Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Rebecca Zhichao Rye

Effects of broodstock diets on egg quality of captive lumpfish (*Cyclopterus lumpus L.*) compared to egg quality of wild caught lumpfish

Master's thesis in MSc Ocean Recources
Supervisor: Elin Kjørsvik
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ABSTRACT

The use of cleaner fish as a biological control to combat salmon lice (*Salmo salar L.*) in the Norwegian aquaculture industry has increased, as salmon lice have become resistant to several chemical treatments. Today lumpfish (*Cyclopterus lumpus L.*) account for the most used cleaner fish species in Norway, but the cleaner fish industry is limited due to lack of knowledge about the optimal diet, rearing procedure and egg quality of lumpfish. Eggs are retrieved from wild caught lumpfish which result in a varying egg quality throughout the year. A varying egg quality will thus affect the further development, survival and delousing efficiency in lumpfish. Good egg quality is known to result in higher fertilization and hatching successes, in addition to survival in marine fish eggs and larvae. It is well established that broodstock diets have an effect on the egg quality of marine fish, but there is no optimal broodstock diet for lumpfish yet.

In this master thesis the effects of three broodstock diets with a varying lipid and carbohydrate content (Diet 1 = high lipid & low carbohydrate, Diet 2 = moderate lipid & moderate carbohydrate, Diet 3 = low lipid & high carbohydrate) on the egg quality of captive lumpfish retrieved from NOFIMA´s research station were tested. The egg quality of the captive lumpfish was compared to the egg quality of wild caught lumpfish which were retrieved from two different locations (Skjerneset and Namdalen). The egg quality of the different groups was evaluated by examining the fertilization and hatching success, the egg diameter, the fatty acid composition in unfertilized eggs, bone analysis and the standard length of the newly hatched lumpfish larvae.

Results from this experiment show that wild caught lumpfish had better egg quality than the captive lumpfish fed the different broodstock diets. Eggs and larvae from the wild caught lumpfish had the highest hatching and fertilization success, higher levels of DHA and EPA in the unfertilized eggs, earlier bone development but more deformities. Diet 1 seemed to result in better egg quality than the other diet types. This diet type resulted in a higher fertilization and hatching success, in addition to higher levels of DHA and EPA in the unfertilized eggs. Higher ARA levels were found in eggs from fish fed the broodstock diets, which resulted in a lower fertilization and hatching success. Lumpfish fed the broodstock diets also had a higher number of normal larvae, but to what extent the broodstock diets had an effect is uncertain and should be investigated further.

SAMMENDRAG

Bruken av rensefisk som en biologisk kontroll for å bekjempe lakselus (*Salmo salar L.*) i norsk havbruksnæring har økt, ettersom at lakselus har blitt resistente mot flere kjemiske behandlingsformer. I dag utgjør rognkjeksen (*Cyclopterus lumpus L.*) den mest brukte rensefiskarten i Norge, men rensefisknæringen er begrenset på grunn av manglende kunnskap om optimalt kosthold, oppdrettsprosedyre og eggkvalitet hos rognkjeks. Egg hentes fra villfanget rognkjeks, noe som gir varierende eggkvalitet gjennom året. En varierende eggkvalitet vil dermed påvirke den videre utviklingen, overlevelsen og avlusingseffektiviteten hos rognkjeks. God eggkvalitet er kjent for å gi høyere befruktnings og klekkesuksesser, i tillegg til overlevelse i marine fiskeegg og larver. Det er veletablert at stamfiskfôr har en effekt på eggkvaliteten hos marine fisk, men det finnes enda ingen optimal stamfiskdiett for rognkjeks.

I denne masteroppgaven ble det testet effekten av tre stamfiskfôr med varierende lipid og karbohydratinnhold (Diett 1 = høyt lipid & lavt karbohydrat, Diett 2 = middels lipid & middels karbohydrat, Diett 3 = lavt lipid & høyt karbohydrat) på eggkvaliteten til rognkjeks fra NOFIMA sin forskningsstasjon. Eggkvaliteten til rognkjeksen ble sammenlignet med eggkvaliteten til villfanget rognkjeks som kom fra to forskjellige lokaliteter (Skjerneset og Namdalen). Eggkvaliteten til de ulike gruppene ble evaluert ved å undersøke befruktnings og klekkesuksessen, eggdiameteren, fettsyresammensetningen i ubefruktede egg, beinanalyse og standardlengden til de nyklekkede rognkjekslarvene.

Resultater fra dette forsøket viser at villfanget rognkjeks hadde bedre eggkvalitet enn rognkjeksen som fikk de forskjellige stamfiskfôrene. Egg og larver fra villfanget rognkjeks hadde størst klekke og befruktningssuksess, høyere nivåer av DHA og EPA i de ubefruktede eggene, tidligere beinutvikling, men flere deformiteter. Diett 1 så ut til å gi bedre eggkvalitet enn de andre stamfiskfôrene. Denne dietten resulterte i en høyere befruktnings og klekkesuksess i tillegg til høyere nivåer av DHA og EPA i de ubefruktede eggene. Høyere ARA-nivåer ble funnet i egg fra fisk som ble fôret på stamfiskfôr, noe som resulterte i en lavere befruktnings og klekkesuksess. Rognkjeks som fikk stamfiskfôr hadde også et høyere antall normale larver, men i hvilken grad stamfiskfôret hadde en effekt er usikkert og bør undersøkes nærmere.

Contents

1 INTRODUCTION

1.1 Use of cleaner fish in the salmon aquaculture industry

Norway is the leading production country of Atlantic Salmon (*Salmo salar L.)* and the aquaculture industry has expanded in the last few decades (Liu, Olaf Olaussen, & Skonhoft, 2011). One of the main challenges in the salmon aquaculture industry today is the combat of salmon lice (*Lepeoptheirus salmonis*). Salmon lice are external marine parasites and target salmonids in the wild (Torrissen et al., 2013). They feed on the fish´s mucus, skin and muscles, and have shown to increase the mortality of their host. Mortality occurs as a result of salmon lice damaging the host´s fins and causing skin lesions, which will expose the fish to physiological stress and increase its susceptibility to other infections (Heuch et al., 2005). A larger production of salmon has resulted in a greater need to find solutions to the salmon lice problem. Several methods have been used to combat salmon lice such as chemical, physical and biological treatments (Kvistad, 2015). In physical or mechanical treatments, the fishes are crowded and the sea lice are then removed mechanically. This method has shown to initiate severe stress responses in the fish, affecting their overall welfare (Barton, Schreck, Ewing, Hemmingsen, & Patiño, 1985). Use of chemical treatments such as chemotherapeutants have caused drug resistance in sea lice, and thus chemical treatments are used less (Aaen, Helgesen, Bakke, Kaur, & Horsberg, 2015).

Cleaner fish is used as a biological control to combat salmon lice, as chemical treatments have ceased (Barrett, Overton, Stien, Oppedal, & Dempster, 2020). Five main cleaner fish species are used in an industrial scale. These species include lumpfish (*Cyclopterus lumpus L.*), corkwing wrasse (*Symphodus melops*), ballan wrasse (*Labrus berggylta*), goldsinny wrasse (*Ctenolabrus rupestris*) and cuckoo wrasse (*Labrus mixtus*) (Norwegian Directorate of Fisheries, 2019a). Use of cleaner fish is less stressful on the salmon. Wild caught cleaner fish species are not adapted to a life in farmed aquaculture. They are vulnerable to increased stress levels which result in a high mortality both during the transportation and within a short time in the sea cages with salmon. There is also a risk of spreading infections from the wild caught cleaner fish in one area to another, and the cleaner fishes are susceptible to other diseases. The egg quality of the wild caught cleaner fish also tend to vary during the spawning season, which affect the further development, survival and delousing efficiency. Therefore the aquaculture industry needs to find ways to do intensive aquaculture of the cleaner fish species rather than depend on wild caught individuals (Nofima, 2022; A. Powell, Treasurer, et al., 2018).

Ballan wrasse is considered to be the most efficient sea lice grazer of the cleaner fish species used in Norway (Nofima, 2022). Since ballan wrasse is sensitive on its feed types (Blanco Gonzalez & de Boer, 2017) and also is sensitive to lower water temperatures

(Imsland et al., 2018; Kelly, Alzaid, Nash, & Gamperl, 2014), lumpfish is more commonly used as a cleaner fish in both low and higher water temperatures (Imsland, Danielsen, Jonassen, Hangstad, & Falk-Petersen, 2019; A. Powell, Pooley, Scolamacchia, & Garcia de Leaniz, 2018). Lumpfish accounted for the largest amount ($>$ 42 millions) of produced cleaner fish in 2019 (Norwegian Directorate of Fisheries, 2019b). Producers have observed a variable survival and larval size, and high mortality in sea cages (Dahle, Hagemann, Attramadal, Kjørsvik, & Bardal, 2017). Unlike ballan wrasse, lumpfish is less sensitive on its feed types and grow rapidly throughout its life. One challenge is that the specie may become too large during the production cycle (Nofima, 2022). Rapid growth requires more feed, which may result in the lumpfish not receiving a sufficient amount of nutrients from the feed (Nofima, 2022). Working with wild caught lumpfish as broodfish leads to challenges regarding transmissions of infection. One challenge with land based farming of juvenile lumpfish is to ensure good water quality in the tanks. Lumpfish has a very low tolerance to bacterial infections, and lumpfish is therefore more sensitive to the water quality than other fish species such as salmon and cod. Thus the water must be treated with UV filters, and a high replacement of water is needed in the tanks. The light intensity is also important for farming juvenile lumpfish, where too much light causes stress and too little light affects the growth (Ulvan, L.J., 2016). In cultivated conditions, larger lumpfish fry in general tend to show aggressive behavior by nibbling on the tail of smaller lumpfish. Therefore lumpfish is sorted by size before it is placed in sea cages with salmon (Nofima, 2022). The body shape of lumpfish makes them a slow-moving fish, and it needs to rest using its suction disk to attach to walls or other accessible surfaces in the cultivation tank. It is also important to add the feed where the lumpfish is located in the tank (Nofima, 2022; A. Powell, Pooley, et al., 2018).

Currently there is a lack of knowledge about the optimal diet, rearing procedure and egg quality of lumpfish (A. Powell, Treasurer, et al., 2018). One of the limiting factors for successful aquaculture of fish fry is a varying egg quality, where a poor egg quality may decrease the survival potential of hatched larvae (Kjørsvik, Mangor-Jensen, & Holmefjord, 1990). Since lumpfish has a broad spawning season, the egg quality of lumpfish seems to be variable throughout the year. Eggs retrieved from lumpfish caught at low temperatures during winter/spring, tend to have the highest hatching success. On the other hand, eggs retrieved from lumpfish caught at higher water temperatures during the start and end of the spawning season tend to have a lower hatching success (Pountney, Lein, Migaud, & Davie, 2020).

1.2 Egg quality

According to Kjørsvik et al. (1990), egg quality is defined as the egg´s potential to produce viable fry. The egg quality is affected by several physical, genetic and chemical parameters and the egg development will fail if one of the essentials factors is lacking or is incomplete (Kjørsvik et al., 1990). Good egg quality is defined as eggs having low mortality during fertilization, eyeing, hatching and first feeding stage (Bromage et al., 1992). In turbot (*Scophthalmus maximus*), poor egg quality has shown to decrease the survival potential of hatched larvae (Kjørsvik, Hoehne-Reitan, & Reitan, 2003). In cod eggs (*Gadus morhua*) and in zebrafish (*Danio rerio*), poor egg quality has shown to decrease the fertilization potential of the eggs (Kjørsvik & Lønning, 1983; Cheung et al., 2019).

The fertilization and hatching success are two common egg quality parameters, where high fertilization and hatching successes indicate good egg quality (Brooks, Tyler, & Sumpter, 1997).) In cod, lipids and highly unsaturated fatty acids (HUFA) such as eicosapantaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA), are important determinants for the egg quality (Salze, Tocher, Roy, & Robertson, 2005). Lipids are important for the gonad formation in female fish and are a source of metabolic energy. They are also important for formation of cell and tissue membranes (Sargent, 1995). It is known that the ratio of DHA, EPA and ARA in sea bass eggs (*Dicentrarchus labrax*) is important for the larval development and hatching success (Bell, Farndale, Bruce, Navas, & Carillo, 1997). By examining whether the eggs have an optimal ratio of these essential fatty acids, one may assess good or poor egg quality.

Egg size is another egg quality parameter. The egg size and larval size has been positively correlated in some fish species, where larger eggs resulted in larger larvae. In addition larger larvae had a better chance of survival than smaller larvae (Hempel & Blaxter, 1967; Araujo-Lima, 1994; Duarte & Alcaraz, 1989). The egg size and initial larval size after hatching may thus be used to indicate if the egg quality is good or poor. Morphological features of larvae can also be an indicator of good or poor egg quality (Kjørsvik, Stene, & Lønning, 1984). Different factors during the larval development can cause development of deformities, which will affect the fry quality and fitness. The most common deformities observed in hatchery-reared fish are in the head, trunk and tail region, where deformities in the head impacts the head shape of the fish (Eissa, Abu-Seida, Ismail, Abu-Elala, & Abdelsalam, 2021). Spinal deformities are found in the trunk region of the fish which include scoliosis (S-shaped lateral side), lordosis (upward curvature of the spine creating a V-shape) or kyphosis (downward curvature of the spine) (Eissa et al., 2021; Arbuatti, Della Salda, & Romanucci, 2013). Deformities in the fin region include

for instance lack of fin rays or fins, connected fins to the body or doubling of twisting of fins (Eissa et al., 2021). Deformities affect the growth of the fish leading to a higher risk of mortality, and there is generally a higher occurrence of deformities in reared fish compared to wild caught fish (Andrades, Becerra, & Fernandez-Llebrez, 1996; Boglione, Gagliardi, Scardi, & Cataudella, 2001a). Findings of deformities in newly hatched larvae may indicate whether the eggs are of good or poor quality.

There is a lack of clarity when lumpfish tend to spawn in the wild. Davenport (1985) suggested that the spawning season for lumpfish females occurred between April and July with no geographic reference (Davenport, 1985). On the other hand, Kennedy et al. (2015) found that lumpfish in Iceland tend to spawn between January and March (Kennedy, Jónsson, Kasper, & Olafsson, 2015). However, the harvesting of eggs from lumpfish in central Norway usually occurs between September and June, and the main catch period is from October to May (Pountney et al., 2020). The Norwegian cleaner fish industry is reliant on eggs retrieved from wild caught lumpfish to meet the egg demand for hatcheries (Nofima, 2022). Due to a varying egg quality of lumpfish throughout the spawning season, one of the goals for farming lumpfish for generations is to ensure a stable egg quality throughout the year. Other reasons for farming lumpfish are to reduce the catching pressure on wild fish and breed for cultivated broodstocks which are more robust with a higher appetite for salmon lice (Nofima, 2022; A. Powell, Treasurer, et al., 2018).

1.3 Broodstock diets affect the egg quality

It is well established that the nutrition of the broodfish has an impact on the egg quality and larval development of marine fish. Nutrition influences the egg yolk composition and thus affect the egg quality, since embryos utilize egg yolk until first feeding. Especially the lipid and fatty acid composition of the diet are major factors which determine successful reproduction and survival of offspring (Izquierdo, Fernandez-Palacios, & Tacon, 2001; Bobe, 2015; Brooks et al., 1997; Furuita, Tanaka, Yamamoto, Suzuki, & Takeuchi, 2002). Highly unsaturated fatty acids (n-3 HUFA) such as eicosapantaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA), are essential fatty acids for many marine fish species (Furuita, Konishi, & Takeuchi, 1999; Luo et al., 2015; Røjbek et al., 2014). Essential fatty acids (EFA) refers to polyunsaturated fatty acids (PUFA) that must be provided by the diet since these can not be synthesized by the body, and the PUFA are divided into omega-3 or omega-6 fatty acids (Kaur, Chugh, & Gupta, 2014).

Sargent et al. (1989), suggested that supplementary DHA in the diet improved resistance to stress in larvae an juvenile fish (Sargent, Tocher, Bell, Halver, & Hardy, 1989). Dietary DHA has also showed to be effective in increasing the stress tolerance in red sea bream larvae (*Pagrus major*) and in juvenile marble sole (*Limundu vokohamae*) (Kanazawa, 1997). High levels of DHA has been found in the eyes and brain in juvenile herring (*Clupea harengus L.*), and plays an important role for maintaining the structure and function of the cell membranes of these tissues (Bell et al., 1997). These findings indicate that DHA is important for the larval development and survival. The DHA/EPA ratio has shown to affect the development of the nervous system such as the brain and visual cells in Japanese flounder larvae (*Paralichthys olivaceus*) and the development of pigmentation in turbot larvae (*Scophthalmus maximus L.*) (Furuita, Takeuchi, & Uematsu, 1998; Reitan, Rainuzzo, & Olsen, 1994). Watanabe et al. (1983), found that DHA is more important as an EFA compared to EPA, and ARA is important in the maturation and ovulation processes in marine fish (Watanabe, 1993). In cod, eggs retrieved from wild fish had a higher ARA content than eggs retrieved from farmed fish (Røjbek et al., 2014). In Atlantic Halibut (*Hippoglossus hippoglossus*), the fertilization and hatching success increased due to a higher amount of ARA in the broodstock diet (Mazorra et al., 2003). On the other hand, the fertilization success of eggs from Japanese flounder (*Paralichthys olivaceus*), decreased when the broodstocks were fed diets with higher levels of supplementary ARA (Furuita, Yamamoto, Shima, Suzuki, & Takeuchi, 2003).

Levels of carbohydrate in the broodstock diet also affect the egg quality (Kjørsvik et al., 1990). Today the broodstock feed for marine fish species mainly contain fishmeal and fish oil, where fishmeal is rich in protein. As fish oil and fishmeal come from wild caught

fish, the aquaculture industry has in the recent years focused on finding more sustainable alternatives. Marine fish generally have a high protein requirement but no specific requirement for dietary carbohydrates (Cowey & Sargent, 1972). It is demonstrated that female rainbow trout (*Oncorhynchus mykiss*) were able to grow and reproduce normally over a reproduction cycle, when fed a diet where the protein was partially replaced with carbohydrates (Callet et al., 2020). Callet et al. (2021), found that a high carbohydrate-low protein diet fed to male and female rainbow trout did not trigger adverse consequences on their offspring (Callet et al., 2021). These findings suggest that carbohydrate may be an additional nutrient in addition to protein in the broodstock feed for marine fish species.

Lumpfish has a broad diet selection and is less sensitive on its feed types. In the wild their diet consists of crustaceans, jellyfish, squids and small fish (Nofima, 2022; Davenport, 1985). The right feed composition and feeding strategy are fundamental prerequisites for good function, health and animal welfare for lumpfish in captivity. Formulation of an optimal broodstock diet for lumpfish is still an ongoing process. The broodstock diet fed to lumpfish nowadays are based on what is assumed minimum requirements for nutrients, experience and knowledge of other fish species and analyzes of wild caught fish (Lein et al., 2021). The nutritional content of the broodstock diet used in this master project is based on existing nutritional studies of lumpfish (FHF 9901562 - CleanLifeCycle project) and their natural diet which is assumed to have a higher content of carbohydrates (Sæle, Bjelland, Kousoulaki, Berge, & Lein, 2021).

1.4 Aim of the study

The main objective of this study is to investigate how broodstock diets with a varying lipid and carbohydrate content affect the egg quality of captive lumpfish (*Cyclopterus lumpus L.*) compared to the egg quality of wild caught lumpfish. The egg quality was examined by the fertilization and hatching success of the eggs, the egg diameter and standard length of larvae, the fatty acid composition of unfertilized eggs and the degree of ossification and deformities in newly hatched larvae. Eggs used in this study originated from wild caught lumpfish from two locations (Skjerneset and Namdalen) and captive lumpfish from NOFIMA´s research station at Sunndalsøra. Based on existing literature, the following hypotheses were examined.

1) The egg quality of wild caught lumpfish will be better than the egg quality off captive lumpfish.

2) The different lipid and carbohydrate content in the broodstock diet will have an effect on the egg quality of captive lumpfish.

2 MATERIALS AND METHODS

This master project was a collaboration between the Norwegian University of Science and Technology (NTNU), SINTEF Ocean and NOFIMA through the STARTRENS project (FHF 901561) funded by the Norwegian Seafood Research Fund. Eggs retrieved from both wild caught and captive lumpfish females were used (N=17). Eggs from wild caught lumpfish were retrieved from Skjerneset Fisk AS in Møre and Romsdal and Namdalen Rensefisk in Trøndelag (N=3 from each location). Eggs from the captive lumpfish were retrieved from 11 lumpfish females at NOFIMA´s research station at Sunndalsøra in Møre and Romsdal. The captive lumpfish were fed three different broodstock diets consisting of a varying carbohydrate and lipid level. An overview of the nutritional composition of the different broodstock diets is presented in Table 9 in Appendix A. Diet 1 consisted of a high lipid and low carbohydrate content (lipid 18,7 %, carbohydrate 7,59 %). Diet 2 had a moderate lipid and carbohydrate content (lipid 13,9 %, carbohydrate 13,50 %), and Diet 3 had a low lipid and high carbohydrate content (lipid 7,3 %, 17,98 % carbohydrate). The lumpfish females used in this experiment were grouped according to place of origin, egg groups (eggs retrieved from females fed the same diet), egg batches (eggs retrieved from different females fed the same diet), dietary differences and date of fertilization of the eggs (Table 1).

The experiment was conducted at the Centre of Fisheries and Aquaculture (NTNU Sealab) and in SINTEF Fisheries and Aquaculture laboratories in Trondheim. Unfertilized eggs from wild caught lumpfish were retrieved from October 2019. The experiment took place from October 2019 to May 2021. A timeline of the sampling of the eggs and larvae, and the conduction of the different analyses is presented in Figure 1. Samples of eggs for the lipid analyses were collected before the fertilization process. Images of the eggs used for measuring the egg diameter (mm) were taken the same day as the fertilization of the eggs. The fertilization success was registered 14 or 15 days post fertilization. Analysis of the hatching success took place at 29 or 30 days post fertilization. Images of newly hatched lumpfish larvae (1-2 days post hatch) were used to measure the standard length (mm). Samples of larvae for the bone analysis were taken 1-2 days post hatch, and the bone analysis was conducted from October 2020 to February 2021. The lipid analysis was conducted from February 2021 to May 2021.

