Sea lice feeding behavior of wild and hatchery lumpfish (*Cyclopterus lumpus*) under hatchery conditions.

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Sammendrag

I 2014 bidro Norge med 1,2 millioner tonn av verdensproduksjonen av oppdrettslaks. Norge er verdens største produsent av oppdrettslaks, og arten er blant de topp fem artene, konsumert kvantum, i de store sjømatmarkedene. Infeksjon med lakselus er den største sykdomsutfordringen som for tiden begrenser produksjonen av atlantisk laks globalt. Lakselus er ansvarlig for et bredt spekter av plager for fisken, fra mild hudskade til stressindusert dødelighet, som kan være en enorm økonomisk belastning for den atlantiske oppdrettsindustrien. Biologisk kontroll av rensefisk har blitt en vanlig tilnærming til avlusing av laks. Rognkjeks er en kaldtvannsfisk som brukes som rensefisk, og laksemerder inneholder 93%-97% færre lakselus med rognkjeks tilstede enn uten. Bruken av rognkjeks som biologisk bekjempelse gir etiske bekymringer for dårlig velferd og høy dødelighet for rensefisken når den lagres i merder. God velferd for rognkjeks er avgjørende fordi det påvirker deres evne til å avluse laks, og en høyere velferd vil ha flere fordeler, blant annet bedre avlusingseffekt og økonomisk verdi ettersom lakselus forårsaker økonomiske tap for næringen. Det er få studier på effektiviteten til rensefisk, og det er viktig å karakterisere atferden til individer som spiser mest aktivt på lakselus, slik at det kan bli mer effektivt å velge rognkjeks som rensefisk i fremtiden. Hovedmålet med denne studien var å observere fôringsatferden til vill og oppdrettsrognkjeks. Dette ble gjort ved å sammenligne antall og type angrep fra vill versus oppdrettsrognkjeks for å avluse laks under settefiskforhold. Mageinnhold fra mageskylling ble også brukt for å undersøke hvor mange lus som ble spist av rognkjeks. Et sekundært mål var å evaluere svømmeaktiviteten til vill og settefisk rognkjeks under to forskjellige lysforhold; 1) når lysene er slått på, og 2) når lysene begynner å dimmes. Fire svømmeaktiviteter vil bli evaluert som er 1) sveving, 2) svømming, 3) tilkoblet svømming og 4) sprengsvømming. Ti oppdrettsrognkjeks og ti ville rognkjeks ble introdusert sammen med åtte laks infisert med lus på ca. 300 g i en 500 l kar over en 3-ukers forsøksperiode. Forsøket ble duplisert i en annen 500 l tank. Antall og type angrep og svømmeaktiviteten til rognkjeksen ble registrert i videoanalyse. I tillegg ble antallet lakselus konsumert av hver rognkjeks undersøkt ved en mageskylling tre ganger i løpet av forsøksperioden. Oppdrettsrognkjeks virket bedre egnet for avlusing basert på det høyere antallet lakselus i magen, 55 lus sammenlignet med 27 av ville individer, og et høyere antall registrerte angrep. Fem individer ble evaluert nærmere, fordi de hadde flest utførte angrep og høyest antall lus i

magen. Individene ble vist å ha 5 til 23 lakselus i magen på totalt antall dager det ble tatt prøver og utførte angrep innenfor de fleste angrepstypene. Oppdrettsrognkjeks viste seg å være dristigere og mer aggressiv enn vill rognkjeks på grunn av dette.

Abstract

In 2014 Norway contributed 1.2 million tons of the world production of farmed salmon. Norway is the world's largest producer of farmed salmon, and the species is among the top five species, quantity consumed, in the major seafood markets. Infection by sea lice is the greatest disease challenge currently limiting the production of Atlantic salmon globally. Sea lice are responsible for a wide range of distresses to the fish, from mild skin damage to stress-induced mortality, which can be a tremendous economic burden for the Atlantic farmed industry. Biological control by cleaner fish has become a common approach to delouse salmon. Lumpfish is a cold-water marine fish used as cleaner fish, and salmon cages contain 93%-97% fewer sea lice with lumpfish present than without them. The use of lumpfish as biological control raises ethical concerns for poor welfare and high mortality of the cleaner fish when stocked in sea cages. Good welfare of lumpfish is essential because it affects their ability to delouse salmon, and a higher welfare will have several advantages, including improving delousing efficacy and having economic value as sea lice cause economic losses to the industry. There are few studies on the effectiveness of cleaner fish, and it is essential to characterize the behavior of individuals eating most actively on sea lice so that choosing lumpfish as cleaner fish in the future can be more efficient. The primary aim of this study was to observe feeding behavior of wild and hatchery lumpfish. This was done by comparing number and type of attacks by wild versus hatchery lumpfish to delouse salmon under hatchery conditions. Stomach content from gastric lavage was also used to examine how many lice were eaten by lumpfish. A secondary aim was to evaluate the swimming activity of wild and hatchery lumpfish under two different light conditions; 1) once lights are turned on, and 2) once the lights start dimming. Four swimming activities will be evaluated which are 1) hovering, 2) swimming, 3) attached swimming, and 4) burst swimming. Ten hatchery and ten wild lumpfish were introduced together with eight sea lice-infested salmon of approximately 300 g in a 500 l tanks over a 3-weeks experimental period. The experiment was duplicated in another 500 l tank. The number and type of attacks and the swimming activity of the lumpfish were recorded for posterior video analysis. Additionally, the number of sea lice consumed by each lumpfish was examined by a gastric lavage three times over the experimental period. Hatchery lumpfish seemed better fit for delousing based on the higher number of sea lice in their stomach, 55 lice compared to 27 by wild individuals, and a higher

number of attacks recorded. Five individuals were evaluated more closely, because they had the highest number of attacks performed and highest number of lice in their stomach. The individuals were shown to have 5 to 23 sea lice in their stomach on total days sampled and performed attacks within most of the attack types. Hatchery lumpfish showed to be bolder and more aggressive than wild lumpfish.

Contents

Preface

I started the master of Coastal Ecology at the University of Agder in 2020. The second year after starting my thesis, I decided to move to Oslo to experience new adventures. I want to thank my supervisor, Enrique Blanco Gonzalez, for making this possible and letting me participate in a project he earlier worked on. Together we found a thesis I could work on without needing to be present in Kristiansand. Enrique has guided me through this thesis with regular Zoom and Microsoft Teams meetings. I am thankful for the articles he has provided me, the additional pressure he has given me to make plans so that I could finish my thesis on time, and all his help with statistical analysis in RStudio.

The experimental work in this thesis was conducted by Enrique at the aquaculture facility of the University of Tromsø at Kårvika. I am also grateful to the University of Tromsø for letting me work on the material Enrique has collected at their facilities.

Oslo, 19.05.22

Seline Brathaug Pedersen

1. Introduction

1.1 Salmon Aquaculture

The global supply of seafood has grown from about 65 million tons in 1970 to 158 million tons in 2012, and the primary growth has been increasing aquaculture production (Asche and Bjorndal, 2011; Rickertsen and Alfnes, 2016). Aquaculture in Norway started in the early 1970s, and by 1995 the output from farmed salmon and trout was more significant than the total meat production of pig, poultry, and cattle (Gjeren and Bentsen, 1997). In 2014 Norway contributed 1.2 million tons of the world production of farmed Atlantic salmon (*Salmo salar*) (Blanco Gonzalez and de Boer, 2017). Norway is the largest producer of farmed fish outside of Asia (Rickertsen and Alfnes, 2016) and the world's largest producer of farmed salmon (Liu *et al.*, 2011). The consumption of seafood has grown due to increased population growth and awareness of eating healthy foods (Claret *et al.*, 2014; Tomić *et al.*, 2017), and salmon is among the top five species, quantity consumed in the significant seafood markets (Asche and Bjorndal, 2011). Given the vital role of salmon aquaculture to the entire world, it is crucial managing the industry sustainably if we want it to last for future generations.

