system in Canada. A previous study by Imsland et al. (2016) investigated the co-existence of the lumpfish and the goldsinny wrasse, a European sea lice cleaner fish, in Norway. Despite their findings of aggression by the large lumpfish on the goldsinny wrasse, the study suggests that such interactions are likely size dependent. The maximum size of the goldsinny has been found to be 15cm (Darwall, Costello, Donnelly, & Lystaght, 1992) whereas the cunners can reach up to 43cm (<https://www.mun.ca/osc/oscedu/tadspersus.php>), so it is possible that cunners will not experience this co-existence issue. A two-species system would be beneficial if attainable because wrasses tend to be more efficient at sea lice foraging, however become torpid in the winter months when temperatures decrease and lumpfish are able to continue to forage during this time (Sayer & Davenport, 1996; Nytro et al., 2014).

A limitation to this study is the small sample size, as I only had 8 cunners and 25 lumpfish to observe shelter preferences. A larger sample size would have given more confidence to my results. Another limitation to this study was the short time span; due to this project being conducted over one semester and a delay in the arrival of the study subjects, it was only feasible to allow the fish to acclimate with shelters in their tanks for one day and conduct observations for seven days. The habitat preferences were not entirely constant over the seven days (**Figure 7**), possibly due to familiarity of shelters used in their previous habitat prior to arrival to the Aqualab. Thus, it would be beneficial for future research to compare habitat preference within and between these two cleaner fish species at a larger scale and over a longer period of time.

Both species of cleaner fishes, the cunners and the lumpfish, require shelter/substrate and show no difference in habitat preference, although the lumpfish were found to be less preferential on the type of shelter they require. These habitat specifications are important because determining optimal aquaculture conditions for Canadian cleaner fishes will lead to the most efficient sea lice foraging. Sea lice infestation issues impact our economy, environment and public health nevertheless sea lice are growing resistance to current control methods. Infestations are expected to be increasingly detrimental because sea lice reproduction is highly dependent on sea temperatures which are rising due to climate change (Costello, 2006). Therefore, Canada must increase the efficiency of cleaner fishes' lice foraging, and fast, if we want to remain competitive in this market.

Acknowledgements

I would like to thank my family and friends for their support throughout this project. I also thank my advisor, E.G. Boulding, for her continual support throughout this project and for providing the idea to focus my research on the

comparison of habitat selection between the lumpfish and cunners, and for editing the final manuscript. The cunners used in this project were provided by K-P Ang and G. McBriarty of Kelly Cove Salmon of the Cooke Aquaculture Inc. The lumpfish used in this project were provided by D. Boyce of the Memorial University of Newfoundland. I would like to acknowledge M. Cornish and A. Sritharan of the University of Guelph Aqualab for their technical support throughout this project. Funding for the AquaLab rental was provided by an NSERC Strategic grant to E.G. Boulding, K-P Ang, J.A.K. Elliot, M. Herlin, F. Powell, and L.R. Schaeffer.

References

- Aaen, S.M., Helesen, K.O., Bakke, M.J., Kaur, K., & Horsberg, T.E. (2015). Drug resistance in sea lice: a threat to salmonid aquaculture. *Trends in Parasitology*, 31(2), 72-81. doi: 10.1016/j.pt.2014.12.006.
- Bjordal, A. (1991). Wrasse as cleaner-fish for farmed salmon. *Progress in Underwater Science*, 16, 17-28. Retrieved from <https://brage.bibsys.no>
- Boyce, D., Ang, K.P., & Prickett, R. (2018). Chapter 23. Cunner and Lumpfish as cleaner fish species in Canada. In: Cleaner fish biology and aquaculture applications. J. Treasurer (editor)_528 pages.
- Bradbury, C., Green, J.M., & Bruce-Lockhart, M. (1995). Home ranges of female cunner, *Tautoglabrus adspersus* (Labridae), as determined by ultrasonic telemetry. *Canadian Journal of Zoology*, 73(7), 1268-1279. doi: 10.1139/z95-151.
- Brauner, C.J., Sackville, M., Gallagher, Z., Tang, S., Nendick, L., & Farrell, A.P. (2012). Physiological consequences of the salmon louse (*Lepeophtheirus salmonis*) on juvenile pink salmon (*Oncorhynchus gorbuscha*): implications for wild salmon ecology and management, and for salmon aquaculture. *Philosophical Transactions of the Royal Society B*, 367, 1770-1779. doi: 10.1098/rstb.2011.0423.
- Costello, M.J. (2006). Ecology of sea lice parasitic on farmed and wild fish. *Trends in Parasitology*, 22(1), 475-483. doi: 10.1016/j.pt.2006.08.006.
- Costello, M. J. (2009). The global economic cost of sea lice to the salmonid farming industry. *Journal of Fish Disease*, 32(1), 115-118. doi:10.1111/j.1365-2761.2008.01011.x.
- Darwall, W.R.T., Costello, M.J., Donnelly, R., & Lystaght, S. (1992). Implications of life-history strategies for a new wrasse fishery. *Journal of Fish Biology*, 41, 111-

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

123. doi: 10.1111/j.1095-8649.1992.tb03873.x.

