Synne Therese Rian

Start-feeding of lumpfish (*Cyclopterus lumpus* L.) larvae with *Artemia* and copepods, focusing on growth effects, survival, live prey selection and larval robustness

Master's thesis in Ocean Resources
Supervisor: Kjell Inge Reitan
May 2019

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Synne Therese Rian
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Abstract

From the 1970s the Norwegian production of Atlantic salmon (*Salmo salar*) has increased considerably. In recent years the production has stagnated due to high occurrences of the marine ectoparasites salmon lice (*Lepoptheirus salmonis*) and the not as abundant *Caligus elongatus*. These parasites are negatively impacting the welfare of both wild and farmed salmon stocks as well as the salmon producers' economical profit. Cleaner fish, like the lumpfish (*Cyclopterus lumpus*), is a biological, non-pharmaceutical and gentle delousing treatment. The use of lumpfish has increased during the past 6-7 years. However, the commercial production is currently characterised by a lack of knowledge in terms of nutritional requirements and optimized start-feeding regimes. In addition, the welfare of lumpfish deployed in the sea cages with salmon are of concern.

The purpose of this study was to optimize the start-feeding regime of lumpfish larvae for use in commercial production. This was accomplished by comparing the effects of different start-feeding regimes with respect to larval growth and survival. In addition, the larvae's prey preference was characterized by feed selection experiments and feeding regime effects was compared with respect to larval quality (robustness) determined by stress tests. Larval growth, survival and robustness was evaluated according to the effects from three different feeding regimes lasting from 2-35 dph. Two groups of lumpfish larvae were fed either copepods (*Acartia tonsa*) or enriched *Artemia* nauplii for 12 days including weaning to formulated feed before fed formulated feed exclusively for 22 days. A third group were fed enriched *Artemia* nauplii for 25 days including weaning to formulated feed which they then were fed for 9 days.

The lumpfish larvae fed with enriched *Artemia* nauplii had higher growth and survival compared to larvae fed copepods. Later weaning to formulated feed did also result in better larval growth and survival. These results indicated that the nutritional content of *Artemia* satisfied the nutritional requirements of lumpfish larvae to a higher degree than the content of copepods and formulated feed. There was no significant difference in the lumpfish larvae's selection of *Artemia* and *A. tonsa*, however, they seemed to occasionally have a slightly higher preference for *Artemia*. Lumpfish larvae fed *Artemia* with late weaning to formulated feed showed the highest robustness, especially towards freshwater, which was likely caused by their larger size and better developed physiological systems crucial for osmoregulation.

Sammendrag

Fra 1970-tallet har den norske produksjonen av Atlantisk laks (*Salmo salar*) økt betydelig. I de siste årene har produksjonen stagnert på grunn av høye forekomster av de marine ektoparasittene lakselus (*Lepoptheirus salmonis*) og den ikke like rikelige, *Caligus elongatus*. Disse parasittene har en negativ innvirkning på velferden til både ville og oppdrettede laksebestander samt lakseprodusentenes økonomiske fortjeneste. Rensefisk, som rognkjeks (*Cyclopterus lumpus*), er en biologisk, ikke-farmasøytisk og skånsom avlusningsbehandling. Bruken av rognkjeks har økt i løpet av de siste 6-7 årene. Den kommersielle produksjonen av rognkjeks er imidlertid preget av mangel på kunnskap når det gjelder næringsbehov og optimalisert startfôring, hvilket hemmer kultiveringen. I tillegg er velferden til rognkjeksen utplassert i havmerdene med laks bekymringsverdig.

Hensikten med denne studien var å optimalisere startfôringsregimet til rognkjekslarver for bruk i kommersiell produksjon. Dette ble oppnådd ved å sammenligne effekten av ulike startfôringsregimer med hensyn til larvevekst og overlevelse. I tillegg ble larvenes byttedyrpreferanse karakterisert ved fôrseleksjonsforsøk og fôringsregimeeffekter ble sammenlignet med hensyn til larvekvalitet (robusthet) bestemt ved stresstester. Larvevekst, overlevelse og robusthet ble evaluert i henhold til effektene av tre ulike fôringsregimer som varte fra 2-35 dph. To grupper av rognkjekslarver ble fôret med enten hoppekreps (*Acartia tonsa*) eller anrikede *Artemia* nauplier i 12 dager, inkludert tilvenning til formulert fôr, før de ble fôret med formulert fôr utelukkende i 22 dager. En tredje gruppe ble fôret med berikede *Artemia* nauplier i 25 dager, inkludert tilvenning til formulert fôr som de ble fôret med i 9 dager.

Rognkjekslarver fôret med anrikede *Artemia* nauplier hadde høyere vekst og overlevelse sammenlignet med larver fôret med hoppekreps. Senere tilvenning fra levende byttedyr til formulert fôr resulterte også i bedre larvevekst og overlevelse. Disse resultatene indikerte at næringsinnholdet i *Artemia* oppfylte næringsbehovet til rognkjekslarver i større grad enn innholdet i hoppekreps og formulert fôr. Det var ingen signifikant forskjell i rognkjekslarvenes seleksjon av *Artemia* og *A. tonsa*, men de syntes noen ganger å ha en litt høyere preferanse for *Artemia*. Rognkjekslarver fôret med *Artemia* med sen tilvenning til formulert fôr viste høyest robusthet, spesielt mot ferskvann, hvilket mest sannsynlig var forårsaket av deres større størrelse og bedre utviklede fysiologiske systemer avgjørende for osmoregulering.