1.2 Challenges with salmon aquaculture

The Atlantic salmon aquaculture undergoes several problems which involve environmental pollution and eutrophication, genetic and ecological interactions between escaped farmed fish and wild stocks, or expansion of parasites and disease (Blanco Gonzalez and de Boer, 2017). Infection of sea lice is the most significant disease challenge currently limiting the production of Atlantic salmon globally (Brooker *et al.*, 2019). Sea lice are copepod ectoparasites that negatively impact farmed salmon production and are responsible for a wide range of distresses to the fish, from mild skin damage to stress-induced mortality, which can be a tremendous economic burden for the Atlantic farmed industry (Costello, 2006; Aaen *et al.*, 2015). Two sea lice copepods mainly cause infestations, *Lepeophtheirus salmonis* and *Caligus elongatus* (Staven *et al.*, 2021). The parasites can attach to the host's surface because of their flattened bodies and appendages (Treasurer, 2018). For 40 years, sea lice have been a troublesome problem to the Atlantic salmon aquaculture (Barrett *et al.*, 2020b), where they thrive due to the high density of hosts (Barrett *et al.*, 2020a). Effective parasite control is challenging, and chemotherapeutics have been the primary delousing strategy for decades. Chemotherapeutants are now used less due to findings of drug resistance in the lice, and spillovers from treatments are potentially harmful to other species like lobsters and shrimps (Aaen *et al.*, 2015; Barrett *et al.*, 2020a). Mechanical and thermal delousing are the most used methods in Norway, but they can be stressful for stocks and raise mortality rates (Overton *et al.*, 2019; Barrett *et al.*, 2020a). Biological control by invertivores cleaner fish is a newer and less stressful approach to delouse salmon, which has become a leading contender (Barrett *et al.*, 2020a).

1.3 Cleaner fish

In the Atlantic salmon industry, cleaner fish as delousing strategy has increased exponentially since 2008 due to a reduction in chemotherapeutics and being potentially less stressful to farmed fish (Powell *et al.*, 2018a). Using cleaner fish was first tested in laboratory trials in 1988, then followed by experiments in sea cages (Bjordal, 1988, 1989, 1991; Skiftesvik *et al.*, 2013). For almost 30 years, wrasse (*Labridae*), mostly ballan (*Labrus bergylta*), corkwing (*Symphodus melops*), and goldsinny (*Ctenolabrus rupestris*) have been used for delousing farmed Atlantic salmon in floating net pens (Blanco Gonzalez and de Boer, 2017). Present-day, the industries in Europe are using wild-caught labrids, which include goldsinny, ballan, corkwing, rockcook (*Centolabrus exoletus*), and cuckoo (*Labrus mixtus*), but also farmed ballan wrasse and lumpfish *(Cyclopterus lumpus*) (Leclercq *et al.*, 2018). Wild wrasses have proven to be effective delousers in commercial salmon sea cages, equivalent are farmed wrasses in experimental tanks studies, while the performance of farmed wrasses in sea cages is uncertain due to changes in environmental conditions and presence of large salmon (Brooker *et al.*, 2020). Although, according to an experiment done by Skiftesvik et al. (2013), wild-caught versus farmed ballan wrasse appeared to be equally efficient at delousing the salmon, despite the cultured ballan wrasse having no contact with salmon and sea lice prior to the experiment (Skiftesvik *et al.*, 2013). However, the problem with utilization of wrasses as cleaner fish is that they tend to become inactive during the winter (Powell *et al.*, 2018a). Ballan wrasse has appeared to have low swimming and foraging activity in earlier studies at temperatures below 9-10°C (Leclercq *et al.*, 2018), while lumpfish are more tolerant to low temperatures (Mortensen *et al.*, 2020).

1.4 Lumpfish

The lumpfish, also called lumpsucker, from the family lumpsuckers (Cyclopteridae), is a cold-water marine fish that occupies habitats dependent on its life stage (Davenport, 1985; Jónsdóttir *et al.*, 2018; Powell *et al.*, 2018b). The distribution of the lumpfish is wide, spread in the boreal region of the east and west North Atlantic coasts (Davenport, 1985; Powell *et al.*, 2018b). The lumpfish is a bony fish from the class Teleostei with a vaguely triangular body (Figure 1) with a flattened ventral surface containing a round, muscular sucking disc (Powell *et al.*, 2018b). Swimming bladder is lacking, which leads them only to swim when necessary to forage for food and avoid danger. The species use their sucking disc to attach to smooth surfaces (Johannesen *et al.*, 2018).

Figure 1 – A lumpfish with a sucking disc on the ventral side. Photo taken by Enrique Blanco Gonzalez.

Lumpfish is an abundant species, with a suggested mean annual abundance of 53-132 million individuals since 1980 in the Barents Sea (Powell *et al.*, 2018b). The diet primarily holds large planktonic organisms in the surface/mid-waters and benthic organisms, especially those dwelling upon weeds. Juveniles and adults have long intestines with plenty of bends and numerous pyloric caeca, which suggests efficient digestion and absorption of food (Powell *et al.*, 2018b). Since the late 1940s, commercial fishery emerged to focus on the roe of female lumpfish to produce caviar, which has continued to the present day. A new focus has appeared on using both male and female broodstock for the emerging cleaner fish industry (Johannesson, 2006; Powell *et al.*, 2018b).

1.4.1 Feeding behavior

Lumpfish have an assertive opportunistic feeding behavior, meaning they will feed on several food items, such as sea lice, salmon pellets, and other organisms in the sea cages (Imsland *et al.*, 2014a; Powell *et al.*, 2017). The species use most of the daylight hours to forage for food (Imsland *et al.*, 2014a). It was also discovered that lumpfish were either resting within floating seaweeds within the cage or hovering underneath them when not feeding or foraging. Additionally, lumpfish that reared alone (without salmon) spent more time resting (Imsland *et al.*, 2014a). Lumpfish attach themselves to suitable substrates to conserve energy (Imsland *et al.*, 2018b). Although the species spend minimal time removing sea lice (Imsland *et al.*, 2014a; Powell *et al.*, 2017), salmon cages contain 93%-97% fewer sea lice with lumpfish present than without them (Imsland *et al.*, 2016b, 2016a, 2018a, 2018b, 2019). A study from Whittaker et al. (2021) showed repeatable behaviors where bold, neophilic lumpfish were more seemingly to inspect salmon, while the most active and social lumpfish were more likely to make the salmon flee. Selecting the best cleaners for delousing salmon would be individuals that are bold enough to approach salmon but not as aggressive to cause them to flee (Whittaker *et al.*, 2021).

1.4.2 Wild and hatchery fish

There are three reasons that differentiate hatchery fish from wild fish. The first reason is that the phenotypes of the hatchery fish may be shaped by the rearing conditions as fish are highly phenotypically plastic (Einum and Fleming, 2001). The intensity and direction of selection between hatchery and wild fish is the second reason. Genetic selection is expected to be different in hatchery compared to wild fish. For example, survival during egg and juvenile stages are higher in hatchery than in the wild. Another example is that hatchery fish may select for behavioral and physiological traits, which are a disadvantage in nature, because of high juvenile density and abundance of food. Multi-generations compared to first-generation hatchery stocks are very likely to differ from wild fish because most genetic changes are likely to be of environmental origin (Einum and Fleming, 2001). A third reason is the use of non-native fish for stocking, which may introduce novel, genetically based characters into the wild population and break up co-adapted gene complexes. Fortunately, releases of non-native fish have decreased (Einum and Fleming, 2001). Hatchery fish has revealed to be more aggressive at higher densities, more disposed to predation, and have less success at foraging for food. Nevertheless, acclimatization and conditioning regimes through the hatchery phase have improved predator response and foraging behavior and decreased stress (Brooker *et al.*, 2020). Therefore, using wild or hatchery lumpfish as cleaner fish can have different outcomes. Wild lumpfish have been found to use one of two modes when foraging; 1) actively search for prey when swimming, or 2) 'sit-and-wait' for prey while being attached to substrate with their suction-disc (Imsland *et al.*, 2014a). An experiment on cultured and wild ballan wrasse revealed that hatchery wrasse was as effective as wild wrasse at removing sea lice from salmon (Skiftesvik *et al.*, 2013). However, the use of cleaner fish, wild or hatchery, as a biological control raises ethical concerns for poor welfare and high mortality of the cleaner fish when stocked in sea cages (Barrett *et al.*, 2020a).

