MASTER THESIS

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BI309F Master thesis in Marine Ecology

Habituation of the lumpsucker (*Cyclopterus lumpus* L.) in interactions with Atlantic salmon (*Salmo salar* L.)

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Preface

This thesis is submitted for the degree of Master of Science in Marine Ecology at Nord University in Bodø, and makes up 60 of 120 credits in total. Fieldwork was done in Trøndelag during summer and autumn 2016, and the thesis was finished in May 2017. Research described herein was conducted under the supervision of Professor Jarle Tryti Nordeide and Associate Professor Torstein Kristensen at Nord University. External supervisors were Professor Albert Kjartansson Imsland and Per Andersen. This work has not been submitted for any other degree or diploma at any other university to the best of the author knowledge.

Fredrik Ribsskog Staven

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Contents

ABSTRACT

Utilizing novel species of cleaner fish for biological delousing in the salmon farming industry has proven to be an important method for promoting sea lice control. In nature, cleaning behaviour often resembles intricate mutualistic relationships, and the interactions between the lumpsucker (*Cyclopterus lumpus* L.) and Atlantic salmon (*Salmo salar* L.) in aquaculture net cages provides an opportunity to study the context of such relationships in a controlled system. The aim of the study was to examine physiological and behavioural changes in lumpsuckers after one month in a net cage coexisting with a normal production unit of salmon. A comparison between lumpsuckers with net cage experience and naive control lumpsuckers was carried out in an experimental tank. Salmon were introduced in ten different trials each consisting of ten experienced lumpsuckers, and in ten different trials each consisting of ten naive lumpsuckers. For analysis of swimming activity, each of the twenty trials was video recorded for ten minutes before and after the introduction of salmon. After each trial, fish were photographed for examination of skin colour before blood was sampled for plasma cortisol measurements in addition to registrations of general morphology.

These results indicate a significant difference in physiological stress responses and behavioural swimming activity between experienced and naive fish. Naive compared to experienced fish increased their swimming activity after salmon were introduced, whereas experienced fish showed no change in swimming activity. Trials of naive and experienced fish showed no difference in swimming activity prior to salmon introduction. Moreover, naive fish had significantly higher plasma cortisol levels at the end of each trial. These results suggests a habituation through learning in experienced fish and the presence of an innate escape response in naive lumpsuckers during first encounter with salmon. Morphological measurements revealed a significant relationship between blue-green colouration of the epidermal skin in both groups of lumpsuckers with mean swimming activity during interaction with salmon. Morphological data in comparison to both swimming activity and plasma cortisol levels should be interpreted with caution, due to variability in rearing conditions and environmental factors between the two groups. It is concluded that naive lumpsuckers express both behavioural and physiological stress responses during first interaction with salmon, which might influence cleaning behaviour. Habituation to salmon presence is present in most individuals after one month of interaction in a net cage environment based on desensitization of escape responses and plasma cortisol levels.

1 INTRODUCTION

1.1 AQUACULTURE

The global aquaculture production has increased from five to sixty-three million tonnes during the last thirty years, and by today cover more than forty percent of global consumption market of marine fish (Beveridge et al. 2013; FAO 2016; World Bank 2013). The necessity of increasing food production is immense, with a population expected to reach nine billion people within 2050 (UNs Population Division 2015). Norway is a global leading producer of Atlantic salmon (*Salmo salar* Linnaeus, 1758) (FAO 2016), with an annual production that exceeded 1.3 million tonnes in 2015 (SSB 2016). Production is administered among nearly one thousand fish farms scattered along the Norwegian coastline and within the fjords systems (Directorate of Fisheries 2016). A surplus of islands and inlets function as shelter against open ocean weather (Gjedrem 1993), and biophysical conditions commonly meet the requirements for productive aquaculture, with strong currents and reliable cold seawater temperatures moderated by the mild Gulf Stream (Paisley et al. 2010). Norway have a history with over forty years of salmon breeding and domestication (Gjedrem 2012), which have contributed to improved utilization of nutrients (Thodesen et al. 1999), faster growth (Gjedrem et al. 1991) and increased pathogen resistance (Kolstad et al. 2005). Aquaculture salmon production is a source for healthy nutrients to human consumption, with a feed conversion ratio lower in comparison to most other domesticated species (Torrissen et al. 2011).

Challenges related to environmental carrying capacity rise when animal biomass production surpasses nature's resilience, and negative impacts are sometimes inescapable (Steffen et al. 2011). The Norwegian Ministry of Trade and Fisheries (2014) highlighted five main challenges which have been considered to influence the nearby environment and biodiversity of aquaculture sites in adversely concerns. These challenges include occupation of coastal areas (Dempster & Sanchez-Jerez 2008), benthic sediment pollution (Bannister et al. 2014; Kalantzi & Karakassis 2006; Mazzola et al. 2000), the use of marine fish feed ingredients (Naylor et al. 2000), genetic introgression in conspecifics (Bolstad et al. 2017; Skaala et al. 2006) and the transmitting of diseases from domesticated fish to wild fish populations (Svåsand et al. 2016). From these challenges, parasitic infections and dispersal from net cages to wild populations have been considered the most prominent in Norwegian aquaculture (Serra-Llinares et al. 2014; Svåsand et al. 2016; Taranger et al. 2015).

In a natural ecosystem, increased host population can be directly related to the prevalence of transmittable macroparasites (Arneberg et al. 1998), where parasitic infections induce a density dependent negative feedback loop with detrimental impacts on the population size (Anderson & May 1991). With aquaculture of salmonids, the risk of parasitic infestations is no exception (Jansen et al. 2012), and the ectoparasitic sea lice copepods *Lepeophteirus salmonis* Krøyer, 1837, and *Caligus elongatus* von Nordmann, 1832, have caused complications concerning animal welfare, economic costs and public reputation since the beginning of the aquaculture production (Costello 2009a; Costello 2009b; Pike 1989; Torrissen et al. 2013). Sea lice feed on the hosts epidermal skin and mucus layer (Boxaspen 2006) and eventually penetrate the protective surface of the fish (MacKinnon 1997) which increase the risk for secondary infections (Denholm et al. 2002; Mustafa et al. 2000). Skin infections can induce complications with osmoregulation (Wootten et al. 2011) and trigger physiological stress responses in salmon (Nolan et al. 1999). If not treated, sea lice infestations have shown to escalate rapidly (Jansen et al. 2012), especially at higher temperatures (Brooks 2005) and may eventually lead to increased mortality (Pike 1989).

A complete sea lice extinctor has remained yet to be discovered, and the salmon aquaculture industry has been dependent on a toolbox with multiple treatment methods, some more successful than others. In general, delousing of salmon has relied on chemotherapeutic drugs, mechanical treatments and bioremediation using cleaner fish. Chemotherapeutic drugs include the use of organophosphates, pyrethroid compounds, avermectins, benzoylphenyl ureas and oxidizing agents (Roth 2000). These drugs have served as important sea lice controllers, and provided good results when implemented in aquaculture. Nevertheless, through time and by repeated monotonic use of specific drugs, sea lice have evolved reduced sensitivity or resistance to mostly all chemotherapeutic drugs (Denholm et al. 2002; Fallang et al. 2004; Igboeli et al. 2012; Sevatdal & Horsberg 2003; Treasurer et al. 2000), summarized in (Aaen et al. 2015).

Recently, the use of mechanical delousing have become more common, which in turn has participated in the reduction of medical treatments by forty-one percent from 2015 to 2016 (Hjeltnes et al. 2017). Some of these treatments have shown promising results in the removal of sea lice, yet challenges related to animal welfare and optimisation is still under development, and published data are lacking.

1.2 CLEANER FISH

In contrast to chemotherapeutic and mechanical treatments, the use of cleaner fish have been considered a less stressful and a more sustainable delousing method (Denholm et al. 2002; Sundt & Jørstad 1998; Treasurer 2002). Cleaner fish are usually small fish species from different taxa that remove ectoparasites, dead skin or mucus from a host fish. The host fish is often referred to as the client (Grutter 2004) while the relationship itself is considered reciprocal altruistic (Trivers 1971), which is a rarely observed interspecific symbiosis among two vertebrates (Bronstein 1994; Grutter 1999). The cleaning behaviour is complex and include different manners of physical signalling from the cleaner and visual recognition by the client, in a series of validations, developed to separate a true cleaner from other mimicking parasitic fish (Cheney et al. 2009; Gingins et al. 2013; Grutter 2004). With focus on the observed cleaning interaction, cleaner fish have earlier been categorized based on whether they perform the behaviour as juveniles or facultatively during their life cycle, or obligately (Côté 2000).

Cleaner symbioses have been observed in a variety of species in different aquatic ecosystems, especially symbioses including different species of wrasse in tropical coral reefs (Bshary & Grutter 2006; Côté 2000). In Norway, six wrasse species live and reproduce (Skiftesvik et al. 2014; Skiftesvik et al. 2015; Sundt & Jørstad 1998; Svåsand et al. 2016). Four of these species, ballan wrasse (*Labrus bergylta* Ascanius, 1767), goldsinny wrasse (*Ctenolabrus rupestris* Linnaeus, 1758*)*, rock cook (*Centrolabrus exolentus* Linnaeus, 1758*)* and corkwing wrasse (*Symphodus melops* Linnaeus, 1758) are considered facultatively cleaner fish, based on their ability to detect and graze ectoparasites from Atlantic salmon in aquaculture (Bjordal 1990; Deady, Sandra et al. 1995; Skiftesvik et al. 2014; Skiftesvik et al. 2015; Treasurer 1994; Treasurer 2002; Tully et al. 1996).

Due to the effectiveness of medical treatments during the nineties, wrasses contributed partially as an alternative delousing method (Bjordal 1990; Deady, S. et al. 1995; Tully et al. 1996). As resistance evolved in sea lice and the biomass of salmon production increased, the demand returned, and from 2006 to 2015, annual catch on wild wrasses accelerated from one to twenty million (Svåsand et al. 2016). Data on how the different wrasse species are affected by amplified fisheries is scarce (Svåsand et al. 2016), however studies on corkwing wrasse have showed how size-selective fisheries have led to changes in sex ratios in wild populations (Halvorsen et al. 2016). Other concerns regarding impacts on wild populations of wrasses are related to disruptions in genetic population structures between subpopulations due to introduction of aquaculture escapees (Blanco Gonzalez et al. 2016; Sundt & Jørstad 1998) and

spread of diseases between wrasse populations and between wrasses and salmonids (Espeland et al. 2010; Svåsand et al. 2016).

From 2010 to 2014, a new species from a completely different taxa, the Lumpsucker (*Cyclopterus lumpus* Linnaeus, 1758) was systematically tested as a possible sea lice grazer (Imsland et al. 2014a; Imsland et al. 2014b; Imsland et al. 2014c). Studies conducted in Northern Norway found significantly reduced sea lice infestations on Atlantic salmon when an intraspecific mix of five to fifteen percent lumpsuckers were used (Imsland et al. 2014a). These studies participated in promoting interest for a species that previously was fished mainly for its roe before discarded (Durif 2014; Holst 1993; Paradis et al. 1975). The production of lumpsuckers through hatching and rearing has expanded due to the growing market interest. In 2016, thirty-three Norwegian production sites produced 17.5 million lumpsuckers (Waatevik 2017), hence, as of today, the lumpsucker is the second most reared marine fish in Norway.

Reared lumpsuckers are introduced to net cage directly from the hatchery without acclimation. Still, its ability to adapt and function as a cleaner fish, have been suggested to be robust when general conditions of good housekeeping and shelter is provided (Imsland et al. 2014b; Imsland et al. 2014c; Imsland et al. 2015a). Lumpsuckers live and migrate in colder waters compared to wrasses, and while ballan wrasse and goldsinny wrasse have shown reduced activity and feeding at temperatures below 6 °C, (Sayer & Davenport 1996; Sayer & Reader 1996), lumpsuckers are considered active lice grazes during winter (Imsland et al. 2014a) and have been observed consuming lice from salmon at temperatures down to 4 °C (Nævdal & Hovland 2014).

1.3 BIOLOGY OF THE LUMPSUCKER

The lumpsucker (*C. lumpus* L.) belong to the family of Cyclopteridae, which currently include seven genera with 28 species (Froese & Pauly 2015; Voskoboinikova 2015). The species is abundant in the North Atlantic, observed from Spain to the Barents Sea in east, and from New Jersey to Hudson Bay in the west (Bañón et al. 2008; Davenport 1985; Froese & Pauly 2015; Stein 1986).

The general appearance of the lumpsucker is characteristic and rarely mistaken with other species (Moen 2014). The lumpsucker has morphological features of a bottom-dwelling species including a ventral sucker, globiform body structure and lack of swim bladder, but is considered semipelagic as most of its life is spent in open waters (Davenport & Thorsteinsson 1990). The

name derives from Greek, *kyklos* meaning circle, and *pteryx* referring to the pelvic fin, which describes the unique physiologically somatic structure of the sucker disc found in all species within the Cyclopteridae family (Budney & Hall 2010; Davenport & Thorsteinsson 1990; Froese & Pauly 2015). The body is compressed, while the anterior dorsal fin is concealed in thick skin forming a crest which increase in height with age (Davenport 1985). Three rows of tubercles run alongside the fish, and the skin is rough in texture. During maturation, males turn red while females stay blue or blue-green (Davenport & Bradshaw 1995). The red colouration in males is considered to play a role in intrasexual competition for territory and during courting (Davenport & Thorsteinsson 1989). Spawning occurs in spring and summer near the coast close to the littoral zone, often at sites with strong currents (Davenport 1985).

Mature fish migrate from open water into coastal sheltered areas (Mitamura 2007), and the egg masses are laid in clusters attached to hard substrate which the male guard and engage with moulding behaviour and pectoral fanning until hatching is completed (Goulet 1985). Juvenile lumpsuckers stay hidden in the benthic parts of the littoral zone for the first 1-2 years, camouflaged in seaweed, kelp and rocky obstacles (Goulet 1985; Moring 2001). Juveniles express a variety in skin colouration; from dull grey to almost clear blue, blue-green or olive green (Davenport & Bradshaw 1995; Moring 1994). The blue-green colouration derives from biliverdin, which is an intermediate by-product in the biochemical conversion of heme (Davenport & Thorsteinsson 1989; McDonagh 2001).

Lumpsuckers are generalists when it comes to feeding preferences, and tends to consume what is available of jellyfish, crustaceans, polychaetes, algae and molluscs (Imsland et al. 2015b; Ingolfsson & Kristjansson 2002), as well as implementing sea lice in its diet (Imsland et al. 2015b), yet studies on lumpsuckers ecological function as cleaner fish in nature is lacking.

1.4 LUMPSUCKERS ADAPTING TO AQUACULTURE

Breeding programmes with artificial selection for desired phenotypic traits is a major cornerstone in the process of animal domestication (Aulstad et al. 1972; Gjedrem 2000; Jarman et al. 1976). Breeding of lumpsuckers is currently in a start phase, and the necessity to gather fundamental knowledge before selecting for preferable traits occur, is crucial. Lumpsuckers used in aquaculture originate from parental wild broodfish, and studies on their ability to cope in a net cage environment comprises on considerations of ontogenetic learning and habituation, observable through the production period for Atlantic salmon.

While changes across multiple generations is known as evolutionary adaptation (Darwin 1859), learning in fish is recognized as an adaptive behaviour to a predictable environment based on experiences during each individuals life cycle (Braithwaite 2010; Brown 2006; Odling-Smee & Braithwaite 2003). Habituation is the reduced response in an organism due to repeated stimulus (Bouton 2007) and is considered adaptive when an organism cease to respond to a repeated stimuli that have no direct consequence (Nilsson et al. 2012). This applies to lumpsuckers living with domesticated salmon with imprinted feeding regimes, presumed disinterested in other food items such as cleaner fish, yet a carnivorous predator with lumpsuckers included in its natural diet (Holst et al. 1993; Jacobsen & Hansen 2001). Imsland et al. (2014c) observed little antagonistic behaviour between the two species, nevertheless, a physiological stress response due to predatory presence, might still occur, as observed in other prey fish species (Barcellos et al. 2007; Barton 2002) and in obligate cleaner fish (Gingins et al. 2017).

Lumpsuckers applied in aquaculture are exposed to different environmental stressors which challenge the fixed heritable behaviour in terms of adaptiveness, and the ability to fine tune through learning to fit the particular environment (Magnhagen et al. 2008). Stress refers to how fish, or other animals respond physiologically in the overcoming, coping and recovering from threatening challenges (Selye 1950). Stress is broadly categorized into three stages, named primary, secondary and tertiary responses (Wendelaar Bonga 1997). The primary response represents the brain activation through perception, which promotes a neuroendocrine reaction that leads to the release of the stress hormones cortisol and cathecholamines (Mazeaud et al. 1977; Wendelaar Bonga 1997). The secondary response describes the activation of metabolic pathways influenced by the release of these hormones into the blood system (Wendelaar Bonga 1997). While the cathecholamine adrenaline express a rapid stress response, plasma cortisol stays elevated and promote the release of plasma glucose for longer periods through glycogenolysis and glucogenesis in the liver (Wendelaar Bonga 1997), and is a common applied measure of secondary stress in aquaculture fish (Iversen et al. 1998; Iversen 2013; Iversen et al. 2015). The tertiary response represents the whole animal response to stress, and referrers to the consequence of not adapting to the stressor, which might lead to chronic stress and morphological changes such as reduced growth or reproduction capacity (Barton & Schreck 1987; Wendelaar Bonga 1997).