Table 1: Captive (LS-BS-1, LS-BS-2 and LS-BS-3) and wild caught (RK and NR) lumpfish females used in experiments grouped into egg groups, egg batches, place of origin, total weight (g), total length (cm), weight of eggs (g), feed composition and date of fertilization of the eggs. Egg groups represent eggs retrieved from lumpfish that were fed the same diet type. Egg batches correspond to eggs from different lumpfish fed the same diet.

Egg group	Egg batch	Origin	Female	Total weight + eggs (g)	Total weight – eggs (g)	Eggs (g)	Standard length (cm)	Total length (cm)	Feed composition	Date of fertilization of eggs
LS-BS-1	LS-BS-1-1		1	2322	1619	703	30	34,5		
LS-BS-1	LS-BS-1-2	Broodstock from Nofima, Tank 1	2	2652	2245	407	31	35,5	18,7 % lipid, 7,6 % carbohydrates	04/02/2020
LS-BS-1	LS-BS-1-3		3	2288	1440	848	30	38		
LS-BS-2	LS-BS-2-1		1	3083	2527	556	33	36,2		
LS-BS-2	LS-BS-2-2		2	2929	2441	488	32	36,2		
LS-BS-2	LS-BS-2-3	Broodstock from Nofima, Tank 2	3	2861	2312	549	30,5	34,2	13,9 %, 13,5 % carbohydrates	04/02/2020
LS-BS-2	LS-BS-2-4		4	3028	2616	412	33	37,2		
LS-BS-2	LS-BS-2-5		5	1987	1765	222	28,5	32,2		20/02/2020
LS-BS-2-6	LS-BS-2-6		6	2525	2055	470	31,5	36,3		
LS-BS-3	LS-BS-3-1		1	3361	2788	573	33,5	37,5		
LS-BS-3	LS-BS-3-2		2	3099	2741	358	33,5	36,5		
LS-BS-3	LS-BS-3-3	Broodstock from Nofima, Tank 3	3	2740	2052	688	31,5	35,2	7,3 % lipid, 18 % carbohydrates	04/02/2020
LS-BS-3	LS-BS-3-4		4	3158	2730	428	34	37,6		20/02/2020
NR	NR-1		1	4986				47,0		
NR	NR-2	Namdalen Rensefisk, wild caught	2	2979				41,0	Natural feed	17/10/2019
NR	NR-3		3	3264				41,0		
RK	RK-1		1	4000				37,6		
RK	RK-2	Skjerneset Fisk, wild caught	2	2500				35,2	Natural feed	06/11/2019
RK	RK-3		3	2320				31,1		

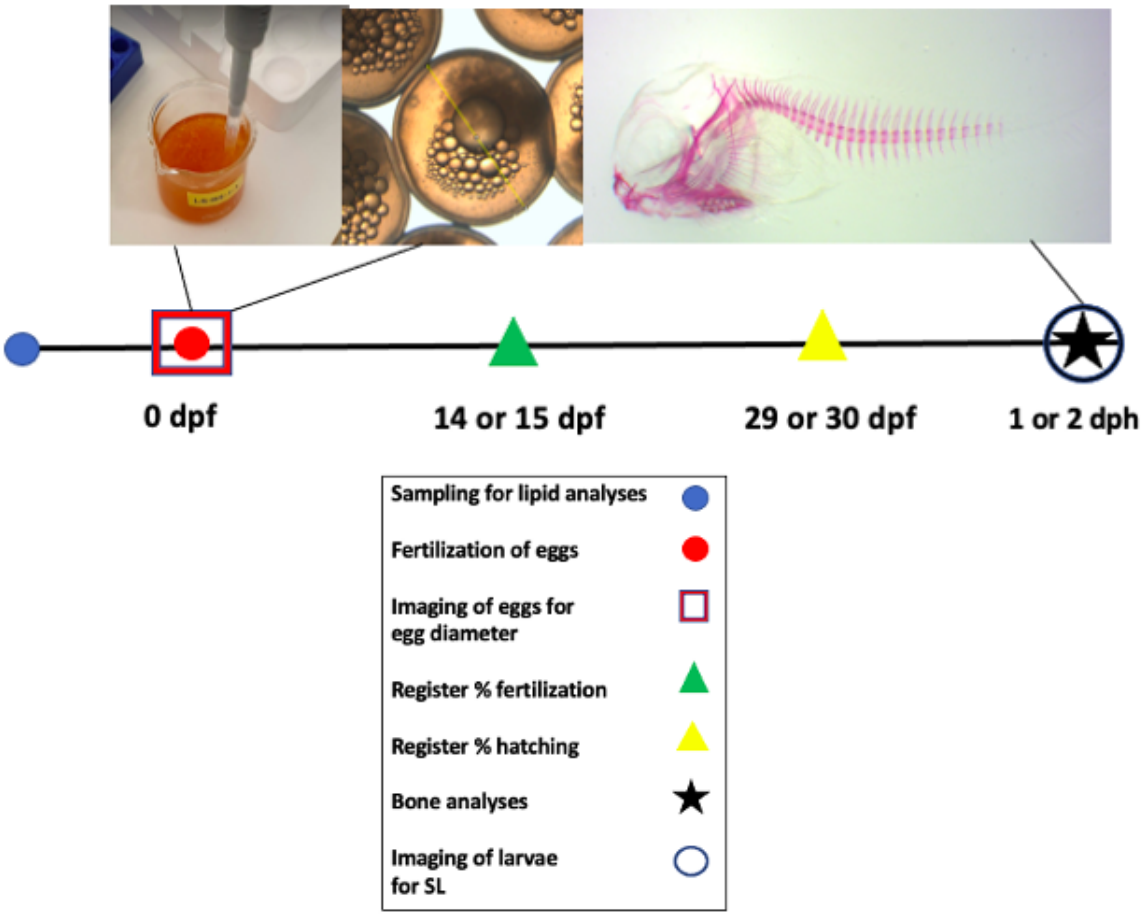


Figure 1: Timeline of sampling of the eggs and larvae, the conduction of the different analyses as days post fertilization (dpf) and days post hatch (dph) for lumpfish eggs retrieved from wild caught and captive lumpfish females. Sampling of eggs for the lipid analyses were done prior to the fertilization of the eggs. Imaging of eggs for the egg diameter was conducted the same day as the fertilization. The fertilization success (%) was registered 14 or 15 dpf, and the hatching success (%) was registered 29 or 30 dpf. Samples of larvae used in the bone analysis and measurement of the standard length (SL) were taken 1-2 days post hatch.

2.1 Strip-spawning of captive lumpfish

Captive lumpfish females from NOFIMA´s research station were retrieved from three different tanks and were fed three different broodstock diets (Table 1). Each broodstock diet was fed to approximately 40-50 individuals. The largest females (3-4) from each tank were selected for the strip-spawning. The lumpfish were caught using a net and placed in a tub with seawater (80 L in total) and anesthesia (FINEQUEL vet. 20 mg/L), see Figure 2. They were kept in the tub for approximately 10 minutes until the anesthesia worked. After the anesthetizing, each lumpfish was identified using a measuring equipment to see which tank they belonged to. The total weight (g), the length measured from the mouth to the start of the notochord (cm) and the total length measured from the mouth to the end of the notochord (cm), were registered for the captive lumpfish (Table 1).

Prior to the strip-spawning, the captive female lumpfish were wiped off around their gonadal area by paper tissues. The sidelines of the fish were pressed towards their gonadal opening and eggs and ovary fluid were collected in a measuring cup (200 mL in total). Total weight (g) of each female after the strip-spawning and the total weight of the eggs (g) were measured (Table 1). Approximately 100 mL of eggs from each individual were stored in ziplock bags which were stored in a styrofoam box with ice. A water-absorbent mat and booble wrap were placed between the ziplock bags and ice. The eggs were kept refrigerated and protected during the transportation from NOFIMA to SINTEF OCEAN´s lab facility in Trondheim by car.

Eggs from the wild caught lumpfish retrieved from Namdalen had a similar strip-spawning process as the captive lumpfish. Eggs retrieved from lumpfish at Skjerneset were sent to the SINTEF OCEAN´s laboratory in Trondheim.

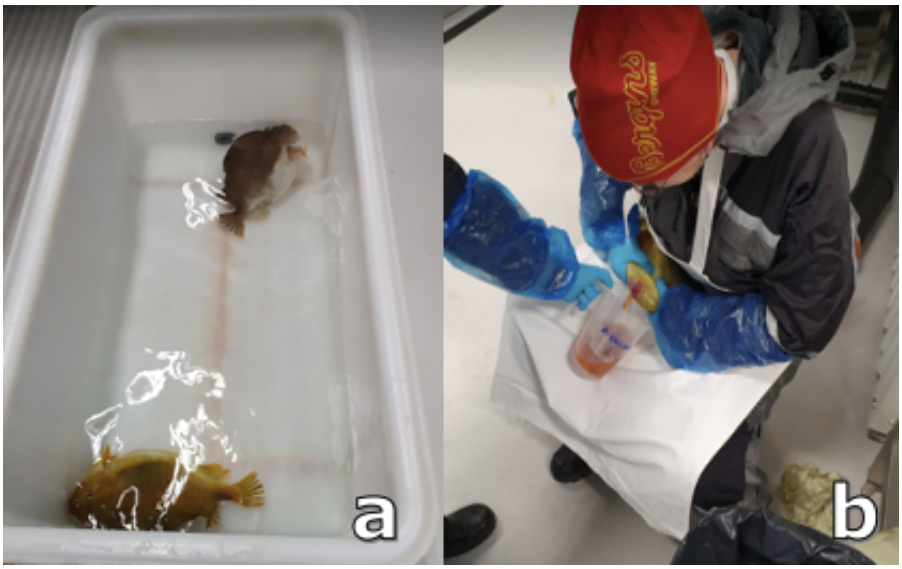


Figure 2: (a) Captive lumpfish females (*Cyclopterus Lumpus L.*) anasthetized in 80 L of seawater in FINQUEL vet 20 mg/L for approximately 10 minutes. (b) Strip-spawning of lumpfish females at NOFIMA´s research station at Sunndalsøra in Møre and Romsdal. Eggs and ovary fluid from each individual were collected in a measuring cup (200 mL in total).

2.2 Fertilization at SINTEF OCEAN in Trondheim

Fertilization of eggs from wild caught and captive lumpfish females was completed at SINTEF Fisheries and Aquaculture laboratory in Trondheim. 50 mL of unfertilized eggs from each egg batch were transferred to a glass beaker and then fertilized with 1 mL cryopreserved milt originating from the same lumpfish male (Cryogenetics AS in Squarepack(c) 12 mL). The cryopreserved milt was stored in liquid nitrogen and thawed in a water tub (20 °C) for 30 seconds before use. Use of the same milt reduced the potential of parental variation to be a confounding factor in the experiments.

The eggs were carefully stirred for 3 minutes before filtered sea water (50 mL) was added, and the mixture was stirred for another 2 minutes. Fertilized eggs were distributed in a plate consisting of circular moulds (approximately 80-90 eggs in each mould), see Figure 3. The eggs hardened to "egg cakes" in the moulds after 15-20 minutes. Several "egg cakes" (7-10) were prepared for each egg batch, transferred to each respective "egg chamber" and then placed in an incubation system (Figure 4).

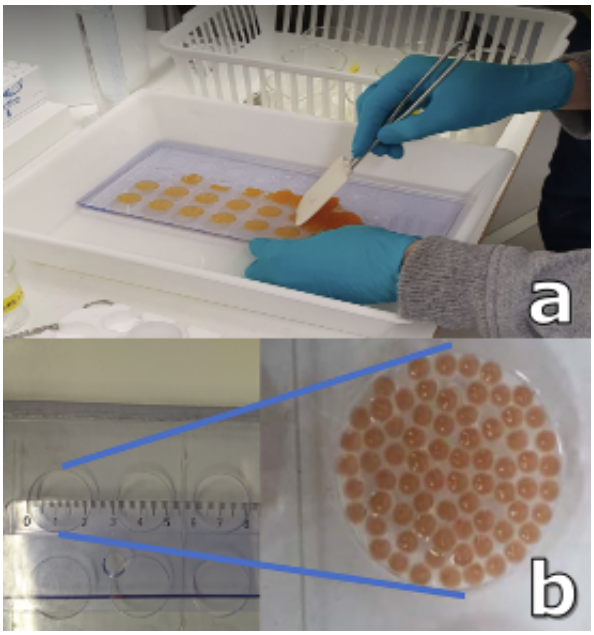


Figure 3: (a) Plate with circular moulds where the fertilized eggs hardened to "egg cakes". (b) The diameter of each circular mould was 2,5 cm and contained approximately 80-90 fertilized eggs.

2.3 Incubation system

The incubation system consisted of "egg chambers" made of several plastic incubation tubes, (50 mL) with a 300 μm plankton mesh (SEFAR NITEX) fixed at the top and bottom of the tubes (Figure 4a). Each "egg chamber" contained approximately 80-90 fertilized eggs. The "egg chambers" were mounted in a flow-through incubator, where the eggs developed until hatching (20-30 days after fertilization). "Egg chambers" were grouped in 16 columns and 7 rows (Figure 4b). A total of 3 incubation systems were used in this experiment. The incubation systems were prefilled with filtered 1 μm sea water with the same salinity. The sea water was kept at 9-10 °C with a constant water flow (10 L/min) through four inlets of water (one close to each corner), which provided an even distribution of flow through the tubes. The incubation system was covered with a plastic lid and kept in a dark room when there was no handling of samples.

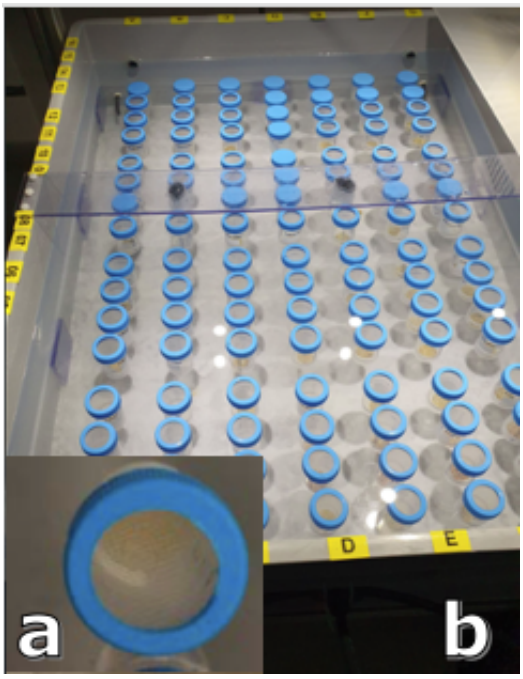


Figure 4: (a) Fertilized eggs were placed in each respective "egg chamber" made of plastic tubes (50 mL) with a 300 μm plankton mesh (SEFAR NITEX) fixed at the top and bottom of the tubes. (b) Each incubation system consisted of 112 "egg chambers" grouped into 16 columns and 7 rows with a constant flow-through (10 L/min) of sea water (9-10 °C) with the same salinity and kept in a dark room.

2.4 Fertilization and hatching success

For assessment of the fertilization success, all "egg cakes" were imaged at 14 or 15 days post fertilization (dpf). Fertilized eggs had eye development and were marked with a red dot. Unfertilized eggs had no eye development and were marked with a blue dot (Figure 5b). Number of "egg cakes" and eggs for the fertilization and hatching success is presented in Table 2.

Table 2: Eggs from captive (LS-BS-1, LS-BS-2, LS-BS-3) and wild caught (RK and NR) lumpfish females grouped into egg group, egg batch, diet type, amount of egg cakes and total number of eggs. Egg group represents eggs retrieved from lumpfish fed the same diet. Egg batch represent eggs retrieved from different lumpfish fed the same diet. Egg cakes are the eggs which hardened in the egg chambers. Total eggs are the number of eggs in each egg cake.

Egg group	Egg batch	Diet type	Egg cakes	Total eggs
LS-BS-1	LS-BS-1-1	D1	7	667
LS-BS-1	LS-BS-1-2	D1	6	527
LS-BS-1	LS-BS-1-3	D1	7	583
LS-BS-2	LS-BS-2-1	D2	6	486
LS-BS-2	LS-BS-2-2	D2	9	748
LS-BS-2	LS-BS-2-3	D2	9	641
LS-BS-2	LS-BS-2-5	D2	13	925
LS-BS-3	LS-BS-3-1	D3	9	516
LS-BS-3	LS-BS-3-2	D3	9	618
LS-BS-3	LS-BS-3-3	D3	6	490
LS-BS-3	LS-BS-3-4	D3	13	1004
RK	RK-1	N1	9	698
RK	RK-2	N1	9	721
RK	RK-3	N1	8	523
NR	NR-1	N2	13	891
NR	NR-2	N2	13	1032
NR	NR-3	N2	12	910
LS-BS-1		D1	20	1777
LS-BS-2		D2	37	2800
LS-BS-3		D3	37	2626
RK		N1	26	1942
NR		N2	38	2833

The fertilization and hatching success (%) were calculated using the following equations.

(1) % fertilization = 100*(total amount of eggs - unfertilized eggs)/total amount of eggs

(2) % hatching = 100*(hatched larvae)/(total amount of eggs - unfertilized eggs)

The mean fertilization and hatching success (see equation 3) and standard deviation (see equation 4) for each egg group were calculated using the combined variance formula, since there was a varying amount of egg cakes. Xc is the combined mean and Sc is the combined variance of n1 + n2... + ni observations. In this case n is the number of egg cakes, S is the standard deviation for each egg batch and X is the mean fertilization or hatching success for each egg batch.

3)

$$Xc = \frac{n1\overline{X1} + n2\overline{X2}}{n1 + n2}$$

4)

$$Sc^2 = \frac{n1\left[S1^2 + (\overline{X1} - \overline{Xc})^2)\right] + n2\left[S1^2 + (\overline{X2} - \overline{Xc})^2)\right]}{n1 + n2}$$

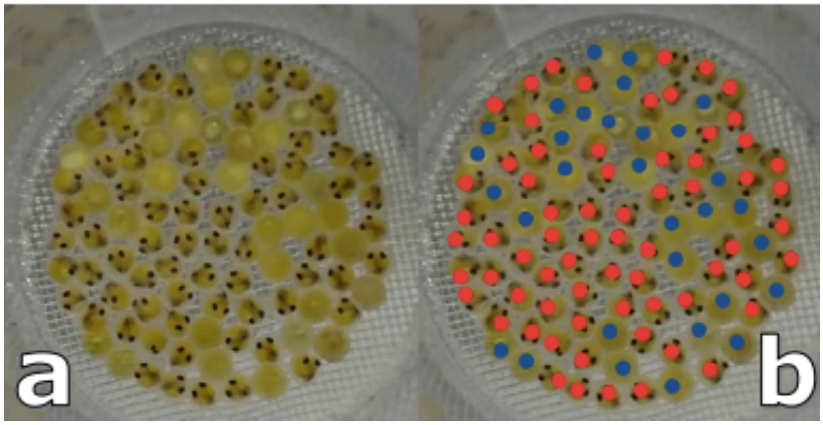


Figure 5: (a) Registration of eye development in lumpfish eggs at 14 days post hatch (dpf). (b) Fertilized eggs were registered if eye development was observed. Eggs with eye development were marked with a red dot. Unfertilized eggs had no eye development and were marked with a blue dot (Kulid, 2020).

2.5 Egg diameter and standard length

The egg diameter was registered by imaging eggs using the Leica MC170 HD camera, zoom Leica Z6 APO and with the light source Leica CLS 150 XD. The egg diameter was measured using the ImageJ (version 1.8.0-112) software for Windows (Figure 6a). Since the number of eggs and larvae varied in the different egg batches, the mean egg diameter and standard length for each egg group were calculated using the combined variance formula (see equation 3 & 4), where n is the number of eggs or larvae.

The ImageJ (version 1.8.0-112) software for Windows was used to measure the standard length (mm) of newly hatched lumpfish larvae from all egg batches. The standard length (mm) was measured from the tip of the snout to the end of the notochord (Figure 6b).

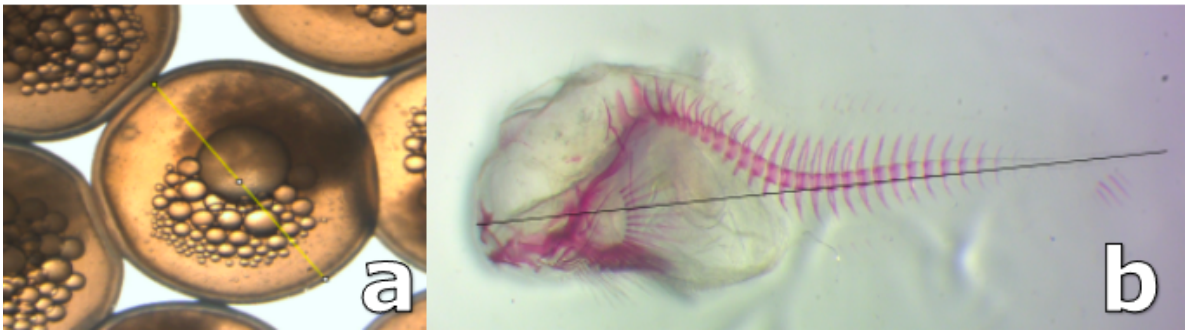


Figure 6: (a) Egg diameter (yellow line) measured of a fertilized lumpfish egg (1 dpf) with the ImageJ (version 1.8.0-112) software program for Windows. (b) Measurement of the standard length (mm) of a newly hatched lumpfish larva using the ImageJ program. The standard length (black line) was measured from the tip of the snout to the end of the notochord.

2.6 Fatty acid composition

Unfertilized eggs (kept in 2 mL cryotubes) from all egg batches were used in the lipid analyses. Lipids were extracted with the Folch method which consisted of three steps; extraction of lipids, methylation and purification of fatty acids on High performance thin layer chromatography (HPTLC), see Appendix B. The HPTLC step was implemented as a cleaning step to remove contaminants from the fatty acid samples. Analysis of the lipid and fatty acid profiles were calculated and classified by a NTNU technical staff member.