1.5 Welfare of lumpfish

The welfare of farmed fish is essential to the industry, not only for the public perception, marketing, and product acceptance but in addition for production efficiency, quantity, and quality (Ashley, 2007). Productivity can be enhanced by improving the welfare, like reducing stress, of farmed fish (Martos-Sitcha *et al.*, 2020). Focusing on the welfare of lumpfish is essential because it affects their ability to delouse salmon (Gutierrez Rabadan *et al.*, 2020). Some of the challenges to the welfare of lumpfish are poor husbandry, disease outbreaks, and stress. Welfare can be tested by physical indicators like fin erosion, body damage, blood parameters, growth, and mortality, to name a few, but also behavioral indicators like loss of appetite, swimming activity, and aggression (Garcia de Leaniz *et al.*, 2021).

Welfare indicators require practical and easy guidelines to be used easily by fish farmers (Garcia de Leaniz *et al.*, 2021). Better welfare will have several advantages, including improving delousing efficacy and having economic value as sea lice cause economic losses to the industry (Gutierrez Rabadan *et al.*, 2020). In addition to increasing the sustainability, social acceptance, and reputation of the salmon farming industry, fish would have higher survival (Garcia de Leaniz *et al.*, 2021). The cleaning rates by lumpfish are variable, and not all individuals graze on sea lice, which escalates the risk of emaciation and has ethical and practical consequences. Therefore, it is crucial to choose good cleaners to make the industry more sustainable. However, there is limited information on what behaviors lumpfish possess to act as effective sea lice cleaners (Whittaker *et al.*, 2021) - examining the behaviors of individual fish grants an estimate of the range of behavioral phenotypes inside a population. Doing so will improve our understanding of the needs of the fish and encourage the industries to improve welfare (Brooker *et al.*, 2020). The present study will investigate behaviors like swimming activity and attacks of both wild and hatchery lumpfish.

1.6 Aim of study

The primary aim of this project is to observe feeding behavior by wild and hatchery lumpfish. This was done by comparing the attacks by wild versus hatchery lumpfish to delouse salmon under hatchery conditions. In addition, stomach content from gastric lavage was also used to examine how many lice were eaten by lumpfish. In this study, wild and hatchery lumpfish were mixed in two tanks along with Atlantic salmon infected with sea lice. While observing feeding behavior, interactions between wild and hatchery lumpfish and both lumpfish and salmon will also be noted. Attacks can give an illusion of how effective the lumpfish are at cleaning the salmon. Comparing wild and hatchery lumpfish may determine which lumpfish is better to use in the Atlantic salmon aquaculture.

A secondary aim is to evaluate the swimming activity of wild and hatchery lumpfish under two different light conditions; 1) once lights are turned on, and 2) once the lights start dimming. Four swimming activities will be evaluated which are 1) hovering, 2) swimming, 3) attached swimming, and 4) burst swimming. Swimming activities can reveal if lumpfish are stressed or whether they are foraging for food.

These aims are to study if the wild or hatchery origin of lumpfish is more beneficial to use as cleaner fish and to characterize the behavior of individuals cleaning salmon. There are few studies on the effectiveness of cleaner fish (Overton *et al.*, 2020), and it is essential to characterize the behavior of individuals eating most actively on sea lice so that choosing lumpfish as cleaner fish in the future can be more efficient.

It is expected that the hatchery lumpfish would graze more and have lower swimming activity because they are familiar with the environmental conditions and because wild lumpfish do not normally graze on sea lice or act as cleaner fish (Whittaker *et al.*, 2021). However, the wild lumpfish would be more familiar with predators than the hatchery lumpfish, which may influence the behaviors of the hatchery lumpfish.

2 Materials and methods

2.1 Fish and research facilities

The experiments were conducted in the autumn 2020 by Enrique Blanco Gonzalez at the aquaculture facilities of UiT (University in Tromsø) at Kårvika in Tromsø under the FOTS permission ID 24318. A total of 66 wild lumpfish were collected from Lyngen on August 11th, 2020, while 78 hatchery individuals were obtained from Senja Akvakultursenter AS on October 21st, 2020. The lumpfish were transported in tanks supplied with oxygen to the experimental tanks at the main campus of UiT in Tromsø. After arrival, the fish were acclimated in separate tanks at 10°C and fed with frozen copepods and commercial pellets (Clean Lumpfish, Skretting AS, Norway).

On October 22nd, wild lumpfish were anesthetized with benzocaine (100 mg/L), measured, and weighed before they were individually PIT-tagged (passive integrated transponder) and marked with colored elastomer (VIE) tags. The same procedures were carried out with the hatchery lumpfish on October 23rd. On October 27th, the two group of fish were transported in tanks supplied with oxygen to the Fish Health Laboratory (FHL) of the Tromsø Aquaculture Research Station at Kårvika (TARS). After arrival, the fish were acclimated in two separate tanks at 9°C and fed with a commercial diet (Clean Lumpfish, Skretting AS, Norway) following routinary practices. On November 10th, ten hatchery and ten wild lumpfish were introduced together with eight salmon of approximately 300 g infested at 12 adult sea lice per fish density in a 500 l tank at 6h light and 18h darkness light regime. The experiment was conducted in 2 replicates, placing the same number of individuals in another 500 l tank and running the experiment simultaneously. Each of the tanks was equipped with 2 Gopro Hero 8 cameras covering the whole volume of the tank and continuously recording the behavior of the lumpfish (Figure 2). After testing the video recording system, the colors of the VIE tags were not possible to be visualized and an additional tagging system was employed. On November 30th, a subgroup of 20 wild and 20 hatchery lumpfish were selected and tagged with an additional tag. Ten individuals in each tank were tagged with FPN 8 mm x 4 mm tags implanted with a thread while the other 10 individuals in the same tank were tagged with fine anchored TBF tags with 10 mm exposed filament using three distinct colors (pink, yellow and green). To identify individual feeding behavior, the tags were placed on various parts of the body, right or left and front or back of the dorsal side of the body, giving four outcomes; right front (RF), left front (LF), right back (RB), and left back (LB) for each of the additional tags in each of the tanks. The experiments were conducted using the same number of fish and under the same conditions as above mentioned over a 3-weeks period. At the beginning of the experiments, three large wild lumpfish were placed in the tanks; however, to avoid any potential bias due to the difference in sizes, those individuals were removed from the tanks after a few days.

Figure 2 – Picture of the environment in tank #10 on the 14th of December, showing salmon and tagged lumpfish co-existing.

2.1.1 Stomach content

In addition to the video recording, gastric lavage was performed on each of the lumpfish for the gut content analysis. Gastric lavage is a method where the fish get anesthetized, then a silicon tube connected to a syringe filled with seawater will be inserted into the stomach cavity. Water from the syringe gets expelled into the stomach cavity, making the sedated fish throw up gut content (Imsland *et al.*, 2014b). Every individual lumpfish from each group was identified by scanning its PIT-tag, measured, and weighed. Then gastric lavage was performed to determine the number of sea lice eaten before returning the fish to the experimental tanks (Figure 3). These gut analyses were conducted three times over the experimental period; on December 3rd, 14th, and 17th. After each gastric lavage, a new group of 8 salmon of approximately 300 g each infested at 12 sea ice per fish density was placed in each of the 500 l tanks. The experiment was performed until December 17th, when all lumpfish were sacrificed.

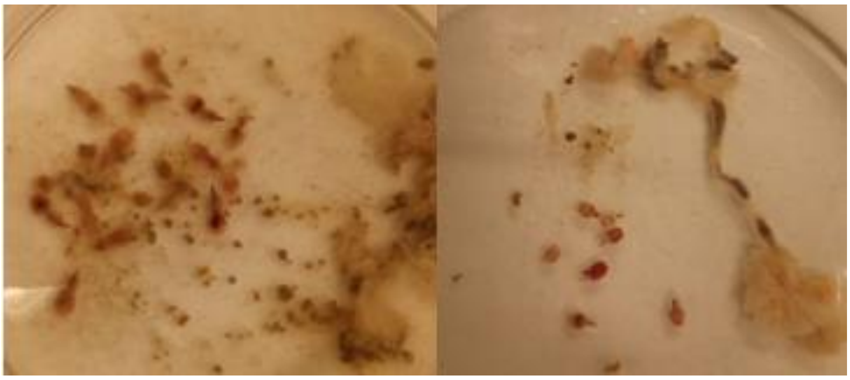

Figure 3 – Two pictures of the gut content of lumpfish after performing gastric lavage. A clear vision of sea lice from the gut content. Photo taken by Enrique Blanco Gonzalez.