In general, stress increases the energy budget to vital organs through the release and transport of plasma glucose into the blood stream. This amplifies the animal's ability to cope with the

stressor (Wendelaar Bonga 1997), and leaves less energy available for other behaviours (Barton & Schreck 1987; Vijayan et al. 1996; Vijayan et al. 1997). The most common observed behavioural changes to acute stress include escape responses (Mommsen et al. 1999), while longer exposures to stress have shown behavioural changes in place preference, locomotion style and swim speed (Clark et al. 2011), as well as impacts on cognitive decision-making, which results in less time spent on feeding and resting (Archard et al. 2012; Harris & Carr 2016).

The interaction with larger potential predator fish through indirect or direct contact is one alternative source of stress lumpsuckers introduced to aquaculture experience. First encounter with Atlantic salmon, include visual cues of a possible predator, and might act as a stressor. Long exposure of repeated stressors have also shown to desensitize fish and moderate the neuroendocrine response to a similar stressor (Barton 2002), which have previously been observed to cause habituation through a supressed cortisol response to predatory presence in zebrafish (*Danio rerio* Hamilton, 1822) (Barcellos et al. 2010). With this exception, studies on habituation by measuring plasma cortisol in fish is scarce, primarily due to the ethical dilemma of keeping prey and predatory fish together (Huntingford 1984). Thus, considering the increased use of lumpsuckers in aquaculture, information about the species ability to cope and adapt to the presence of salmon is of interest.

1.5 AIM OF THE STUDY

The aim of the study was to examine how lumpsuckers with experience from living in an open net cage with Atlantic salmon for one month expressed measurable changes in behaviour and physiological responses by comparison with control replicates of naive lumpsuckers with no such experience. This emphasise a relatively broad question whether behavioural and physiological adaptation influence lumpsuckers function as a cleaner fish. It was hypothesized that experienced lumpsuckers would differ from the naive fish during controlled interaction with Atlantic salmon in (1) swimming activity and (2) secondary stress response measured in plasma cortisol as a result of habituation to salmonids. In addition, the effect of morphological (3) colouration and (4) growth was compared between experienced and naive lumpsuckers swimming activity and plasma cortisol measurements.

2 MATERIAL AND METHODS

2.1 FISH STOCK AND EARLY ONTOGENY

All lumpsuckers used during this study originated from roe and milt extracted from wild broodfish captured in Flatanger (64°30’20.4"N, 10°50’40.8"E, WSG84), Nord-Trøndelag, Norway in September 2015. Mature males and females from the same group of broodfish were later transported to Nordland Rensefisk at Lovund (66°22’1.1"N, 12°22’36.4"E, WSG84). Lumpsuckers from Lovund are hereafter referred to as naive, while lumpsuckers from Flatanger are referred to as experienced. Naive fish would not interact with Atlantic salmon nor a net cage environment until trial period, while experienced fish lived in net cages with Atlantic salmon for one month prior to first trial.

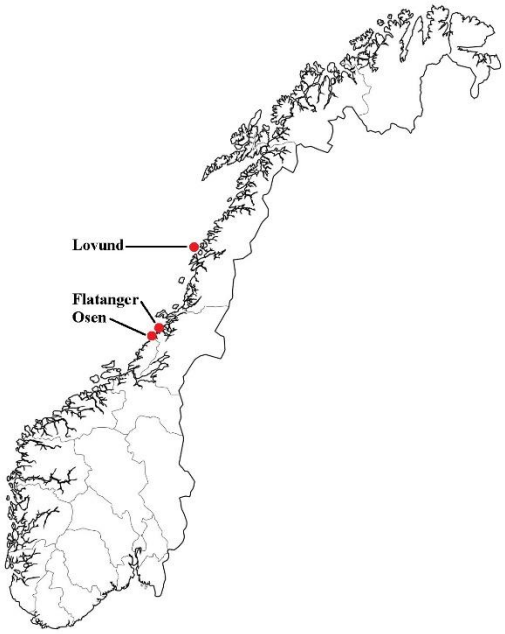

Figure 1. Map shows broodfish capture site, location of hatchery- and rearing facilities and the fish farm site where trials were conducted. Broodfish capture and hatching of lumpsuckers took place in Flatanger, before the experimental population was split and reared both in Flatanger and at Lovund. Trials were conducted at the fish farm location nr.30817 Raudøya in Osen.

At Namdal Rensefisk in Flatanger incubation started during the first week of October, while at Lovund, incubation started four weeks later in early November. Incubation occurred in 40 x 40 cm hatching trays (Sterner Fish Tech, Ski, Norway), and at both locations, larvae started hatching between 280 and 300 day degrees, respectively on November 15 at Namdal Rensefisk

and on December 18 at Lovund Rensefisk. At both locations, feeding regime throughout early ontogeny, and until departure, consisted of dry feed pellets. During the first two months, lumpsuckers were fed with Micro 300, 0.3 mm pellets (Skretting, Stavanger, Norway) and Gemma Wean Diamond, 0.5 mm (Skretting, Stavanger, Norway). In the following months and until departure, lumpsuckers were fed with Gemma Diamond 0.8 mm, 1.5 mm, Silk 1.5 mm and 1.8 mm (Skretting, Stavanger, Norway). At both locations, lumpsuckers were kept in circular green tanks measuring 2.5 m^3 for most of the period during rearing, before fish was relocated to 5.5 m^3 respectively at similar size during size-selective sorting.

Daily monitored oxygen saturation were stable above eighty percent at both locations. At Lovund Rensefisk, mean water temperature was 8.0 °C, S.D. ± 0.95, with maximum temperature at 9.5 °C and minimum temperature at 7.5 °C. At Namdal Rensefisk, mean water temperature was 7.4 °C, S.D. ± 0.95, with maximum temperature at 9.6 °C and minimum temperature at 6.3 °C. Lumpsuckers at Lovund Rensefisk were vaccinated with Alpha Marine® (Pharmaq AS, Overhalla, Norway) on June 1, 2016. The same vaccine was used at Namdal Rensefisk on June 6, 2016.

2.2 ATLANTIC SALMON

Atlantic salmon used during the study originated from the AquaGen strain, hatched and reared at Flatanger Settefisk. Salmon were translocated to open net cages at Raudøya in December 2015. Here, salmon coexisted with lumpsuckers at an approximate 5-10 % percent mix intervention from December 2015 and until experiment start in August 2016. Eighty salmon assigned to the experiment were selected based on similarity in size, and had a mean weight of 1258.5 g, S.D. ± 152.12.

2.3 TRANSPORTATION

2.3.1 LUMPSUCKERS FROM LOVUND

On July 5, 12600 lumpsuckers were transported with trailer from Lovund to the fish farm location number 30817 Raudøya (64°21’59.5"N, 10°26’40.9"E, WSG84), in Osen. Mean oxygen saturation during transport was 87.5 %, mean pH was 8.3 and mean temperature was 13.2 °C. In Osen, fish were unloaded and transported further by boat for half an hour until arrival at Raudøya. Mean oxygen saturation during boat transport was 89.3 %, S.D. ± 0.56 and mean temperature was 12.5 °C, S.D. ± 0.15. Lumpsuckers were added to open net cages, fifty meters in diameter and eighteen meters deep, containing a normal production unit of Atlantic

salmon. Net cages had artificial kelp installations made of plastic, used as shelter for cleaner fish. Fifteen lumpsuckers were measured. Mean weight was 24.2 g, S.D. ± 7.64 and mean length was 7.8 cm, S.D. ± 0.97.

2.3.2 LUMPSUCKERS FROM FLATANGER

On July 29, 14800 lumpsuckers were transported from Namdal Rensefisk to Raudøya with well boat. Mean oxygen saturation during transport was 88.9 %, and mean temperature was 13.9 °C. Fish were split between three net cages containing Atlantic salmon, separate from net cages with lumpsuckers from Lovund. In addition, 100 lumpsuckers were placed in a holding tank (2 x 2 x 1.5 m) at the feed barge at Raudøya. In the holding tank, seawater was supplied from 4 m depth with an 24V immersion pump (Metabo®, Nürtingen, Germany) connected to a 50 mm hose. Seawater was filtered through a net, which resembled that of a net cage. From this group, fifteen lumpsuckers were measured. Mean weight was 37.6 g, S.D. ± 6.50 and mean length was 9.5 cm, S.D. ± 1.00.

2.4 EXPERIMENTAL PREPARATION

2.4.1 GENERAL SETUP

Experienced lumpsuckers were situated in net cages with Atlantic salmon from June 5 and until first trial conducted on August 2 2016, while naive lumpsuckers were kept in a holding tank at the feed barge from the July 27 and throughout the trial period until September 6. Naive fish had no previous interaction with salmon.

From August 2 and until September 3 2016, twenty trials were conducted at the stationary feed barge at Raudøya fish farm in Osen. Ten replicates contained experienced lumpsuckers while the remaining ten replicates contained naive lumpsuckers. Each replicate included ten lumpsuckers and the introduction of four Atlantic salmon (Figure 2). Trials were spatially distributed throughout the month, partially affected by weather and available logistical support. Lumpsuckers were tagged and taken off feeding while stationed in the holding tank two days prior to each trial. Next, replicates of lumpsuckers were moved to the experimental tank and acclimated for one day, before behaviour was video recorded, prior to, and during interaction with salmon. After video recordings had ended, lumpsuckers were euthanized before physiological and morphological measurements were sampled.

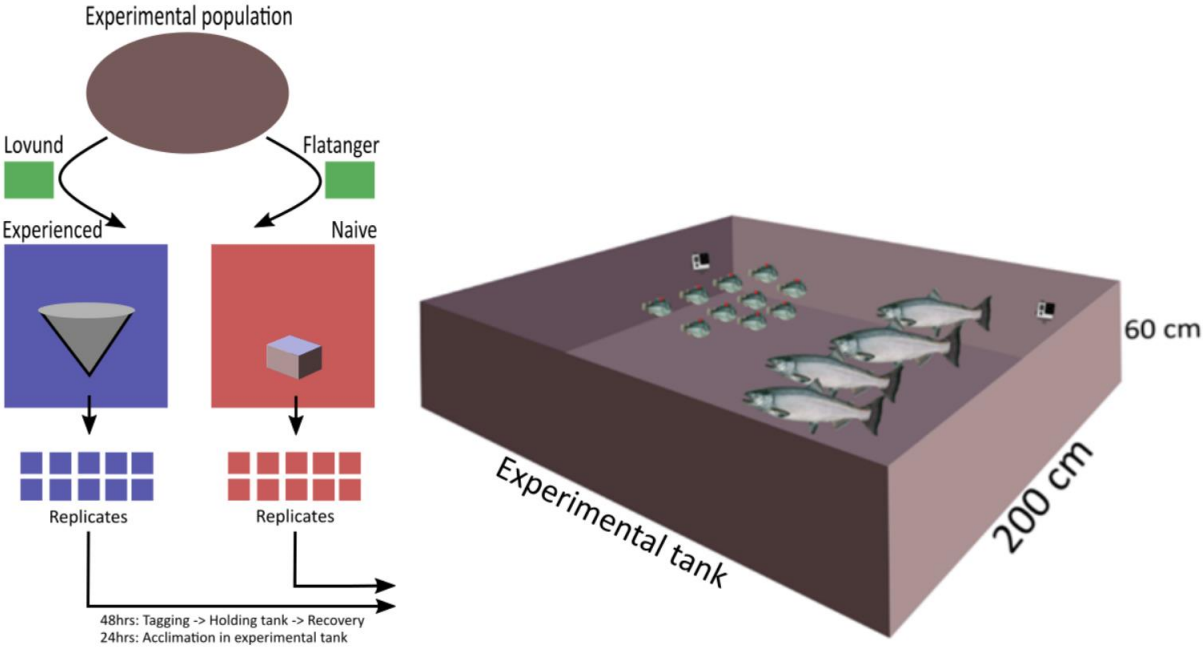

Figure 2. Figure shows logistic flow of lumpsuckers. Experimental population originated from broodfish captured in Flatanger. Lumpsuckers were reared at two different hatcheries: in Flatanger and at Lovund. Lumpsuckers were transported to the fish farm location Raudøya, and for one month, experienced fish coexisted in a net cage with Atlantic salmon, while naive fish from Flatanger were kept in a holding tank. Twenty trials were conducted in the experimental tank from August 2 until September 3, with ten replicates of naive and ten replicates of experienced lumpsuckers, each replicate containing ten lumpsuckers. Four Atlantic salmon were used for each trial.

2.4.2 FEEDING

Every second day, both groups were fed with Lumpfish Grower 2.0 mm pellets (Biomar, Karmøy, Norway). In addition, 9 mm salmon feed pellets (Skretting, Stavanger, Norway) were added to the holding tank to simulate the feeding regime conducted in net cages.

2.4.3 COLLECTING AND MOVING EXPERIENCED FISH

Experienced lumpsuckers were collected and transported from the net cage to the barge fleet two days prior to each trial of experienced fish. A randomized selection of fish were hand netted when lumpsucker shelters were raised to the surface. Lumpsuckers were put in a bucket before a blinded selection of ten individuals were picked. Fish with visible wounds or physical abnormalities were excluded. During the transportation from the net cage to the barge fleet, lumpsuckers were kept in a 1 m^3 tank with water supply and oxygen monitoring. Oxygen saturation was above eighty percent during the whole transport period. Naive fish located at the barge fleet were not moved any further after arrival in the holding tank, with exception of hand netting before tagging and transfer to the experimental tank located next to it.

2.4.4 TAGGING

In advance to the trial period in August and September, tagging with Peterson discs (Petersen 1896) were tested at the research facilities of Nord University in March 2016 and during a pilot study in April 2016 in Osen. Tags showed no clinical harm, nor any effect on appetite, behaviour or plasma cortisol levels (Appendix III Table I).

Prior to tagging, lumpsuckers were kept in a bucket with 1 ml/10 L Benzoak Vet (ACD Pharmaceuticals AS, Leknes, Norway) mixed into 13 °C seawater. When clinical symptoms of anaesthesia occurred, lumpsuckers were moved to a 30 x 40 cm tray containing only seawater at 13 °C, covering mouth and gills (Figure 3). With sterile DS 24 suture (Resolon®, Nürnberg, Germany) and a surgical scissor, Petersen discs were quickly attached at both lateral sides at the tip of the dorsal crest. Each pair of discs had unique symbols, to recognize individuals during video monitoring. A fishing knot secured the discs. After tagging, lumpsuckers were put in the holding tank for recovery under observation.

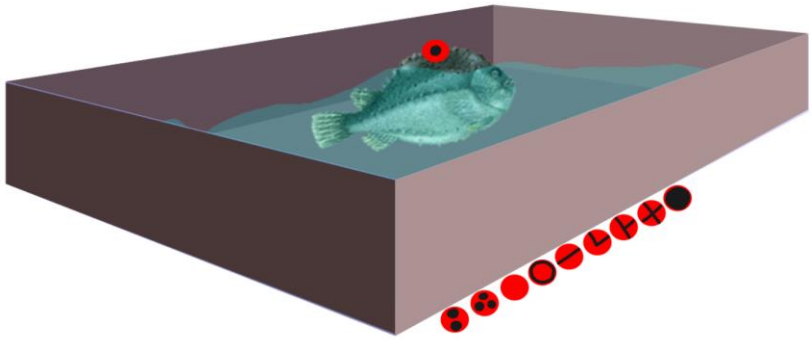

Figure 3. During tagging, lumpsuckers were kept in a tray with seawater covering mouth and gills, while Petersen discs with distinctive symbols (1-10) were attached to the dorsal crest for visual identification.

2.4.5 FISH ACCLIMATION

After 24 hours in the holding tank without feeding, the batch of ten lumpsuckers devoted for each experiment were hand netted and moved to the experimental tank, where an adjusted acclimation period of 24 hours followed.

2.4.6 EXPERIMENTAL TANK SETUP

The experimental tank was located next to the holding tank, and measured 2 x 2 x 0.7 m. The experimental tank stored 2600 l of water with a drain pipe regulating water depth to 65 cm. Seawater was supplied through an 24V immersion pump (Metabo®, Nürtingen, Germany) at 4 m depth connected to a 50 mm hose. Seawater was filtrated through a 3 mm sieve before

entering the experimental tank to reduce turbidity. Water exited through a 5 mm sieve in the middle bottom of the tank through a 40 mm hose. The tank was dark green-grey in colour, with a tarpaulin attached two meters above to avoid surface tension from rain droplets and direct sunlight. Underwater cameras were installed the evening in advance to each trial, while a net protected the replicate from predators during night.

2.4.7 COLLECTING AND STORING OF ATLANTIC SALMON WITH SEA LICE

Atlantic salmon (1258.5 g, S.D. ± 152.12) were collected based on similarity in size, in collaboration with personnel at Raudøya. Salmon were anesthetized with 15-20 ml/100 l Benzoak Vet (ACD Pharmaceuticals AS, Leknes, Norway) before Salmon lice (*L. salmonis*) were counted, replaced and manipulated onto fish assigned for experimental use. This was done with the aim to give lumpsuckers the opportunity to graze sea lice. Eight to ten sea lice were positioned between the dorsal and the caudal fin.