2.7 Bone analyses

2.7.1 Ossification score of vertebrae, suction disk and gills

Segments in the vertebrae are not fully ossified in newly hatched larvae. The ossification score of vertebrae segments (n = 28) in newly hatched lumpfish larvae from different egg groups were counted and scored according to degree of ossification (Figure 7). The segments were classified as Compact (C) = ossified and fully saturated color, Moderate (M) = ossified but not fully saturated color, Partly (P) = partly ossified segments or Transparent (T) = no visible bone ossification. The ossification score of the suction disk was classified as Score 2 = ossified and fully saturated color, Score 1 = partly ossified or Score 0 = no visible ossification. The ossification score of the gills was classified as Score

1 = ossified gill arches or Score 0 = no visible ossification.

The amount of Compact (C), Moderate (M), Partly (P) and Transparent (T) vertebrae segments was multiplied with a factor to get the Vertebrae score, see equation (1). The Suction disk score was calculated by multiplying the number of suction disk segments classified as either Score 2, Score 1 or Score 0 with a factor, see equation (2). Number of gill arches classified as Score 1 or Score 0 was multiplied with a factor to find the Gill score, see equation (3). Egg groups with a higher Vertebrae score, Suction disk score and Gill score were considered more developed than egg groups with a lower score.

(1) Vertebrae score = (C segments*1,00) + (M segments*0,66) + (P segments*0,33) + (T segments*0,00)

(2) Suction disk score = (Score 2 * 1,0) + (Score 1 * 0,5) + (Score 0 * 0,0)

(3) Gill score = (Score 1 * 1,0) + (Score 0 * 0,0)

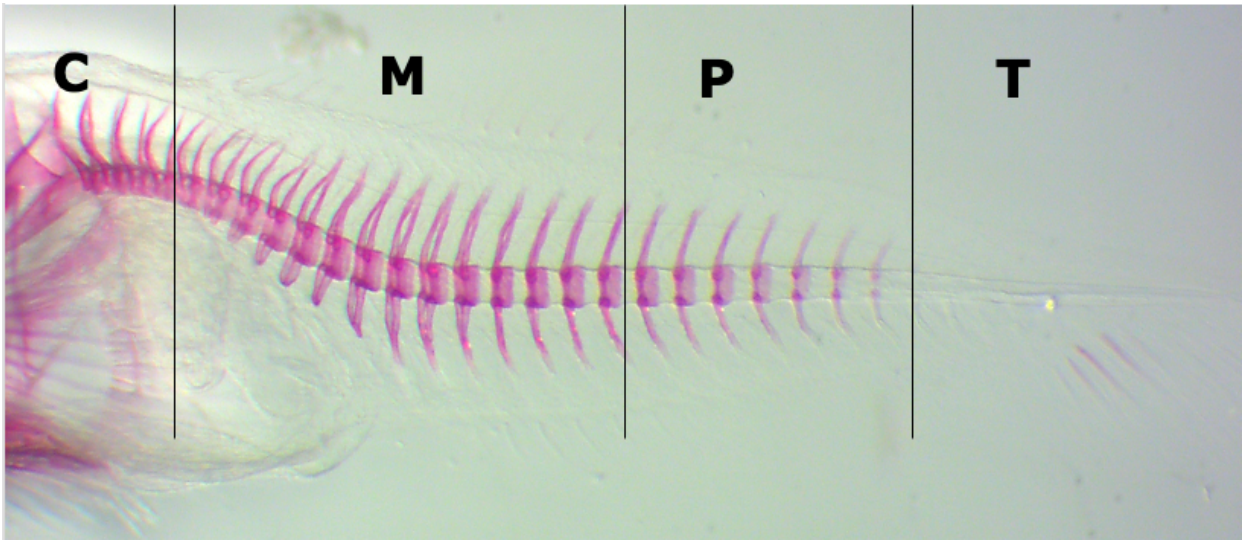


Figure 7: Ossification score of vertebrae segments in a newly hatched lumpfish larva classified as Compact (C) = ossified and fully saturated color, Moderate (M) = ossified but not fully saturated color, Partly (P) = partly ossified segments or Transparent (T) = no visible bone ossification. The classification was based on the the staining saturation and coverage in each verebralsegment.

2.7.2 Ossification score of fins

Since the amount of fin rays varied between larvae, neither the number of colored segments in the fins was counted nor classified by color saturation. However, the amount of newly hatched larvae in the different egg groups with ossified segments in the 1. dorsal fin, 2. dorsal fin, caudal fin and pelvic fin was counted. The distribution of different fin types is presented in Figure 8.

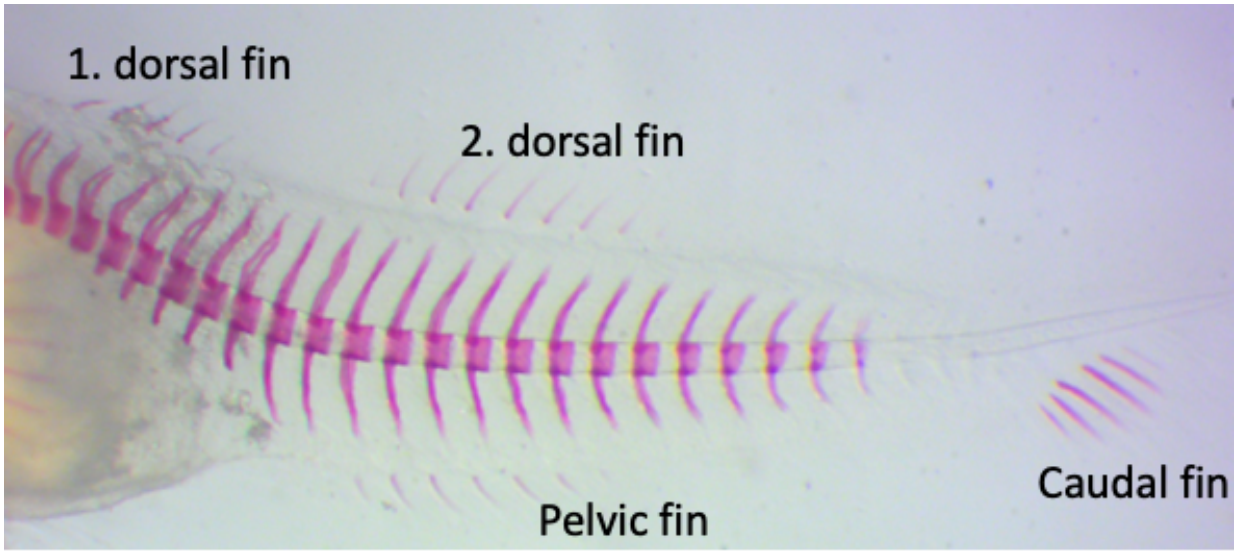


Figure 8: Ossification of 1. dorsal fin, 2. dorsal fin, pelvic fin and caudal fin of a newly hatched lumpfish larva belonging to the NR egg group.

2.7.3 Deformities

Deformities which are deviations from the normal shape, body part or organ (Heiberg, 2020), in the lumpfish larvae were categorized and counted. The deformities observed in newly hatched lumpfish larvae were grouped into three main categories which consisted of axial, vertebral and craniofacial deformities, see Figure 9.

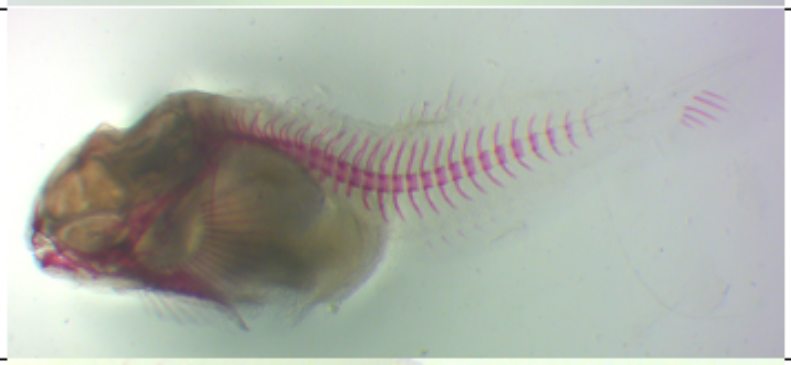
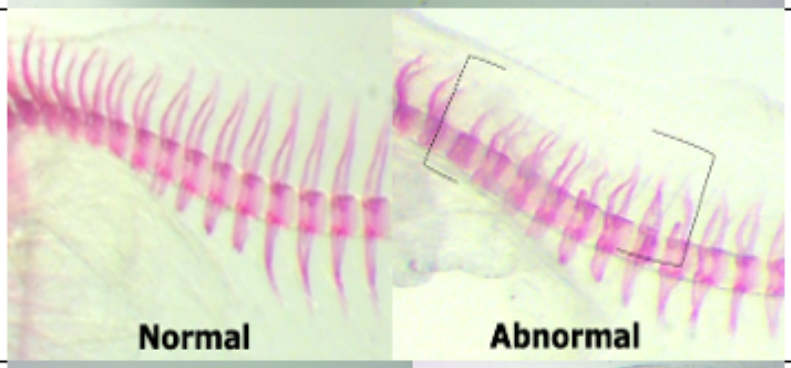
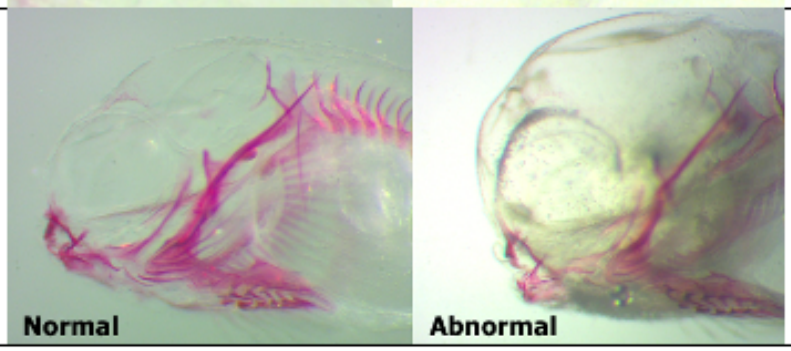
Deformities	Picture	Description
Normal larva		Larva with no deformities
Axial		Axis deviation such as lordosis, creating a curvature (V-shape of the spine)
Vertebral		Stunted or shorter neural arches in the vertebrae
Craniofacial		Underdeveloped jaw where the jaw area is pulled further back in relation to the mouth

Figure 9: Categorization of different deformities with explanations, observed in newly hatched lumpfish larvae.

2.8 Statistical analyses

Statistical analyses and graphs were made using Microsoft Excel and Graphpad Prism version 9.0. The normal distribution of the data was tested by a Shapiro-Wilk test. For normal distributed data, significant differences between groups were tested by a One-way ANOVA with an assumption that the SD values were equal. A post-hoc test such as the Tukey test was used to determine which groups were significantly different from each other. For non normal distributed data, significant differences between groups were tested by a Kruskal Wallis test. A post-hoc test such as Dunn´s multiple comparison test was used to test which groups were significantly different from each other. A Pearson correlation test was used to test correlations between different analyses for normal distributed data, and a Spearman correlation test was used for non parametric data. Correlation plots were made in Excel as scatterplots with respective trendlines.

3 RESULTS

3.1 Fertilization and hatching success

Eggs from wild caught lumpfish (NR and RK) had the highest mean fertilization success compared to eggs from captive lumpfish (LS-BS-1, LS-BS-2 and LS-BS-3), see Table 3 and Figure 10A. NR had the highest mean fertilization success compared to the other egg groups and had a significantly higher mean fertilization success compared to LS-BS-2 (p-value = 0,023). The mean fertilization success of eggs from captive lumpfish had a high variability, where eggs from egg group LS-BS-2 was most variable, see Table 3. Most egg batches had a low fertilization success ($<$ 10 %), while egg batch LS-BS-2-5 had a fertilization success above 70 %. The mean fertilization success of eggs from egg group LS-BS-3 was also variable. Egg batches LS-BS-3-2 and LS-BS-3-3 had a much lower fertilization success compared to egg batches LS-BS-3-1 and LS-BS-3-4. Eggs from egg group LS-BS-1 had a less variable fertilization success, with the exception of egg batch LS-BS-1-3 which had a lower fertilization success.

Egg group NR had the highest mean hatching success and had a significantly higher mean hatching success than LS-BS-2 (p-value = 0,037), see Table 2 and Figure 10B. Egg group LS-BS-1 had the second highest mean hatching success compared to the other egg groups, while egg group LS-BS-2 had the lowest mean hatching success. The hatching success of egg group LS-BS-2 was most variable. Egg batches LS-BS-2-1 and LS-BS-2-3 had a hatching success of 0 %, while egg batches LS-BS-2-2 and LS-BS-2-5 had a higher hatching success.

Table 3: Overview of egg groups, egg batches, number of egg cakes and amount of eggs used for the fertilization and hatching success with respective SD from captive (LS-BS-1, LS-BS-2 and LS-BS-3) and wild caught (RK and NR) lumpfish females. The mean fertilization and hatching success in addition to the standard deviation for each egg group were calculated according to equations in section 2.4 Fertilization and hatching success. Egg groups represent eggs retrieved from lumpfish fed the same diet type. Egg batches correspond to eggs from different lumpfish fed the same diet. Egg cakes are eggs which hardened in the egg chambers. Total eggs are the number of eggs in each egg cake. Significant differences between egg groups are labeled with different letters (A & B). Egg groups with no significant difference are labeled with the same letters (AA or BB). Egg groups that are not significant from neither A or B are marked with AB.

Egg group	Egg batch	Diet type	Egg cakes	Total eggs	% fertilization ± SD	% hatching ± SD
LS-BS-1	LS-BS-1-1	D1	7	667	75 ± 5	91 ± 13
LS-BS-1	LS-BS-1-2	D1	6	527	72 ± 5	89 ± 16
LS-BS-1	LS-BS-1-3	D1	7	583	46 ± 7	66 ± 46
LS-BS-2	LS-BS-2-1	D2	6	486	8 ± 6	0 ± 0
LS-BS-2	LS-BS-2-2	D2	9	748	4 ± 3	37 ± 40
LS-BS-2	LS-BS-2-3	D2	9	641	2 ± 3	0 ± 0
LS-BS-2	LS-BS-2-5	D2	13	925	71 ± 6	65 ± 10
LS-BS-3	LS-BS-3-1	D3	9	516	50 ± 38	92 ± 13
LS-BS-3	LS-BS-3-2	D3	9	618	16 ± 8	72 ± 29
LS-BS-3	LS-BS-3-3	D3	6	490	2 ± 1	33 ± 41
LS-BS-3	LS-BS-3-4	D3	13	1004	84 ± 6	85 ± 27
RK	RK-1	N1	9	698	84 ± 9	84 ± 20
RK	RK-2	N1	9	721	84 ± 4	79 ± 4
RK	RK-3	N1	8	523	67 ± 7	44 ± 11
NR	NR-1	N2	13	891	99 ± 1	91 ± 4
NR	NR-2	N2	13	1032	92 ± 2	95 ± 3
NR	NR-3	N2	12	910	92 ± 3	88 ± 5
LS-BS-1		D1	20	1777	64 ± 14 (AB)	82 ± 43 (AB)
LS-BS-2		D2	37	2800	21 ± 33 (B)	26 ± 35 (B)
LS-BS-3		D3	37	2626	38 ± 36 (AB)	75 ± 30 (AB)
RK		N1	26	1942	79 ± 11 (AB)	48 ± 28 (AB)
NR		N2	38	2833	94 ± 4 (A)	91 ± 5 (A)

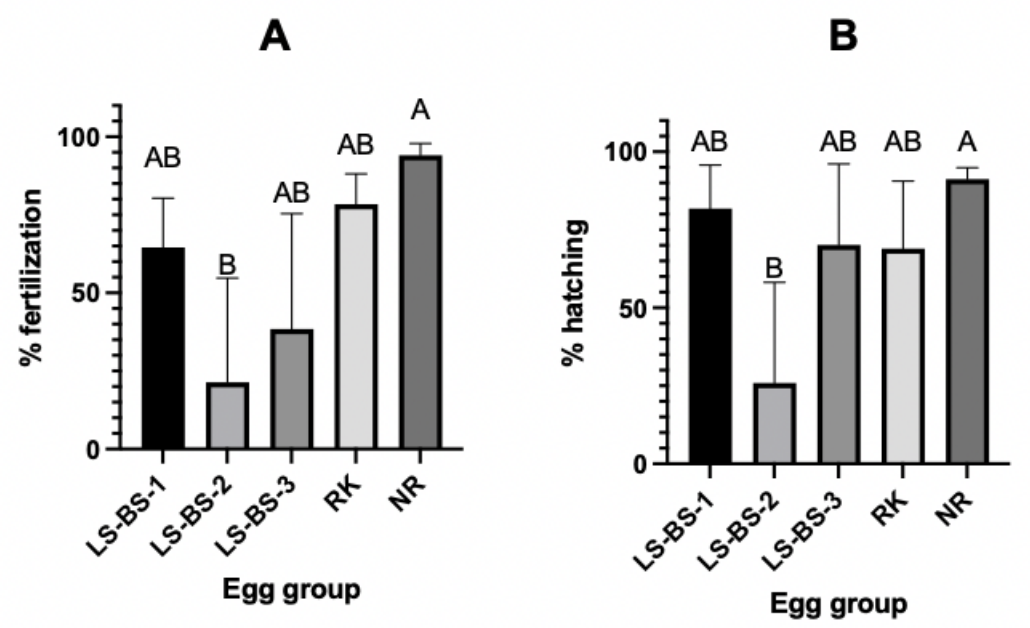


Figure 10: (A) Fertilization success and (B) hatching success of fertilized lumpfish eggs given in %. The data are expressed as mean number of eggs from each egg group. Significant differences between egg groups are labeled with different letters (A & B). Egg groups with no significant difference are labeled with the same letters (AA or BB). Egg groups that are not significant from neither A or B are marked with AB. NR had significantly higher fertilization success (p-value = 0,023) and hatching success (p-value = 0,037) than LS-BS-2.

There was a positive correlation between % fertilization and % hatching (r = 0,553), where a higher fertilization success resulted in a higher hatching success (Figure 11). The NR egg group had the highest fertilization and hatching success compared to the other egg groups, while LS-BS-2 had the lowest % fertilization and % hatching.

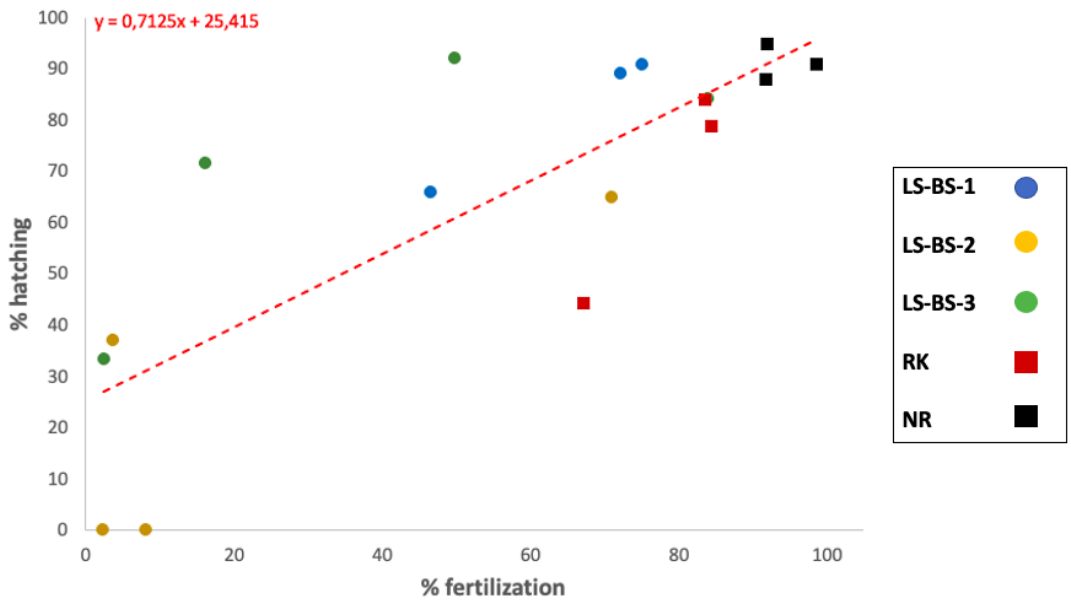


Figure 11: Correlation between % fertilization and % hatching of fertilized lumpfish eggs from all egg groups. From the Spearman correlation test there was a positive correlation between % fertilization and % hatching (p- value $< 0{,}05$, r $= 0{,}553$, $\alpha = 0,05$).

3.2 Egg diameter and standard length

Eggs from group RK had a significantly larger mean egg diameter than LS-BS-1 (p-value $= 0{,}022$), see Table 4 and Figure 12A. No significant differences between the other egg groups were found. RK and LS-BS-3 had the highest mean standard length (mm) compared to the other egg groups, while LS-BS-2 had the lowest mean standard length (Table 4, Figure 12B). Larvae from group LS-BS-2 had the most variable standard length due to a lower number of egg batches with a varying number of larvae in each egg batch. This resulted in a higher standard deviation for the egg group. No significant difference regarding the standard length was found between larvae from the different egg groups. From the Spearman correlation test, no correlation between the egg diameter and the standard length of the larvae was found, nor any correlation between the egg diameter and fertilization success or egg diameter and hatching success.

Table 4: Overview of egg groups, egg batches, diet types and amount of eggs used for measuring the egg diameter (mm) and amount of larvae used for measuring the standard length (mm) with respective SD from captive (LS-BS-1, LS-BS-2 and LS-BS-3) and wild caught (RK and NR) lumpfish females. Egg groups represent eggs retrieved from lumpfish fed the same diet type. Egg batches correspond to eggs from different lumpfish fed the same diet. Significant differences between egg groups are labeled with different letters (A & B). Egg groups with no significant difference are labeled with the same letters (AA or BB). Egg groups that are not significant from neither A or B are marked with AB.