2.3 Video analysis

2.3.1 Swimming activity

Initially, I decided to study the activities under three different light conditions; 1) once lights were turned on, 2) middle of light conditions, and 3) once lights started dimming. Four types of swimming behavior were evaluated; 1) swimming, 2) attached swimming, 3) hovering, and 4) burst swimming under these three different light conditions (Table 1). Swimming behaviors were observed for 8 minutes and 51 seconds in each lighting condition because that was the duration of each of the video segments recording with the GoPro cameras. After observing several video fragments, I realized I realized that due to some technical problems during the recording, some dates were either not recorded or did not have a clear visualization of the three light conditions. Therefore, I decided to focus only on light conditions 1 (turning the light on) and 3 (lights starting dimming). The days analyzed were the 1st, 2nd, 3rd, 10th, 14th, and 15th of December. Type of swimming behavior was noted along with the time when swimming behavior occurred and ended, the tag of the lumpfish, videorecording number observed, and light condition, date of video recording, type of tank (#9 or #10), the number of the camera (#1, #2, #3, or #4), and the IDs of the lumpfish. Henceforward, "W" is wild, and "H" is hatchery lumpfish when evaluating IDs of lumpfish.

Table 1 – Classification of the diverse types of swimming activity by the lumpfish.

Score	Swimming Activity	Description
1	Swimming	Swimming horizontal or vertical, or in rounds.
2	Attached	Swimming while attached to substrate with the sucker disc.
3	Hovering	Hovering performance, neither horizontal nor vertical motion.
4	Burst	Rapid swimming in any directions.

2.3.2 Attacks

Lumpfish attacks were analyzed from all video records available over the 3-weeks experimental period, totaling approximately 160 hours of video recordings. Every date recorded was examined to get as much data on attacks as possible. Observing under light conditions like the swimming activities would give too little result. Every attack was noted along with the time of the attack, type of tag, ID, and video recording number. Date of video recording, type of tank (#9 or #10), and type of camera (#1, #2, #3, or #4) were also noted. Every attack was noted as a combination of three digits; a big letter, a number, and a small letter (see Table 2). The big letter could be "A," "B," or "C"; A meaning that the lumpfish were hovering along the side or on the dorsal or ventral part of the salmon before the attack, and B being a direct attack. Interactions were written as a C, either salmon attacking a lumpfish, lumpfish attacking each other, or the tag of another lumpfish. Numbers 1 or 2 were how long the lumpfish attacked or was attached to the salmon; 1) being attached for over 1 second, while 2) was a shorter attack. Small letters (a or b) were how many attacks occurred on the same salmon before the attack discontinued. The days analyzed were the 1st, 2nd, 3rd, 8th, 9th, 10th, 11th, 14th, and 15th of December, three more dates examined than in the swimming activity.

Table 2 – An overview of the possible combinations of attacks performed by lumpfish.

A Hovering before attack	1 Long attachment	a-	Single attack
B Direct attack	2 Short attack	b-	Multiple attacks on the same salmon
C Interaction			
Possible combinations: A1a, A1b, A2a, A2b, B1a, B1b, B2a, B2b, C			

2.4 Data analysis

The data were analyzed in RStudio Team (2021) using the tidyverse- package (Wickham *et al.*, 2019). Three-way analysis of variance (ANOVA) tests were performed to examine the significance of the various swimming activities with dependent variables as tank, origin, and days observed, or tags, tank, and days observed (Herzog *et al.*, 2019). One-way (ANOVA) test was also used to examine the significance of the total number of attacks with dependent variables such as tank, origin, or days observed. A t-test was performed to test the difference in total attacks in each of the tanks (#9 and #10), and another t-test was run to test the difference in attacks between wild and hatchery lumpfish. A significance level was set as $p < 0.05$, examining both attacks and swimming activities. The graphical representation of the analyses was made by using the package ggplot2 (Wickham, 2016).

3 Results

3.3 Swimming activity

In Rstudio, the four different swimming activities were split into separate datasets to examine each activity individually in a three-way ANOVA test. A three-way ANOVA test revealed that swimming was the only activity that showed significance between the various dependent variables (origin, tank, date, and tank), see Table 3. Hovering, attached, and burst swimming showed low significance in all dependent variables, and were excluded from Table 3 and Figures 4, 5, and 6 to show a more presentable result. Light conditions were also excluded from Table 3 and Figures 4, 5, and 6 given low significance in swimming activities, but can be observed in Figures 9 and 10 in Appendix 2. The three-way ANOVA test showed that there are significant differences in the activity “swimming” and origin, date, and tank by date (p-value 0.002), between origin:date (p-value 0.03), date:tank (p-value 0.0004), origin:date:tank (p-value 0.02). There was also difference discovered in “swimming” and tags, date, and tank by date (p-value 0.001), and between tags:tank (p-value < 2e-16).

Table 3 – Formulas examined in a three-way ANOVA test. Testing significance within the swimming activity “swimming” in origin, date, tank, and tags.

Formula	**f-value**	**p-value**
Swimming ~ Origin * Date * Tank		

Origin	1.5	0.3
Date	3.8	0.002 **
Tank	0.9	0.3
Origin:Date	2.5	0.03 *
Origin:Tank	2.1	0.2
Date:Tank	4.5	0.0004 ***
Origin:Date:Tank	2.8	0.02 *
Swimming ~ Tags * Date * Tank		
Tags	1.6	0.2
Date	4.1	0.001**
Tank	0.4	0.6
Tags:Date	6.5	< 2e-16 ***
Tags:Tank	0.2	0.1
Tags:Date:Tank	0.8	0.8
Swimming ~ Light condition		
Light condition	0.4	0.5
Level of significance: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 ´ ´		

To lower number of figures in result section, I decided to examine some of the variables with significant difference. Since swimming showed difference in origin, date, and tank all together (p-value 0.02); Figures 4 and 5 were made to evaluate "swimming" between the two origins in the separate tanks in every date observed.

Figures 4 and 5 reveals that the total seconds spent swimming was higher the three last observed days (10th, 14th, and 15th of December) in tank 9 than tank 10. Tank 9 show that wild and hatchery lumpfish spent over 40 minutes swimming the last two observed days (14th and 15th of December). Tank 10 show that wild lumpfish spent approximately 30 minutes swimming the last observed day (15th of December), and that hatchery lumpfish spent approximately 20 minutes swimming on the 14th and 15th of December.

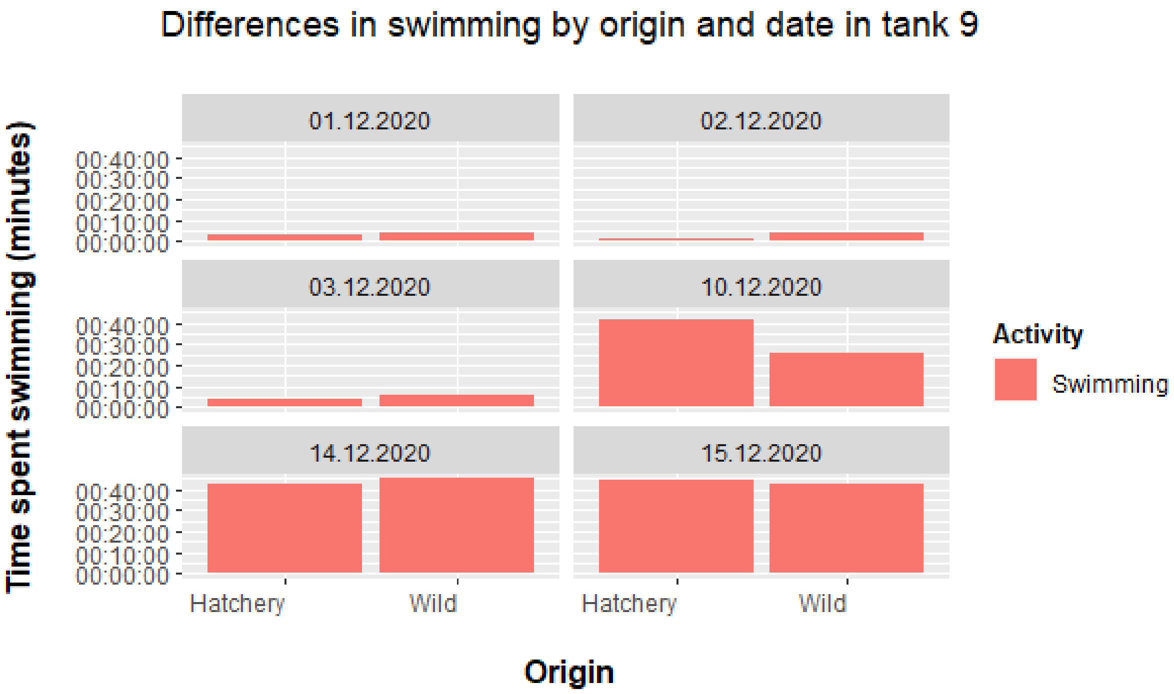

Figure 4 – The figure shows differences in swimming by wild and hatchery lumpfish in tank 9, split into dates observed. Differences is shown how time (minutes) each origin spent swimming each day.