1. *L. salmonis*: 5 adult females with egg strings
2. *L. salmonis:* 3 to 5 adult males

Salmon were transported to the feed barge, and kept in a 1 m^3 tank with an 24V immersion pump (Metabo®, Nürtingen, Germany) connected to a 50 mm hose, which provided the tank with water from four meters depth, supplying six-thousand litres per hour.

2.5 EXPERIMENTAL EXECUTION

2.5.1 VIDEO RECORDING

To reduce diurnal variation in hormonal outputs (Lorenzi et al. 2008), all twenty trials were carried out between noon and 2 p.m. during the trial period. In each trial, five high definition GoPro Hero 3^+ cameras (Gopro™, California, USA) with tape-covered indicator lights were attached with suction cups, one in each corner the experimental tank, and a fifth camera above the tank to cover the surface in 2D perspective. GoPro™ cameras have previously been implemented in a number of methodologies for marine research, i.e. in Assis et al. (2013), Harasti et al. (2014) and Letessier et al. (2015). Inside the tank, video was shot in 1080p, with 60 fps and ultra-wide field of view, while the surface camera was shot in 1080p with 60 fps at medium field of view.

Ten minutes prior to filming started, water supply was disconnected and the experimental tank drainpipe was elevated to reduce turbulence, particle flow and provide a clearer view. At the same time, a 5 ml/100 l dosage of Benzoak Vet (ACD Pharmaceuticals AS, Leknes, Norway)

was added to the tank with four Atlantic salmon assigned for each trial, to induced a light sedative effect. Optimal sedation occurred when salmons no longer responded to waving action above the tank. The sedative was crucial in calming the fish, since moving salmonids by hand nets from the salmon holding tank to the experimental tank would cause stress, and influence behavioural- and physiological responses in lumpsuckers.

After ten minutes, cameras were synchronously started with a GoPro Wi-Fi remote. Recording continued for ten minutes until four Atlantic salmon were carefully added to the tank by hand net from behind a cover. Filming continued for ten minutes, until recording was stopped. An additional interaction-window of twenty minutes were added to ensure a solid primary stress response in plasma cortisol (Iversen et al. 2015) before fish were euthanized.

2.5.2 EUTHANIZATION

Forty minutes after filming had started, a 20 l bucket with premixed Aqua calm™ (Western Chemical inc, Canada) containing metomidate, was added in the experimental tank, providing a dosage of 3 mg/ l. Metomidate was used due to its biochemical ability to block cortisol synthesis (Iversen et al. 2003) and a dosage at 3 mg/ l would provide such an effect, as previously shown for fish at similar size (Olsen et al. 1995). After five minutes, lumpsuckers were hand netted to a 10 l bucket containing 10 mg/ l metomidate, which caused cessation of movement and breathing reflexes within ten to twenty seconds. When the replicate of ten lumpsuckers were fully anaesthetized, each fish was killed with a blow to the head. Salmon were euthanized with an overdose of 100 ml/ l Benzoak Vet (ACD Pharmaceuticals AS, Leknes, Norway), due to regulations on use of metomidate in fish intended for consumption (Bowker & Trushenski 2013).

2.5.3 HYDROLOGICAL DATA

At Raudøya, hydrological measurements were logged using a CTDOX-instrument (SAIV AS, Bergen, Norway). This included measurements of conductivity, temperature, oxygen, fluorescence and turbidity. The CTDOX-instrument was installed in the holding tank and set to measure every tenth minute throughout the experimental period (see Appendix I Section 6.2 Table I).

2.6 MORPHOLOGICAL MEASUREMENTS

2.6.1 GENERAL PHYSIOLOGY

After each experiment, lumpsuckers were photographed before weight, length and general external health conditions were registered. Next, fish was dissected to determine stomach contents, sex and internal health conditions.

2.6.2 COLOURATION

To measure the quantity of blue and blue-green skin colouration, all lumpsuckers were photographed using a Canon 550d (Canon, Tokyo, Japan) with a Canon 20mm f/2.8 lens. Settings were manually pre-set with fixed ISO-800, 1/200 shutter speed and 6000 Kelvin. An internal build in flash was used. The camera was placed on a stand, and each fish was photographed on a white plate with a piece of blue cardboard included to use for colouration correction. Images were stored in jpeg format, and Red-Blue-Green (RGB) values were later extracted using ImageJ software (Schindelin et al. 2015). Methods were based on Villafuerte and Negro (1998), previously implemented in studies on colouration in fish, i.e. three-spined sticklebacks (*Gasterosteus aculeatus* Linnaeus, 1758) (Amundsen et al. 2015), Eurasian perch (*Perca fluviatilis* Linnaeus, 1758) (Eckmann et al. 2017) and Atlantic salmon (*Salmo salar* L.) (Houde et al. 2015). In ImageJ, a line was drawn from the root of the caudal fin along the lateral side to the edge of the pectoral fin, and back along the lower crest (Figure 4). The mean pixel density for red, green and blue were quantified and adjusted based on the mean value from the blue cardboard. Next, the intensity of blue (I_b) and blue-green ($I_b + I_g$) was calculated using the following formulas:

$$I_b = \frac{\mathrm{B}}{(\mathrm{R} + \mathrm{G} + \mathrm{B})} \qquad I_b + I_g = \frac{(\mathrm{B} + \mathrm{G})}{(\mathrm{R} + \mathrm{G} + \mathrm{B})}$$

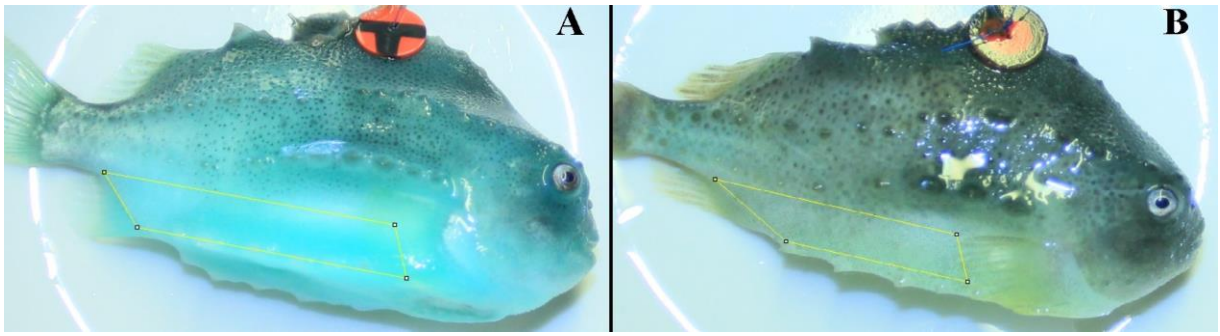

Figure 4. Photos describing observed variation in colour between lumpsuckers, and the area used to calculate RGB. Figure 4A illustrates a stronger blue and blue-green colouration with an I_b index of 0.43 and an $I_b + I_g$ index of 0.83, whereas Figure 4B illustrated less colouration with an I_b index of 0.34 and an $I_b + I_g$ index of 0.72.

2.6.3 BLOOD SAMPLING

Blood samples were collected within fifteen minutes after lumpsuckers had been euthanized. Blood was extracted from the ventricle using a 0.33 x 12.7 mm syringe (BD Micro-fine™) containing anticoagulating heparin. Next, samples were put in Eppendorph tubes and centrifuged at six thousand rpm (rounds per minute) for five minutes in a Mini Star centrifuge (VWR™, UK). After centrifugation, plasma was collected with a pipette, transferred to a Cryotube, and stored in a freezer at -30 °C. Samples were later analysed by personnel at Nord University in Bodø, Norway.

2.6.4 PLASMA CORTISOL ANALYSIS

Plasma cortisol concentrations were analysed with Radioimmunoassasy (RIA), as earlier described by Iversen et al. (1998). As a tracer, [1, 2, 6, 7, -3H] Cortisol (Amersham plc, Oslo, Norway), treated with 250 mCi (9.25 MBq) and diluted in 25 mL of absolute alcohol (Activity of about 10 mCi / mL) was used. Hydrocortisone (H 4001, Sigma-Aldrich, Oslo, Norway) was applied to produce a standard range from 0-137.5 nmol/L. The antibody was obtained from Sheep Anti-Cortisol, code: S020 (Guildhay Ltd, Surrey, UK). Samples were incubated at 1-2 °C for 24 hours before centrifuged with a Haraeus sepatech Omnifuge 2.ORS radius 154 mm, rotor 3360. Antibody-antigen complex was counted in a scintillation counter type Packard Tri Carb 1900 TR. The sensitivity in the assay was 1.68 nmol/L. Samples under "detection limit" were set equal to the sensitivity of the assay. Intra-assay was below 10 % and inter-assay was 12.5 % at 80 nmol/L. NSB ranged from 2.1 % to 4.8 % of the total activity. Previously executed recovery tests at the laboratory of Nord University gave the following results: Measurement of 4, 17, 34 and 69 nmol/L radiolabelled cortisol with added plasma, showed a recovery of 90, 94, 96 and 95 %.

2.7 STATISTICAL METHODS

All statistical work was completed with R software™ R.3.2.2 (R Development Core Team 2013). A Shapiro-Wilk test (Shapiro & Wilk 1965) was used to test normality of distributions for both physiological and behavioural data (See Appendix II Table III and Table IV). A Student's t-test was used when Levene's F-test assumption of homogeneity in variances between populations was met (See Appendix II Table V). Non-parametric data was tested with Wilcoxon rank sum test, and Spearman's rank correlation test (See Appendix II Table VI). A chi-square test was used to compare observed counts of swimming activity with expected values. A significance level of $\alpha = 0.05$ was used, if not specified otherwise.

It is not obvious whether or not observations of swimming activity and plasma cortisol levels of each of the 10 individuals in each trial can be regarded as independent, of the other 9 individuals in the tank in each trial. This is important, since statistical tests require independent observations. Thus, swimming activity and plasma cortisol were both tested in two different ways: with replicates (section 2.7.1 and 2.7.2) and with individual fish (section 2.7.3 and 2.7.4).

2.7.1 SWIMMING ACTIVITY CALCULATED FROM MEAN VALUE OF EACH REPLICATE

For comparison of swimming activity between naive and experienced replicates (Figure 6), a mean was calculated from 10 fish in each trial, for each of the 9 replicates of naive and each of the 9 replicates of experienced fish. Next, a mean was calculated from these 9 replicates of naive, and a mean was calculated from these 9 replicates of experienced fish at each given minute, 10 minutes before and 10 minutes after the introduction of salmon. Means at each given minute were presented with 95 % confidence intervals for comparison of experienced and naive replicates.

2.7.2 PLASMA CORTISOL CALCULATED FROM MEAN VALUE OF EACH REPLICATE

For comparison of plasma cortisol between naive and experienced replicates (Figure 8), a mean was calculated from 10 fish in each trial, for each of the 10 replicates of naive and each of the 10 replicates of experienced fish. Mean values of 10 naive replicates and mean values of 10 experienced replicates were statistically tested using Wilcoxon rank sum test.

2.7.3 SWIMMING ACTIVITY INDEX CALCULATED FROM VALUES OF EACH FISH

Swimming activity index (SAI) in naive (n = 90) and experienced (n = 89) lumpsuckers was calculated from mean swimming activity after the introduction of Atlantic salmon minus mean swimming activity before the introduction of Atlantic salmon. Mean swimming activity included 1 observation of swimming behaviour for every minute, 10 observations before and 10 observations after salmon introduction in total.

$$SAI = \bar{x}\ SA\ post\ salmon\ introduction - \bar{x}\ SA\ pre\ salmon\ introduction$$

SAI produced a positive index value if swimming activity increased after salmon introduction or a negative number if swimming activity had decreased. SAI for each fish was statistically compared to explore individual differences between all experienced lumpsuckers and all naive lumpsuckers. A comparison of SAI between naive and experienced fish was statistically tested using Wilcoxon rank sum test.

2.7.4 PLASMA CORTISOL LEVELS OF EACH FISH

Plasma cortisol levels of each individual fish was compared between naive (n = 100) and experienced (n = 99) lumpsuckers with a Wilcoxon rank sum test.

2.7.5 VIDEO RECORDINGS

Swimming activity was registered every minute for each fish for twenty minutes, ten minutes before and ten minutes after the introduction of Atlantic salmon. Types of swimming activity was categorized based on previous work by Tully et al. (1996) and Imsland et al. (2014c), with simplification of distinguishable locomotion divided into four categories where each category represented a specific score from 1 to 4 (Table 1), used to calculate swimming activity index. Video recordings were analysed at Nord University from September to November 2016, using VLC media player (VideoLAN 2017). Trials were blinded before video analysing were conducted.

Table 1. Classification of lumpsucker swimming activity based on distinguishable locomotion with given description and ranked score used to calculate swimming activity index (SAI).

Score	Swimming activity	Description
1	Attached	Attached to substrate with sucker disc
2	Hovering	Hovering performance with no horizontal or vertical motion
3	Normal swimming	Locomotion between hovering and burst swimming activity
4	Burst swimming	Clearly distinguishable rapid acceleration in any direction

2.8 ETHICAL AND SAFETY NOTES

The study was conducted according to the Animal Welfare Act (LOV-2009-06-19-97) and the Norwegian law on Regulation of Animal Experimentation (FOR-1996-01-15-23). Handling of live fish was managed by personnel with FELASA-C course, based on the policies by the Federation of European Laboratory Animal Science Association. Lumpsuckers and Atlantic salmon used during the field experiment were assigned to project FDU 7835, accepted by the Norwegian Food Safety Authority under the regulation of the Research Animal Act (FOR-2015-06-18-761). Safety and working conditions during field experiment was based on the internal routines and regulations of Bjørøya AS.

3 RESULTS

3.1 SWIMMING ACTIVITY

3.1.1 DISTRIBUTION AND CHANGE IN SWIMMING ACTIVITY COUNTS

The largest alteration in swimming activity was observed in naive lumpsuckers (n = 90), where the ratio of burst swimming increased from 1 % to 25.44 % after the introduction of Atlantic salmon, while in experienced lumpsuckers (n = 90), burst swimming activity increased from 0.56 % to 3.07 % (Figure 5). This difference in increased burst swimming was larger for naive compared to experienced lumpsuckers (x^2 = 8.05, p = 0.005, d.f. = 1, Chi-square test).

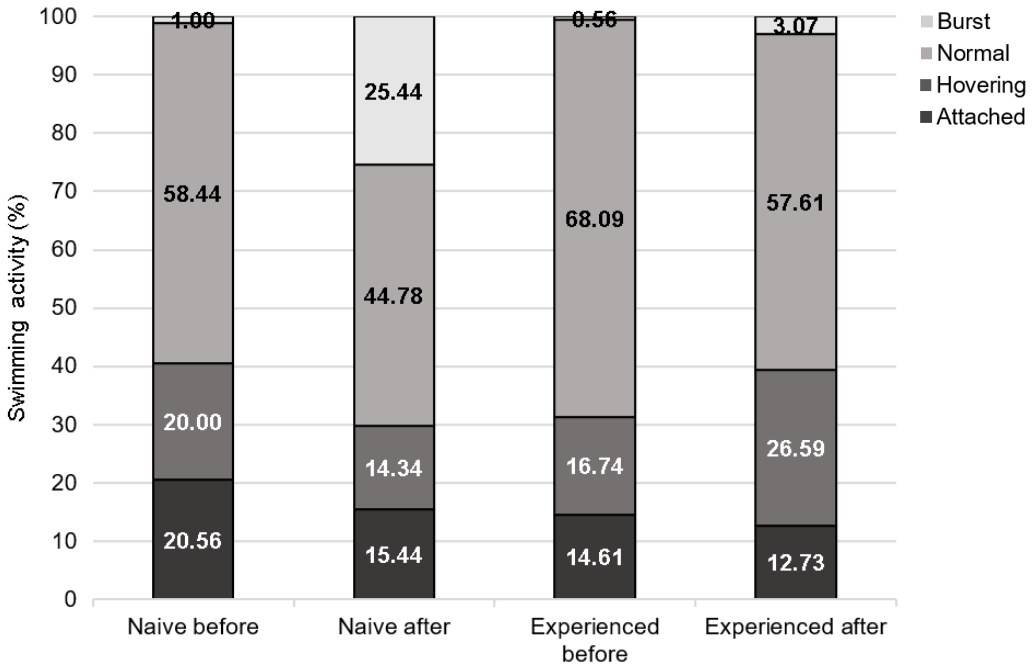

Figure 5. Swimming activity of naive (n = 90) and experienced (n = 90) lumpsuckers. Each bar shows observations from ten minutes before and ten minutes after the introduction of Atlantic salmon based on 3580 observations. Largest alteration was observed in naive fish (n=90), which showed increased burst swimming activity after salmon were introduced. Each scale of grey represents categorized swimming locomotion.

3.1.2 EFFECTS ON SWIMMING ACTIVITY FROM SALMON INTERACTION

Before the introduction of Atlantic salmon, naive (9 replicates) and experienced (9 replicates) of lumpsuckers showed small differences in mean swimming activity, and in eight of ten recorded minutes, the 95 % confidence intervals of the two groups overlapped (Figure 6). Mean swimming activity in naive fish increased from the first observations after the introduction of Atlantic salmon whereas swimming activity of experienced fish remained unchanged. In total, the mean swimming activity of the naive group was higher in all the measurements after salmon introduction, and the 95 % confidence intervals for the two groups did not overlap in nine of the ten measurements (Figure 6).