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Abbreviations

Art 1	Lumpfish larvae fed with *Artemia* for 12 days
Art 2	Lumpfish larvae fed with *Artemia* for 25 days
Cop	Lumpfish larvae fed with copepods (*Acartia tonsa*) for 12 days
Dph	Days post hatch
DHA	Docosahexaenoic acid (22:6*n*-3). Essential omega-3 fatty acid for marine fish larvae.
DW	Dry weight (mg). Individual body mass of the fish dried at least 48 hours in 60 degrees.
DWI	Daily weight increase in percentage during a specific time interval.
d°	Degree-days. Days after fertilisation multiplied with the degrees (temperature in °C).
EFA	Essential fatty acid. Fatty acids that the fish need to obtain through their diet.
EPA	Eicosapentaenoic acid (20:5*n*-3). Essential omega-3 fatty acid for marine fish larvae.
FW	Freshwater
HUFA	Highly unsaturated fatty acid. Polyunsaturated fatty acid with at least 20 carbon atoms.
NL	Neutral lipid. Hydrophobic molecules with no charged groups.
PL	Phospholipid. Consist of two hydrophobic fatty acids and a hydrophilic phosphate group. Main constituent of cell membranes.
PUFA	Polyunsaturated fatty acid. Contains at least one double bond.
SE	Standard error. The sample population's standard deviation.
SGR	Specific growth rate. Increase in dry weight during a specific time interval.
SL	Standard length (mm). Length measured from snout to notochord end.
SW	Sea water

1. Introduction

1.1 Cleaner fish in salmon aquaculture

The global aquaculture production of Atlantic salmon (*Salmo salar*) has since the 1970s been characterized as a rapid growing industry, and Norway is currently considered as the world's leading producer of Atlantic salmon contributing with more than 1.3 of about 2.5 million tons produced globally (Davidsen, 2018; FAO, 2019a; FAO, 2019b). 95 % of the Norwegian production of salmon is exported, mainly to the EU, but also to other countries all over the world (FAO, 2019b).

Alongside the increased production and densities of salmon, there have also been an increased occurrence of the pathogenic marine ectoparasites salmon lice *Lepoptheirus salmonis* as well as the not as abundant *Caligus elongatus* (Jansen *et al.*, 2012). Salmon lice is one of the most comprehensive challenges Norwegian salmon aquaculture is currently subjected to as it impairs both fish health and welfare as well as the producer's economical profit with regard to salmon lice treatment, control management and lost stock (Costello, 2009; Skiftesvik *et al.*, 2013; Torrissen *et al.*, 2013). The salmon lice are naturally occurring with salmon in sea water, and high fish densities connected to intensive salmon production have demonstrated to create ideal conditions for transmission and reproduction of these parasites (Johansen *et al.*, 2011). Salmon lice attach to the salmonids and consume mucus, skin and blood, creating erosions which reduce the feed conversion efficiency, are susceptible to secondary infections and cause osmoregulatory failure which can lead to fish death (Wootten *et al.*, 1982; Costello, 1993; Skiftesvik *et al.*, 2013). The salmon lice have also experienced to spread to wild stocks of salmon and sea trout located in close proximity to sea cages (Johansen *et al.*, 2011; Torrissen *et al.*, 2013; Vollset *et al.*, 2017). In order to manage further growth of the salmon production without negative effects on natural stocks of salmon, the Norwegian government introduced in 2017 a regulatory framework, termed the "traffic light system". This implies that potential production capacity increase of salmon along the Norwegian coast will depend on the salmon lice's impact on wild salmonid stocks (Vollset *et al.*, 2017). Protecting the salmon against salmon lice and preventing outbreaks is therefore crucial for the salmon industry in order to sustain a further increase in aquaculture production, but also to enhance fish welfare and accomplish a sustainable and financially profitable development.

There are mechanical, chemical and medicinal treatments as well as other methods and technological features to combat the salmon lice. However, through the years the lice have developed resistance towards many of the most actively used chemotherapeutic treatments (Treasurer *et al.*, 2000; Denholm *et al.*, 2002; Fallang *et al.*, 2004; Aaen *et al.*, 2015). Some chemical treatments have also experienced to have negative impact on non-targeted species situated nearby the sea cages and production sites, changing the local biodiversity (Burridge *et al.*, 2010). Freshwater treatments, mechanical removal of the lice from the salmon through hydrolicers (creates water turbulence brushing off the lice) and thermal treatments performed by thermolicers (creates unfavourable fluctuations in temperature for the lice) requires crowding and pumping of the salmon (Overton *et al.*, 2018). This can cause stress, physical damage and even death, impairing fish welfare (Overton *et al.*, 2018). The need for more sustainable alternatives is strongly present, and the deployment of cleaner fish, like ballan wrasse (*Labrus bergylta*) (Skiftesvik *et al.*, 2013) and lumpfish (*Cyclopterus lumpus*) in sea cages with salmon have been presented as a biological, non-pharmaceutical and gentle delousing solution (Treasurer, 2002; Skiftesvik *et al.*, 2013; Torrissen *et al.*, 2013).