Egg group	Egg batch	Diet type	Number of eggs	Egg diameter (mm) ± SD	Amount of larvae	SL (mm) ± SD
LS-BS-1	LS-BS-1-1	D1	12	2,11 ± 0,06	12	5,33 ± 0,31
LS-BS-1	LS-BS-1-2	D1	12	2,20 ± 0,07	12	5,67 ± 0,22
LS-BS-1	LS-BS-1-3	D1	12	2,16 ± 0,10	12	5,57 ± 0,16
LS-BS-2	LS-BS-2-1	D2	5	2,22 ± 0,10	5	4,77 ± 0,31
LS-BS-2	LS-BS-2-5	D2	12	2,27 ± 0,09	12	5,77 ± 0,29
LS-BS-3	LS-BS-3-1	D3	12	2,25 ± 0,06	12	5,65 ± 0,25
LS-BS-3	LS-BS-3-2	D3	12	2,33 ± 0,07	12	5,71 ± 0,19
LS-BS-3	LS-BS-3-4	D3	12	2,22 ± 0,08	12	5,65 ± 0,09
RK	RK-1	N1	12	2,23 ± 0,05	12	5,75 ± 0,17
RK	RK-2	N1	12	2,36 ± 0,06	12	5,92 ± 0,21
RK	RK-3	N1	5	2,48 ± 0,03	5	5,53 ± 0,10
NR	NR-1	N2	12	2,28 ± 0,07	12	5,49 ± 0,11
NR	NR-2	N2	12	2,19 ± 0,06	12	5,39 ± 0,24
NR	NR-3	N2	12	2,26 ± 0,07	12	5,45 ± 0,14
LS-BS-1		D1	36	2,16 ± 0,08 (B)	36	5,52 ± 0,28
LS-BS-2		D2	17	2,26 ± 0,09 (AB)	17	5,27 ± 0,58
LS-BS-3		D3	36	2,27 ± 0,08 (AB)	36	5,67 ± 0,19
RK		N1	29	2,33 ± 0,11 (A)	29	5,73 ± 0,23
NR		N2	36	2,24 ± 0,08 (AB)	36	5,45 ± 0,18

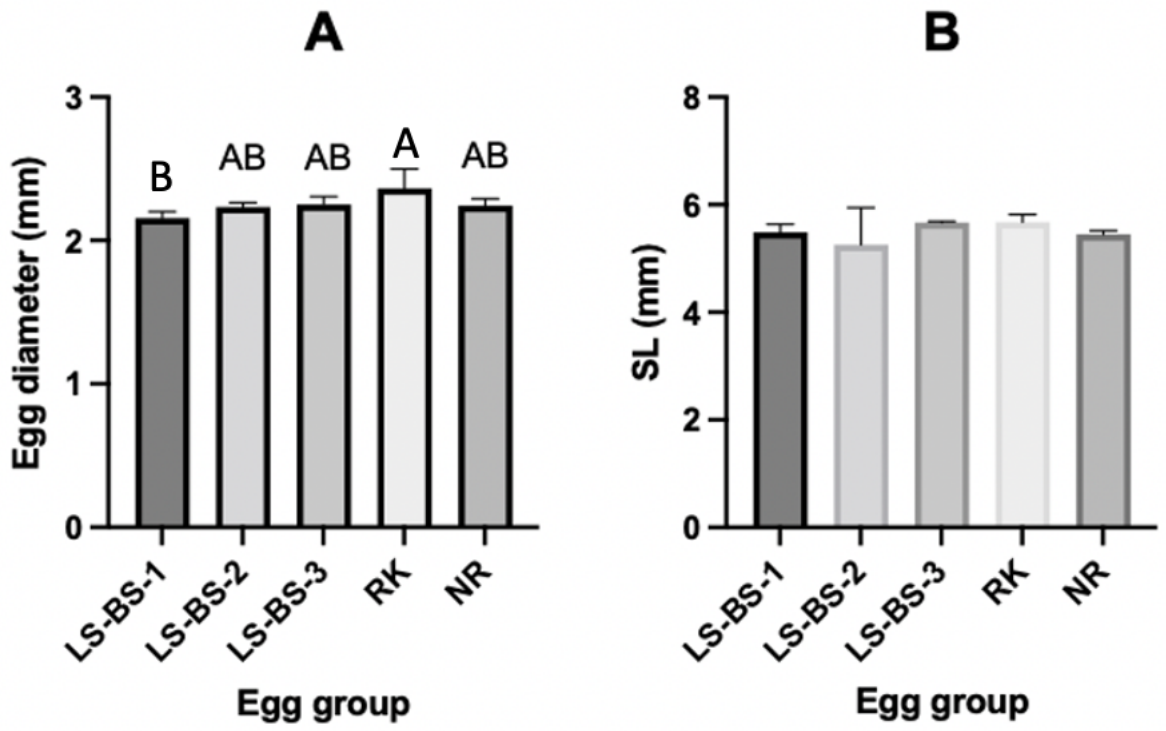


Figure 12: (A) Mean egg diameter (mm) of fertilized lumpfish eggs and (B) mean standard length (mm) of newly hatched lumpfish larvae between egg groups. Significant differences between egg groups are labeled with different letters (A & B). Egg groups with no significant difference are labeled with the same letters (AA or BB). Egg groups that are not significant from neither A or B are marked with AB. RK had a significantly higher mean egg diameter (mm) than LS-BS-1 (p-value = 0,022). No significant difference was observed between egg groups for standard length.

3.3 Fatty acid composition and egg quality

Overview of the fatty acid composition of the different egg groups is presented in Table 5. Eggs from the wild caught lumpfish had the highest % total lipid content compared to eggs retrieved from captive lumpfish, where the RK group had a significantly higher % total lipid content than LS-BS-1 and LS-BS-2. No significant difference between egg groups was found for saturated fatty acids (SFA). A greater content of mono unsaturated fatty acids (MUFA) was found in eggs from captive lumpfish, where LS-BS-2 had a significantly higher content of MUFA than RK. The amount of poly unsaturated n-6 fatty acids (PUFA n-6) was also greater in eggs from captive lumpfish than wild caught lumpfish, where the LS-BS groups had a significantly higher PUFA n-6 content than the RK and NR group. Eggs from NR and RK had a significantly higher content of poly unsaturated n-3 fatty acids (PUFA n-3) than LS-BS-2 and LS-BS-3. The DHA/EPA ratio was higher in eggs from captive lumpfish than eggs from wild caught lumpfish, but no significant difference between egg groups was found.

Eggs from captive lumpfish (LS-BS groups) had a higher content of arachidonic acid (C20:4n6, ARA) than eggs from wild caught lumpfish, where LS-BS-1 and LS-BS-2 had a significantly higher ARA content than the RK group. The eicosapentaenoic acid (C:20:5n3, EPA) content was significantly higher in eggs from wild caught lumpfish, and RK and NR had a significantly higher EPA content than the LS-BS groups. Eggs from the wild caught lumpfish also had a higher content of docosahexanoic acid (C22:6n3, DHA) than the LS-BS-groups, where RK had a significantly higher DHA content than the LS-BS groups.

Data for the egg weight (g) for all egg batches (Table 10), the distribution of % saturated fatty acids (Table 11), % mono unsaturated fatty acid (Table 12), % total lipid content (Table 14) and % poly unsaturated fatty acids (Table 13) for unfertilized lumpfish eggs are presented in Appendix B.

Table 5: Fatty acid profile of unfertilized lumpfish eggs presented as % of total Fatty Acid Methyl Esters (FAME) content from captive (LS-BS-1, LS-BS-2 and LS-BS-3) and wild caught (RK and NR) lumpfish females. Data are expressed as mean ± SD. Significant differences between egg groups are labeled with different letters (A & B). Egg groups with no significant differences are labeled with the same letters (AA or BB). Egg groups that are not significantly different from neither A or B are marked with AB. Data for all egg batches are presented in tables in Appendix B.

Fatty acid	Name	LS-BS-1	LS-BS-2	LS-BS-3	RK	NR
C14:0	Myristic acid	1,13 ± 0,15	1,08 ± 0,05	1,07 ± 0,46	1,38 ± 0,35	1,63 ± 0,25
C14:1n5	Myristoleic acid	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
C15:0	Pentadecanoic acid	0,30 ± 0,00	0,25 ± 0,06	0,27 ± 0,12	0,36 ± 0,05	0,30 ± 0,00
C16:0	Palmitic acid	17,53 ± 0,40 (A)	17,48 ± 0,59 (A)	16,12 ± 0,79 (AB)	16,12 ± 0,36 (B)	15,90 ± 0,20 (B)
C16:1n7	Palmitoleic acid	2,23 ± 0,21 (A)	2,23 ± 0,13 (A)	1,80 ± 0,53 (A)	1,22 ± 0,08 (B)	1,43 ± 0,15 (B)
C17:0	Heptadecanoic acid	0,30 ± 0,00	0,30 ± 0,00	0,37 ± 0,21	0,48 ± 0,08	0,43 ± 0,06
C17:1n7	Heptadecanoic acid	2,23 ± 0,06	0,40 ± 0,00	0,40 ± 0,00	1,22 ± 0,05	0,33 ± 0,06
C18:0	Stearic acid	3,33 ± 2,46	5,0 ± 0,22	5,00 ± 0,50	4,74 ± 0,34	4,50 ± 0,10
C18:1n7	Vaccenic acid	4,27 ± 0,15	4,48 ± 1,50	4,23 ± 1,50	4,23 ± 0,69	2,90 ± 0,36
C18:1n9	Oleic acid	19,43 ± 0,93 (AB)	20,23 ± 0,98 (AB)	20,90 ± 2,98 (A)	17,60 ± 0,17 (B)	17,80 ± 0,46 (AB)
C18:2n6	Linoleic acid	5,67 ± 0,15 (AB)	6,58 ± 0,26 (A)	7,43 ± 5,51 (A)	1,04 ± 0,09 (B)	1,17 ± 0,06 (B)
C18:3n3	α-linoleic acid	0,67 ± 0,06	0,68 ± 0,05	0,80 ± 0,10	0,62 ± 0,20	0,70 ± 0,17
C18:3n6	ϒ-Linoleic acid	0,10 ± 0,00	0,13 ± 0,05	0,13 ± 0,06	0,16 ± 0,05	0,13 ± 0,06
C18:4n3	Stearidonic acid	0,60 ± 0,10 (B)	0,60 ± 0,00 (B)	0,63 ±± 0,23 (B)	0,98 ± 0,16 (A)	0,97 ± 0,15 (A)
C20:0	Arachidic acid	0,10 ± 0,00	0,10 ± 0,00	0,10 ± 0,00	0,10 ± 0,00	0,10 ± 0,00
C20:1n9	Gondoic acid	2,60 ± 0,10 (AB)	2,15 ± 0,19 (AB)	1,80 ± 0,70 (A)	2,56 ± 0,38 (B)	2,97 ± 0,21 (AB)
C20:2n6		0,30 ± 0,00 (AB)	0,30 ± 0,00 (AB)	0,33 ± 0,06 (AB)	0,26 ± 0,05 (B)	0,30 ± 0,10 (A)
C20:3n3	Eicosatrienoic acid	0,10 ± 0,00	0,10 ± 0,00	0,13 ± 0,06	0,14 ± 0,05	0,13 ± 0,06
C20:4n3		1,13 ± 0,06	1,00 ± 0,08	1,03 ± 0,21	1,12 ± 0,22	1,17 ± 0,21
C20:4n6 (ARA)	Arachidonic acid (ARA)	0,83 ± 0,06 (A)	0,85 ± 0,06 (A)	0,73 ± 0,06 (AB)	0,66 ± 0,09 (B)	0,70 ± 0,00 (AB)
C20:5n3 (EPA)	Eicosapentaenoic acid (EPA)	13,63 ± 0,68 (B)	13,10 ± 0,59 (B)	13,07 ± 3,58 (B)	17,66 ± 0,86 (A)	18,10 ± 0,78 (A)
C22:0	Behenic acid	0,10 ± 0,00	0,10 ± 0,00	0,07 ± 0,06	0,10 ± 0,00	0,10 ± 0,00
C22:1n9	Eruric acid	0,17 ± 0,12	0,10 ± 0,00	0,07 ± 0,12	0,12 ± 0,04	0,20 ± 0,00
C22:5n3 (DPA)	Docosapentaenoic acid (DPA)	2,37 ± 0,40 (A)	2,18 ± 0,22 (A)	1,87 ± 0,40 (AB)	1,54 ± 0,11 (B)	1,60 ± 0,10 (B)
C22:5n6		0,30 ± 0,00	0,40 ± 0,00	0,30 ± 0,00	0,30 ± 0,00	0,30 ± 0,00
C22:6n3 (DHA)	Docosahexanoic acid (DHA)	20,90 ± 0,70 (B)	20,28 ± 0,38 (B)	20,40 ± 6,53 (B)	27,22 ± 1,35(A)	26,00 ± 0,95 (AB)
C24:1	Nervonic acid	0,13 ± 0,12	0,25 ± 0,06	0,23 ± 0,06	0,20 ± 0,00	0,23 ± 0,06
Sum SFA	Saturated fatty acid	22,79 ± 6,40	24,31 ± 6,42	23,08 ± 5,93	23,28 ± 5,98	22,96 ± 5,78
Sum MUFA	Mono unsaturated fatty acid	31,06 ± 6,46 (AB)	29,84 ± 6,84 (A)	29,43 ± 7,10 (AB)	27,15 ± 5,92 (B)	25,86 ± 6,01 (AB)
Sum PUFA(n-6)		7,20 ± 0,17 (A)	8,25 ± 0,33 (A)	8,93 ± 5,52 (A)	2,42 ± 0,18 (B)	2,70 ± 0,00 (B)
Sum PUFA(n-3)		39,40 ± 1,15 (AB)	37,93 ± 0,5 (B)	37,93 ± 10,11 (B)	49,80 ± 1,45 (A)	48,67 ± 0,31 (A)
(n-3)/(n-6)		0,18 ± 0,01 (AB)	0,22 ± 0,01 (A)	0,27 ± 0,19 (A)	0,05 ± 0,00 (B)	0,06 ± 0,00 (B)
Sum DHA/EPA		1,53 ± 0,09	1,54 ± 0,09	1,56 ± 0,09	1,54 ± 0,18	1,43 ± 0,10
% total lipid		2,99 ± 0,59 (B)	2,96 ± 0,45 (B)	3,98 ± 0,87 (AB)	5,28 ± 0,41 (A)	4,14 ± 0,68 (AB)

There was a positive correlation between the fertilization success in fertilized lumpfish eggs and the DHA content (r = 0,68, p-value < 0.05) and EPA content (r = 0,68, p-value = 0,05) in the unfertilized lumpfish egg. On the other hand, a negative correlation between the fertilization success and the ARA content (r = -0,59, p-value $<$ 0,05) was found. A higher DHA content resulted in a higher fertilization success. NR had the highest DHA content and fertilization success compared to the other egg groups (Figure 13). Eggs with a higher content of EPA resulted in a higher fertilization success (Figure 14). NR and RK seemed to have the highest % fertilization and EPA content compared to the other egg groups. A lower ARA content resulted in a higher % fertilization (Figure 15). NR had the lowest ARA content in the eggs and the highest fertilization success. No correlation between % fertilization and % total lipid content in the eggs was found.

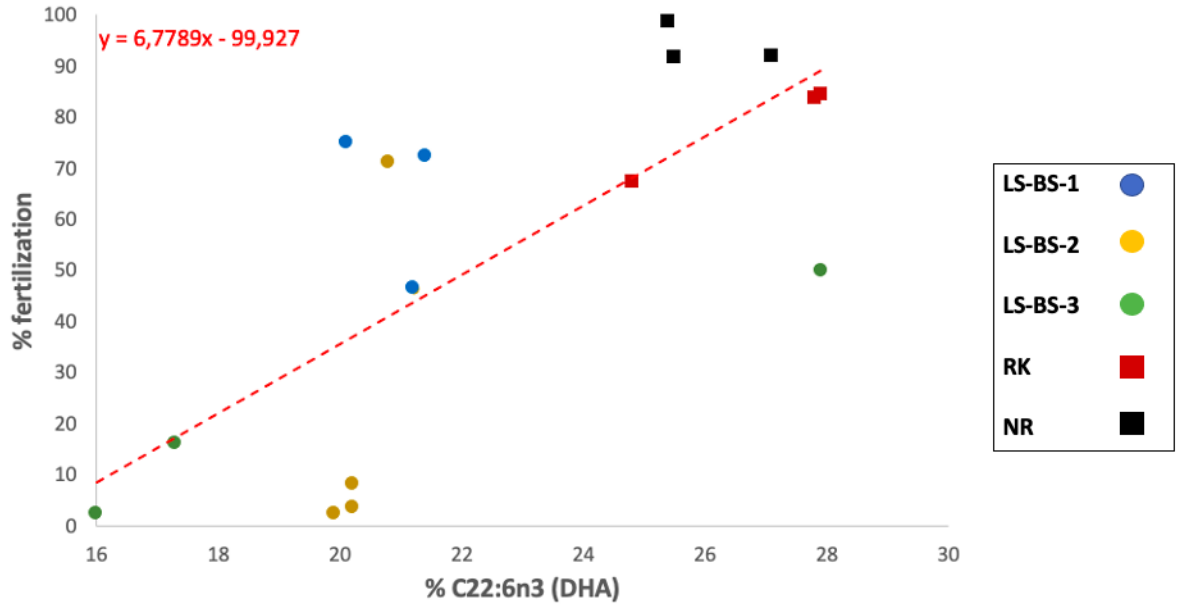


Figure 13: Correlation between the fertilization success in fertilized lumpfish eggs and % DHA content in unfertilized lumpfish eggs. From the Spearman correlation test there was a positive correlation between % fertilization and % DHA (p-value $< 0{,}05$, r $= 0{,}71$, $\alpha = 0,05$).

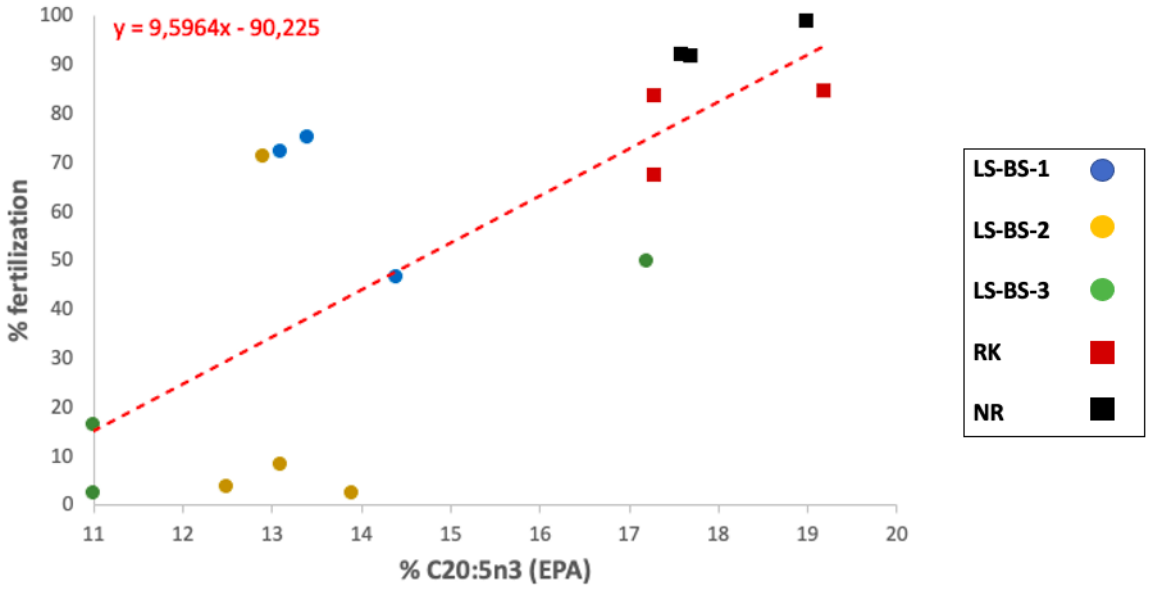


Figure 14: Correlation between the fertilization success in fertilized lumpfish eggs and % EPA content in unfertilized lumpfish eggs. From the Spearman correlation test there was a positive correlation between % fertilization and % EPA (, p-value $< 0{,}05$, r $= 0{,}68$,$\alpha = 0,05$).

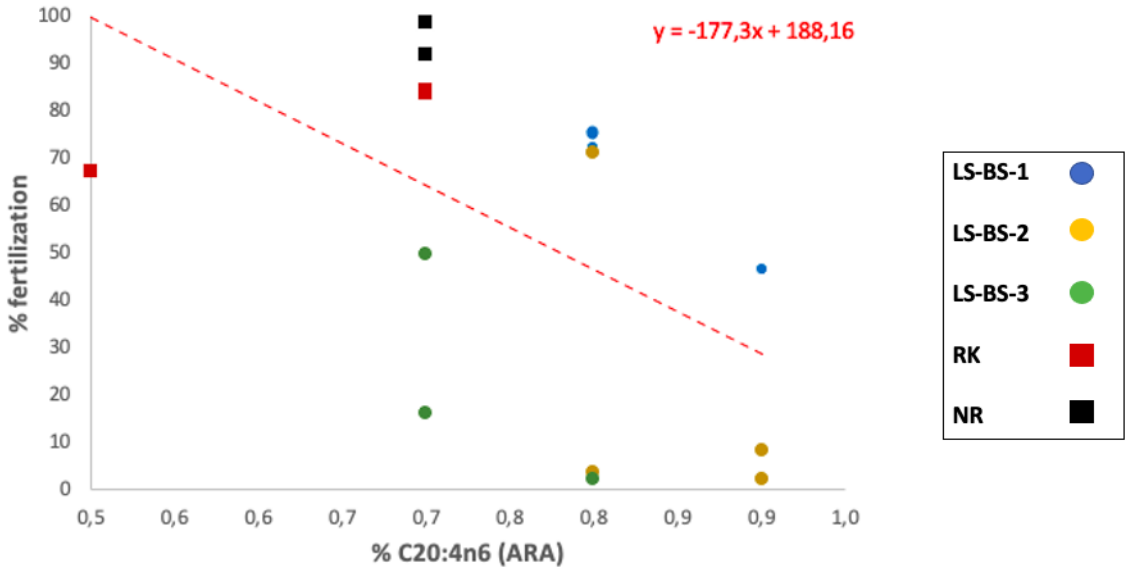


Figure 15: Correlation between the fertilization success in fertilized lumpfish eggs and % ARA content in unfertilized lumpfish eggs. From the Spearman correlation test there was a negative correlation between % fertilization and % ARA (p-value $<$ 0,05, r = -0,59, $\alpha = 0,05$).