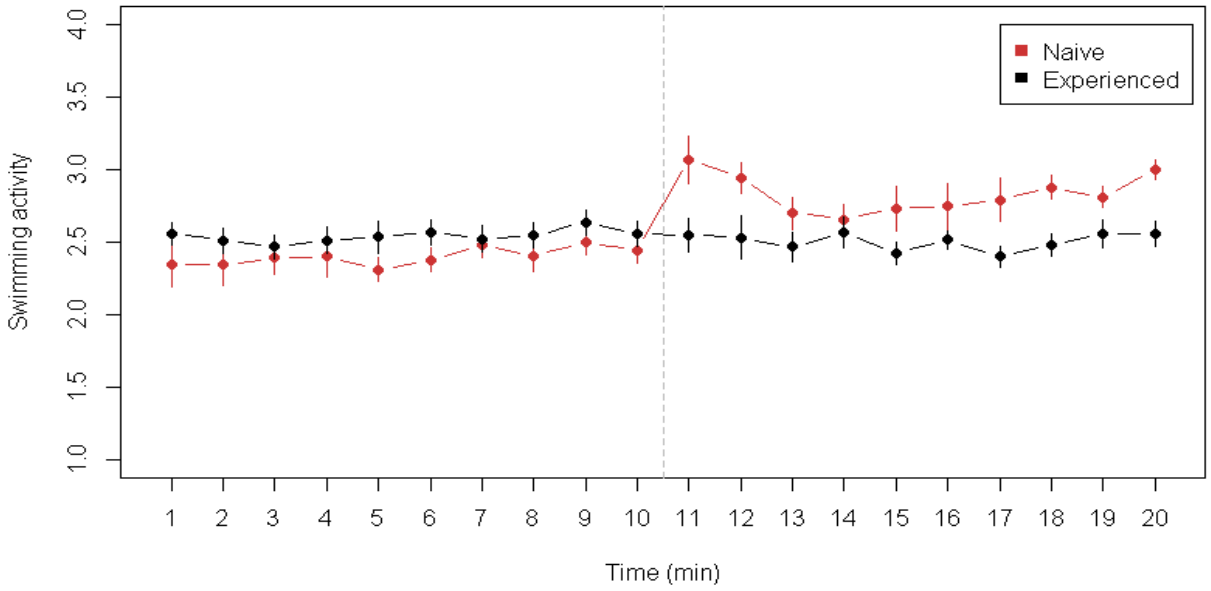

Figure 6. Swimming activity (mean ± 95 % confidence intervals) for naive (9 replicates) and experienced (9 replicates) lumpsuckers during twenty minutes of video observations. Vertical dotted line shows introduction of Atlantic salmon. Y-axis shows mean total swimming activity from the four categories (1) attached, (2) hovering, (3) normal swimming and (4) burst swimming. Trial 1 and trial 2 were not included due to high water turbidity, which impeded swimming activity measurements visibility.

3.1.3 SWIMMING ACTIVITY INDEX CALCULATED BASED ON VALUES OF EACH FISH

Swimming activity index (SAI) from all naive lumpsuckers (Mdn = 0.40, n = 90) and all experienced lumpsucker (Mdn = -0.10, n = 89) was significantly different (W = 1827.5, $p<0.001$, Wilcoxon rank sum test). SAI above zero indicated that mean swimming activity increased after the introduction of Atlantic salmon (Figure 7). In naive lumpsuckers, 73 fish had a positive SAI, 4 fish showed no change and 13 fish had a negative SAI. In experienced lumpsuckers, 34 fish had a positive SAI, 9 showed no change and 46 fish had negative SAI.

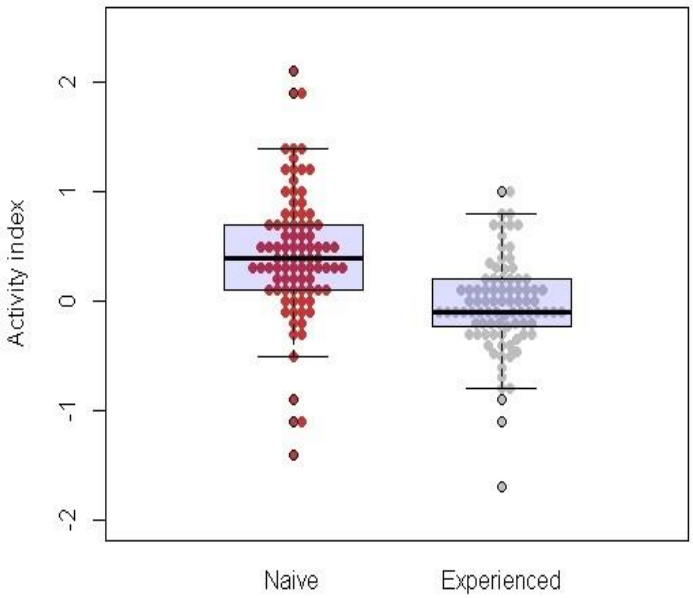

Figure 7. Swimming activity index described with a Box-and-Whiskers plot and Beeswarm dots showing the distribution of naive (n = 90) and experienced (n = 89) lumpsuckers. SAI above zero showed that mean swimming activity increased after the introduction of Atlantic salmon.

3.2 PLASMA CORTISOL

3.2.1 PLASMA CORTISOL LEVELS COMPARISON OF REPLICATES

Plasma cortisol levels in naive lumpsucker replicates constituted 8 of the top 10 highest median values out of 20 trials (Figure 8), and the difference was significant after pooling naive fish ($\bar{x}$ = 114.6, S.D. ± 30.02), and experienced fish ($\bar{x}$ = 57.9, S.D. ± 35.37), and calculating the mean of each of the 10 replicates from each group (t = 3.67, p<0.01, d.f. = 18, Student's t-test).

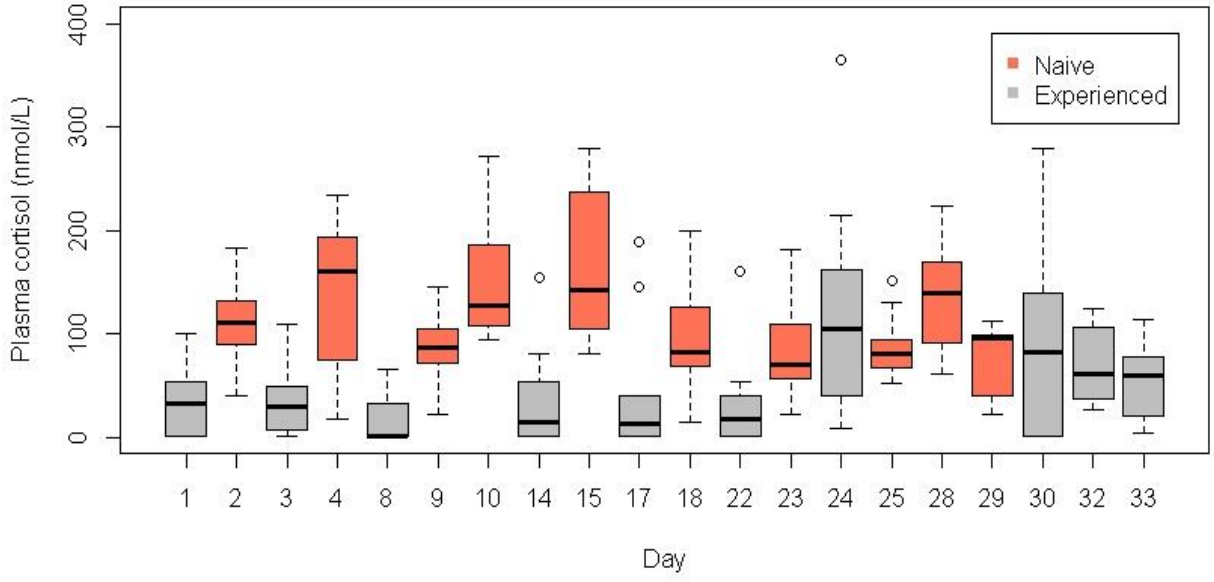

Figure 8. A Box-and-Whiskers plot show the difference in plasma cortisol levels in 10 replicates of naive lumpsuckers and 10 replicates of experienced lumpsuckers after interacting with Atlantic salmon. Replicates included 10 new lumpsuckers (except n = 9 at day 14). Samples were collected 30 minutes after Atlantic salmon was introduced to the lumpsuckers. Plasma cortisol (nmol/L) was measured using radioimmunoassay (RIA). Day refers to the time from trial 1 conducted on August 2, and until trial 20 at day 33 conducted on September 3.

3.2.2 PLASMA CORTISOL LEVELS COMPARISON OF INDIVIDUAL FISH

The primary stress response measured in plasma cortisol in all naive lumpsuckers (Mdn = 105.30, n = 100) and all experienced lumpsuckers (Mdn = 37.75, n = 99) was significantly higher in naive fish (W = 2018, p<0.0001, Wilcoxon rank sum test) (Figure 9). Control group of naive lumpsuckers (n = 10) from the holding tank and experienced lumpsuckers (n = 10) from the net cage did not differ (W = 65, p = 0.145, Wilcoxon rank sum test) (Table 2).

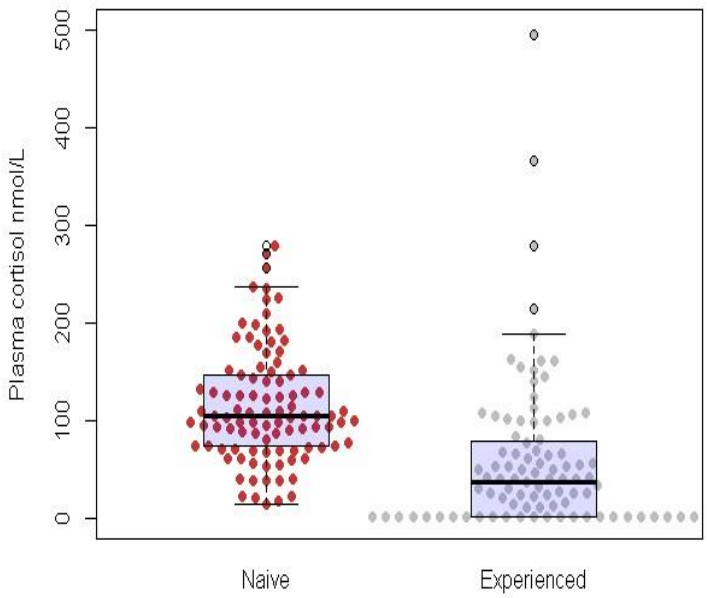

Figure 9. Box-and-Whiskers plot and Beeswarm dots show difference in primary stress response in naive (n = 100) and experienced (n = 99) lumpsuckers. Samples were collected 30 minutes after Atlantic salmon was introduced to the lumpsuckers. Plasma cortisol (nmol/L) was measured using radioimmunoassay (RAS).

Table 2. Plasma cortisol measurements in naive and experienced fish after each conducted experiment, and control measurements from the holding tank and the net cage. Means ± S.D. are listed for each parameter. Trials include all 100 naive fish and all 99 experienced fish, while each control group include 10 fish. W is the test statistics for Wilcoxon rank sum test.

Cortisol (nmol/L)	**N**	**Naive**	**N**	**Experienced**	**P**	**W**
20 Trials	100	114.9 ± 58.91	99	58.1 ± 77.56	<0.001	2018
Control groups	10	11.0 ± 16.69	10	3.9 ± 6.99	0.145	65

3.3 COLOURATION

3.3.1 BLUE-GREEN COLOURATION CALCULATED FROM REPLICATES

Blue-green colouration comparison of mean values from 9 replicates of naive (Mdn = 0.82) and 9 replicates of experienced (Mdn = 0.79) lumpsuckers did not significantly differ (W = 59.5, p = 0.098, Wilcoxon rank sum test) (Figure 10).

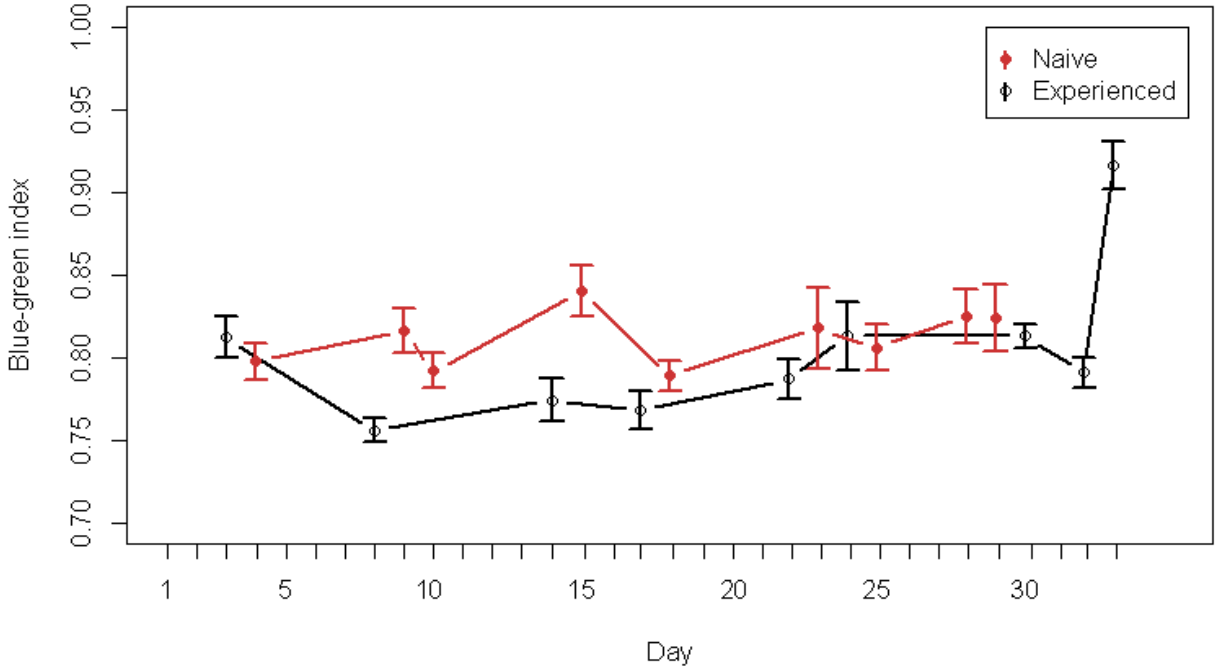

Figure 10. A line plot of the mean blue-green colouration in 9 replicates of naive and 9 replicates of experienced lumpsuckers during the trial period. Means were calculated from n = 10 lumpsuckers in each replicate. Day refers to the time from trial 1 conducted on August 2, and until trial 20 at day 33 conducted on September 3. Whiskers and error bars show standard error (S.E.).

3.3.2 BLUE-GREEN COLOURATION CALCULATED FROM INDIVIDUAL FISH

Blue-green colouration measured from the epidermal skin of naive lumpsuckers (Mdn =0.81, n = 89) and experienced lumpsuckers (Mdn = 0.97, n = 89) did not significantly differ (W = 4567, p = 0.077, Wilcoxon rank sum test). There was no difference in blue colouration between naive (Mdn = 0.43, n = 89) and experienced lumpsuckers (Mdn = 0.42, n = 89) (W = 4414, p = 0.1862, Wilcoxon rank sum test).

3.3.3 BLUE-GREEN COLOURATION AND SWIMMING ACTIVITY

Mean swimming activity from ten observation of lumpsuckers prior to the introduction of Atlantic salmon, showed a significant weak positive correlation to skin blue-green colouration in naive fish (r_{sp} = 0.21, p = 0.046, n = 89, Spearman's rank correlation rho) while no correlation was observed in experienced fish (r_{sp} = 0.10, p = 0.309, n = 89, Spearman's rank correlation rho) (Figure 11A). Mean swimming activity from ten observation of each individual lumpsucker after the introduction of Atlantic salmon, showed a significant weak positive correlation to skin blue-green colouration in naive fish (r_{sp} = 0.28, p = 0.007, n = 89, Spearman's rank correlation rho), and in experienced fish (r_{sp} = 0.23, p = 0.028, n = 89, Spearman's rank correlation rho) (Figure 11B).