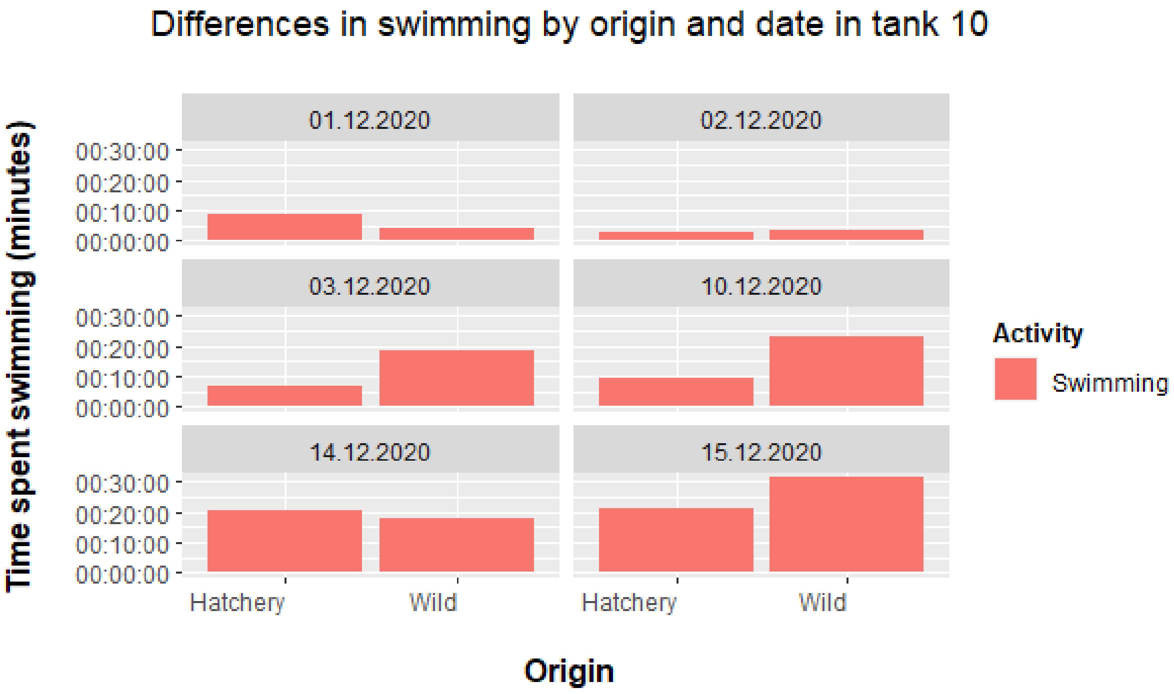

Figure 5 – The figure shows differences in swimming by wild and hatchery lumpfish in tank 10, split into dates observed. Differences is shown how time (minutes) each origin spent swimming each day.

Table 3 also showed that tags and date together had significant difference in swimming. A new figure was made to show how much time each individual tag was swimming each day observed (Figure 6). The figure shows an increase in swimming in each tag from the 1st of December to 10th of December. Time spent swimming were higher on the 10th, 14th, and 15th of December, compared to the first three days (1st, 2nd, and 3rd of December). Green and pink sticker tag seem to have spent less time swimming the last three observed days than the other tags, giving slightly lower bars in the figure.

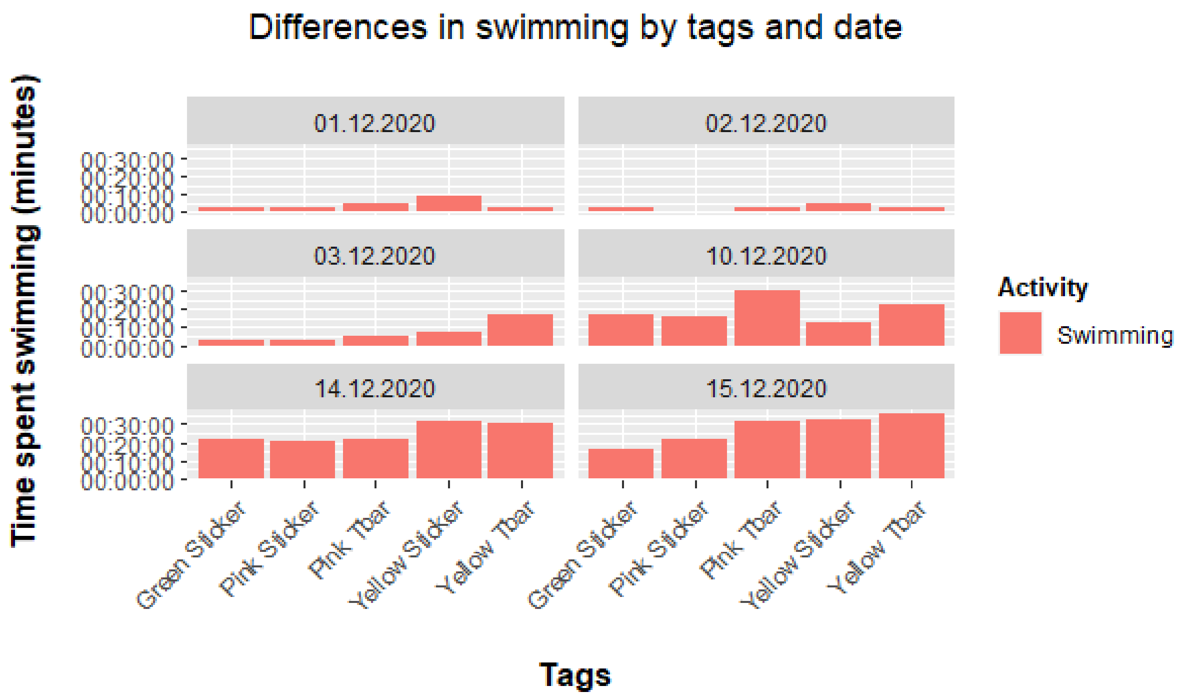

Figure 6 – The figure shows differences in swimming by individual tags split into dates observed. Differences is shown how time (minutes) each tag spent swimming each day.

3.4 Attacks

One-way ANOVA test was performed to look at possible differences between the total attacks, tank, origin, and date. Tanks were the only variables significant ($p < 0.01$) for the total number of attacks (table 4). T-test was also tested to examine difference in total attacks between tanks, and origins. T-test showed that the two tanks had difference in total attacks, tank 9 with a mean of 29 attacks and tank 10 with a mean of 3 attacks. The difference in tanks had a 0.02 p-value which makes it significant. Difference in origins showed not to be significant. Interactions (C) were excluded from the analysis as it was not found relevant to the performance of attacks. An individual table was made for interactions but put in the appendix because I did not find it relevant for feeding behavior of wild and hatchery lumpfish (Table 9, Appendix 3).

Table 4 – Showing values from the formula in the one-way ANOVA test and t-test.