There was a positive correlation between the hatching success in fertilized lumpfish eggs and the DHA content (r=0,62) and a negative correlation between the hatching success and the ARA content (r = -0,53) in the unfertilized lumpfish eggs. A higher DHA content in the eggs resulted in a higher hatching success (Figure 16). A lower content of ARA in the eggs resulted in a higher hatching success (Figure 17). From the Spearman correlation test, no correlation between the hatching success and the EPA content was found nor any correlation between the hatching success and % total lipid content. No correlations between the egg diameter and EPA, DHA or ARA content in the unfertilized eggs were found.

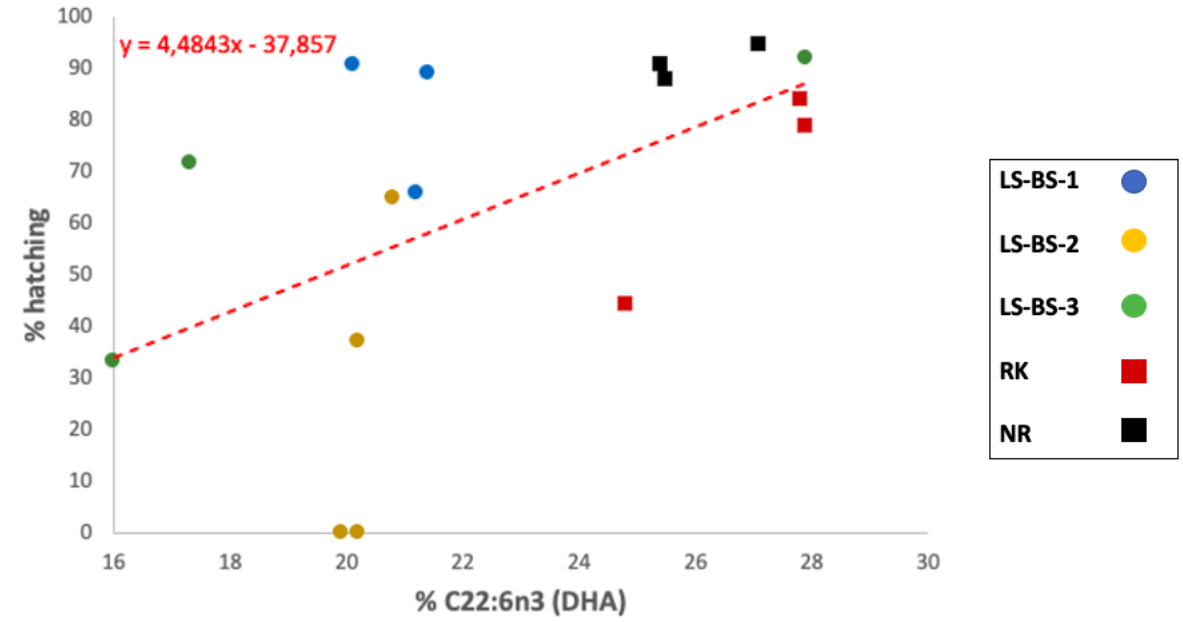


Figure 16: Correlation between the hatching success in fertilized lumpfish eggs and % DHA content in unfertilized lumpfish eggs. From the Spearman correlation test there was a positive correlation between % hatching and % DHA (p-value $<$ 0,05, r = 0,62, $\alpha = 0,05$).

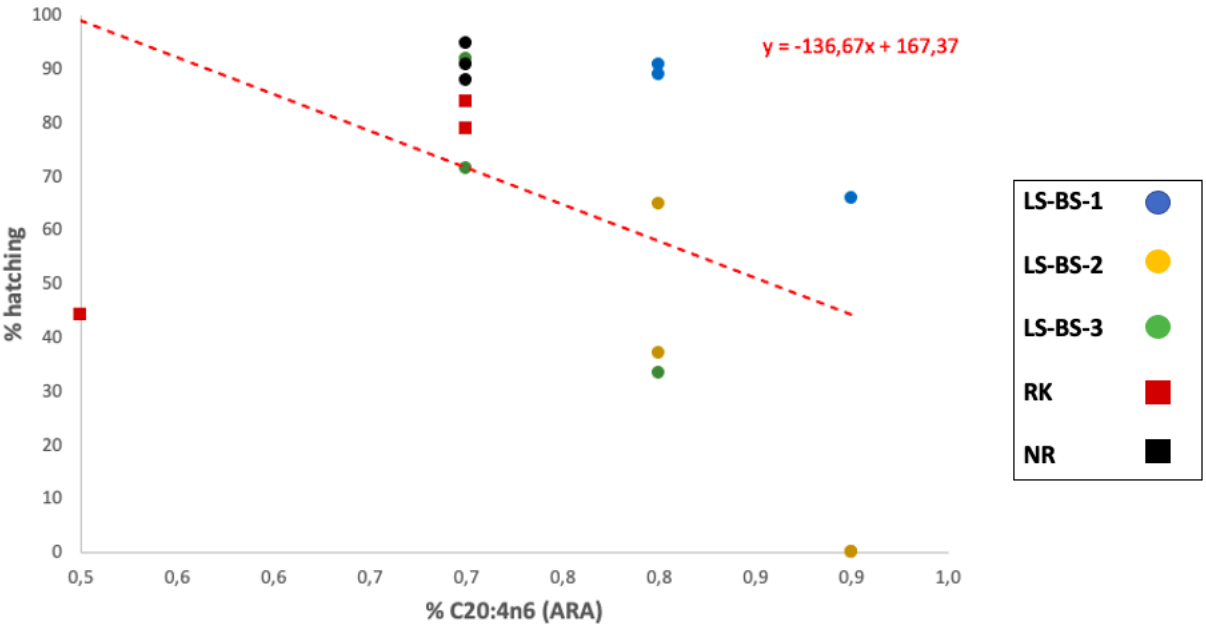


Figure 17: Correlation between the hatching success in fertilized lumpfish eggs and % ARA in unfertilized lumpfish eggs. From the Spearman correlation test there was a negative correlation between % hatching and % ARA (p-value $<$ 0,05, r = -0,53, $\alpha = 0,05$).

3.4 Bone analyses

3.4.1 Ossification score of vertebrae, suction disk and gills

Larvae from the NR egg group had the highest mean Vertebrae score compared to the other egg groups, and LS-BS-2 had the lowest. The highest mean Suction disk score was found in larvae from egg group LS-BS-1, and LS-BS-2 had the lowest score. LS-BS-2 had two egg batches (LS-BS-2-2 and LS-BS-2-5) where LS-BS-2-2 had less larvae than LS-BS-2-5. Therefore the SD for LS-BS-2 is larger than the other egg groups for both the Vertebrae score and Suction disk score. The Gill score was similar for all egg groups. No significant difference was found between egg groups for neither the Vertebrae score, Suction disk score or Gill score. Overviews of the mean Vertebrae score, Suction disk score and Gill score for the different egg groups are presented in Figure 18 and Table 6.

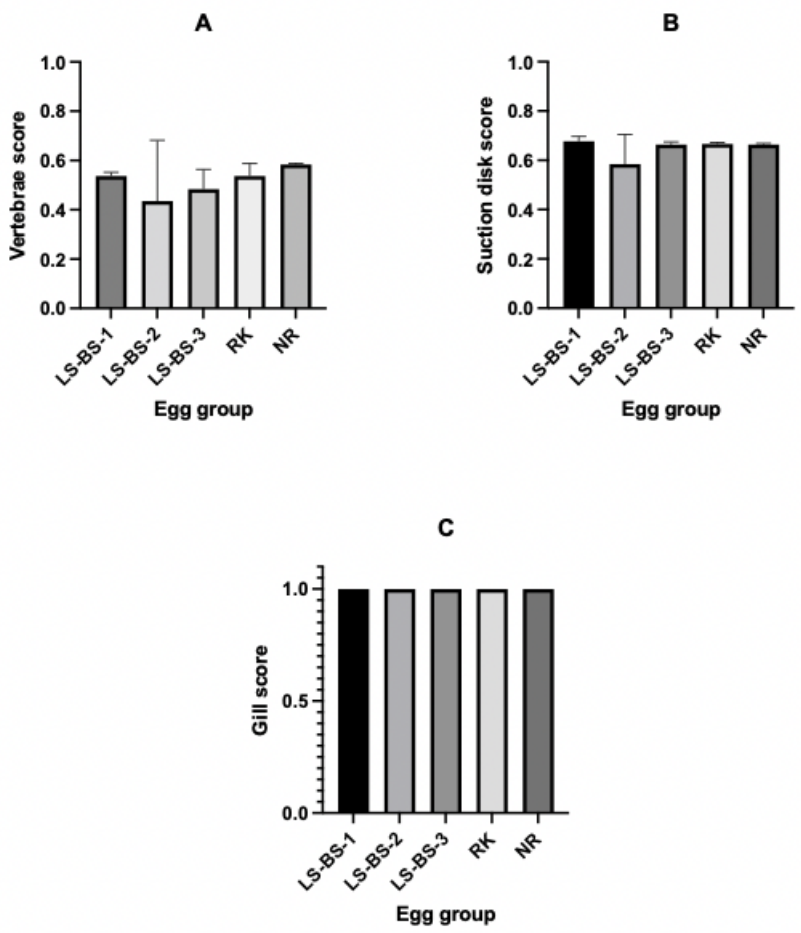


Figure 18: Ossification score of (A) vertebrae segments, (B) suction disk and (C) gill arches observed in newly hatched lumpfish larvae between different egg groups. There was a varying amount of larvae from each egg group, and the data are expressed as the mean number. No significant differences between egg groups were observed.

Table 6: Ossification score of vertebrae, suction disk and gills of newly hatched lumpfish larvae between different egg groups and egg batches retrieved from captive (LS-BS-1, LS-BS-2 and LS-BS-3) and wild caught (RK and NR) lumpfish females. Egg groups represent larvae from lumpfish the same diet type. Egg batches correspond to larvae retrieved from different lumpfish fed the same diet. The score is from 0-1 where 0 is the lowest score and 1 is the highest. No significant differences between egg groups were found.

Egg group	Egg batch	Female	Diet type	Amount of larvae	Vertebrae score	Suction disk score	Gill score
	LS-BS-1-1	1		12	0,52 ± 0,03	0,70 ± 0,10	1,00 ± 0,00
LS-BS-1	LS-BS-1-2	2	D1	13	0,54 ± 0,04	0,66 ± 0,02	1,00 ± 0,00
	LS-BS-1-3	3		15	0,55 ± 0,04	0,67 ± 0,00	1,00 ± 0,00
LS-BS-2	LS-BS-2-2	1	D2	5	0,26 ± 0,04	0,50 ± 0,00	1,00 ± 0,00
	LS-BS-2-5	2		14	0,61 ± 0,04	0,67 ± 0,00	1,00 ± 0,00
	LS-BS-3-1	1		15	0,39 ± 0,016	0,65 ± 0,04	1,00 ± 0,00
LS-BS-3	LS-BS-3-2	2	D3	15	0,53 ± 0,10	0,67 ± 0,00	1,00 ± 0,00
	LS-BS-3-4	3		15	0,53 ± 0,00	0,67 ± 0,00	1,00 ± 0,00
	RK-1	1		14	0,53 ± 0,01	0,67 ± 0,01	1,00 ± 0,00
RK	RK-2	2	N1	15	0,49 ± 0,01	0,66 ± 0,01	1,00 ± 0,00
	RK-3	3		5	0,59 ± 0,00	0,67 ± 0,00	1,00 ± 0,00
	NR-1	1		13	0,58 ± 0,02	0,66 ± 0,02	1,00 ± 0,00
NR	NR-2	2	N2	12	0,58 ± 0,03	0,66 ± 0,04	1,00 ± 0,00
	NR-3	3		15	0,59 ± 0,03	0,67 ± 0,00	1,00 ± 0,00
LS-BS-1			D1	40	0,54 ± 0,04	0,68 ± 0,00	1,00 ± 0,00
LS-BS-2			D2	19	0,52 ± 0,16	0,63 ± 0,01	1,00 ± 0,00
LS-BS-3			D3	45	0,48 ± 0,02	0,66 ± 0,00	1,00 ± 0,00
RK			N1	34	0,52 ± 0,00	0,67 ± 0,00	1,00 ± 0,00
NR			N2	40	0,58 ± 0,00	0,66 ± 0,00	1,00 ± 0,00

3.4.2 Ossification score of fins

The highest mean number of larvae with ossified 1. dorsal fin segments was observed in the NR and RK groups. Egg group LS-BS-3 had the lowest mean number of larvae with ossified 1. dorsal fin segments. Larvae from the NR egg group had more ossified 2. dorsal fin segments than the other egg groups, and larvae from egg group LS-BS-3 had the lowest number of ossified 2. dorsal fin segments. The highest mean number of larvae with ossified pelvic fin segments was found in egg group LS-BS-2, and the lowest mean number was found in egg group LS-BS-3. Larvae from the NR group had more ossified caudal fin segments than the other egg groups, while larvae from LS-BS-2 had less ossified caudal fin segments. Overview of the mean number of larvae (%) with ossified 1. dorsal fin, 2. dorsal fin, pelvic fin and caudal fin is presented in Figure 19 and Table 7.

There was a varying amount of larvae from egg group LS-BS-2, where egg batch LS-BS-2-2 had 5 larvae and LS-BS-2-5 had 14 larvae. In addition there was a lower number of larvae in LS-BS-2 than the other egg groups, which resulted in a larger SD than the other egg groups. No significant differences between the egg groups were observed for the ossification of the 1. dorsal fin, 2. dorsal fin, pelvic fin or caudal fin.

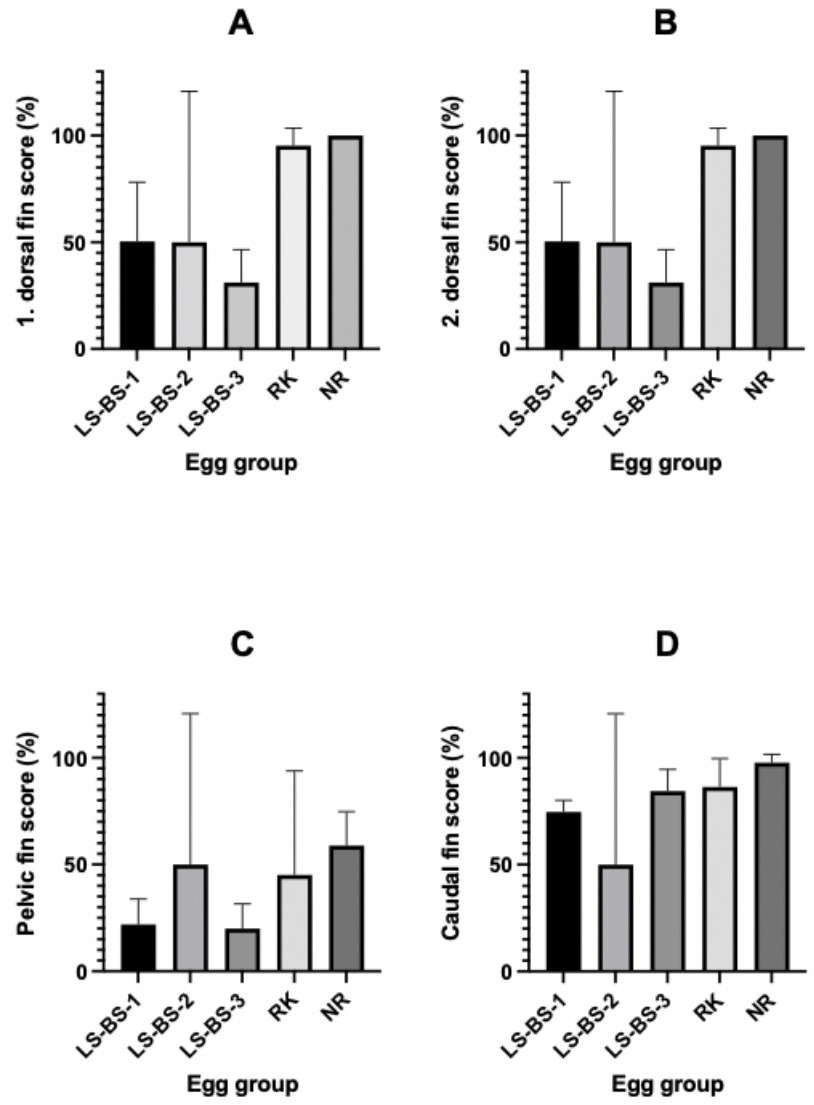


Figure 19: Ossification score of fins in newly hatched larvae retrieved from captive (LS-BS-1, LS-BS-2 and LS-BS-3) and wild caught (RK and NR) lumpfish females. Data are expressed as mean number (%) of newly hatched lumpfish larvae with ossified segments in (A) the 1. dorsal fin, (B) 2. dorsal fin, (C) pelvic fin and (D) caudal fin. Number of ossified segments and staining saturation in each fin are not taken into account, but the number of larvae observed with staining in the respective fins was counted. There were no significant differences between egg groups.

Table 7: Overview of egg groups, egg batches, diet type, amount of larvae used for measuring standard length (mm) and the mean number of larvae (%) with ossified segments in the 1. dorsal fin, 2. dorsal fin, pelvic fin and caudal fin ± SD. Egg groups represent larvae retrieved from lumpfish fed same diet type. Egg batches correspond to larvae retrieved from different lumpfish fed the same diet. The amount of ossified segments in each fin and the staining saturation are not taken into account, but the number of larvae observed with staining in the different fin types was counted. No significant differences between egg groups for the different fin types were found.

Egg group	Egg batch	Female	Diet type	Amount of larvae	Mean SL (mm) ± SD	Mean (%) 1.dorsal fin Score ± SD	Mean (%) 2.dorsal fin Score ± SD	Mean (%) pelvic fin score ± SD	Mean (%) caudal fin score ± SD
LS-BS-1	LS-BS-1-1	1	D1	12	5,3 ± 0,3	25 ± 50	17 ± 40	8 ± 30	75 ± 50
LS-BS-1	LS-BS-1-2	2	D1	13	5,6 ± 0,3	46 ± 50	54 ± 50	31 ± 50	69 ± 50
LS-BS-1	LS-BS-1-3	3	D1	15	5,5 ± 0,2	80 ± 40	80 ± 40	27 ± 50	80 ± 50
LS-BS-2	LS-BS-2-2	1	D2	5	4,8 ± 0,3	0 ± 00	0 ± 0	0 ± 0	0 ± 0
LS-BS-2	LS-BS-2-5	2	D2	14	5,7 ± 0,3	100 ± 00	100 ± 0	100 ± 0	100 ± 0
LS-BS-3	LS-BS-3-1	1	D3	15	5,6 ± 0,2	40 ± 50	40 ± 50	33 ± 50	73 ± 50
LS-BS-3	LS-BS-3-2	2	D3	15	5,7 ± 0,2	40 ± 50	27 ± 50	13± 40	87 ± 40
LS-BS-3	LS-BS-3-4	3	D3	15	5,7 ± 0,2	13 ± 40	13 ± 50	13 ± 40	93 ± 30
RK	RK-1	1	N1	14	5,7 ± 0,2	86 ± 40	36 ± 50	29 ± 50	86 ± 40
RK	RK-2	2	N1	15	5,8 ± 0,3	100 ± 0	13 ± 40	7 ± 30	73 ± 50
RK	RK-3	3	N1	5	5,5 ± 0,1	100 ± 0	100 ± 0	100 ± 0	100 ± 0
NR	NR-1	1	N2	13	5,5 ± 0,1	100 ± 0	77 ± 40	62 ± 50	100 ± 0
NR	NR-2	2	N2	12	5,4 ± 0,2	100 ± 0	75 ± 50	42 ± 50	100 ± 0
NR	NR-3	3	N2	15	5,5 ± 0,3	100 ± 0	73 ± 50	73 ± 50	93 ± 30
LS-BS-1			D1	40	5,5 ± 0,3	53 ± 23	53 ± 17	23 ± 9	75 ± 5
LS-BS-2			D2	19	5,5 ± 0,5	74 ± 44	74 ± 44	74 ± 44	74 ± 44
LS-BS-3			D3	45	5,7 ± 0,2	31 ± 13	27 ± 11	20 ± 9	84 ± 8
RK			N1	34	5,7 ± 0,3	94 ± 7	35 ± 29	29 ± 31	82 ± 26
NR			N2	30	5,5 ± 0,2	100 ± 0	75 ± 2	60 ± 13	98 ± 3

3.4.3 Deformities

Deformities in newly hatched lumpfish larvae were observed in all egg groups (Figure 20, Table 8). The captive lumpfish (LS-BS-1, LS-BS-2 and LS-BS-3) had the highest number of normal larvae, while larvae from the wild caught lumpfish (RK and NR) had a lower number of normal larvae (Figure 20A). Egg group LS-BS-3 had a significantly higher number of normal larvae than the RK group (p-value $<$ 0,05).

The axial deformity that was observed in the newly hatched lumpfish larvae was lordosis (V-shape of the spine). Axial deformities were observed in newly hatched in all egg groups, except LS-BS-2 (Figure 20B). Larvae from the RK egg group had more axial deformities than the other egg groups, and LS-BS-2 had the lowest number of larvae with axial deformities. Stunted or shorter neural arches in the vertebrae were categorized as vertebral deformities. Larvae with vertebral deformities were only observed in egg group and LS-BS-1 and LS-BS-2, where LS-BS-1 had the highest number of larvae with vertebral deformities (Figure 20C). Larvae with craniofacial deformities had an underdeveloped jaw and were only observed in egg group RK and NR, see Figure 20D. No significant differences between egg groups regarding axial, vertebral or craniofacial deformities were found. The large standard deviations for the different egg groups regarding deformity types are due to an uneven amount of larvae, see Table 8. Some egg groups and egg batches had more larvae than the others, which influenced the standard deviation.

No correlations between the number of normal larvae (%) and the EPA, ARA or DHA content in the unfertilized eggs were found. There was not found a correlation between the egg diameter (mm) and number of normal larvae (%), nor any correlation between the number of normal larvae (%) and standard length (mm).