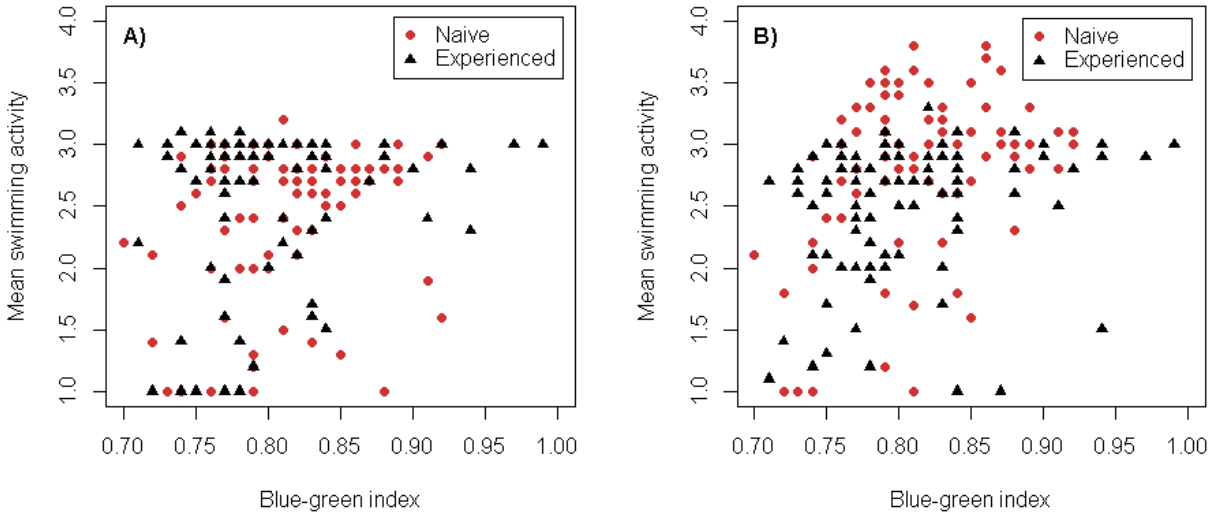

Figure 11. Scatter plot with blue-green colouration from lumpsucker skin and the relationship with mean swimming activity prior to salmon introduction (Figure 11A), and after salmon introduction (Figure 11B) in naive (n = 89) and experienced (n = 89) lumpsuckers. Blue-green colouration was calculated from RGB-values with the formula (Ig +Ib)/(Ir+Ig+Ib). Mean swimming activity was based on 10 observations of each fish, prior to or after the introduction of Atlantic salmon. Experienced lumpsuckers are presented with black triangles, while naive lumpsuckers are presented as circled red dots

3.3.4 BLUE-GREEN COLOURATION AND PLASMA CORTISOL LEVELS

Plasma cortisol levels in experienced fish showed a significant weak positive correlation with measured blue-green colouration from epidermal skin (r_{sp} = 0.32, p = 0.001, n = 89, Spearman's rank correlation rho), while no such relationship was observed with blue-green colouration in naive fish (r_{sp} = 0,03, p = 0.777, n = 89, Spearman's rank correlation rho) (Figure 12).

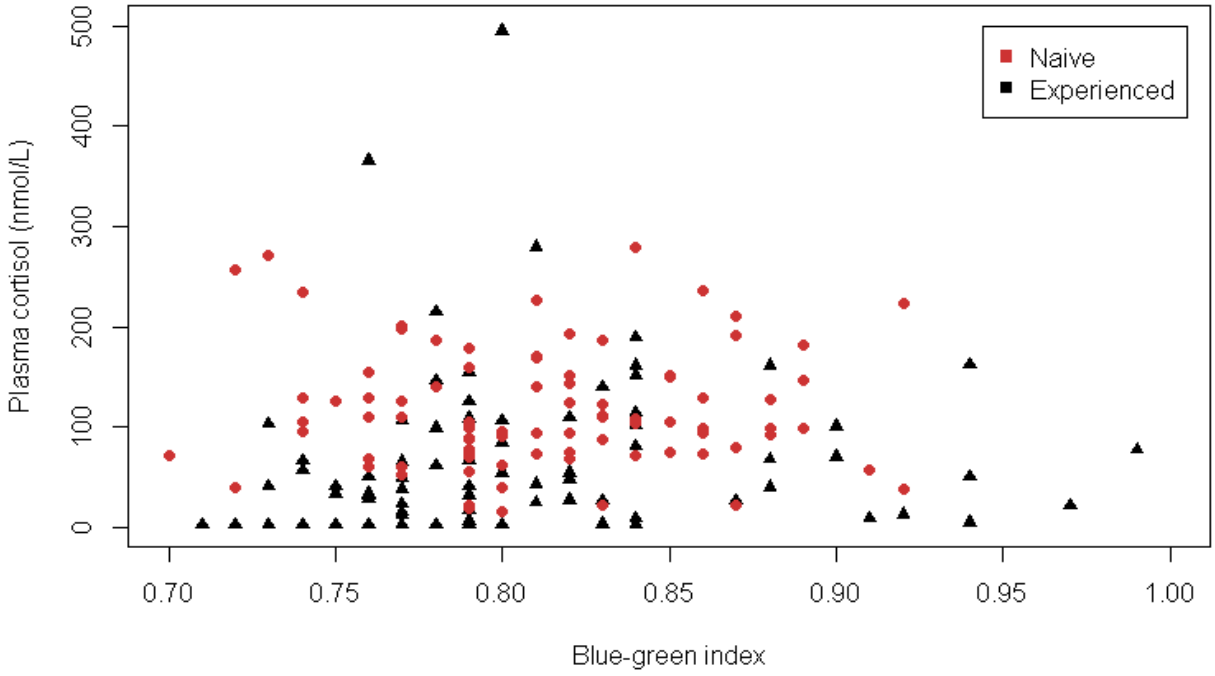

Figure 12. Scatter plot with blue-green colouration from lumpsucker skin in relationship with plasma cortisol levels measured after each trial of naive (n = 89) and experienced (n = 89) fish. Blue-green colouration was calculated from RGB-values with the formula (Ig +Ib)/(Ir+Ig+Ib). Plasma cortisol (nmol/L) was measured using radioimmunoassay (RAS). Experienced lumpsuckers are presented with black triangles, while naive lumpsuckers are presented as circled red dots

3.3.5 BLUE-GREEN COLOURATION AND WEIGHT

No correlation was observed between skin blue-green colouration and weight in naive fish (r_{sp} = 0.15, p = 0.157, n = 89, Spearman's rank correlation rho) or skin blue-green colouration and weight in experienced fish (r_{sp} = 0.02, p = 0.812, n = 89, Spearman's rank correlation rho) (Figure 13). There was no correlation between skin blue-green colouration and length measurements in either naive (r_{sp} = 0.15, p = 0,164, n = 89, Spearman's rank correlation rho) or experienced fish (r_{sp} = 0.08, p = 0,479, n = 89, Spearman's rank correlation rho).

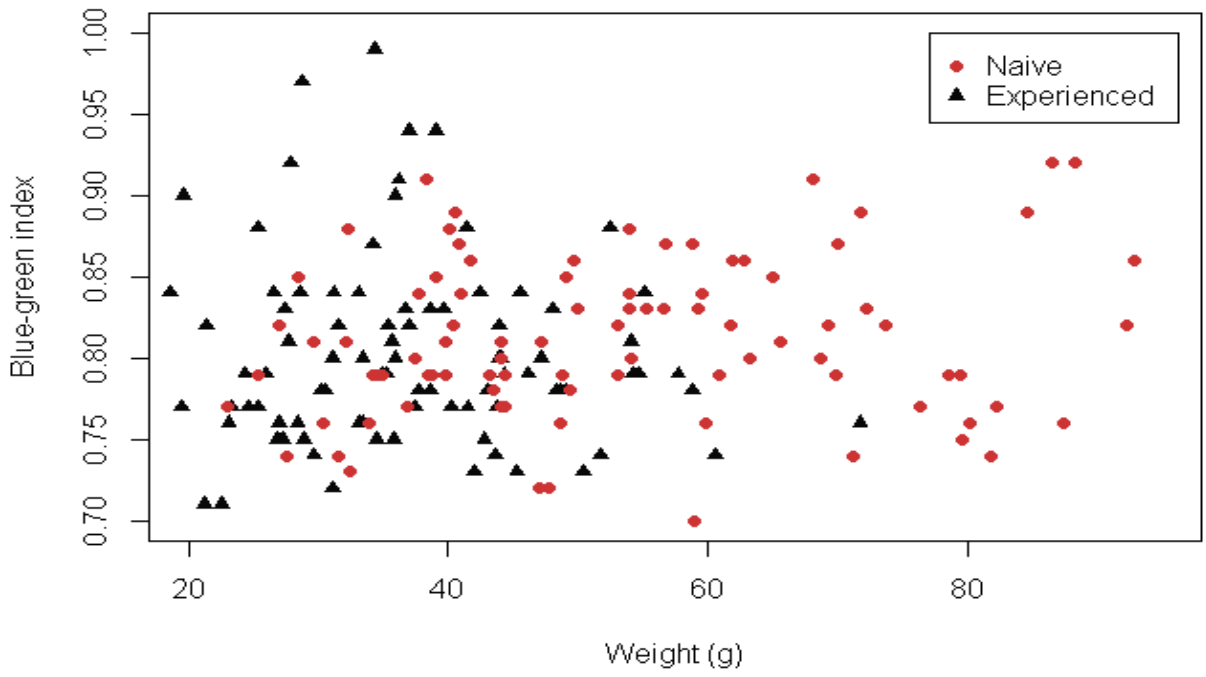

Figure 13. Scatter plot of blue-green colouration in epidermal skin in lumpsuckers and the relationship with fish weight. Experienced (n = 89) lumpsuckers are presented with black triangles, while naive (n = 89) lumpsuckers are presented as circled red dots. Each plot include data from 9 replicates. Blue-green colouration was calculated from RGB-values with the formula (Ig +Ib)/(Ir+Ig+Ib). Experienced lumpsuckers are presented with black triangles, while naive lumpsuckers are presented as circled red dots

3.3.6 BLUE-GREEN COLOURATION AND SEX

Blue-green colouration between male (Mdn = 0.80, n = 89) and female (Mdn = 0.79, n = 89) lumpsuckers did not significantly differ (W = 3699, p = 0.455, Wilcoxon rank sum test) (Figure 14).

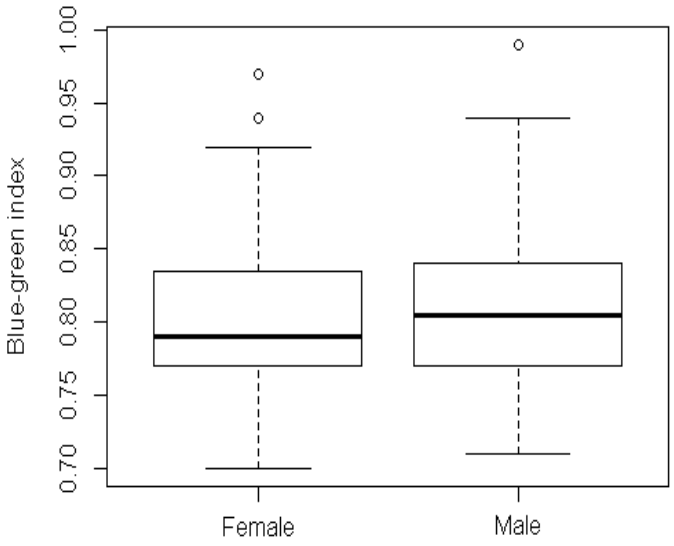

Figure 14. Boxplot with blue-green colouration comparison between male (n = 101) and female (n = 99) lumpsuckers. Gender was determined based on gonadal inspection during dissection. Blue-green colouration was calculated from RGB-values with the formula (Ig +Ib)/(Ir+Ig+Ib).

3.4 WEIGHT

In the holding tank, mean weight (± S.E.) in each replicate containing 10 naive fish increased from 38.3 g (± 3.91) to a final weight of 62.4 g (± 4.73) during a period of 27 days, while in the net cage, mean weight (± S.E.) in each replicate containing 10 experienced fish, increased from 30.2 g (± 2.50) to a final weight of 32.2 g (± 2.13) during a period of 33 days (Figure 15).

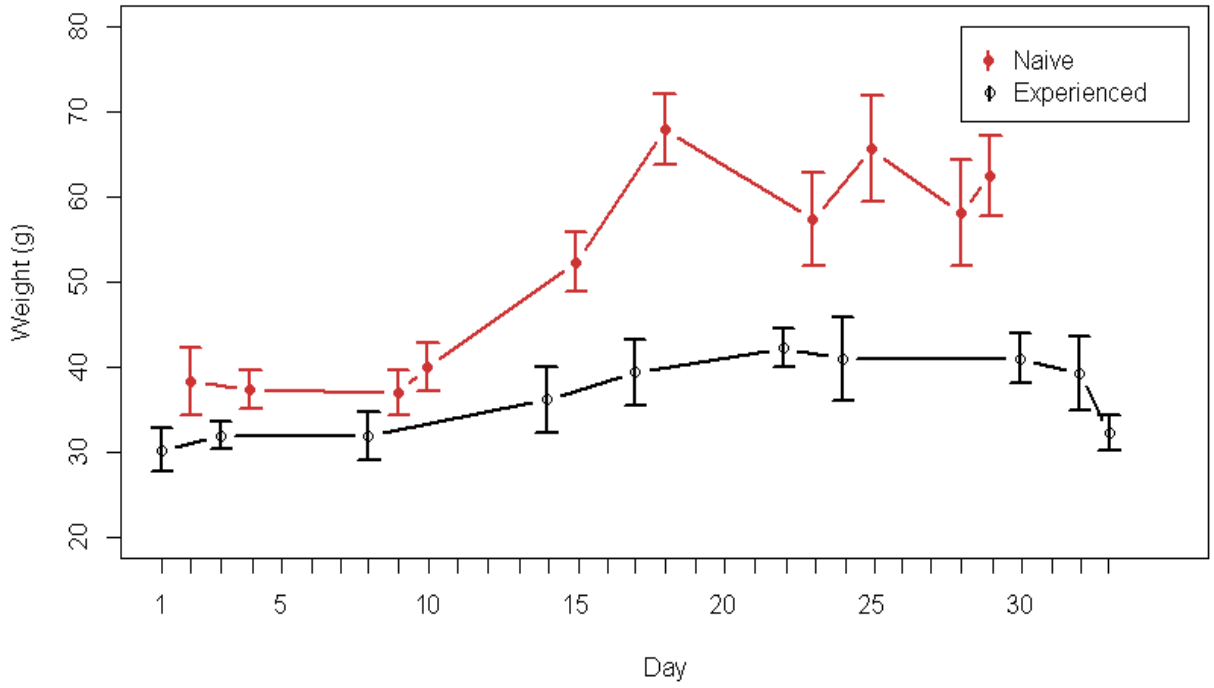

Figure 15. Line plot with mean weight (± S.E.) from each replicate of naive and experienced lumpsuckers measured during the experimental period from August 2 to September 3. Each replicate included measurements of n = 10 fish. Whiskers and error bars show standard error. Day 1 refers to August 2 while day 33 refers to September 3. Red line show naive fish and black line show experienced fish.

Table 3. Morphological data on naive and experienced lumpsuckers. Means (± S.D.) are listed for each parameter. Start and final weight and length of n = 10 experienced fish was measured August 2 and on September 3, while start and final weight and length of n = 10 naive fish was measured on August 3 and on August 28.

Background	n	Naive	Period (days)	Experienced	Period (days)
Start weight (g)	10	38.2 ± 3.91	27	30.2 ± 2.5	33
Final weight (g)	10	62.4 ± 4.73	27	32.2 ± 2.13	33
Start length (cm)	10	9.5 ± 0.31	27	9.1 ± 0.25	33
Final length (cm)	10	12.0 ± 0.34	27	9.7 ± 0.23	33

3.4.1 WEIGHT AND SWIMMING ACTIVITY

Mean swimming activity prior to the introduction of Atlantic salmon, showed a significant weak positive correlation with weight in naive fish (r_{sp} = 0.22, p = 0.037, n = 89, Spearman's rank correlation rho) while no correlation was observed with weight of experienced fish (r_{sp} = 0.01, p = 0.309, n = 89, Spearman's rank correlation rho) (Figure 16A). Mean swimming activity after the introduction of salmon did not correlate with weight of naive fish (r_{sp} = 0.02, p = 0.788, n = 89, Spearman's rank correlation rho) or with weight of experienced fish (r_{sp} = -0.03, p = 0.743, n = 89, Spearman's rank correlation rho) (Figure 16B).

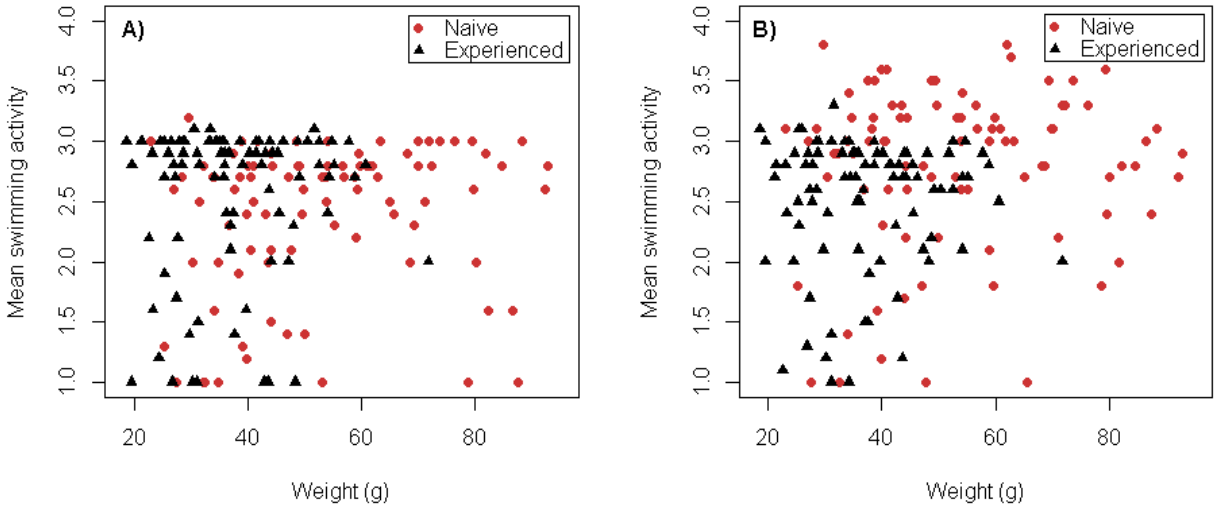

Figure 16. Scatter plot with weight in relationship with mean swimming activity prior to salmon introduction (Figure 16A), and after salmon introduction (Figure 16B) in naive (n = 89) and experienced (n = 89) lumpsuckers. Mean swimming activity was based on 10 observations of each fish, prior to or after the introduction of Atlantic salmon. Experienced lumpsuckers are presented with black triangles, while naive lumpsuckers are presented as circled red dots

3.4.2 WEIGHT AND PLASMA CORTISOL LEVELS

A significant weak positive correlation was observed when comparing weight with plasma cortisol levels in experienced fish (r_{sp} = 0.26, p = 0.008, n = 99, Spearman's rank correlation rho) while no significant correlation was observed between weight and plasma cortisol levels in naive fish (r_{sp} = -0.04, p = 0.649, n = 99, Spearman's rank correlation rho) (Figure 17).