Davenport, J., & Kjorsvik, E. (1986). Buoyance in the Lump sucker *Cyclopterus Lumpus*. *Journal of the Marine Biological Association of the United Kingdom*, 66(1), 159-174. doi: 10.1017/S0025315400039722.

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(1-2), 73-90. doi: 10.1016/0044-8486(94)00331-H.

Fairchild, W., Doe, K., Jackman, P., Arsenault, J., Aube, J., Losier, M., & Cook, A. (2010). Acute and chronic toxicity of two formulations of the pyrethroid pesticide Deltamethrin to an amphipod, sand shrimp and lobster larvae. Canadian Technical Report of Fisheries and Aquatic Sciences, No. 2876, Oceans and Science Branch, Fisheries and Oceans Canada, Moncton, N.B. Retrieved from <http://www.dfo-mpo.gc.ca>.

Fisheries and Oceans Canada. (2011). Utilization of the Cunner (*Tautogolabrus adspersus*), a Wrasse, as a Means of Sea Lice Removal in Commercial Salmon Farms. Retrieved from <http://www.dfo-mpo.gc.ca>

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytro, A.V., Foss, A., Vikingstad, E., & Elvegard, T.A. (2014). Notes on the behaviour of lumpfish in sea pens with and without Atlantic salmon present. *Journal of Ethology*, 32, 117-122. doi: 10.1007/s10164-014-0397-1.

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytro, A.V., Foss, A., Vikingstad, E., & Elvegard, T.A. (2015). Assessment of suitable substrates for lumpfish in sea pens. *Aquaculture International*, 23(2), 639-645. doi: 10.1007/s10499-014-9840-0.

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Jonsdottir, O.D.B., Elvegard, T.A., Lemmens, S.C.A., Ryland, R., & Nytro, A.V. (2016). Investigation of behavioural interactions between lumpfish (*Cyclopterus lumpus*) and gold sinny wrasse (*Ctenolabrus rupestris*) under controlled conditions. *Aquaculture International*, 24(5), 1509-1521. doi: 10.1007/s10499-016-0008-y.

Johnson, S.C., Constible, J.M., & Richard, J. (1993). Laboratory investigations on the efficacy of hydrogen peroxide against the salmon louse, *Lepeophtheirus salmonis*, and its toxicological and histopathological effects on Atlantic salmon, *Salmo salar*, and chinook salmon, *Oncorhynchus tshawytscha*. *Diseases of*

Aquatic Organisms, 17, 197-204. doi: 10.3354/dao017197.

Jones, M.W., Sommerville, C., & Wootten, R. (1992). Reduced sensitivity of the salmon louse, *Lepeophtheirus salmonis*, to the organophosphate dichlorvos. *Journal of Fish Disease*, 15(2), 197-202. doi: 10.1111/j.1365-2761.1992.tb00654.x.

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. doi:10.1111/j.1365-2656.2007.01237.x.

MacKinnon, B.M. (1995). The poor potential of cunner, *Tautogolabrus adspersus*, to act as a cleaner fish in removing sea lice (*Caligus elongatus*) from farmed salmon in eastern Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 52(S1), 175-177. doi: 10.1139/f95-524.

Memorial University (MUN). (2015). Cunner. Faculty of Science: Ocean Sciences. Retrieved from: <https://www.mun.ca/osc/oscedu/tadspersus.php>.

Mittelbach, G.G. (1981). Foraging efficiency and body size: a study of optimal diet and habitat use by Bluegills. *Journal of Ecology*, 62(5), 1370-1386. doi: 10.2307/1937300.

Nytro, A.V., Vikingstad, E., Foss, A., Hangstad, T.A., Reynolds, P., Eliassen, G., Elvegard, T.A., Falk-Petersen, I., & Imsland, A.K. (2014). The effect of temperature and fish size on growth of juvenile lumpfish (*Cyclopterus lumpus* L.). *Aquaculture*, 434, 296-302. doi: 10.1016/j.aquaculture.2014.07.028.

Olla, B.L., Bejda, A.J., & Martin, A.D. (1975). Activity, movements, and feeding behavior of the cunner, *Tautogolabrus adspersus*, and comparison of food habits with young tautog, *Tautoga onitis*, off Long Island, New York. *Fishery Bulletin*, 73(4). Retrieved from: www.osti.gov/.

Rice, J., & Owsley, B. (2005). Habitat selection. Iowa State University. Retrieved from: <http://www.public.iastate.edu/>.