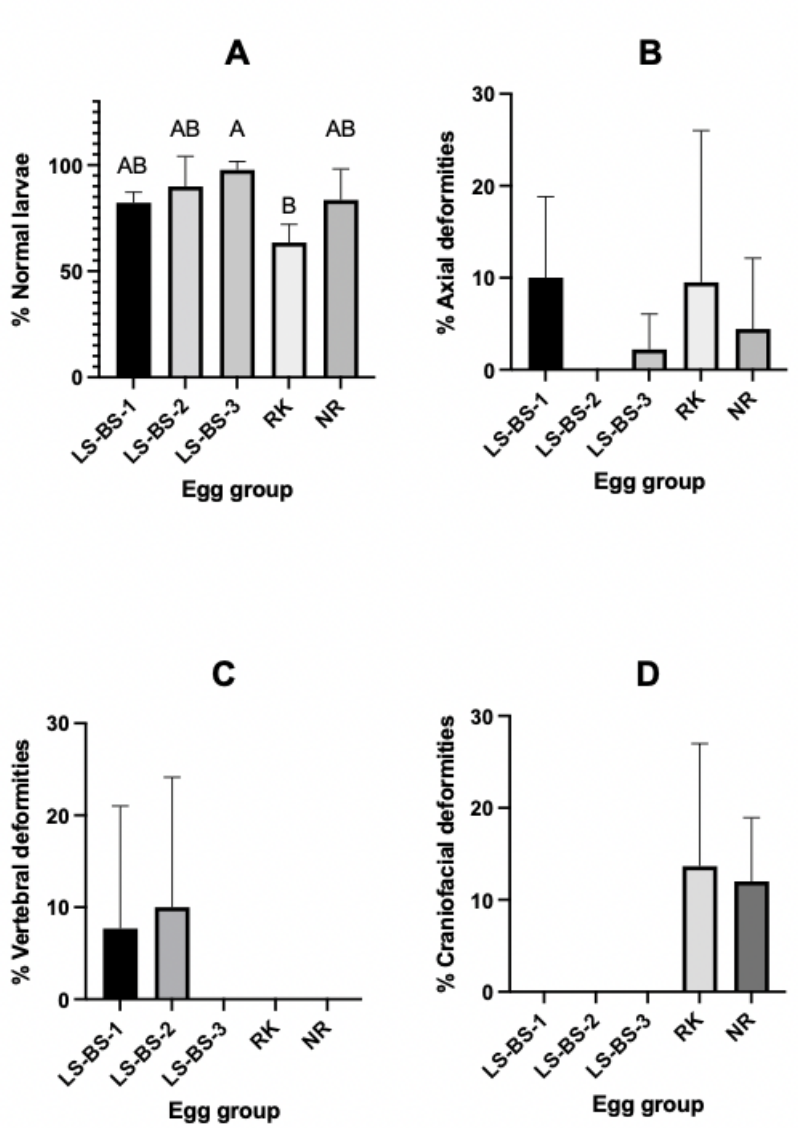


Figure 20: (A) Mean number (%) of normal newly hatched larvae in the different egg groups. Significant differences between egg groups are labeled with different letters (A & B). Egg groups with no significant differences are labeled with the same letters (AA or BB). Egg groups that are not significant from either group A or B are marked with AB. (B) The axial deformity that was observed in the newly hatched lumpfish larvae was lordosis (V-shape of the spine). (C) Stunted or shorter neural arches in the vertebrae were categorized as vertebral deformities. (D) Larvae with craniofacial deformities had an underdeveloped jaw. No significant differences between egg groups were found for axial, vertebral or craniofacial deformities.

Table 8: Overview of egg groups, egg batches, diet type and mean number (%) of newly hatched lumpfish larvae observed with no or different deformities ± SD. Egg groups represent larvae from lumpfish fed the same diet type. Egg batches correspond to larvae retrieved from different lumpfish fed the same diet. Axial deformities that were observed was lordosis (V-shape of the spine). Stunted or shorter neural arches in the vertebrae were categorized as vertebral deformities, and larvae with craniofacial deformities had an underdeveloped jaw. Significant differences between egg groups are labeled with different letters (A & B). Egg groups with no significant differences are labeled with the same letters (AA or BB). Egg groups that are not significantly different from neither A or B are marked with AB.

Egg group	Egg batch	Female	Diet type	Amount of larvae	Mean number of normal larvae (%) ± SD	Axial deformities (%) ± SD	Vertebral deformities (%) ± SD	Craniofacial deformities (%) ± SD
LS-BS-1	LS-BS-1-1	1	D1	12	83 ± 39	17 ± 39	0 ± 0	0 ± 0
LS-BS-1	LS-BS-1-2	2	D1	13	77 ± 44	0 ± 0	23 ± 44	0 ± 0
LS-BS-1	LS-BS-1-3	3	D1	15	87 ± 35	13 ± 35	0 ± 0	0 ± 0
LS-BS-2	LS-BS-2-2	1	D2	5	80 ± 45	0 ± 0	20 ± 45	0 ± 0
LS-BS-2	LS-BS-2-5	2	D2	14	100 ± 0	0 ± 0	0 ± 0	0 ± 0
LS-BS-3	LS-BS-3-1	1	D3	15	93 ± 26	26 ± 0	0 ± 0	0 ± 0
LS-BS-3	LS-BS-3-2	2	D3	15	100 ± 0	0 ± 0	0 ± 0	0 ± 0
LS-BS-3	LS-BS-3-4	3	D3	15	100 ± 0	0 ± 0	0 ± 0	0 ± 0
RK	RK-1	1	N1	14	57 ± 51	29 ± 47	0 ± 0	14 ± 36
RK	RK-2	2	N1	15	73 ± 46	0 ± 0	0 ± 0	27 ± 46
RK	RK-3	3	N1	5	60 ± 55	0 ± 55	0 ± 0	0 ± 0
NR	NR-1	1	N2	13	92 ± 28	0 ± 0	0 ± 0	8 ± 28
NR	NR-2	2	N2	12	92 ± 29	0 ± 0	0 ± 0	8 ± 29
NR	NR-3	3	N2	15	67 ± 49	13 ± 35	0 ± 0	20 ± 41
LS-BS-1			D1	40	83 ± 40 (AB)	10 ± 31	8 ± 27	0 ± 0
LS-BS-2			D2	19	95 ± 25 (AB)	0 ± 0	5 ± 25	0 ± 0
LS-BS-3			D3	45	98 ± 15 (A)	2 ± 15	0 ± 0	0 ± 0
RK			N1	34	65 ± 50 (B)	12 ± 39	0 ± 0	18 ± 39
NR			N2	30	83 ± 39 (AB)	5 ± 22	0 ± 0	13 ± 34

3.5 Summary of findings

Summary of the different analyses are presented in Figure 21. Egg groups colored green have a higher score than egg groups colored red and yellow. Eggs belonging to the wild caught lumpfish (NR and RK) had the highest fertilization and hatching success compared to eggs retrieved from the captive lumpfish (LS-BS-1, LS-BS-2 and LS-BS-3). Eggs from the NR group had a significantly higher fertilization and hatching success than LS-BS-2. Eggs and larvae from RK had the largest egg diameter and standard length (mm) compared to the other egg groups, where RK had a significantly larger egg diameter than the LS-BS-1 group.

Eggs from wild caught lumpfish had the highest % total lipid content than eggs from captive lumpfish, where the RK group had a significantly higher % total lipid content than LS-BS-1 and LS-BS-2. The highest % content of MUFA was found in eggs from captive lumpfish, where LS-BS-1 had the highest MUFA content. Egg group LS-BS-2 had a significantly higher content of MUFA than the RK group. Eggs from captive lumpfish had a significantly higher content of PUFA(n-6) fatty acids than eggs from wild caught lumpfish. On the other hand, eggs from the RK and NR group had a significantly higher content of PUFA(n-3) fatty acids than LS-BS-2 and LS-BS-3. The (n-3)/(n-6) ratio was higher in eggs from captive lumpfish, where LS-BS-2 and LS-BS-3 had a significantly higher (n-3)/(n-6) ratio than RK and NR. The EPA and DHA content were higher in eggs from wild caught lumpfish compared to captive lumpfish. RK and NR had a significantly higher content of EPA than the LS-BS groups. RK also had a significantly higher DHA content than the LS-BS-groups. Since the fertilization and hatching success were positively correlated with higher levels of EPA and DHA, eggs with a higher EPA and DHA content were given a higher score and are colored green. On the other hand, a higher content of ARA was found in eggs from captive lumpfish compared to eggs from the wild caught lumpfish. The fertilization and hatching success were negatively correlated with a higher ARA content in the eggs, and thus egg groups with a lower ARA content were given a higher score and are colored green.

No significant differences between egg groups were found for the ossification score of the vertebrae, suction disk, gills or different fin types. However, larvae from the NR group seem to have more ossified vertebralsegments and fins than larvae from the other egg groups. Captive lumpfish had a greater proportion of normal larvae (%) compared to larvae from the wild caught lumpfish, where LS-BS-3 had a significantly higher proportion of normal larvae (%) than the RK group.

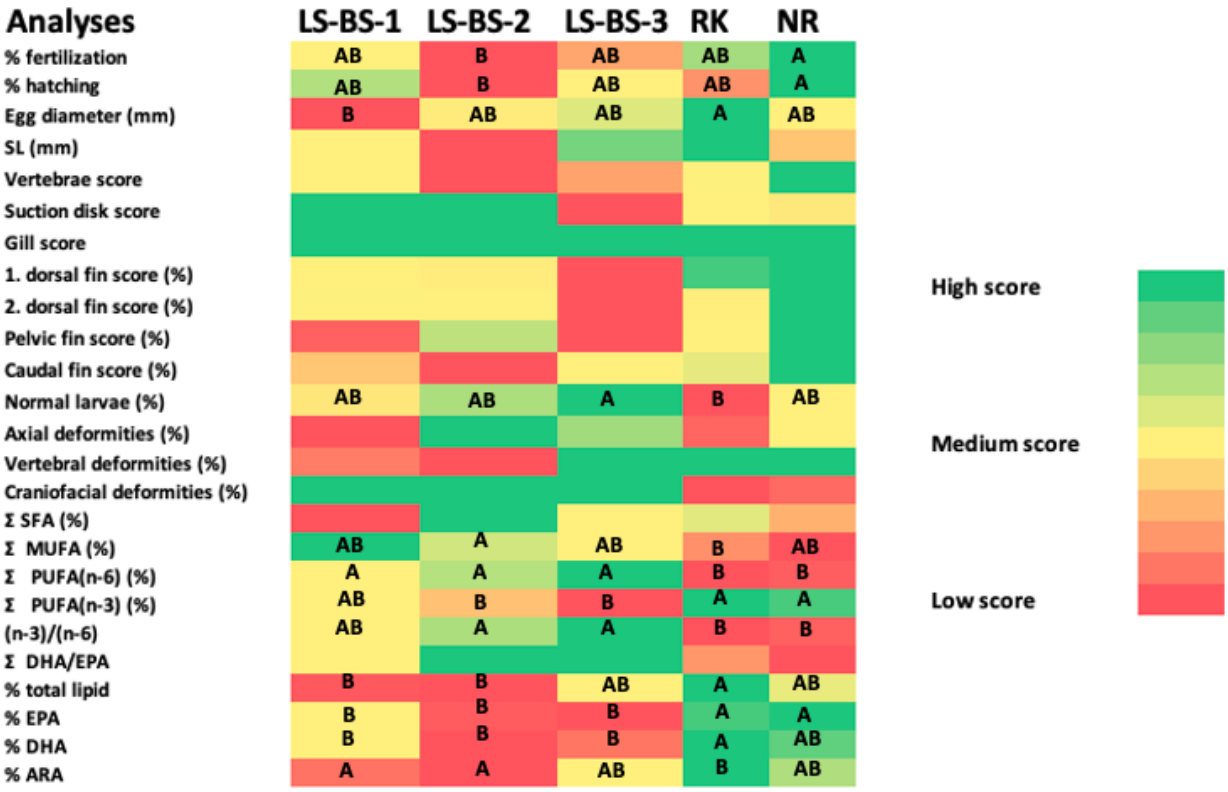


Figure 21: Summary of the different analyses presented in a Heat map. The scale is measured from Low score (red), Moderate score (yellow and orange) to High score (green), where egg groups with a High score are more desirable than egg groups with a Moderate or Low score. Significant differences between egg groups are labeled with different letters (A & B). Egg groups with no significant differences are labeled with the same letters (AA or BB). Egg groups that are not significantly different from neither A or B are marked with AB.

4 DISCUSSION

4.1 Egg quality of wild caught VS captive lumpfish eggs

In this master project the overall egg quality was better in wild caught lumpfish than captive lumpfish, even though the captive lumpfish were fed broodstock diets with a varying lipid and carbohydrate content. A higher fertilization and hatching success were found in eggs from wild caught lumpfish than captive lumpfish, where the NR group had a significantly higher fertilization and hatching success than the LS-BS-2 group. Wild lumpfish females tend to spawn from February and onwards in the spring, and the fertilization success for wild caught lumpfish tend to be above 90 % (Brown, Somerton, Methven, & Watkins, 1992; Imsland et al., 2019). Since the female lumpfish used in this experiment were strip-spawned in October (NR group), November (RK group) and February (LS-BS-1, LS-BS-2 and LS-BS-3), it was conceivable that the fertilization success of the lumpfish eggs would be high. Higher fertilization successes in wild caught fish than captive fish have also been found in longfin yellowtail (*Seriola rivoliana*) and cod (*Gadus morhua*) (Quiñones-Arreola et al., 2015; Salze et al., 2005). According to Jonassen et al. (2018), eggs from wild caught lumpfish tend to have a high hatching success ranging from 80 - 100 %. In farmed conditions, the hatching success for lumpfish eggs is variable and tend to be relatively low compared to the total eggs spawned (Jonassen, Lein, & Nytrø, 2018). Lower hatching successes have also been found in farmed European sea bass (*Dicentrarchus labrax L.*) (Carrillo, Bromage, Zanuy, Serrano, & Prat, 1989) and Atlantic halibut (*Hippoglossus hippoglossus*) compared to the wild fish (Norberg et al., 1991). Findings of a variable hatching success in eggs from the captive lumpfish and a higher hatching success in the wild caught lumpfish, are in line with existing literature. The fertilization and hatching success were positively correlated, where a higher fertilization success resulted in a higher hatching success. Positive correlations between the fertilization and hatching success have also been found in turbot (*Scophthalmus maximus L.*) (Kjørsvik et al., 2003) and in European sea bass (*Dicentrarchus labrax*) (Saillant, Chatain, Fostier, Przybyla, & Fauvel, 2001). These findings help to clarify that the egg quality was better in wild caught lumpfish eggs than captive lumpfish eggs, since eggs from wild caught lumpfish had the highest fertilization and hatching successes.

Whether a high or low content of total lipids in the eggs is an indicator of good egg quality is species specific. Kamler et al. (1982), found that a high content of lipids in vendace (*Coregonus albula*) indicated good egg quality (Kamler, Zuromska, & Nissinen, 1982). On the other hand, a higher total lipid content generally resulted in low viability in eggs from turbot, sole and seabass. In addition the eggs from the wild caught fish had a higher content of proteins and lipids than eggs from the captive fish (Devauchelle, Brichon, Lamour, & Stephan, 1982). In this experiment a higher % total lipid content

was found in eggs from the wild caught lumpfish, where the RK group had a significantly higher % total lipid content than LS-BS-1 and LS-BS-2. Higher lipid contents in eggs from wild caught fish than captive fish have also been found in great amberjack (*Seriola dumerili*) (Rodríguez-Barreto et al., 2012) and Japanese eel (*Anguilla japonica*) (Furuita, Ohta, et al., 2003; Furuita et al., 2006). Even though no correlation between the % total lipid content in the unfertilized lumpfish eggs and the fertilization or hatching success were found, a higher lipid content in the eggs seem to be more preferable. It is reasonable to believe that eggs with a higher total lipid content also have a higher content of essential fatty acids such as docosahexaenoic acid (22:6n-3; DHA), eicosapentaenoic acid (20:5n-3; EPA) and arachidonic acid (C20:4n6, ARA).

Highly unsaturated n-3 fatty acids (n-3 HUFA) are known to be important for the physiological role in marine fish. Especially DHA, EPA and ARA play an important role in the fish development, and fish have a high dietary requirement for these essential fatty acids (Sargent, 1995; Sargent et al., 1989). In this experiment eggs from the wild caught lumpfish had a significantly higher content of EPA and DHA than eggs from the captive lumpfish, where the RK and NR group had a significantly higher EPA content than the LS-BS groups. Higher EPA levels have also been found in eggs from wild caught sole than the captive broodstock (*Solea Solea L.*) (Lund, Steenfeldt, Suhr, & Hansen, 2008). A positive correlation between the EPA content in the unfertilized lumpfish eggs and the fertilization success, suggests that higher EPA levels in the eggs result in better egg quality. A higher DHA content was also found in eggs from the wild caught lumpfish, where the RK group had a significantly higher DHA content than the LS-BS groups. DHA has showed to be a more important essential fatty acid (EFA) than EPA for larval development (Watanabe, 1993). In juvenile herring (*Clupea harengus L.*), high levels of DHA has been found in the eyes and brain. In addition the DHA plays an important role for maintaining the structure and function of the cell membranes of these tissues (Bell et al., 1997; Sargent, 1995). Positive correlations between the DHA content in the unfertilized lumpfish eggs and the fertilization and hatching success, suggest that a higher DHA content in the eggs result in better egg quality. Higher DHA levels in eggs from Japanese eel (*Anguilla japonica*) and wild common snook *Centropomus undecimalis*) also resulted in a higher fertilization and hatching success (Furuita et al., 2006; Yanes-Roca, Rhody, Nystrom, & Main, 2009). Given the above, findings in this experiment suggest that higher levels of EPA and DHA which were found in eggs from wild caught lumpfish, result in a higher fertilization and hatching success and thus a better egg quality.

On the other hand eggs from the wild caught lumpfish had a lower ARA content than eggs from the captive lumpfish, where the LS-BS-1 and LS-BS-2 group had a significantly higher ARA content than the RK group. In contrast, the ARA content was higher in

eggs from wild caught cod and common sole than eggs from the captive broodstock fish (Røjbek et al., 2014; Lund et al., 2008). There was found a negative correlation between the ARA content in the unfertilized lumpfish eggs and the fertilization and hatching success, where a higher ARA content in the eggs resulted in a lower fertilization and hatching success. High contents of ARA in cobia eggs (*Rachycentron canadum*) have also resulted in a lower fertilization success (Nguyen, Tran, Reinertsen, & Kjørsvik, 2010). High dietary ARA levels have shown to reduce egg and larval quality in Japanese flounder (Furuita, Yamamoto, et al., 2003) and Japanese eel (*Anguilla japonica*) (Furuita et al., 2006). On the other hand, a higher ARA content in eggs resulted in a higher fertilization and hatching success in cod and European eel (Røjbek et al., 2014; Kottmann et al., 2020). Findings from the different studies indicate that the effect of ARA on the fertilization and hatching success may be species specific. According to Bell et al. (1994), spawning, egg and larval quality are reliant on sufficient amount of ARA in the broodstock diet, but too high levels of ARA may cause negative effects (Bell, Tocher, & Sargent, 1994). As the eggs from the wild caught lumpfish had a lower ARA content, but a higher fertilization and hatching success than eggs from the captive lumpfish, may indicate that higher levels of ARA have negative effects on the fertilization and hatching success. Thus higher levels of ARA in the unfertilized lumpfish eggs resulted in a poor egg quality.

Larvae from wild caught lumpfish had the highest mean Vertebrae score than larvae from the captive lumpfish. On the other hand, the Suction disk score did not vary much between the different egg groups, expect egg group LS-BS-2 which had the lowest score. In addition the Gill score was similar for all egg groups. According to Voskoboinikova & Kudryavtseva (2013), the development of bone elements in lumpfish is similar to other teleost fishes. Especially the suction disk, gills and bone elements of the skull appear early in the development, which tend to grow rapidly (Voskoboinikova & Kudryavtseva, 2014). Gills are important for several critical functions such as respiration, osmoregulation, excretion of nitrogenous waste and pH regulation (Herrero, Thompson, Ashby, Rodger, & Dagleish, 2018). Lumpfish is also a slow moving fish and uses its suction disk to attach to surfaces to rest (Nofima, 2022). Given that the vertebrae, gills and suction disk are important for the physiology of the lumpfish, these body parts also tend to be noticeable in an early larval stage. Since the vertebrae, suction disk and gills had a similar ossification score in the different egg groups and given their physiological importance, these findings may suggest that lumpfish develop these body parts at an early stage regardless of whether they are in captivity and fed different broodstock diets or in the wild. Thus there were no differences between the larvae from the wild caught and captive lumpfish regarding the development of these body parts.

A higher number of larvae from the wild caught lumpfish had ossified segments in the 1.

dorsal fin, 2. dorsal fin, pelvic fin and caudal fin than larvae from the captive lumpfish. The NR group had the highest number of larvae with ossified segments in the different fin types, while LS-BS-3 had the lowest number. Dorsal fins help the fish to do quick turns, help the fish against rolling and they are often located on the top or back of the body. Pelvic fins are located below and behind the pectoral fins and fish generally use pelvic fins for mowing upwards and downwards in the water. The fins also function to stabilize the fish while swimming or slow down the swimming speed. Caudal fin also known as the tail fin, is used for locomotion in many fishes (Biologyeducare, 2019). Given the function of the fins in fish, the results in this experiment show that lumpfish develop these body parts at an early larval stage since all egg groups had larvae with ossified segments in the different fin types. Even though no significant differences between egg groups regarding the ossification score of the fins were found, the results suggest that larvae from the wild caught lumpfish had more developed fins at hatching than larvae from captive lumpfish.