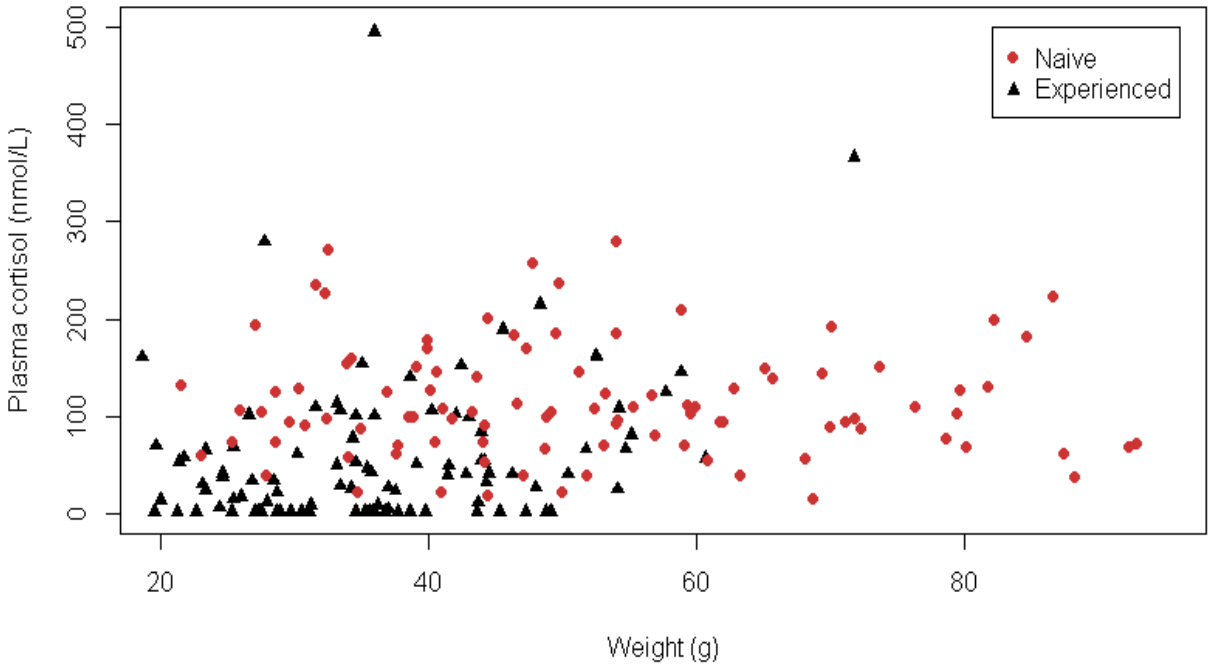

Figure 17. Scatter plot with weight in relationship with plasma cortisol levels measure in naive (n = 99) and experienced (n = 99) lumpsuckers. Plasma cortisol (nmol/L) was measured using radioimmunoassay (RAS). Experienced lumpsuckers are presented with black triangles, while naive lumpsuckers are presented as circled red dots.

3.4.3 WEIGHT AND BLUE-GREEN COLOURATION

No significant correlation was observed between weight and blue-green colouration from epidermal skin of naive lumpsuckers (r_{sp} = 0.15, p = 0.157, n = 89, Spearman's rank correlation rho) or weight and blue-green colouration from epidermal skin of experienced lumpsuckers (r_{sp} = 0.02, p = 0.812, n = 89, Spearman's rank correlation rho) (Figure 18).

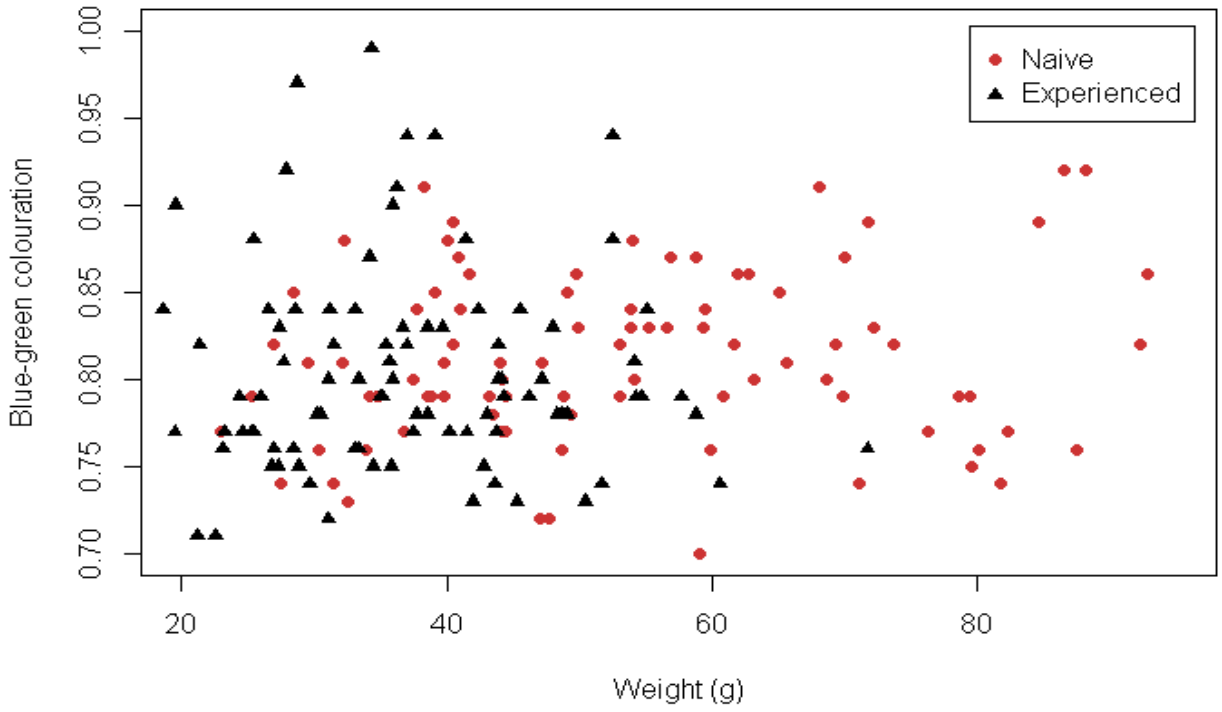

Figure 18. Scatterplot with the relationship between weight of naive (n = 89) and experienced (n = 89) lumpsuckers and blue-green colouration from skin. Blue-green colouration was calculated from RGB-values with the formula (Ig +Ib)/(Ir+Ig+Ib). Experienced lumpsuckers are presented with black triangles, while naive lumpsuckers are presented as circled red dots.

3.5 ADDITIONAL BEHAVIOURAL OBSERVATIONS AND PHYSIOLOGICAL DATA

No lumpsuckers were observed grazing sea lice from Atlantic salmon during video recording in the experimental tank. Recounts of sea lice (n = 183) from eighty salmon used during the twenty trials, showed that 97 % of sea lice were still attached to salmon.

No mortality was observed in the holding tank or the experimental tank during the study period. One lumpsucker (fish 8.10) was removed from the experimental tank due to abnormal behaviour. No mortality was observed in the tank containing Atlantic salmon.

Stomach contents showed that 7 % of experienced lumpsuckers (n = 99) had consumed sea lice (*L. salmonis* and *C. elongatus*) prior to experimental use, while no sea lice were found in naive lumpsuckers (n = 100) (Table 4). Most commonly ingested food items were crustaceans, which were found in 47 % of the experienced lumpsuckers and in 44 % of the naive lumpsuckers.

Table 4. Percentage values (%) of food items ingested by experienced (n = 99) and naive (n = 100) lumpsuckers prior to experimental use. Stomach content was examined after each trial.

	Feed fragments	Crustacean spp.	Hydrozoan spp.	Sea lice	Mollusca	No content
Experienced	11	47	19	7	2	36
Naive	30	44	1	0	2	36

4 DISCUSSION

This study revealed a significant difference in measured physiological stress responses and stress-induced behavioural swimming activity between naive and experience lumpsuckers (*Cyclopterus lumpus* Linnaeus, 1758) when introduced to Atlantic salmon (*Salmo salar* Linnaeus, 1758). It was suggested that experienced lumpsuckers change in both physiological and behavioural responses after interspecific living in net cages indicated a habituation to salmon presence. Naive lumpsuckers' increased stress and flight response during salmon interaction indicated that an innate escape response is present within the species. Thus, the process of desensitizing to the stressor before ideal grazing of sea lice might require additional learning through perception and repeated interactions.

Additional observations on morphology in relationship with swimming behaviour and plasma cortisol levels revealed a significant positive correlation between mean swimming activity during interaction with Atlantic salmon and the blue-green colouration of epidermal skin in both groups of lumpsuckers. A significant positive correlation was also observed between mean swimming activity measured prior to salmon interaction and blue-green colouration in naive fish, while this relationship was absent in experienced fish. Relationships between both blue colouration and blue-green colouration with plasma cortisol levels were observed in experienced fish from the net cage, while no such relationship was observed with plasma cortisol measurements from naive fish. Conspicuous blue-green colouration in lumpsuckers skin might be associated with both behavioural swimming activity and plasma cortisol levels, yet these relationships are weak and could be interfered by environmental differences between the two groups of fish.

The study design was built around a full scale production of Atlantic salmon to resemble the commercial use of lumpsuckers in aquaculture. In an ideal experimental design, every parameter except the parameter under study should be identical between the experimental groups. The aim of the present experiment was to study the effect of the parameter "no experience versus experience with salmon", on behavioural swimming activity and physiological stress measured as plasma cortisol in lumpsuckers. In addition, morphological measurements of colouration and weight was tested against swimming activity and plasma cortisol levels in both groups. In the original plan, the intention was to rear one group of lumpsuckers together from the same parents. Fish from this group should then be split into two subgroups and reared under as identical environments as possible before and during the trial

period, except that one of the groups should be reared together with salmon. Unfortunately, the original group of naive lumpsuckers ceased (Appendix I section 6.2), and another group of lumpsuckers had to be used as naive fish. Despite this challenge, the two groups used in the experiment still originated from the same catch of broodfish, were reared under similar conditions in terms of tank environment, water parameters (see Material and methods section 2.1) and feeding regimes prior to the trial period. In addition, water parameters and feeding regime were similar during the trial period in both naive and experienced fish (see Material and methods section 2.4). Differences between the two groups were the location of hatcheries, the length of transportation, and the naive fish living in a holding tank at the barge fleet whereas the experienced coexisted with salmon in a net cage 300 meters away. These deviations from an ideal experiment have to be taken into account when interpreting the results. From a scientific point of view the present study cannot claim that the difference in behaviour and stress between the two groups are solely due to their different experience with salmon.

4.1 SWIMMING ACTIVITY

Locomotion of lumpsuckers has previously been considered to resemble low swimming speed and a higher favoured manoeuvrability (Davenport & Kjorsvik 1986), as observed among conspecifics in the holding tank and in the experimental tank prior to interspecific interaction. Naive fish showed increased swimming activity observed as escape behaviour (Figure 6) and a reduction in attachment to the substrate (Figure 5) during first interaction with Atlantic salmon. Prey fish escape behaviour from predators is a fundamental defensive adaptation to reduce mortality and increase fitness in fish (Domenici 2010). Davenport and Thorsteinsson (1990) observed how lumpsuckers would quickly detach from the substrate and flee when a predator approached, which also coincided with observation on naive fish swimming behaviour in the current study. A rapid escape response from environmental stressors has also been observed in larval lumpsuckers (Hale 2000). Escape behaviour is activated through physiological mechanisms of the Mauthner neuron cells, which is triggered through visual, acoustic or tactile stimulus (Zottoli 1977) referred to as the C-start response (Eaton et al. 1988). Mauthner cells are found in most teleost (Bierman et al. 2009), but have not been observed in lumpsuckers (Hale 2000). Yet, increased swimming activity as a measurement of escape behaviour is without difficulty applied to lumpsuckers, since the species show a similar behavioural response to the C-start response, most likely due to the presence of a homologous physiological structure with comparable functions as the Mauthner cells (Hale 2000).

Escape behaviour and high burst swimming activity are both energetically costly (Endler 1991) and affects the overall fitness in regards to time spent on other tasks (Helfman 1989). In nature, lumpsuckers experience different stimulus that trigger antipredator behaviours at an everyday basis and the ability to habituate and quickly learn how to distinguish danger from safety is essential to adapt and cope with the changing environment (Brown & Godin 1999; Magurran & Girling 1986).

Experienced lumpsuckers that coexisted with Atlantic salmon did not significantly change their swimming activity from the moment of reintroduction in the experimental tank and during the interaction-window (Figure 6), and the swimming activity index for all experienced fish were lower in comparison to naive fish (Figure 7). This indicates that lumpsuckers recognized salmon as harmless through cognitive perception, learning and memory (Salas et al. 2006). Learning of predator recognition from sensory cues, including odour, the lateral line system and visual perception, require experience through the ontogeny of fish (Brown 2003; Chivers & Smith 1998; Pitcher et al. 1986). Yet, prey fish have appeared to be predisposed to certain visual predator cues including shape, colour and size of the body as well as mouth structure (Karplus & Algom 1981; Karplus et al. 1982; Magurran & Girling 1986).

While naive lumpsuckers did not differ in escape performance to salmon compared to many other prey teleosts interacting with predators (Eaton et al. 1977; Endler 1991), the obligate cleaner fish bluestreak cleaner wrasse (*Labroides dimidiatus* Valenciennes, 1893) expresses a similar fast-start escape performance (Gingins et al. 2017), which indicates that lumpsuckers might not differ in terms of first response to predators compared to obligate cleaner fish.

4.2 PHYSIOLOGICAL STRESS RESPONSE

Plasma cortisol measured during the experimental period was significantly higher in naive lumpsuckers over experienced lumpsuckers, both at replicate (Figure 8) and individual level (Figure 9). In Figure 8, a clear distinguishable difference was observed between fish from the two different backgrounds during the first thirteen replicates, while trial 14 at day 24 and trial 18 at day 30 showed irregularities in terms of increased plasma cortisol levels among experienced lumpsuckers. Environmental data did not differ during this period (see Appendix I Figure II), but routinely *in situ* cleaning of nets with high-pressure underwater washing was conducted at day 23 (Figure 8) in the respective net cage housing experienced lumpsuckers, and proceeded for the following week in nearby net cages. Anthropogenic noise is known to induce stress in marine fish (Nichols et al. 2015) and a high pressure washer tracing along the

net cage over extended periods, could possibly increase plasma cortisol levels measured in lumpsuckers.

In naive lumpsuckers, primary stress response measurements of plasma cortisol were similar to values measured during crowding stress on lumpsuckers (Iversen et al. 2015). Iversen et al. (2015) showed that the mean plasma cortisol reached a peak of 200 nm/L one hour after exposure to the stressor, while the present study observed a mean value of 114.94 nm/L thirty minutes after the introduction of salmon in naive fish. These values interrelated with the time perspective, which indicated that naive lumpsuckers showed a strong primary stress response due to the presence of Atlantic salmon.

Stress in lumpsuckers is scarcely studied, and no published data on measurements of plasma cortisol levels in lumpsuckers during interspecific interaction in terms of habituation is known to the best of the author's knowledge. Similar studies on other species have observed habituation in prey zebrafish (*Danio rerio*) after repeated interactions with predatory cichlids (*Parachromis managuensis*) (Barcellos et al. 2010), yet with a different method of exposure and length of the stressor. Barcellos et al. (2010) observed habituation in zebra fish after five days of interaction with predator fish, while other species including Eurasian perch (*Perca fluviatillis*) and rainbow trout (*Oncorhynchus mykiss*), showed habituation to mechanical stressors after eight weeks (Jentoft et al. 2005). In contrast, studies on catfish (*Rhamdia quelen*) did not observe habituation to imposed stressors after three weeks of stress exposure (Koakoski et al. 2013). Thus, habituation to predatory presence could be considered species specific. Yet, it is uncertain if cleaner fish and other species that partly include ectoparasites in their diet, have shorter habituation to predatory client fish, or share comparable behavioural and physiological traits that distinguish them from pure predator versus prey interactions.