Formula	**Mean**	**Mean**	**f-value**	**p-value**
Total attacks ~Tank				
Tank	-	-	10.9	0.01**
Total attacks ~ Origin				
Origin	-	-	0.25	0.6
Total attacks ~ Date				
Date	-	-	1.9	0.2
t-test				
Total attacks ~ Tank	29 – Tank9	3 – Tank10	-	0.02
Total attacks ~ Origin	18 – H	13 – W	-	0.63
Level of significance: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1 ´ ´				

Even though origin and group were not significant for the total number of attacks, tables 5 and 6 evaluate the differences in attacks between origin, group, and tanks. This could answer why the other variables were not significant and continue working on the aim of the study which was to evaluate the feeding behavior by examining the attacks performed by the two origins.

Dates observing attacks were split into groups in Table 5 to make the table more presetable. Group 1 included December 1st, 2nd, and 3rd, group 2 was December 8th, 9th, and 10th, and finally, group 3 included December 11th, 14th, and 15th. Every date observed had various amounts of hours; the number of attacks from each tank and the date observed was therefore divided by the number of hours observed to give each group a correct value (Table 5).

Table 5 – Number of total attacks on salmon per hour by wild and hatchery lumpfish per date examined. Total attacks were divided by the number of hours observed on each date.

Date	Wild	Hatchery
Tank 9		
Group 1	0.98	0.60
Group 2	0.54	0.77
Group 3	1.43	4.07
Tank 10		
Group 1	0.02	0.19
Group 2	0	0.05
Group 3	0.12	0.19

To further analyze attacks, Table 6 indicates which individual lumpfish attacked salmon in each of the tanks and show what type of attacks each of the individual performed. This could give an idea of the behavior of how individuals clean salmon for lice. A summation of all the attacks was also included in the table to show what type of attack was most common among the species, both between the origins and in total. Every hour observed from video recordings found that 49% of all 41 lumpfish were seen attacking salmon. Table 6 show that the most common attack was swimming directly toward the salmon, followed by a single short attack (B2a). The second typical attack was hovering before a single short attack (A2a). Hatchery lumpfish had the highest number of attacks, 113 in total. Wild lumpfish had 79 attacks.

Table 6 – Number of attacks of each category performed by wild and hatchery lumpfish. Each fish is sorted by its ID. The table also includes a summation of the number of attacks performed by wild and hatchery lumpfish in each attack category. In addition to a summation of every attack type at the end in "total attacks."

ID	A1a	A1b	A2a	A2b	B1a	B1b	B2a	B2b	Total
Tank 9									
H9	-	-	1	-	-	-	-	-	1
H12	-	-	1	-	-	-	2	-	3
H19	-	-	-	-	1	-	-	-	1
H30	4	-	6	-	9	6	15	15	55

H38	-	-	-	-	2	-	-	-	2
H67	3	2	9	4	4	1	11	3	37
W6	3	1	24	5	4	-	15	-	52
W7	1	-	-	-	-	-	-	-	1
W8	-	-	-	-	-	-	1	-	1
W10	-	-	1	-	-	-	-	-	1
W17	-	-	4	4	-	1	3	8	20
W34	-	-	-	-	-	-	1	-	1
Tank 10									
H11	3	-	1	1	1	-	3	1	10
H13	-	-	1	-	-	-	-	-	1
H28	1	-	-	-	-	-	-	-	1
H34	-	-	-	-	-	-	1	-	1
H36	-	-	-	-	-	1	-	-	1
H56	-	-	-	-	-	-	1	-	1
W16	-	-	-	-	-	-	1	-	1
W66	-	-	-	1	-	-	-	-	1
Tank 9									
Hatchery	7	2	17	4	16	7	28	18	99
Wild	4	1	29	9	4	1	20	8	76
Tank 10									
Hatchery	4	-	1	1	1	1	5	1	14
Wild	-	-	1	1	-	-	1	-	3
Total attacks	15	3	48	15	21	9	54	27	192

3.5 Stomach content

The gastric lavage method gave an overview of individuals who had consumed the most sea lice. On the 3rd of December, the first sampling date revealed that 5% of all lumpfish had lice in their stomach. Second sampling, 14th of December, 24% of all lumpfish were found to have lice in their stomach. Last sampling 17th of December, 20% of the lumpfish were found to have lice in their gut content. In total, from all three sampling dates, 32% of all 41 lumpfish were found to have lice in their stomach. Table 7 shows an increase of lice from stomach content in sampling days, finding 2 lice first sampling day (3rd of December), 17 lice the second sampling day (14th of December), and 63 lice the last sampling day (17th of December).

Table 7 – Number of lice in stomach per lumpfish from the gastric lavage performed 3rd, 14th, and 17th of December. The table only views the lumpfish which had lice in their stomach.

ID	**3rd of December**	**14th of December**	**17th of December**
H11	-	-	5
H30	-	3	18
H38	1	-	-
H41	-	1	1
H56	-	1	2
H67	-	3	20
W6	-	2	7
W13	-	1	2
W17	-	3	8
W33	-	1	-
W34	1	-	-
W50	-	1	-
W65	-	1	-
Total	2	17	63

Five individuals (W6, W17, H11, H30, and H67) excelled from Tables 6 and 7 for having high number of attacks and lice in the stomach. Figure 8 was made to look at the difference in swimming between these five individuals (W6, W17, H11, H30, and H67). Individual H11 and H30 seem to spend less time swimming than other individuals. W17

W6 and H67

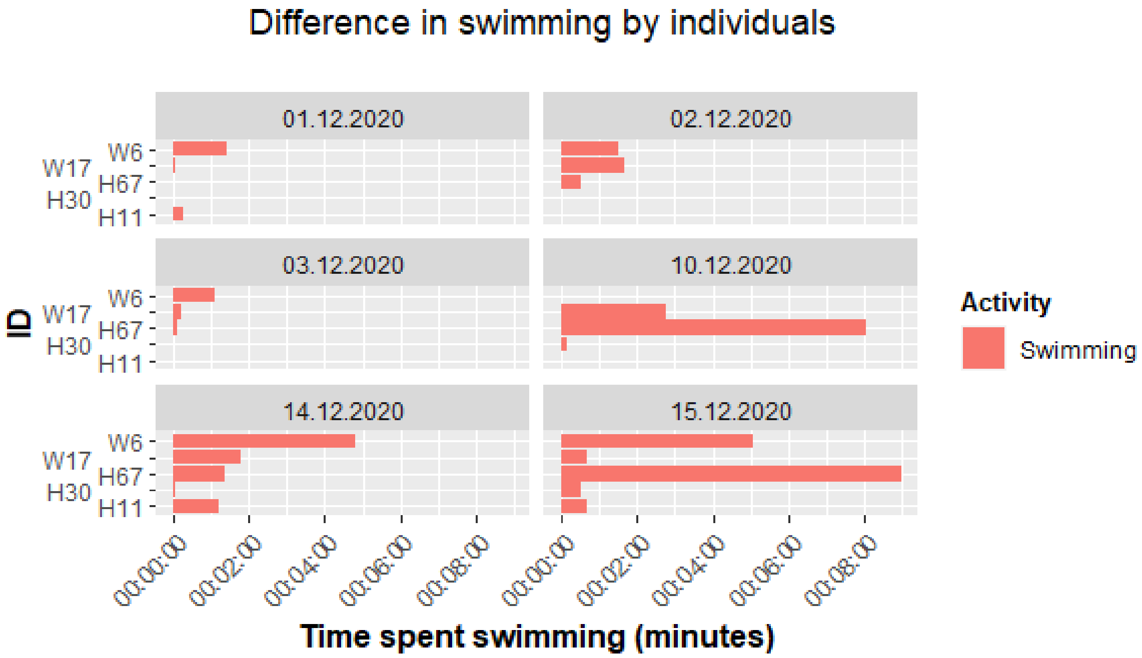

Figure 8 – Time spent swimming in minutes by five individuals (W6, W17, H11, H30, and H67) each observed day (1st, 2nd, 3rd, 10th, 14th, and 15th of December).

4 Discussion

In this study, five individuals showed to be bolder and more aggressive than the remaining 41 lumpfish, meaning that they attacked more and had a higher number of lice in their stomachs. Three of the individuals were of hatchery origin, while the remaining two were wild. Swimming activities were also examined among the five individuals.