The captive lumpfish had the highest number of normal larvae compared to larvae from the wild caught lumpfish, where the LS-BS-3 group had a significantly higher number of normal larvae than the RK group. Skeletal deformities are seen as a major problem in fish aquaculture, since they are known to affect the external morphology, growth and survival of the fish. Cultivated fish generally tend to have a higher occurrence of deformities than wild caught fish (Boglione, Gagliardi, Scardi, & Cataudella, 2001b; Fjelldal, van der Meeren, Jørstad, & Hansen, 2009). In contrast, the results in this experiment differentiate from previous literature. However Fjelldal et al. (2020), found a higher number of vertebral deformities in wild caught lumpfish than cultured lumpfish (Fjelldal et al., 2021). Because lumpfish is a slow-swimming fish with few natural predators (Davenport, 1985), it is able to find food and avoid being eaten. Fjelldal et al. (2020) thus suggested that the reason why lumpfish is able to survive in the wild despite having severe vertebral deformities, was due to its swimming speed and lack of natural predators (Fjelldal et al., 2021). Axial deformities such as lordosis (V-shape of the spine) were found in all egg groups except LS-BS-2. In gilthead seabream (*Sparus aurata*), about 27 % of the larvae showed different axial deformities at hatching, where most of them had lordosis. The gilthead seabream larvae with deformities had a low survival, and only a small percentage of these larvae were able to reach juvenile and adult stages (Andrades et al., 1996). Lordosis has also been observed Japanese sea bream (*Chrysophrys major*) (Kitajima, Watanabe, Tsukashima, & Fujita, 1994), gilthead seabream (Paperna, 1978) and sea bass (*Dicentrarchus labrax*) (Chatain, 1994). Findings from literature may suggest that axial deformities in marine fish are common. Observations of lordosis in lumpfish larvae from almost all egg groups may suggest that axial deformities also are common in lumpfish whether they are in captivity fed different broodstock diets or in the wild. Craniofacial deformities such as an underdeveloped jaw were only observed in larvae from wild caught lumpfish. Cran-

iofacial deformities have also been observed in cultured common carp (*Caprynus carpio L.*) and in adult African catfish (*Clarias gariepinus*). Any jaw deformity will most likely affect the eating abilities of the fish, where a high degree of a deformed jaw could reduce the feed intake. Thus it is conceivable that jaw deformities will have a negative impact on the survival of the lumpfish larvae. Given the above, the results in this experiment suggest that more larvae from the wild caught lumpfish had deformities than larvae from the captive lumpfish.

4.2 Effects of broodstock diets on egg quality of captive lumpfish

It is known that the nutritional content in broodstock diets have major effects on the spawning, fertilization and hatching success in fish (Izquierdo et al., 2001). Lumpfish fed Diet 1 which consisted of a high lipid and low carbohydrate content (lipid 18,7 %, carbohydrate 7,59 %) had eggs (egg group LS-BS-1) with the highest fertilization and hatching success than lumpfish fed Diet 2 and Diet 3. Diet 2 consisted of a moderate lipid and carbohydrate content (lipid 13,9 %, carbohydrate 13,50 %), and fish fed this diet had the lowest fertilization success (egg group LS-BS-2). Diet 3 consisted of a low lipid and high carbohydrate content (lipid 7,3 %, carbohydrate 17,98 %) and lumpfish fed this diet had a lower fertilization and hatching success than the LS-BS-1 group, but higher than LS-BS-2. These results may indicate that the broodstock diet may affect the fertilization and hatching success of the eggs. A higher lipid level in the broodstock diets fed to rabbitfish (*Siganus guttatus*) resulted increased fecundity and hatching (Duray, Kohno, & Pascual, 1994). Increased dietary n-3 HUFA in the broodstock diets have also shown to increase the fecundity in gilthead seabream (*Sparus aurata*) and red sea bream (*Pagrus major*) (Fernández-Palacios et al., 1995; Watanabe, Arakawa, Kitajima, & Fujita, 1984). The results from this experiment may indicate that the broodstock diet with a higher lipid content is more favorable than diets with a lower lipid content. Thus the broodstock diet with a higher lipid content resulted in a better egg quality in lumpfish.

However, there is a high risk of obtaining eggs that are not finished maturing if the lumpfish females are strip-spawned too early, and thus this can affect the fertilization and hatching success (Jonassen et al., 2016). In this experiment the captive lumpfish were strip-spawned in early February and at the end of February, since the eggs retrieved from fish fed Diet 2 and Diet 3 in early February were not mature. Therefore the date of the strip-spawning may also affect the fertilization and hatching success. If a diet with a higher lipid content seems to result in a better egg quality in lumpfish eggs, Diet 2 with a moderate lipid content should perform better than Diet 3 consisting of a low

lipid content. Since eggs from captive lumpfish fed Diet 3 had a higher fertilization and hatching success than eggs from fish fed Diet 2, suggests that the egg quality most likely also is affected by the time of the strip spawning of the lumpfish females. Eggs from the LS-BS-2 group varied the most in all the analyzes that were taken, which is probably due to the fact that fish from this group were not sexually mature at strip-spawning. The fertilization and hatching success are not always correlated with good survival and development in later embryonic stages for many marine fish species (Avery, Killen, & Hollinger, 2009; Kjørsvik et al., 1984). In red snapper (*Lutjanus campechanus*), the fertilization and hatching successes were poor predictors of survival of the larvae from hatching to start feeding (Bardon-Albaret & Saillant, 2017). Findings from previous studies show that although eggs have good fertilization and hatching successes, these egg quality parameters can not predict good development and survival in a later larval stage (Kjørsvik et al., 1990; Bardon-Albaret & Saillant, 2017)

The larval performance is known to be affected by the quantity and fatty acid composition in the yolk sac, and this often reflects the nutritional status of the feed fed to maternal fish before spawning (Morais, Narciso, Dores, & Pousao-Ferreira, 2004; Kjørsvik et al., 1990; Rainuzzo, Reitan, & Olsen, 1997). Broodstock diets with increased n-3 HUFA have shown to increase fecundity, egg size, fertilization and hatching success, fry viability and survival of the broodstock in some marine fish species (Izquierdo et al., 2001; Hardy, 1983; Springate, 1985). On the other hand, excessive n-3 HUFA in the broodstock diets have resulted in poor egg quality in gilthead seabream (Fernández-Palacios et al., 1995), turbot (Lavens et al., 1999) and Japanese flounder (Furuita et al., 2002). Eggs from the LS-BS-1 group had the highest EPA and DHA content compared to the other egg groups from the captive lumpfish. LS-BS-1 also had the highest fertilization and hatching success. A positive correlation between the DHA level in the eggs and hatching success and larval survival has also been found in wild common snook eggs (*Centropomus undecimalis*) (Yanes-Roca et al., 2009). The fatty acid composition in the broodstock diet also affect the ARA content in the eggs. In European eel (*Anguilla anguilla*), broodstock diets with a higher ARA content resulted in more viable eggs and larvae in addition to a higher content of ARA in the unfertilized eggs (Støttrup et al., 2016). The fatty acid composition of the diet fed to Japanese eel (*Anguilla japonica*) affected the ARA levels in the eggs (Furuita, Hori, Sugita, Yamamoto, et al., 2007). In this experiment higher levels of ARA in the unfertilized lumpfish eggs were negatively correlated with the fertilization and hatching success. The LS-BS-3 group had the lowest % ARA content compared to the other egg groups from the captive lumpfish, but had a lower fertilization and hatching success than LS-BS-1. The results in this experiment may suggest that Diet 1 resulted in a better egg quality than the other broodstock diets, despite having eggs with a higher ARA content.

Although lumpfish may be able to survive in the wild despite having different deformities, as a cleaner fish the lumpfish must be able to cope with strong tidal currents and hunt for sea lice on the swimming salmon in the sea cages. Any deformities in the fish structure may affect its swimming ability, and reduce its overall grazing capabilities (Basaran, Ozbilgin, & Ozbilgin, 2007; M. D. Powell, Jones, & Lijalad, 2009). Thus it is conceivable that the survival of lumpfish in sea cages will be considerably reduced if it has deformities. Broodstock diets have shown to have an effect on the larval quality in studies of other fish species. The broodstock diet with a high lipid content fed to rabbitfish resulted in a higher percentage of normal hatchlings than broodstock diets with a lower lipid content (Duray et al., 1994). In this experiment newly hatched lumpfish larvae from fish fed the broodstock diets, had the highest number of normal larvae. The LS-BS-3 group had a significantly higher number of normal larvae than the RK group. These findings may suggest that the broodstock diet may have an effect. The extent to which the broodstock diet may have an effect on the amount of deformities in the larvae is uncertain, and should be investigated further.

4.3 Limitation of the study and further research

The sample size in this master project is quite small where only 3 females from each egg group were used. For the bone analysis and standard length, 5-15 larvae from each female were examined. This greatly affects the results of the different analyzes. A larger sample size of fish and larvae (eg. 10-20 females and 40-50 larvae from each egg batch), would give more precise results when testing the effect of broodstock diets on the egg quality of captive lumpfish, and also comparing the egg quality to the wild caught lumpfish. LS-BS-2 was most variable in all analyses that were taken, due to a lower amount of eggs and larvae compared to other egg groups. Since LS-BS-2 was most variable, the standard deviation in some analyzes was very large. Therefore the results in this experiment may be somewhat uncertain. For example there may be correlations or significant differences between groups by chance, or correlations and significant differences were not found due to a too small sample set. By increasing the sample size one can avoid large standard deviations, get more precise significant differences between groups and correlations between different factors.

It is known that the maternal genetics such as the DNA provided by the female have an impact on the egg quality in fish (Reinitz, Orme, & Hitzel, 1979; Withler, 1987). In Atlantic halibut (*Hippoglossus hippoglossus*), the hatching success was strongly influenced by the paternal effects especially the mother (Ottesen & Babiak, 2007). Maternal contribution to the egg quality is transferred via the yolk reserves, which in turn depends on the

nutritional status of the female before spawning (Ottesen & Babiak, 2007). The egg quality is also influenced by the sperm quality (Astuarino et al., 2001; Campbell, Pottinger, & Sumpter, 1992). The sperm motility has shown to correlate with the fertilization success in yellowtail flounder (*Pleuronectes ferrugineus*), rainbow trout (*Oncorhynchus mykiss*) and African catfish (*Clarias gariepinus*) (Clearwater & Crim, 1998; Lahnsteiner, Berger, Weismann, & Patzner, 1998; Rurangwa, Volckaert, Huyskens, Kime, & Ollevier, 2001). In this experiment, milt from the same male lumpfish was used to reduce the potential of parental variation to be a confounding factor. On the other hand, it is uncertain how much influence the genetics of the different lumpfish females have on the egg quality. Especially the wild caught lumpfish, as there was no information about which environmental factors they were exposed to before spawning. Therefore the uncertainty about the maternal effects on the egg quality may be taken into account when evaluating the results in this experiment.

Not all larvae used for the bone staining analysis were sampled at the same time, which will also affect the level of ossification of the vertebrae, suction disk, gills and the different fin types. Marine fish undergo major changes in transformation from the larval stage to the juvenile stage (Osse & Van den Boogaart, 1995). Thus it is conceivable that larvae that are 2 days post hatch have come further in the development than larvae that are 1 day post hatch. This difference must also be taken into account when evaluating the results of the bone staining analysis.

The results in this experiment show that broodstock diets with a varying lipid and carbohydrate content had an impact on the egg quality of captive lumpfish. The broodstock diet with a high lipid and low carbohydrate content seemed to result in better egg quality. However, different contents of the lipid and carbohydrate in the broodstock diet were tested simultaneously. Whether it was the increased or decreased contents of the lipids or carbohydrates that had the greatest impact on the egg quality remain uncertain. In further studies it may be appropriate to investigate the effect of one nutrient at a time. By testing a broodstock diet with a varying lipid or carbohydrate level, one can discover which nutrient has the greatest effect on the egg quality. The nutritional content of the diet for the wild caught lumpfish is also unknown. Thus it becomes difficult to know with certainty whether it was the diet of the wild caught lumpfish that affected the egg quality to the greatest extent, or if the egg quality could be affected by temperature, age of the female or other environmental factors. This must also be taken into account when evaluating the egg quality of the wild caught lumpfish.

5 CONCLUSION

To conclude the wild caught lumpfish had better egg quality than the captive lumpfish fed broodstock diets with a varying lipid and carbohydrate content. Eggs and larvae from wild caught lumpfish had the highest fertilization and hatching success, higher levels of DHA and EPA in the unfertilized eggs, earlier bone development but more deformities. Other findings in this experiment may suggest that lumpfish fed the high lipid and low carbohydrate diet gave better egg quality. Fish fed this diet type had a higher fertilization and hatching success, in addition to higher levels of DHA and EPA in the unfertilized eggs. Higher levels of ARA were also found in eggs from captive lumpfish than wild caught lumpfish, which was due to the broodstock diet. The fertilization and hatching success were reduced at higher levels of ARA in the eggs, and thus high levels of ARA result in poor egg quality in lumpfish. Minor deformities were also found in larvae from captive lumpfish, but whether the broodstock diet had an impact on the amount of deformities is uncertain and should be investigated further.

An improvement for further research would be to increase the sample size to prevent large standard deviations, which in turn will result in more precise findings of significant differences between groups and correlations between different factors. Since the paternal effects may also influence the egg quality, it is uncertain to what extent the broodstock diets have the greatest effect. To test broodstock diets focusing on either a varying lipid or carbohydrate level, will provide a better indication to what the degree the specific nutrient affects the egg quality which is recommended to investigate in further studies.

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6 APPENDIX

A Diet composition of broodstock feed

Table 9: Dietary composition of the broodstock diet fed to captive lumpfish females (*Cyclopterus Lumpus L.*) at NOFIMA´s research station at Sunndalsøra in Møre and Romsdal. Diet 1 was fed to lumpfish in egg group LS-BS-1, Diet 2 was fed to egg group LS-BS-2 and egg group LS-BS-3 was fed Diet 3.

Composition of diet	Diet 1	Diet 2	Diet 3
Diet no			
	%	%	%
Codfish powder 8/19 + 13/19	10,00	10,00	10,00
Krill hydrolysat 17/16	2,00	2,00	2,00
FM 23/18 + 11/19	39,05	36,57	36,91
Krillmel P75/17	4,00	4,00	4,00
Biomos T3/17 + MOS P44/19	4,00	4.00	4,00
Stay-C 35 % T9/18	0,27	0,27	0,27
Cholesterol T14/19	0,50	0,50	0,50
Choline chloride 19/18	0,50	0,50	0,50
Fiskeolje 11/18	10,00		
Fiskeolje til grunnblanding 11/18	2,09	7,30	0,5
Hvetegluten	19,38	18,15	18,31
Krill oil 25/18 + P48/19	1,00	1,00	1,00
Hvetemel 7/19	4,00	12,50	18,80
Vitamin mix T 1/19	3,00	3,00	3,00
Yttrium oksyd T19/17	0,01	0,01	0,01
Mineral premiks T10/18	0,50	0,50	0,50
Lysin T4/18	0,60	0,60	0,60
Carop. Pink (10 %) T 6/18	0,10	0,10	0,10
Taurine	0,20	0,20	0,20
MSP T10/19	2,40	2,40	2,40
Sum	100,000	100,000	100,000
Calculated chemical composition in the feed (% in diet)			
Protein	54,3	52,7	53,9
Lipid	18,7	13,9	7,3
Carbohydrate	7,59	13,50	17,98
Ash	12,5	12,1	12,3
Water	7,0	7,7	8,5
Sum	100,0	100,0	100,0

B Lipid analyses

B.0.1 Materials

- Kimax tubes 100 x 15 mm with teflon-coated screw lid (VWR, art.nr. KIML45066A-1600)
- GC tubes with 8 mm cap/lids, chromacol (VWR, art. nr. 548-1208)
- Graduated pipette glasses 0,5 mL and 1,0 mL (VWR, art.nr. 612-1104 and 612-1105)
- Plastic pipettes 2 mL, 150 mm (VWR, art.nr. 612-1701)
- Hamilton micro syringe
- Eppendorf research automatic-pipette (100 – 100 micro liter)
- Pipette tips
- Whatman qualitative filter paper, grade 1
- Nitrile gloves (VWR, art.nr. 112 – 2373)
- Glass funnel
- Measuring cylinder

B.0.2 Instruments

- Heating plate
- Weight mg (Mettler Toledo UMX2)
- Weight g (Precisa 180 A)
- Ultra Turrax T8 homogenizer
- Perkin Elmer AutoSystem XL Gas chromatograph PSS-injector, PPC FID-detector, WCOT Fused Silica, coating CP-wax 52 CP capillarycolumn (Holger CP7713)
- Vortex mixer (Heidolph reax top)
- Centrifuge (Hettich universal 32R
- Pipette filler Pipetboy (VWR, art.nr. 612-0927)
- Water treatment system (Elix5)
- Freeze-drying (intake lock, Sealab rom 145)

B.0.3 Chemicals

- Chloroform pro analysis (VWR, art.nr. 1.02445.1000)
- Methanol pro analysis (VWR, art.nr. 20847.295)
- Sulfuric acid
- Isooctane pro analysis (VWR, art.nr. 1.04727.1000)

- Sodium chloride, NaCl pro analysis (VWR, art.nr. 1.06404.1000)
- Potassium chloride (KCl) pro analysis
- External standard 68D from NuChecPrep, 4 ampoules 25 mg (Chiron, art.nr.)
- Internal standard 23:0 / 19:0 from NuChecPrep (Chiron, art.nr.)

B.0.4 Solvents

- Chloroform:Methanol (2:1), 2 parts chloroform mixed with 1 part methanol
- 1 % H2SO4:MeOH (methylation reagent), order: 1 mL H2SO4 added to 99 mL methanol
- Internal standard (IS 23:0), IS 23:0 was made, the IS added to the sample should correspond to 10 % of total lipid (less than 50 ng/ micro liters initiated on GC)
- External standard (68 D)
- Saturated NaCl, minimum 20 g NaCl (s) mixed with 100 mL dH2O, gives 20 % NaCl (aq)

B.0.5 Day 1 - Weighing sample

Approximately 0,5 g of unfertilized eggs from each egg batch were transferred to its respective kimaxtube, and then weighed using the Precisa 180A weight. It was important that the eggs stayed cold prior to and after the weighing, and thus a glass beaker with ice was used for this purpose. A total of 10 mL CHCL3:MeOH (2:1), was added to each sample. First, 5 mL was added to the kimaxtubes. Then, the samples were homogenized using Ultra-turrax T8 homogenizer with a stainless steel tip (IKA T 10 basic ULTRA-TURRAX). In samples with clear precipitates, the remaining 5 mL of CHCl3: MeOH (2:1) was added. If the total volume did not correspond to 10 mL, more CHCl3:MeOH (2:1) was added to achieve the desired amount. In this experiment, there was no need for filtration. 2,5 mL of 0,88 % KCl (aq), 1/4 of the volume of 10 mL was added to its respective kimaxtube. The samples were mixed using a vortex mixer (Heidolph reax top), followed by centrifugation (Hettich universal 32R) at 4000 rpm, 4 minutes and 4°C. The lipids were in the lower phase of the sample, and this phase was extracted by a glass pipette and then transferred to a pre-weighed kimaxtube (15 mL). It was important not to add any water from the lower phase of the sample to the pre-weighet kimaxtube. The lipids were evaporated under N2-gas. When most of the liquid had evaporated, a small amount of chloroform was added to remove the remaining water content in the sample. The samples were transferred to a desiccator for 1 hour to remove any excess water. After the drying step, the weight (g) of the samples was registered and the total amount of

lipids for each sample was calculated. All samples were dissolved in CHCl3:MeOH (2:1) to a concentration of 10 mg/mL.

For the methylation step, 15 mL kimaxtubes were used. The desired amount of lipid from a known concentration of 10 mg/mL was transferred to a new kimaxtube. 1 mL CHCl3 and 2 mL 1 % H2SO4: MeOH (methylation reagent) were added in the order indicated. The samples were mixed for 5 seconds using the vortex mixer and then flushed with N2-gas. After this step the screw lids were tightened on the kimaxtubes, and the kimaxtubes were placed on a heating plate inside the fume hood. The screw lids were tightened after 10 minutes and the samples were incubated at 50°C for 16-18 hours.

B.0.6 Day 2 - After methylation

After 16-18 hours, the samples were cooled down until they reached room temperature. 5 mL isooctane and 5 mL saturated NaCl were added. The samples were mixed on the vortex mixer and centrifuged at 4000 rpm, for 4 minutes at 4°C. Lipids were in the upper phase of the samples, and this phase was transferred to a new 15 mL kimaxtube. The samples were evaporated under N2-gas, and (40 μL) isooctane were added to the samples for further processing of the HPTLC disc. After the cleaning step, the samples were transferred to GC glasses using a pointed insert. The upper layer of the samples were gently flushed with nitrogen gas before the lid of the tubes were tightened. Then, the samples could be run on the GC machine at NTNU Sealab. If the samples were not placed on the GC machine directly, they were stored in the freezer at 20°C.

B.0.7 Cleansing phase - High performance thin layer chromatography (HPTLC)

For the cleansing phase by using a HPTLC disc, a mobile phase of hexane:ether:acetic acid (90:10:1) was added to the chroma tank (0,5 cm over the bottom of the chroma tank). A lid was put on top of the chroma tank, and the chroma tank was left untouched for 30 minutes until the atmosphere inside the tank was saturated. The HPTLC disc was drawn on similar to Figure 22. The samples were applied to the disc using a 10 µL Hamilton syringe, and the Hamilton syringe was cleaned with the solvent used in the method. The amount of sample added to the HPTLC disc was 20, 40, 60 and 80 µL respectively. Evaporated lipid extract was dissolved in 30 µL hexane, and the sample material was transferred at small points beyond the drawn line for the current sample. An external standard NuchecPrep 68D (28 µg in 21,43 mL isooctante) was applied to the HPTLC disc. After the appli-

cation, the HPTLC disc was placed in the chroma tank. The plate was removed when the mobile phase was 1 cm from the top edge, and then set to evaporate in the fume hood.

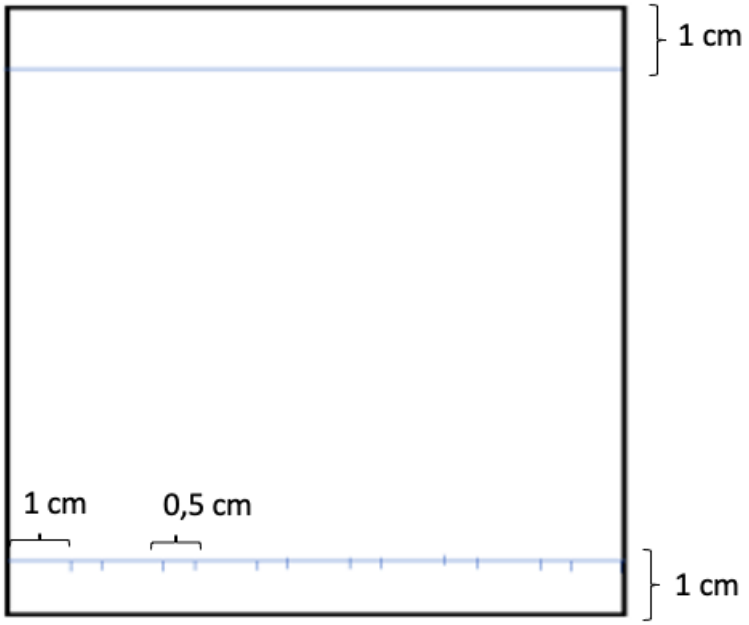


Figure 22: Making of HPTLC disc and placement of sample. The horizontal lines were 1,0 cm from the bottom and top of the disc, and the first point 1,0 cm from the outside of the disc. Each point was 0,5 cm and the distance between the points of the sample was 1,0 cm. In total there was room for 6 samples on the HPTLC disc.