4.3 COLOURATION

In marine fish, colouration is commonly less fixed and more effectively regulated by morphological (Leclercq et al. 2010; Sugimoto 2002) and physiological mechanisms (Aspengren et al. 2008) compared to higher vertebrate taxa. which is strictly beneficial in quickly adapting to various niches and stimuli (Nilsson Sköld et al. 2013). This is true for the lumpsucker, which regulate light and dark skin tone through melanophores, and blue-green colouration through the degradation of heme into biliverdin that is stored in the epidermal skin (Davenport & Thorsteinsson 1989; Davenport & Bradshaw 1995; Mudge & Davenport 1986). In comparison to the more frequently cryptic colouration change in flatfish and bottom dwelling

fish (Saidel 1988), this ability is less common among pelagic teleost species, and might reflect the lumpsuckers adaptation to two different environments including benthic living at juvenile age and during pelagic migration and feeding as adolescents and adults (Davenport & Bradshaw 1995; Mitamura 2007).

Naive and experienced fish showed no significant difference in blue-green colouration (Figure 10), and no relationship was observed between blue-green colouration and sex (Figure 14) or weight (Figure 18). A significant but weak positive correlation between swimming activity during interaction with salmon and blue-green colouration was observed in both naive and experienced fish (Figure 11B). Additionally, a weak positive correlation was observed between swimming activity prior to salmon introduction and blue-green colouration in naive fish (Figure 11A). Last, a weak positive correlation was observed between blue-green colouration and plasma cortisol levels in lumpsuckers, yet this was only seen in the experienced group.

Blue-green colouration from biliverdin is found in multiple marine fish species (Fang & Bada 1990), but its function have rarely been explained (Gagnon 2006). In lumpsuckers, blue-green colouration from biliverdin stored in epidermal skin is suggested to play a role in cryptic colouration used by juveniles hiding in benthic sea weed, or as crypsis against the matching green background of sea water in later ontogeny when fish migrate and feed in pelagic waters (Davenport & Bradshaw 1995). In addition, biliverdin has been proposed to have a function in sexual signalling in mature male lumpsuckers (Mudge & Davenport 1986). Nevertheless, how biliverdin might affect swimming activity and plasma cortisol levels in lumpsuckers is not answered in previous studies, and as far as what is known, this is the first report on the relationship between blue-green colouration and swimming activity. A possible explanation on how increased biliverdin concentrations might be linked to increased swimming activity in lumpsuckers, is through bilirubin oxidase (2 bilirubin + $0_2 \rightleftharpoons$ 2 biliverdin H_20) (Murao & Tanaka 1981). Lumpsuckers have a low detection of myoglobin in the myofibrils (Driedzic & Stewart 1982) which have crucial functions as both intracellular storage and diffusion of oxygen in fish muscle (Wittenberg & Wittenberg 2003). Thus, the biochemical properties of bilirubin reductase might promote an alternative access to oxygen for metabolic work.

4.4 GROWTH

Growth of naive and experienced lumpsuckers differed during the experimental period. The naive group was reared in a land-based tank with *ab libitum* feeding whereas the experienced group was reared in sea pens with additional feeding. Hence, the comparison of growth is

confounded by the difference in rearing conditions and not elaborated further here. Growth in lumpsuckers in general have previously been discussed by Imsland et al. (2016), which observed slow growth rate in small lumpsuckers (22.6 ± 7 g) during the first 57 days coexisting with salmon before growth increased again, while other studies on growth in juvenile lumpsuckers reared in lab showed that small lumpsuckers generally exhibited higher growth rate than larger (Nytrø et al. 2014). While growth in experienced fish was slower and more homogenously among individuals in the present study, growth in naive fish increased faster through the experimental period. In addition, naive fish individual growth varied more with time, as shown in Figure 15. Larger fish would quickly swim to the surface during feeding, while distinguishable smaller fish delayed for feed to sink to the bottom. These observations corroborated with previous studies on growth in lumpsuckers in tanks, and might indicate social ranking among conspecifics (Nytrø et al. 2014).

5 CONCLUSION

In salmon aquaculture, the necessity to implement cleaner fish as a method for delousing salmon is growing and estimated to reach 50 million fish per year in 2020 (Powell et al. 2017). Breeding programmes require more knowledge about lumpsuckers, both in terms of behavioural and physiological traits, with the aim to promote survivability, welfare and sea lice grazing.

The present study suggests two methods of measuring stress responses in lumpsuckers during interaction with salmon, both through changes in swimming activity and changes in plasma cortisol levels. Results revealed indications on the presence of an innate stress response during first encounter with salmon, and a habituation to salmon after one month with interspecific coexistence in a net cage. However, the possibility that other differences between the groups than exposure to salmon, prior to the experiment, may explain part of this difference. Still, it is unlikely that lumpsuckers express cleaning behaviour when plasma cortisol measurements are levelled and while escape responses are high, yet this study did not tell how long this response is latent, only that habituation in individual fish is common after thirty days. The additional observed weak association between blue-green colouration of epidermal skin and swimming activity in naive and experienced fish during interaction with salmon raise questions regarding the biochemical properties of biliverdin, which might have other evolutionary functions in addition to adaptive camouflage.

For further studies, habituation and progressive learning in adapting to salmon predatory cues could be applied earlier in ontogeny to promote a better transition from rearing facilities to net cages. This might be especially important during summer when sea lice reproduction is faster, and the necessity to have a lumpsuckers grazing parasites quickly on the arrival in net cages is crucial. In addition, the relationship between blue-green colouration from biliverdin and swimming activity lack explanations, and should be further examined.

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7 APPENDIX I – NOTES ON MATERIAL AND METHODS

7.1 THE LUMPSUCKER

Appendix I Figure 1. Illustrations of the lumpsucker (*C. lumpus*) at different stages through their ontogeny. From left: a juvenile lumpsucker at 8 g, a six month old lumpsucker at 30 g used in the present study, and a mature male lumpsucker at 180 g. Photo of mature lumpsucker obtained from (Rehn 2007).

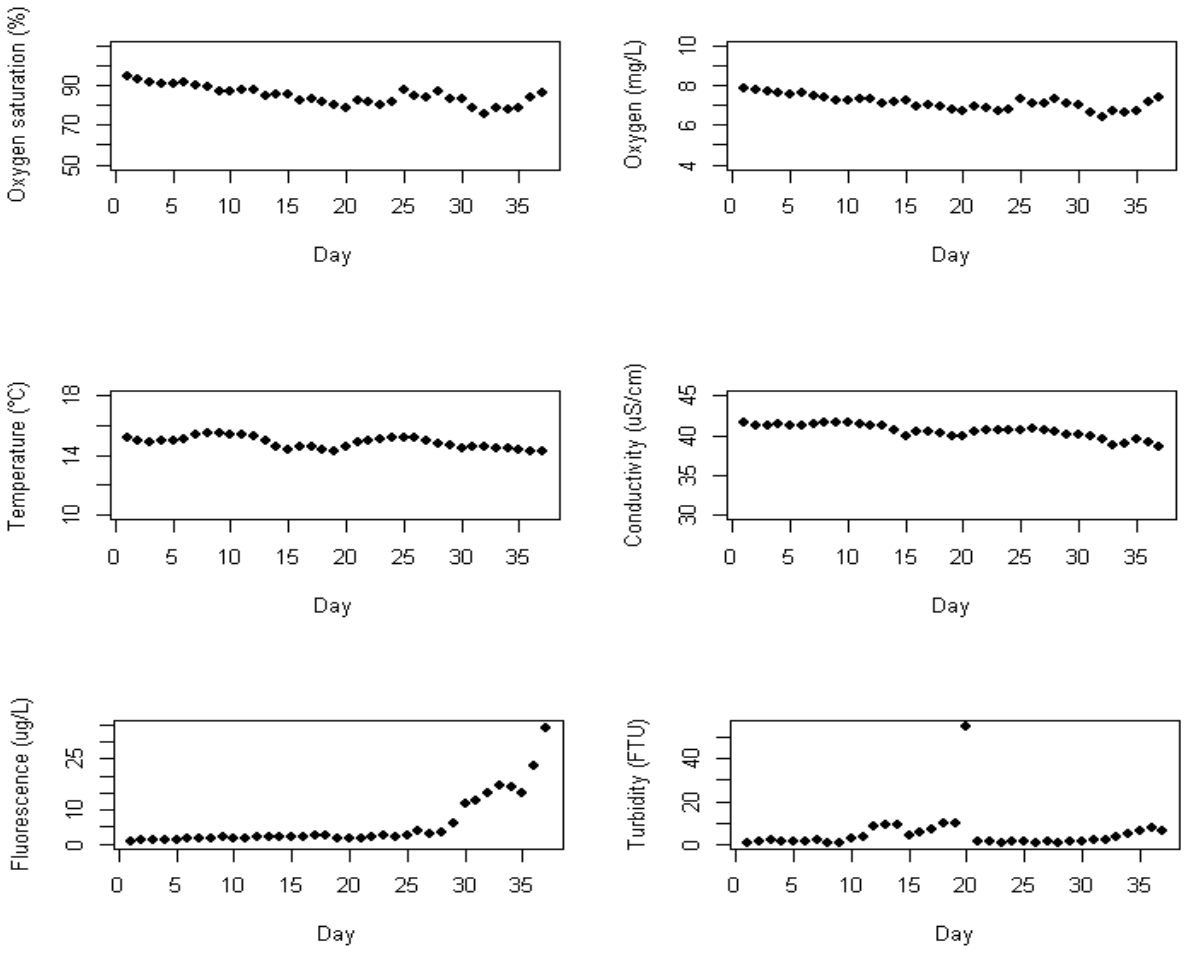
7.2 FISH STOCK AND MORTALITY

Naive and experienced lumpsuckers originated from two different rearing locations. At July 5, 2016, lumpsuckers from Lovund arrived at Raudøya to be used as both naive and experienced fish. Naive fish stationed in the holding tank seized as water supply failed the night of July 16, and a new supply of naive lumpsuckers was assembled and transported from Namdal Rensefisk on July 29.

7.3 ENVIRONMENTAL DATA

Appendix I Figure I show a summary of environmental water parameters including oxygen, temperature, conductivity, fluorescence and turbidity during the experimental period from August 2 to September 3. Mean oxygen saturation was 83.7 % S.D. ± 8.70 with max and min saturation of 103.1 % and 51.2 %. Mean dissolved oxygen was 7.05 mg/L, with max and min value of 10.32 mg/L and 4.38 mg/L. Mean water temperature was 14.8 °C with T_{max} 16.17 °C and T_{min} 13.54 °C. Mean conductivity was 40.47 uS/cm, with max and min value of 42.16 uS/cm and 35.20 uS/cm. Mean fluorescence was 5.71 ug/L with max and min value of 74.79

ug/L and a minimum value of 0.61 ug/L. Fluorescence measurements were unchanging during the first thirty days, before an increase was observed during the following six days. Mean turbidity was 5.06 FTU with a max and min value of 772.70 FTU and 0.31 FTU. Turbidity was relatively stable through the experimental period.



Appendix I Figure II. Daily mean oxygen saturation, dissolved oxygen levels, temperature, conductivity, fluorescence and turbidity during the experimental period from 02.08.16 to 03.09.16 measured at the feed barge at Raudøya.

8 APPENDIX II – TABLES OF DESCRIPTIVE STATISTICS

8.1 PHYSIOLOGICAL DATA

Appendix II Table I. Descriptive statistics on measurements of weight (g), length (cm), swimming activity index (SAI), plasma cortisol levels (nmol/L), blue-green colouration and blue colouration for epidermal skin in all experienced and naive lumpsuckers. Day 1 was on August 02, 2016.

Day	Trials	Background	Fish	Weight	Length	SAI	Cortisol	Blue-green colouration	Blue colouration
1	1	Experienced	1	34.5	10.1	NA	52.88	NA	NA
1	1	Experienced	2	24.6	9.3	NA	41.41	NA	NA
1	1	Experienced	3	35.9	9.1	NA	1.68	NA	NA
1	1	Experienced	4	27.5	8.5	NA	1.68	NA	NA
1	1	Experienced	5	23.3	8.5	NA	23.77	NA	NA
1	1	Experienced	6	35.6	9.6	NA	1.68	NA	NA
1	1	Experienced	7	34.5	9.7	NA	100.88	NA	NA
1	1	Experienced	8	21.7	8.3	NA	57.12	NA	NA
1	1	Experienced	9	44.5	10	NA	41.06	NA	NA
1	1	Experienced	10	19.9	7.6	NA	13.73	NA	NA
2	2	Naive	11	52.3	10.6	NA	108.67	NA	NA
2	2	Naive	12	25.9	7.7	NA	105.61	NA	NA
2	2	Naive	13	51.2	10.4	NA	146.75	NA	NA
2	2	Naive	14	46.3	9.6	NA	182.96	NA	NA
2	2	Naive	15	21.5	8.6	NA	132.40	NA	NA
2	2	Naive	16	51.8	10.6	NA	40.05	NA	NA
2	2	Naive	17	46.6	10.5	NA	114.07	NA	NA
2	2	Naive	18	28.5	9.2	NA	125.38	NA	NA
2	2	Naive	19	27.8	9.2	NA	39.61	NA	NA
2	2	Naive	20	30.7	8.7	NA	90.21	NA	NA
3	3	Experienced	21	24.4	9	0.8	6.38	180.67	0.44
3	3	Experienced	22	35.7	9.7	-0.5	42.59	132.49	0.47
3	3	Experienced	23	35.4	9.5	-0.3	46.71	147.73	0.48
3	3	Experienced	24	26	9.2	0.2	17.03	166.13	0.45
3	3	Experienced	25	31.5	9.4	0.5	108.72	138.30	0.48
3	3	Experienced	26	36.2	10.1	0.1	8.86	125.71	0.56
3	3	Experienced	27	41.5	9.6	-0.2	49.46	168.36	0.42
3	3	Experienced	28	30.2	9.5	0.2	61.45	175.86	0.44
3	3	Experienced	29	31.1	9.1	0.1	1.68	165.91	0.44
3	3	Experienced	30	27.4	8.5	0	2.71	133.42	0.49
4	4	Naive	31	40.4	10.5	1	74.21	195.65	0.45
4	4	Naive	32	27	8.6	0.5	193.62	180.38	0.46
4	4	Naive	33	39.1	10.2	0.7	151.50	176.68	0.46
4	4	Naive	34	31.5	9.5	0.6	234.92	188.99	0.40
4	4	Naive	35	39.8	10.9	0.2	178.04	168.99	0.41
4	4	Naive	36	32.2	9.8	0.3	226.11	191.21	0.44
4	4	Naive	37	27.5	9.2	0	105.42	205.49	0.41

4	4	Naive	38	44	10.6	0.3	73.09	186.71	0.45
4	4	Naive	39	47.2	11	0.2	170.39	184.02	0.44
4	4	Naive	40	44.4	10.5	0.5	18.00	197.59	0.42
8	5	Experienced	41	19.5	7.7	1	1.68	212.45	0.43
8	5	Experienced	42	35.8	9.5	-0.6	1.68	219.12	0.40
8	5	Experienced	43	28.4	8.7	0.2	33.12	178.98	0.41
8	5	Experienced	44	33.1	10.2	-0.1	50.17	203.62	0.42
8	5	Experienced	45	35.2	10	0	1.68	229.71	0.43
8	5	Experienced	46	28.9	8.7	0	1.68	202.71	0.42
8	5	Experienced	47	30.5	9.3	-0.7	1.68	192.18	0.44
8	5	Experienced	48	21.2	7.2	-0.3	1.68	154.11	0.32
8	5	Experienced	49	34.5	9.1	-0.3	1.68	211.70	0.37
8	5	Experienced	50	51.7	10.8	-0.2	66.08	208.36	0.35
9	6	Naive	51	40.5	10.1	0.3	146.16	207.52	0.44
9	6	Naive	52	25.3	8.3	0.5	74.54	233.62	0.41
9	6	Naive	53	38.5	10.2	0.5	99.25	219.53	0.40
9	6	Naive	54	43.2	10.7	0.8	105.51	216.79	0.39
9	6	Naive	55	43.5	10.6	1.3	140.54	243.03	0.41
9	6	Naive	56	48.8	11.5	0.7	100.33	230.56	0.41
9	6	Naive	57	40.9	10.5	0.8	22.59	244.84	0.46
9	6	Naive	58	23	8.7	0.1	60.86	212.35	0.40
9	6	Naive	59	28.5	9.4	0.4	73.96	235.56	0.45
9	6	Naive	60	37.7	9.8	0.4	71.00	226.42	0.44
10	7	Naive	61	34.2	10.1	0.7	159.53	193.02	0.39
10	7	Naive	62	38.8	10.5	0.5	99.19	247.31	0.40
10	7	Naive	63	53.9	11.3	0.5	186.14	249.86	0.43
10	7	Naive	64	49.4	11.3	1.1	186.04	249.38	0.39
10	7	Naive	65	36.8	10.7	0.3	125.85	219.77	0.39
10	7	Naive	66	41	11.3	0.1	108.30	239.00	0.44
10	7	Naive	67	53.1	11	0.5	124.43	240.66	0.42
10	7	Naive	68	29.6	10.2	0.6	94.21	222.19	0.41
10	7	Naive	69	30.3	9.8	0.7	128.75	227.00	0.37
10	7	Naive	70	32.5	10.4	0	271.25	221.99	0.36
14	8	Experienced	71	47.2	11	0.1	1.68	151.38	0.37
14	8	Experienced	72	25.3	8.1	-0.2	1.68	197.32	0.38
14	8	Experienced	73	25.4	8.7	0.4	15.08	165.80	0.39
14	8	Experienced	74	26.8	9.5	0.3	33.50	190.61	0.39
14	8	Experienced	75	44.1	10.5	0.7	53.91	210.72	0.41
14	8	Experienced	76	35	10.5	-0.1	154.04	215.58	0.40
14	8	Experienced	77	55.1	11.3	-0.1	80.73	249.38	0.42
14	8	Experienced	78	43.6	10.6	0.2	1.68	204.13	0.34
14	8	Experienced	79	22.6	8.1	-1.1	1.68	128.75	0.30
14	8	Experienced	80	NA	NA	NA	NA	NA	NA
15	9	Naive	81	56.8	11.7	0.4	80.03	232.45	0.46
15	9	Naive	82	49.7	11.1	0.7	236.72	234.87	0.48
15	9	Naive	83	69.3	12.4	1.2	143.81	232.62	0.44
15	9	Naive	84	32.3	9.5	1.9	98.23	229.36	0.48
15	9	Naive	85	47.7	10	-1.1	256.86	165.04	0.35