4.3 Swimming activity

Three-way ANOVA tested that swimming was significant for tank, origin, and days observed. Figures 4 and 5 expressed that wild and hatchery lumpfish increased swimming activity from day 1 (1st of December) of the experiment to the last day (15th of December). According to Table 3, time spent swimming was significant for date observed (p-value 0.002), which means that swimming was different in each day. The ANOVA test did not show any significant difference in swimming in tanks or origin. Tags and date also appeared to be significant for swimming (Table 3) with a p-value of < 2e-16. Evaluating figure 6, each tag spent more time swimming the last three observed days compared to the three first days.

An experiment with naïve and experienced lumpfish in tanks showed that naïve individuals increased swimming activity when introduced to salmon, while experienced individuals showed no change in swimming activity (Staven *et al.*, 2019). In the present study, wild lumpfish would be experienced with predators compared to the hatchery individuals. However, hatchery lumpfish would be more experienced with the environment than the wild individuals. This could be a reason for both origins increasing swimming activity (Figure 4 and 5), hatchery being introduced to predators, and wild introduced into an unaccustomed environment. Lumpfish has shown to spend 65% of the daytime foraging and 25% swimming, and only 8% resting (Imsland *et al.*, 2014a; Leclercq *et al.*, 2018). Considering this, evaluating swimming activity from Figures 4 and 5 can be compared with the number of lice consumed in table 7, which increases over time and can indicate increased swimming activity because of foraging.

As mentioned, wild and hatchery lumpfish did not show significant differences in in swimming with a p-value 0.3 (Table 3). However, wild lumpfish did spend more time hovering and burst swimming than hatchery lumpfish, examining the counts from Table 8 in appendix 1.

Even though light conditions showed low significance for swimming in Table 3 (p-value 0.5), the result from figures 10 and 11 in appendix 2 can indicate similarities to other experiments. As expressed in other research papers, swimming activity is higher at day than at night (Imsland *et al.*, 2014a; Leclercq *et al.*, 2018), which also applies to this study where swimming is higher under light condition 1 (once lights were turned on) and 3 (once lights started dimming) in tank #9 (figures 10 and 11).

4.4 Attacks

Table 4 revealed that the tanks showed significant difference for the total number of attacks and was further analyzed in two tables (Tables 5 and 6) along with origins, dates observed, and IDs. Out of all 41 lumpfish, 49% were observed to attack the salmons. Tank #9 appeared to have a much higher number of attacks per hour and in total compared to tank #10 (Tables 5 and 6). Table 5 showed that hatchery lumpfish in tank #9 increased the number of attacks per hour per group observed, while the wild population decreased from group 1 to group 2 but increased again at the end of the trial. Tank #10 had few attacks in both hatchery and wild populations, but especially in the wild lumpfish, and table 6 was made to examine this further. Only one individual in tank #10, H11, had consumed more than one sea lice (Table 6). The table also revealed that tank #9 showed just a slight difference in the total number of attacks performed by hatchery and wild lumpfish but showed more of an individual difference and that hatchery and wild lumpfish were equally numbered in performing attacks. The same is not to be seen in tank #10, where hatchery lumpfish have both the highest number of attacks and fish performing attacks. The total number of hatchery lumpfish attacks was 113 attacks, while 79 for wild lumpfish, indicating that hatchery population are bolder and more aggressive than wild population. Although, total attacks by origin did not show any significant differences with a p-value of 0.6, with hatchery lumpfish having a mean of 18 attacks, while wild lumpfish had 13 (Table 4). Total attacks did not show any significantly differences in days observed (p-value 0.2), evaluating Table 5, attacks per day seem various. Hatchery lumpfish in tank #9 seem to increase attacks per hour when the dates are split into group 1, 2, and 3 (Table 5).

Tanks were the only dependent variables with significant difference (p-value 0.01) in total number of attacks. The high f-value of 10.9 indicates that the variance between the two tanks is different (Herzog *et al.*, 2019). A t-test also confirmed difference in attack between the two tanks, with 29 attacks in tank #9 and only 3 attacks in tank #10.

Bold and aggressive behavior in the present study was shown in lumpfish performing within most of the attack types and consuming more than five sea lice. Individuals that performed less than three of the attack types in total and with less than five lice in the stomach are presented with shy behavior. Evaluating the two origins as populations from Table 6, hatchery population performed higher numbers of attacks where individuals directly swim and attacks salmon, with short or long, single, or multiple attacks (B1a, B1a, B2a, and B2b). The wild population had a higher score of hovering before a short single or multiple attacks (A2a, and A2b). Giving the hatchery population have a higher performance of every attack type (Table 6), except for two, indicates a slightly bolder and more aggressive behavior than the wild population.

Table 9 from appendix 3 showed that 9 out of 41 lumpfish (22%) were chased or attacked by salmon. An earlier trial declared no antagonistic behavior between lumpfish and salmon and that the two species seemed to co-exist in the sea pens (Imsland *et al.*, 2014a).

As the present study experimented in tanks, space could be a stressing factor for the fish. Although salmon attacked 22% of all lumpfish, the interaction did not occur often (Table 9), and no physical signs of the attacks was observed in the lumpfish.

Lumpfish were observed eating food from the free water columns and grazing on pellets fed to salmon. This was only visually observed, not noted and analyzed further, but it can confirm that the lumpfish studied has the opportunistic feeding behavior described in other studies (Imsland *et al.*, 2014a, 2014c, 2015; Powell *et al.*, 2017).

4.5 Stomach content

Table 7 shows that only 13 out of 41 lumpfish had lice in the stomach from the three sampling dates, which is 32% of all lumpfish. On the first sampling date, the 3rd of December, only 5% of all lumpfish had lice in their stomach. This increased to 24% on the second sampling day and decreased to 20% on the last sampling day. Imsland et al. (2015) observed that 13-17% of all lumpfish kept in open net pens had sea lice in their stomach at day 11 increasing to 33%-38% of the lumpfish at day 70. It has also been seen from a semi-commercial trial that sea lice were grazed by only 10% of the lumpfish at the beginning, increasing to 36% at the end of the trial (Imsland *et al.*, 2016b). It was estimated that if 30% or more lumpfish introduced into commercial cages consumed sea lice, the sea lice infestations levels would be significantly suppressed (Imsland *et al.*, 2015; Powell *et al.*, 2017). The present study has shown a lower percentage of sea lice consummation than the trials mentioned, yet it showed similar trends with an increasing consummation over time. Although the number of lumpfish consuming sea lice decreased from 24% to 20% on the last sampling day, Table 7 showed that the number of sea lice in the stomach increased per sampling day. A total of 32% of lumpfish graze sea lice in the present study, which reaches the calculated estimation of 30%. Cleaning rates of lumpfish are very variable, and it has been discovered that not all individuals eat sea lice (Whittaker *et al.*, 2021), this is also seen in present study, see table 7.

It is essential to keep in mind that the present study has been experimented in tanks and not in sea cages like the trials mentioned earlier and may therefore not be as relatable. The present experiment ended after 17 days in tanks, while the study with up to 38% active delousers ended after 77 days (Imsland *et al.*, 2015), which also makes the timeframe different. Although, the efficiency of delousing by ballan wrasse from a tank-scale trial revealed 90-99% efficacy regardless of size and the presence of supplementary feeding (Leclercq *et al.*, 2014; Overton *et al.*, 2020).

Five individuals excelled in the present trial for having consumed the highest number of sea lice and performed the highest number of attacks (table 6 and 7), indicating bold and aggressive behavior. Figure 8 expresses time spent swimming by each of the individuals. Viewing figure 8, individual H30 and H11 showed little swimming activity compared to the other individuals. Individual W6 and H67 showed to spend no swimming at some days and a lot of swimming other days. The individuals seem not to have any similarities in time spent swimming, other than H30 and H11 showing low activity. Table 6 shows that 55 attacks in total was performed by H30, 37 by H67, 52 by W6, 20 by W17, and 10 by H11. By examining Table 7 it is estimated that 21 lice were found in the stomach of H30, 23 in H67, 9 in W6, 11 in W17, and 5 in H11. Lumpfish has a long intestine with many bends, suggesting efficient digestion, lumpfish may absorb sea lice quickly (Powell *et al.*, 2018b). Therefore, to perform a perfect correlation between the number of attacks and the number of lice found in their stomach, the number of attacks should be observed no longer than a day before sampling gut content from performing gastric lavage. Days observing attacks did not occur day before sampling of stomach content and can therefore not be compared.