The chroma tank was placed in a water bath with lukewarm water. A box of iodine was placed inside the chroma tank, the lid of the chroma tank was put on and the system was left untouched for 30 minutes. After the 30 minutes, the HPTLC disc was placed in the chroma tank. The iodine colored the lipid yellow. When a faint yellow color appeared, the HPTLC disc was removed from the chroma tank and the yellow areas were marked with a pencil. The marked areas were scraped off using a knife, and the sample was transferred to a kimaxtube using a glass funnel. The kimaxtube with silica was added 2x2 mL hexane:ether (1:1). 2 mL of hexane:ether (1:1) was first added. Then, the sample was vortex mixed and centrifuged at 4000 rpm at 4°C for 4 minutes. The upper layer was transferred to a new, clean kimaxtube, and the procedure was repeated. Samples were then evaporated to dryness and dissolved in isooctane. The samples were then transferred to GC tubes and the samples were run on the GC machine at NTNU Sealab performed by one of NTNU´s technical staff members. Lipid and fatty acid profiles were calculated and classified by a NTNU technical staff member.

Table 10: Date of experiment, weight of kimaxtube with eggs and without lid, in addition to weight of dry sample (g) and amount of CHCl3:MeOH (mL)

Date	**Egg batch**	**Tube number**	**Weight with eggs (g)**	**Tube without lid (g)**	**Dry sample g()**	**CHCl3:MeOH (mL)**
22/03/2021	LS-BS-1-1	1.1 (1)	0,5442	10,5424	10,5569	1,45
	LS-BS-1-2	1.2 (2)	0,5971	10,9230	10,9449	2,19
	LS-BS-1-3	1.3 (3)	0,5403	11,0175	11m0317	1,42
	NR-1	N-1 (4)	0,5359	11,073	11,0803	2,3
	NR-2	N-2 (5)	0,3820	11,5914	11,6044	1,3
	NR-3	N-3 (6)	0,4852	11,1165	11,1395	2,3
23/03/2021	LS-BS-2-1	2.1 (7)	0,5908	11,1246	11,1400	1,54
	LS-BS-2-2	2.2 (8)	0,6611	10,7122	10,7357	2,35
	LS-BS-2-3	2.3 (9)	0,5547	11,0605	11,0776	1,72
	LS-BS-2-4	2.4 (10)	0,6425	10,9593	10,9760	1,67
	LS-BS-3-1	3.1 (11)	0,6229	10,9244	10,9540	2,96
	LS-BS-3-2	3.2 (12)	0,5764	11,1258	11,1433	1,75
24/03/2021	LS-BS-3-3	3.3 (13)	0,7146	10,9486	10,9782	2,96
	RK2-1-2	R-1 (14)	0,7182	11,1027	11,1428	4,01
	RK2-1-3	R-2 (15)	0,8575	11,6658	11,7077	4,19
	RK2-2-3	R-3 (16)	0,8700	10,9112	10,9529	4,17
	RK2-2-4	R-4 (17)	0,6491	10,8054	10,8410	3,56
	RK2-3-6	R-5 (18)	0,5684	10,9111	10,9411	3,0
	RK2-3-1	R-6 (19)	0,4196	11,4655	11,4893	2,38

Table 11: Distribution of percent saturated fatty acid (SFA) content in the different egg groups and egg batches retrieved from captive (LS-BS-1, LS-BS-2 and LS-BS-3) and wild caught (RK and NR) lumpfish females. Egg groups represent eggs retrieved from lumpfish fed the same diet. Egg batches represent eggs retrieved from different lumpfish fed the same diet.

Egg group	Egg batch	Female	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C22:0
LS-BS-1	LS-BS-1-1	1	1	0,3	18	0,3	4,9	0,1	0,1
LS-BS-1	LS-BS-1-2	2	1,3	0,3	17,3	0,3	4,6	0,1	0,1
LS-BS-1	LS-BS-1-3	3	1,1	0,3	17,3	0,3	0,5	0,1	0,1
LS-BS-2	LS-BS-2-1	1	1,1	0,3	17	0,3	5,2	0,1	0,1
LS-BS-2	LS-BS-2-2	2	1,1	0,2	17,1	0,3	4,8	0,1	0,1
LS-BS-2	LS-BS-2-3	3	1,1	0,3	18,3	0,3	5	0,1	0,1
LS-BS-2	LS-BS-2-4	4	1	0,2	17,5	0,3	4,7	0,1	0,1
LS-BS-3	LS-BS-3-1	1	1,6	0,4	16,0	0,6	4,5	0,1	0,0
LS-BS-3	LS-BS-3-2	2	0,8	0,2	17,2	0,2	5	0,1	0,1
LS-BS-3	LS-BS-3-3	3	0,8	0,2	17,5	0,3	5,5	0,1	0,1
RK	RK-1-2	1	1	0,3	16,5	0,4	5,1	0,1	0,1
RK	RK-1-3	2	1	0,3	16,5	0,4	5,1	0,1	0,1
RK	RK-2-3	3	1,7	0,4	15,7	0,5	4,6	0,1	0,1
RK	RK-2-4	4	1,6	0,4	15,9	0,5	4,4	0,1	0,1
RK	RK-3-1	5	1,6	0,4	16	0,6	4,5	0,1	0,1
NR	NR-1	1	1,9	0,3	15,9	0,4	4,4	0,1	0,1
NR	NR-2	2	1,4	0,3	16,1	0,5	4,5	0,1	0,1
NR	NR-3	3	1,6	0,3	15,7	0,4	4,6	0,1	0,1

Table 12: Distribution of percent mono unsaturated fatty acid (MUFA) content in the different egg groups and egg batches retrieved from captive (LS-BS-1, LS-BS-2 and LS-BS-3) and wild caught (RK and NR) lumpfish females. Egg groups represent eggs retrieved from lumpfish fed the same diet. Egg batches represent eggs retrieved from lumpfish fed the same diet.

Egg group	Egg batch	Female	C14:1n5	C16:1n7	C17:1n7	C18:1n9	C18:1n7	C20:1n9	C22:1n9	C24:1
LS-BS-1	LS-BS-1-1	1	0,0	2,3	0,4	19,7	4,4	2,5	0,1	0,2
LS-BS-1	LS-BS-1-2	2	0,0	2,4	0,3	20,2	4,3	2,6	0,3	0,0
LS-BS-1	LS-BS-1-3	3	0,0	2	0,4	18,4	4,1	2,7	0,1	0,2
LS-BS-2	LS-BS-2-1	1	0,0	2,2	0,4	19,7	4,4	2,3	0,1	0,3
LS-BS-2	LS-BS-2-2	2	0,0	2,4	0,4	21,1	4,6	2,3	0,1	0,2
LS-BS-2	LS-BS-2-3	3	0,0	2,1	0,4	19,1	4,3	2,1	0,1	0,3
LS-BS-2	LS-BS-2-5	4	0,0	2,2	0,4	21,0	4,6	1,9	0,1	0,2
LS-BS-3	LS-BS-3-1	1	0,0	1,2	0,4	17,6	2,5	2,6	0,2	0,2
LS-BS-3	LS-BS-3-2	2	0,0	2,2	0,4	23,4	5,2	1,3	0,0	0,2
LS-BS-3	LS-BS-3-3	3	0,0	2,0	0,4	21,7	5,0	1,5	0,0	0,3
RK	RK-1-2	1	0,0	1,3	0,3	17,5	3,8	2,2	0,1	0,2
RK	RK-1-3	2	0,0	1,3	0,3	17,5	3,7	2,2	0,1	0,2
RK	RK-2-3	3	0,0	1,2	0,4	17,9	2,5	3,1	0,2	0,2
RK	RK-2-4	4	0,0	1,2	0,4	17,6	2,5	2,6	0,1	0,2
RK	RK-3-1	5	0,0	1,1	0,4	17,5	2,5	2,7	0,1	0,2
NR	NR-1	1	0,0	1,6	0,3	17,3	3,2	2,9	0,2	0,2
NR	NR-2	2	0,0	1,3	0,3	17,9	2,5	3,2	0,2	0,2
NR	NR-3	3	0,0	1,4	0,4	18,2	3,0	2,8	0,2	0,3

Table 13: Distribution of percent poly unsaturated fatty acid (PUFA) content in unfertilized lumpfish eggs in the different egg groups and egg batches retrieved from captive (LS-BS-1, LS-BS-2 and LS-BS-3) and wild caught (RK and NR) lumpfish females. Egg groups represent eggs retrieved from lumpfish fed the same diet. Egg batches represent eggs retrieved from different lumpfish fed the same diet.

Egg group	Egg batch	Female	C18:3n3	C18:4n3	C20:3n3	C20:4n3	C20:5n3 (EPA)	C22:5n3 (DPA)	C22:6n3 (DHA)	C18:2n6	C18:3n6	C20:2n6	C20:4n6 (ARA)	C22:5n6
LS-BS-1	LS-BS-1-1	1	0,6	0,5	0,1	1,2	13,4	2,6	20,1	5,8	0,1	0,3	0,8	0,3
LS-BS-1	LS-BS-1-2	2	0,7	0,7	0,1	1,1	13,1	1,9	21,4	5,5	0,1	0,3	0,8	0,3
LS-BS-1	LS-BS-1-3	3	0,7	0,6	0,1	1,1	14,4	2,6	21,2	5,7	0,1	0,3	0,9	0,3
LS-BS-2	LS-BS-2-1	1	0,7	0,6	0,1	1,1	13,1	2,5	20,2	6,7	0,2	0,3	0,9	0,4
LS-BS-2	LS-BS-2-2	2	0,7	0,6	0,1	1,0	12,5	2,1	20,2	6,6	0,1	0,3	0,8	0,4
LS-BS-2	LS-BS-2-3	3	0,7	0,6	0,1	0,9	13,9	2,1	19,9	6,8	0,1	0,3	0,9	0,4
LS-BS-2	LS-BS-2-5	4	0,6	0,6	0,1	1,0	12,9	2,0	20,8	6,2	0,1	0,3	0,8	0,4
LS-BS-3	LS-BS-3-1	1	0,7	0,9	0,2	1,2	17,2	1,5	27,9	1,1	0,2	0,3	0,7	0,3
LS-BS-3	LS-BS-3-2	2	0,8	0,5	0,1	0,8	11,0	1,8	17,3	10,1	0,1	0,3	0,7	0,3
LS-BS-3	LS-BS-3-3	3	0,9	0,5	0,1	1,1	11,0	2,3	16,0	11,1	0,1	0,4	0,8	0,3
RK	RK-1-2	1	0,4	0,8	0,1	0,9	17,3	1,6	27,8	1,0	0,1	0,2	0,7	0,3
RK	RK-1-3	2	0,4	0,8	0,1	0,9	17,3	1,5	27,9	0,9	0,1	0,2	0,7	0,3
RK	RK-2-3	3	0,8	1,1	0,1	1,4	19,2	1,7	24,8	1,1	0,2	0,3	0,5	0,3
RK	RK-2-4	4	0,8	1,1	0,2	1,2	17,3	1,4	27,8	1,1	0,2	0,3	0,7	0,3
RK	RK-3-1	5	0,7	1,1	0,2	1,2	17,2	1,5	27,8	1,1	0,2	0,3	0,7	0,3
NR	NR-1	1	0,6	0,8	0,1	1,0	19	1,7	25,4	1,2	0,1	0,4	0,7	0,3
NR	NR-2	2	0,6	1,0	0,1	1,1	17,6	1,5	27,1	1,2	0,1	0,4	0,7	0,3
NR	NR-3	3	0,9	1,1	0,2	1,4	17,7	1,6	25,5	1,2	0,2	0,3	0,7	0,3

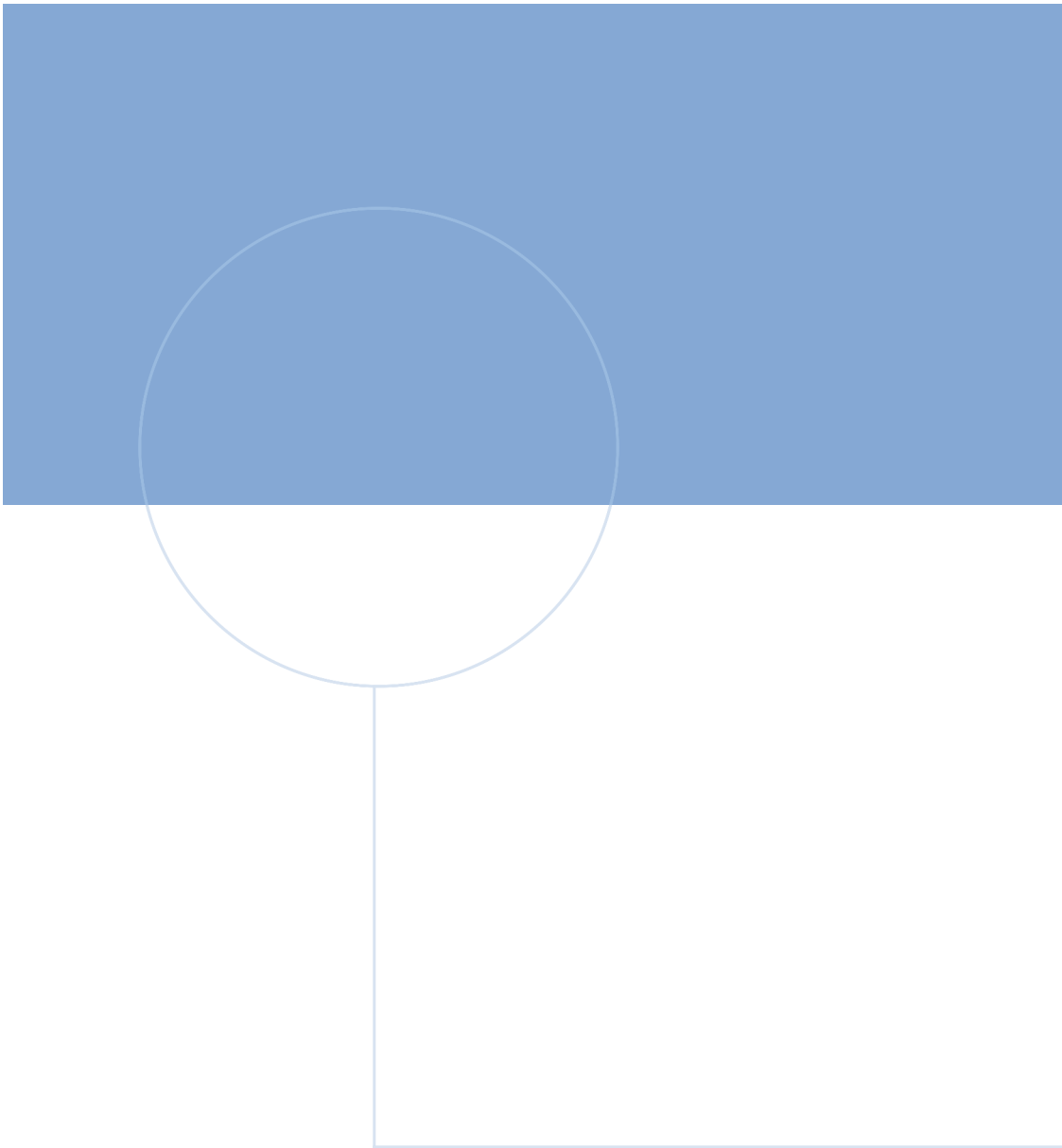
Table 14: Percent total lipid content of eggs in the different egg groups and egg batches retrieved from captive (LS-BS-1, LS-BS-2 and LS-BS-3) and wild caught (RK and NR) lumpfish females. Egg groups represent eggs retrieved from lumpfish fed the same diet. Egg batches represent eggs retrieved from different lumpfish fed the same diet.

Egg group	Egg batch	Female	% total lipid
LS-BS-1	LS-BS-1-1	1	2,66
LS-BS-1	LS-BS-1-2	2	3,67
LS-BS-1	LS-BS-1-3	3	2,63
LS-BS-2	LS-BS-2-1	1	2,61
LS-BS-2	LS-BS-2-2	2	3,55
LS-BS-2	LS-BS-2-3	3	3,08
LS-BS-2	LS-BS-2-4	4	2,6
LS-BS-3	LS-BS-3-1	1	4,75
LS-BS-3	LS-BS-3-2	2	3,04
LS-BS-3	LS-BS-3-3	3	4,14
RK	RK-1-2	1	5,58
RK	RK-1-3	2	4,89
RK	RK-2-3	3	4,79
RK	RK-2-4	4	5,48
RK	RK-3-1	5	5,67
NR	NR-1	1	4,29
NR	NR-2	2	3,4
NR	NR-3	3	4,74

C Bone analyses

Egg batch	Larvae (n)	Mean SL (mm) ± SD	Mean number of ossified vertebrae larva^{-1} ± SD					Mean ossification score of suction disk larva^{-1} ± SD				Mean ossification sore of gills larva^{-1} ± SD		
			C	M	P	T	Total	Score 2	Score 1	Score 0	Total	Score 1	Score 0	Total
LS-BS-1-1	12	5,3 ± 0,3	4,3 ± 0,7	10,9 ± 2,2	9,4 ± 2,1	3,3 ± 1,0	28	4,8 ± 2,4	7,2 ± 8,2	0 ± 0	12	6 ± 0	0 ± 0	6
LS-BS-1-2	13	5,6 ± 0,3	4,6 ± 0,8	11,5 ± 2,6	8,7 ± 2,8	3,2 ± 0,8	28	3,8 ± 0,6	8,2 ± 0,6	0 ± 0	12	6 ± 0	0 ± 0	6
LS-BS-1-3	15	5,5 ± 0,2	4,4 ± 0,6	13,1 ± 2,9	7,1 ± 2,6	3,3 ± 1,0	28	4,4 ± 0,0	8,0 ± 0,0	0 ± 0	12	6 ± 0	0 ± 0	6
LS-BS-2-2	5	4,8 ± 0,3	0,0 ± 0,0	1,0 ± 2,2	19,8 ± 2,6	6,6 ± 0,7	28	0,0 ± 0,0	12,0 ± 0,0	0 ± 0	12	6 ± 0	0 ± 0	6
LS-BS-2-5	14	5,7 ± 0,3	4,1 ± 1,5	17,6 ± 2,1	3,9 ± 1,5	2,4 ± 0,7	28	4,0 ± 0,3	8,0 ± 0,0	0 ± 0	12	6 ± 0	0 ± 0	6
LS-BS-3-1	15	5,6 ± 0,2	2,0 ± 2,6	7,2 ± 5,4	12,9 ± 7,3	5,9 ± 4,9	28	3,6 ± 0,8	8,4 ± 0,8	0 ± 0	12	6 ± 0	0 ± 0	6
LS-BS-3-2	15	5,7 ± 0,2	3,3 ± 4,5	13,5 ± 4,5	7,8 ± 5,3	3,4 ± 0,8	28	4,0 ± 0,0	8,0 ± 0,0	0 ± 0	12	6 ± 0	0 ± 0	6
LS-BS-3-4	15	5,7 ± 0,2	3,1 ± 1,4	14,2 ± 1,2	6,8 ± 1,6	3,9 ± 0,6	28	4,0 ± 0,0	8,0 ± 0,0	0 ± 0	12	6 ± 0	0 ± 0	6
RK-1	14	5,7 ± 0,2	3,6 ± 1,3	13,4 ± 2,1	7,3 ± 3,1	3,7 ± 0,6	28	3,9 ± 0,3	8,1 ± 0,3	0 ± 0	12	6 ± 0	0 ± 0	6
RK-2	15	5,8 ± 0,3	3,0 ± 2,0	11,5 ± 4,5	9,7 ± 6,1	3,8 ± 0,7	28	3,9 ± 0,4	8,1 ± 0,4	0 ± 0	12	6 ± 0	0 ± 0	6
RK-3	5	5,5 ± 0,1	4,6 ± 0,5	16,2 ± 0,8	4,0 ± 1,2	3,2 ± 0,4	28	4,0 ± 0,0	8,0 ± 0,0	0 ± 0	12	6 ± 0	0 ± 0	6
NR-1	13	5,5 ± 0,1	4,2 ± 0,9	16,1 ± 1,5	4,2 ± 1,5	3,5 ± 0,5	28	3,8 ± 0,4	8,2 ± 0,4	0 ± 0	12	6 ± 0	0 ± 0	6
NR-2	12	5,4 ± 0,2	4,2 ± 0,7	4,2 ± 1,2	4,3 ± 1,1	3,6 ± 0,5	28	3,8 ± 0,4	8,0 ± 0,0	0 ± 0	12	6 ± 0	0 ± 0	6
NR-3	15	5,5 ± 0,3	4,2 ± 0,6	16,9 ± 1,1	3,8 ± 0,8	3,1 ± 0,8	28	4,0 ± 0,0	8,0 ± 0,0	0 ± 0	12	6 ± 0	0 ± 0	6

Figure 23: Mean number of ossified vertebrae, suction disk and gill segments per larva in newly hatched lumpfish larvae (n) ± SD, between different egg batches. Egg batches represent larvae retrieved from different females fed the same diet. The larvae are classified according to degree of ossification. Compact (C) = ossified and fully saturated color. Moderate (M) = ossified but not fully saturated color. Partly (P) = ossification starting from the arches root. Transparent (T) = no visible bone ossification. The ossification score for suction disk segments is classified as Score 2 = ossified and fully saturated color, Score 1 = ossified but not fully saturated color or . Score 0 = no visible ossification. The ossification score for the gill arches is classified as Score 1 = ossified gill arches or Score 0 = no visible ossification.



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