15	9	Naive	86	65.6	12.5	-1.4	139.81	223.62	0.42
15	9	Naive	87	49.1	11.1	0.7	105.32	226.58	0.46
15	9	Naive	88	58.8	11.8	0.3	210.00	232.45	0.46
15	9	Naive	89	53.9	10.9	0.1	279.05	200.22	0.42
15	9	Naive	90	40.1	10.5	-0.5	126.57	233.66	0.46
17	10	Experienced	91	45.5	11.2	0	189.33	217.18	0.46
17	10	Experienced	92	23.1	8.5	-0.1	30.48	174.55	0.37
17	10	Experienced	93	42.8	10.5	0.7	40.69	232.43	0.38
17	10	Experienced	94	58.8	12.7	0.1	145.70	200.93	0.40
17	10	Experienced	95	54.1	12	0.3	24.76	242.17	0.43
17	10	Experienced	96	45.3	10.1	-0.1	1.68	161.06	0.33
17	10	Experienced	97	27	9.3	0.1	1.68	171.39	0.36
17	10	Experienced	98	31.1	9.7	0.4	1.68	156.22	0.33
17	10	Experienced	99	27.3	9	-0.1	1.68	221.77	0.39
17	10	Experienced	100	38.6	10	-0.2	1.68	198.61	0.39
18	11	Naive	101	82.2	12.7	1.4	198.30	246.47	0.39
18	11	Naive	102	72.2	11.8	0.7	87.27	255.96	0.43
18	11	Naive	103	79.6	11.7	-0.2	126.32	241.70	0.38
18	11	Naive	104	78.6	12.9	0.8	77.09	253.71	0.41
18	11	Naive	105	59.5	10.4	-1.1	102.69	283.42	0.43
18	11	Naive	106	44.4	11.2	-0.1	200.27	241.43	0.39
18	11	Naive	107	53	10.1	2.1	69.81	261.44	0.41
18	11	Naive	108	80.1	12.5	0.9	68.83	240.61	0.37
18	11	Naive	109	60.8	11.6	0.3	54.71	233.49	0.39
18	11	Naive	110	68.6	11.6	0.9	15.24	263.84	0.41
22	12	Experienced	111	52.5	11.4	-0.4	160.95	242.08	0.46
22	12	Experienced	112	37.7	10.1	0.5	1.68	255.28	0.40
22	12	Experienced	113	43.9	10.5	-0.1	54.33	287.99	0.42
22	12	Experienced	114	37.5	10.3	-0.9	23.27	217.77	0.38
22	12	Experienced	115	46.2	11	-0.3	40.61	199.21	0.40
22	12	Experienced	116	33.4	10.5	-0.1	28.38	211.60	0.37
22	12	Experienced	117	29.7	10.1	0.7	1.68	240.10	0.36
22	12	Experienced	118	43.7	10.7	0.1	11.35	255.91	0.39
22	12	Experienced	119	48.7	11	-0.8	1.68	208.84	0.39
22	12	Experienced	120	49.1	11.9	-0.1	1.68	222.55	0.42
23	13	Naive	121	34.7	9.9	1.2	22.52	197.98	0.43
23	13	Naive	122	63.2	11.7	0	39.77	199.31	0.44
23	13	Naive	123	38.3	10.6	1.2	NA	241.97	0.52
23	13	Naive	124	59.8	11.3	0.4	109.45	209.15	0.42
23	13	Naive	125	84.6	13.5	0	181.29	241.23	0.51
23	13	Naive	126	76.3	12.7	0.3	109.82	225.13	0.42
23	13	Naive	127	55.2	11.4	0.3	109.52	227.03	0.47
23	13	Naive	128	34	10.5	-0.2	59.14	NA	NA
23	13	Naive	129	59	11.5	-0.1	70.66	199.56	0.35
23	13	Naive	130	68.1	12.8	-0.1	56.97	236.24	0.50
24	14	Experienced	131	48.3	11	1	214.71	171.42	0.41
24	14	Experienced	132	18.6	8.5	0.1	161.17	188.41	0.44
24	14	Experienced	133	33.4	10.6	-0.3	105.56	205.29	0.42

24	14	Experienced	134	71.7	13.4	0	365.83	217.91	0.41
24	14	Experienced	135	52.5	11.7	0.2	162.20	211.12	0.52
24	14	Experienced	136	31.2	10.2	-0.5	8.43	205.18	0.45
24	14	Experienced	137	50.4	12.6	-0.4	40.60	194.44	0.39
24	14	Experienced	138	42	11	-0.3	102.75	175.40	0.39
24	14	Experienced	139	34.2	10.6	-1.7	25.67	222.09	0.47
24	14	Experienced	140	26.6	9.1	-0.2	101.09	202.89	0.46
25	15	Naive	141	73.6	13	0.5	151.12	230.68	0.44
25	15	Naive	142	61.7	12.4	0.2	94.13	218.98	0.44
25	15	Naive	143	54	11.7	0.2	92.51	238.88	0.48
25	15	Naive	144	92.8	12.8	0.1	72.43	239.82	0.47
25	15	Naive	145	48.6	11.2	-0.3	67.59	232.30	0.41
25	15	Naive	146	44.1	10.8	-0.1	52.81	225.32	0.42
25	15	Naive	147	69.9	12	0.1	88.58	238.54	0.42
25	15	Naive	148	81.7	13.5	-0.9	129.63	208.36	0.37
25	15	Naive	149	37.5	10.3	0.6	61.99	237.96	0.44
25	15	Naive	150	92.2	14	0.1	69.00	233.68	0.46
28	16	Naive	151	86.5	13.5	1.4	223.36	244.06	0.49
28	16	Naive	152	70	12.4	0.3	191.45	236.17	0.47
28	16	Naive	153	33.9	10.2	0.3	154.98	232.93	0.40
28	16	Naive	154	62.7	12.1	1	128.74	227.78	0.45
28	16	Naive	155	34.9	10.6	1.9	87.17	238.06	0.43
28	16	Naive	156	56.6	10.5	0.5	122.51	241.85	0.44
28	16	Naive	157	39.8	9.7	1.2	169.10	177.32	0.39
28	16	Naive	158	87.4	12.7	1.4	61.04	206.17	0.39
28	16	Naive	159	44.1	10.8	0.1	91.33	225.23	0.42
28	16	Naive	160	65	12.1	0.2	149.65	224.68	0.44
29	17	Naive	161	79.4	13.2	0.6	102.97	265.87	0.42
29	17	Naive	162	71.7	12.1	0.3	98.10	223.49	0.46
29	17	Naive	163	41.7	10.8	0.3	98.04	212.72	0.44
29	17	Naive	164	49.9	10.9	0.8	21.93	213.56	0.43
29	17	Naive	165	71.1	12.9	-0.3	95.16	222.76	0.38
29	17	Naive	166	54.1	11.5	0.4	95.93	226.75	0.42
29	17	Naive	167	61.9	11.3	1	93.68	228.46	0.46
29	17	Naive	168	88.2	14	0.1	38.13	252.05	0.51
29	17	Naive	169	59.3	12.5	0.5	111.62	256.83	0.45
29	17	Naive	170	47	11.2	0.4	39.35	225.40	0.37
30	18	Experienced	171	36.7	9.9	-0.34	1.68	196.92	0.44
30	18	Experienced	172	44.3	10.8	0.1	31.43	162.20	0.41
30	18	Experienced	173	42.4	11	-0.47	151.77	188.02	0.45
30	18	Experienced	174	43	10.5	-0.12	98.49	94.82	0.35
30	18	Experienced	175	38.6	10.6	0	139.66	186.79	0.44
30	18	Experienced	176	54.7	11.5	0	66.15	151.34	0.38
30	18	Experienced	177	27.7	9.9	0.36	279.25	217.31	0.42
30	18	Experienced	178	28.6	9.5	-0.23	1.68	186.88	0.43
30	18	Experienced	179	39.7	10	0.29	1.68	203.55	0.45
30	18	Experienced	180	54.2	11.1	-0.48	108.50	150.98	0.41
32	19	Experienced	181	40.2	10.1	0	105.75	181.99	0.37

32	19	Experienced	182	35.9	9.9	-0.4	494.66	303.30	0.42
32	19	Experienced	183	60.6	12.7	-0.3	56.56	273.28	0.38
32	19	Experienced	184	24.6	8.5	-0.1	37.75	201.50	0.37
32	19	Experienced	185	48	12.2	0.6	26.50	230.31	0.43
32	19	Experienced	186	21.4	8.1	-0.2	53.40	195.16	0.41
32	19	Experienced	187	23.3	9	0.8	65.08	235.77	0.38
32	19	Experienced	188	57.7	12.2	-0.1	124.75	256.30	0.41
32	19	Experienced	189	43.9	10.9	-0.1	84.18	274.40	0.41
32	19	Experienced	190	37	10	0.7	26.63	249.76	0.43
33	20	Experienced	191	35.9	10.2	0.1	100.48	165.64	0.51
33	20	Experienced	192	33.1	9.5	-0.1	113.66	186.45	0.47
33	20	Experienced	193	39.1	11.2	0.1	50.23	221.06	0.57
33	20	Experienced	194	34.3	10	0	77.54	156.72	0.60
33	20	Experienced	195	19.6	9.1	0.2	69.97	169.55	0.50
33	20	Experienced	196	41.4	9.6	-0.1	39.77	206.45	0.51
33	20	Experienced	197	37	10	-0.8	4.53	179.23	0.54
33	20	Experienced	198	27.9	9.6	-0.2	12.33	186.17	0.55
33	20	Experienced	199	28.7	8.6	-0.1	21.05	170.32	0.57
33	20	Experienced	200	25.4	9	0.1	67.59	176.06	0.49

8.2 SWIMMING ACTIVITY COUNT

Appendix II Table II. Swimming activity count for all lumpsuckers prior to and after the introduction of Atlantic salmon. Swimming activity was registered in each lumpsucker every sixty second for twenty minutes, giving a total swimming activity count of 3580, two hundred for each lumpsucker. Categories of swimming activity are (A) attached, (H) hovering, (N) normal swimming and (B) burst swimming.

Background	Data	Swimming activity	Score	Count
Naive	Before	A	1	185
Naive	Before	H	2	180
Naive	Before	N	3	526
Naive	Before	B	4	9
Naive	After	A	1	139
Naive	After	H	2	129
Naive	After	N	3	403
Naive	After	B	4	229
Experienced	Before	A	1	130
Experienced	Before	H	2	149
Experienced	Before	N	3	606
Experienced	Before	B	4	5
Experienced	After	A	1	114
Experienced	After	H	2	236
Experienced	After	N	3	513
Experienced	After	B	4	27

8.3 SHAPIRO TEST FOR NORMALITY

Appendix II Table III. Test results from Shapiro test for normality. Table show swimming activity index (SAI) for comparison of replicates and individuals of naive and experienced fish in addition to plasma cortisol levels.

	SAI replicates			SAI individuals			Cortisol replicates			Cortisol individuals		
Background	N	W	P	N	W	P	N	W	P	N	W	P
Naive	9	0.99	0.987	90	0.96	0.007	10	0.89	0.174	100	0.95	0.002
Experienced	9	0.91	0.322	89	0.95	0.003	10	0.86	0.079	99	0.7	<0.001

Appendix II Table IV. Test results from Shapiro test for normality. Table shows plasma cortisol levels measured in control group in addition to blue-green colouration and blue colouration for epidermal skin and weight from all naive and all experienced lumpsuckers.

	Control cortisol			Blue-green colouration			Blue colouration			Weight		
Background	N	W	P	N	W	P	N	W	P	N	W	P
Naive	10	0.63	<0.001	79	0.91	0.018	79	0.98	0.392	100	0.96	0.004
Experienced	10	0.36	<0.001	79	0.99	0.887	79	0.95	0.003	99	0.96	0.02

8.4 LEVENE'S TEST FOR HOMOGENEITY OF VARIANCE

Appendix II Table V. Test results for Levene's test for homogeneity of variance, performed for comparison of swimming activity index (SAI), cortisol, blue-green colouration and blue colouration between naive and experienced lumpsuckers.

Comparison of variances	Comparison level	d.f.	F	P
Naive SAI & Experienced SAI	Replicates	8	3.84	0.07
Naive SAI & Experienced SAI	Individuals	88	1.87	0.003
Naive cortisol & Experienced cortisol	Replicates	9	0.72	0.633
Naive cortisol & Experienced cortisol	Individuals	98	0.55	0.004
Naive control cortisol & Experienced control cortisol	Individuals	9	5.7	0.016
Naive blue-green colouration & Experienced blue-green colouration	Individuals	88	0.69	0.085
Naive blue colouration & Experienced blue colouration	Individuals	88	0.38	<0.001

8.5 SPEARMAN'S RANK CORRELATION

Appendix II Table VI. Test results for Spearman rank correlation (R_{sp}) analysis between comparison factors. Significant correlations are marked as bold.

Background	Comparison factors	r_{sp}	P
Naive	SAI & Cortisol	-0.02	0.8
Experienced	SAI & Cortisol	0.03	0.719
Naive	Activity pre salmon introduction & Cortisol	-0.09	0.36

Experienced	Activity pre salmon introduction & Cortisol	0.2	0.052
Naive	Activity post salmon introduction & Cortisol	0	0.99
Experienced	Activity post salmon introduction & Cortisol	**0.29**	**0.004**
Naive	Activity pre salmon introduction & Blue-green colouration	**0.21**	**0.046**
Experienced	Activity pre salmon introduction & Blue-green colouration	0.10	0.309
Naive	Activity post salmon introduction & Blue-green colouration	**0.28**	**0.007**
Experienced	Activity post salmon introduction & Blue-green colouration	**0.23**	**0.028**
Naive	Blue-green colouration & Cortisol	0.03	0.777
Experienced	Blue-green colouration & Cortisol	**0.32**	**0.001**
Naive	Blue colouration & Cortisol	-0.04	0.7
Experienced	Blue colouration & Cortisol	**0.24**	**0.021**
Naive	Activity pre salmon introduction & Weight	**0.22**	**0.037**
Experienced	Activity pre salmon introduction & Weight	0.01	0.309
Naive	Activity post salmon introduction & Weight	0.02	0.788
Experienced	Activity post salmon introduction & Weight	-0.03	0.743
Naive	Weight & Cortisol	-0.04	0.649
Experienced	Weight & Cortisol	**0.26**	**0.008**
Naive	Weight & Blue-green colouration	0.15	0.157
Experienced	Weight & Blue-green colouration	0.02	0.812

9 APPENDIX III – PILOT STUDY

A pilot study was conducted in Osen in April 2016. Five trials were executed with experienced lumpsuckers, ten fish in each (Table Appendix III Table I). Experimental procedures and execution were performed following the same processes as described in the present study (See section 2 Material and Methods). Lumpsuckers used in each pilot trial, had coexisted in a net cage with a normal production unit of Atlantic salmon at Raudøya for sixty days. The pilot revealed that secondary stress responses in experienced fish, measured in plasma cortisol levels, were low after two hours from Atlantic salmon interaction, and absent after 24 hours from Atlantic salmon.

Appendix III Table I. Data on mean weight (g), plasma cortisol (nmol/L) and plasma cortisol median from five pilot trials conducted in April 2016. Time for sampling of plasma cortisol was 24 hours and 2 hours after each trial was conducted.

Trial	N	Weight	S.D.	Cortisol	S.D.	Median	Sampling
P1	10	55.49	15.11	20.95	28.16	3.74	24 hrs
P2	10	55.56	23.74	1.68	0.00	1.68	24 hrs
P3	10	38.6	14.12	12.10	19.58	1.68	24 hrs
P4	10	41.65	15.43	62.84	61.60	44.14	2 hrs
P5	9	42.13	21.53	78.57	75.37	44.96	2 hrs