5 Conclusion and future directions

As expected, hatchery lumpfish were the best fit as cleaner fish in present study because of higher number of sea lice in their stomach and higher number of attacks. Hatchery lumpfish were bolder and more aggressive, than the wild population. Reasons for this could be that the hatchery lumpfish are more shaped by the hatchery conditions since fish are highly phenotypically plastic, and giving the trial was performed under hatchery conditions, the environmental condition was in their favor. There were no significant differences time spent swimming and attacks performed between the origins. Five individuals were seen to be more aggressive than other individuals by having high numbers of attacks and lice in their stomach. For future trials, it would be interesting to look at the genes of individuals that are bolder and aggressive, to examine if there are similarities in genes. For future directions concerning the present trial, a more extended experiment period with more replicates and several gastric lavages could give more evidence-based use of cleaner fish to increase their efficacy, and further help to alleviate economic, environmental, and ethical concerns (Overton *et al.*, 2020). Make sure that days are recorded correlated to samplings of stomach content, so it would be possible to compare attacks with lice in the stomach and evaluate success of attacks. Having a single camera viewing the whole tank so that the activity of the fish will not be counted several times would also be beneficial. Lumpfish would often disappear out of sight, not knowing if the individuals were moving the tank upward and out of sight from the camera or into the other side of the tank in the second camera. The swimming activity should also be evaluated differently to correlate it to attacks performed by lumpfish.

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7 Appendices

7.1 Appendix 1 – Count of every swimming activity by origin and tank

Table 8 – Total count of each swimming activity through the experimental period, done by both wild and hatchery lumpfish.

	Swimming	**Attached**	**Hovering**	**Burst**
Tank 9				
Hatchery	545	78	3	5
Wild	506	52	22	7
Tank 10				
Hatchery	228	49	1	2
Wild	356	52	15	8

7.2 Appendix 2 – Swimming activity by origin in tank 9 comparing light condition

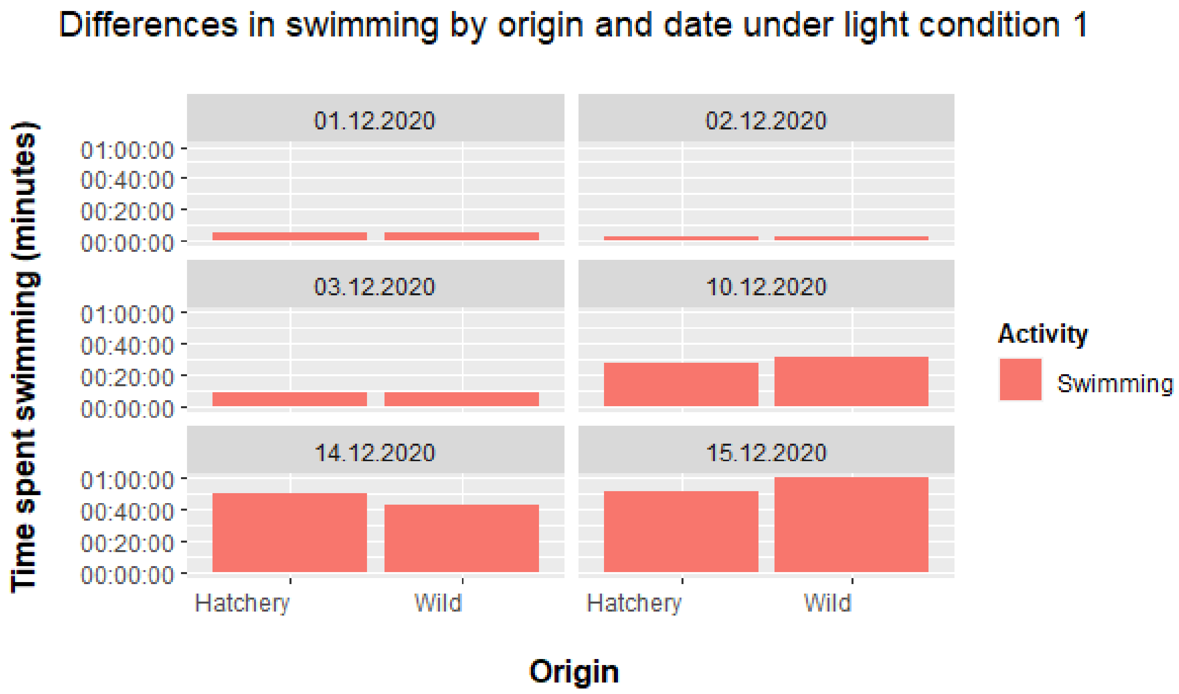

Figure 9 – Differences in swimming by the two origins (hatchery and wild lumpfish) under light condition 1 (once lights were turned on), split into days observed.

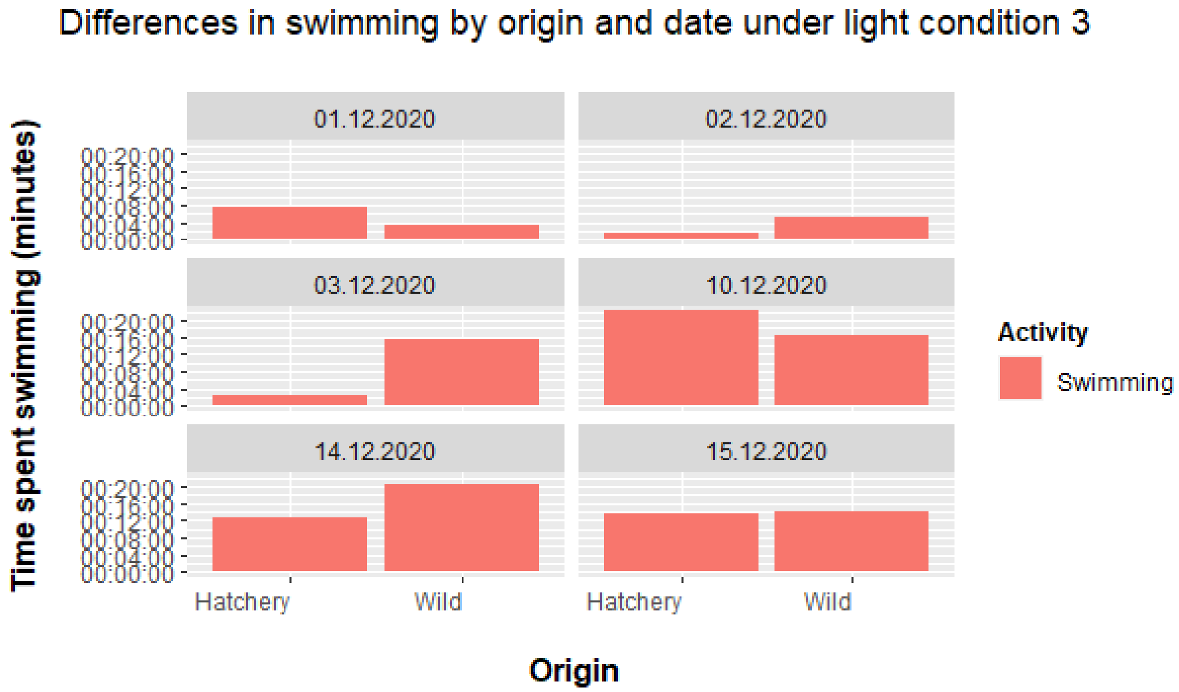

Figure 10 – Differences in swimming by the two origin (hatchery and wild lumpfish) under light condition 3 (once lights started dimming), split into days observed.

7.3 Appendix 3 - Interactions

Table 9 – Number of interactions in each group by IDs of lumpfish. Comments showing type of interaction.

ID	Interactions - C	Comments
Group 1		
H9	2	Attacked by salmon Attacked by salmon
H38	1	Attacked tag of another lumpfish
H64	1	Chased by salmon
W17	1	Attacked by slamon
W32	1	Chased by salmon
Group 2		
H13	1	Attacked by salmon
H64	1	Attacked by salmon
H56	1	Attacked by salmon
W17	1	Attacked by salmon
W22	1	Attacked by salmon
Group 3		
H5	1	Attacked by salmon
H18	1	Attacked by salmon
H56	1	Attacked by salmon
W22	1	Attacked by salmon
W50	1	Attacked by salmon