Roth, M., Richard, R.H., Dobson, D.P., & Rae, G.H. (1996). Field trials on the efficacy of the organophosphorus compound azamethiphos for the control of sea live (*Copepoda: Caligidae*) infestations of farmed Atlantic

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

salmon (*Salmo salar*). *Aquaculture*, 140(3), 217-239.
doi: 10.1016/0044-8486(95)01181-1.

Sayer, M.D.J., & Davenport, J. (1996). Hypometabolism in torpid goldsinny wrasse subjected to rapid reductions in seawater temperature. *Journal of Fish Biology*, 49(1), 64-75. doi:10.1111/j.1095-8649.1996.tb00005.x.

Skiftesvik, A.B., Blom, G., Agnalt, A., Durif, C.M.F., Browman, H.I., & Bjelland, R.M. (2013). Wrasse (Labridae) as cleaner fish in salmonid aquaculture – The Hardangerfjord as a case study. *Marine Biology Research*, 10(3), 289-300. doi: 10.1080/17451000.2013.810760.

Tacon, A. (2005). State of information on salmon aquaculture and the environment. Salmon Aquaculture Dialogue. Aquatic Farms Ltd. HI, USA.

Torrissen, O., Jones, S., Asche, F., Guttormsen, A.G., Skilbrei, O., Nilsen, F., Horsberg, T.E., & Jackson, D. (2013). Salmon lice- impact on wild salmonids and salmon aquaculture. *Journal of Fish Disease*, 36(3), 171-194. doi: 10.1111/jfd.12061.

Treasurer, J.W., Wadsworth, S., & Grant, A. (2000). Resistance of sea lice, *Lepeophtheirus salmonis* (Kroyer), to hydrogen peroxide on farmed Atlantic salmon, *Salmo salar* L. *Aquaculture Research*, 31(11), 855-860. doi: 10.1046/j.1365-2109.2000.00517.x.

Tupper, M., & Juanes, F. (2017). Testing foraging arena theory: The effects of conspecific density and habitat type on time and energy budgets of juvenile cunner. *Journal of Experimental Marine Biology and Ecology*, 487, 86-93. doi: 10.1016/j.jembe.2016.12.001.

Tables and Figures

Figure 1. The three types of materials I used to create shelters for the cunners and lumpfish. **a.** artificial grass turf to mimic seagrass ("Grass"), **b.** PVC piping to imitate cavities in rocky reefs ("Pipe") and **c.** shredded pieces of reinforced polyethylene tarp to mimic kelp ("Plant").

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

Figure 2. Boxplots of the four habitat preferences of the cunners. The Kruskal-Wallis test found a significant difference in the number of cunners that selected the habitats ($N=28$, $t=21.244$, $df=2$, $p<0.001$). The post-hoc Dunn's test with Bonferroni corrections found that there was only significant difference between the number of fish that selected the control and the number of fish that selected the plant habitat with means of 0 and 5, respectively ($t=-4.608$, $p<0.001$). ** indicates $p<0.001$.

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

Figure 3. Boxplots of the four habitat preferences of the lumpfish. The Kruskal-Wallis test found a significant difference in the number of lumpfish that selected the habitats ($N=28$, $t=17.902$, $df=2$, $p<0.001$). The post-hoc Dunn's test with Bonferroni corrections found that there were significant differences between the number of fish that selected the grass turf habitat ($M=1$) and the number of fish that selected the other three habitats, control ($M=8$, $t=2.833$, $p=0.028$), pipe ($M=9$, $t=-4.110$, $p<0.001$) and kelp plant ($M=6$, $t=-2.685$, $p=0.043$). * indicates $p<0.05$ ** indicates $p<0.001$.

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

Figure 4. Boxplots showing the difference in proportion of cunner and lumpfish that selected the kelp plant. The proportion of fish was Arcsine SQRT transformed to normalize the data. The independent-samples t-test showed there was significantly higher proportion of cunners that resided in the plant than lumpfish ($t(12)=3.793$, $p=0.003$). * indicates $p<0.05$.

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

Figure 5. Boxplots showing the difference in proportion of cunner and lumpfish that selected the pipe. The proportion of fish was Arcsine SQRT transformed to normalize the data. The independent-samples t-test showed there was no significant difference in the proportion of cunners and lumpfish that resided in the pipe ($t(12)=-2.137$, $p=0.054$)

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

Figure 6. An example of the two species selecting the same habitat, but utilizing it differently to suit their requirements. (a) A cunner who selected the pipe, burrowing underneath it for shelter (b) A lumpfish who selected the pipe, attaching to the smooth top of it with its ventral sucker.

Habitat Selection by Two Species of Cleaner Fishes That May be Beneficial in Removing Sea Lice From Cultured Salmon (Charmley)

Figure 7. The change in habitat selection over the seven-day observation period. Each line represents a different species-shelter combination. The total number of lumpfish per day was 25 and the total number of cunners per day was 8.