The connection between salmon and cleaner fishes is classified as mutualistic where both parties benefit from the relationship. Application of cleaner fish reduces the use of chemicals and medicines and is believed to be more cost-effective compared to chemotherapeutants (Treasurer, 2002). Deploying labrid species in sea cages with salmon started in the late 1980s and has become a more popular approach in recent years, but there have been some challenges. The goldsinny wrasse (*Ctenolabrus rupestris*) was the first to be applied in 1988 (Bjordal, 1991) and the ballan wrasse some years later as it has convenient size, is robust and possesses characteristics suitable for large-scale delousing in sea cages with large salmon (Muncaster, 2008). However, many of the currently applied species of wrasse are temperature sensitive and less effective delousers at temperatures below 6 °C as they become inactive and exhibit dormancy (Sayer and Reader, 1996; Kelly *et al.*, 2014). As water temperatures can reach levels below this point during winter along the coast of Norway the wrasse species are not an optimal option alone in year-around intensive salmon production (Treasurer, 2002). The lumpfish is a cold-water species native in the east, north and western parts of the North Atlantic Ocean (Davenport, 1985). It has proven to be a suitable alternative to labrid species as it is an effective delouser at lower temperatures (Imsland *et al.*, 2014). Nytrø *et al.* (2014) have reported lumpfish feeding at as low as 4 °C. Additionally, lumpfish are ready for deployment in sea cages already at 4 months while ballan wrasse usually require 1.5 years (Helland *et al.*, 2014;

Powell *et al.*, 2018), which is a key factor to why this species have received increased focus (Brooker *et al.*, 2018). The commercial production of lumpfish has increased from 431 000 in 2012 to over 30 million in 2017 (Norwegian Directorate of Fisheries, 2019), and is currently the cleaner fish most commonly used (Powell *et al.*, 2018), while production of ballan wrasse is low in comparison and have stagnated (Figure 1.1). However, along with the increased lumpfish production there have been disclosed several challenges regarding cultivation and use of lumpfish in aquaculture.

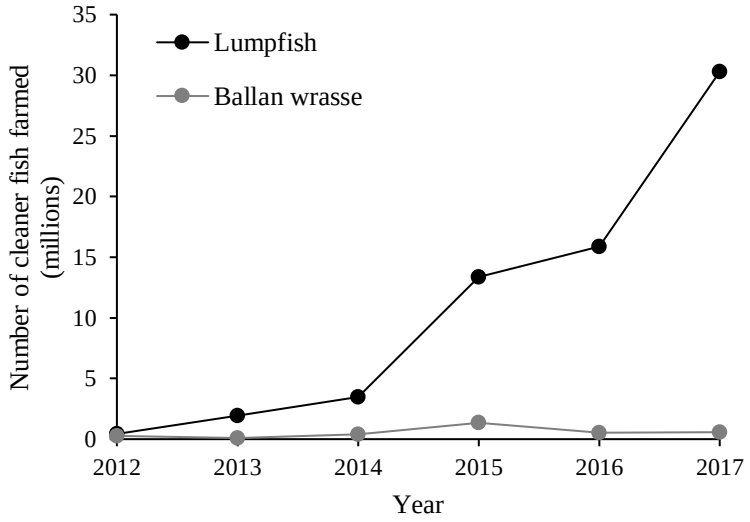

Figure 1.1. *Sale of farmed lumpfish (Cyclopterus lumpus) and ballan wrasse (Labrus bergylta) to producers of Atlantic salmon and Rainbow trout in millions from year 2012-2017 in Norway. Source: Norwegian Directorate of Fisheries (2019).*

1.2 Challenges regarding lumpfish production and welfare

The main challenges associated with lumpfish are currently identified as the dependence on wild broodstock for farming, inadequate start-feeding regimes, high mortality during specific stages of the larval rearing, pathogens and lack of efficient vaccines, along with impaired welfare and stress after deployment in the sea cages (Powell *et al.*, 2018). All these challenges are founded in the lack of knowledge regarding the natural biology of lumpfish. In order to increase animal welfare and achieve an efficient and sustainable lumpfish production these knowledge gaps need to be filled through research. The problems regarding inadequate start-feeding regimes, larval rearing mortality and stress will be investigated in this master's thesis.

1.2.1 Commercial lumpfish rearing

The first studies concerning controlled production of lumpfish and lumpfish larvae feeding behaviour dates back to the mid-1980s (Benfey and Methven, 1986; Brown, 1986). At this time the roe of the fish was of commercial interest (Benfey and Methven, 1986), while at present commercial production have shifted focus towards the lumpfish as a delouser in salmon aquaculture. The species is intensively farmed in tanks of land-based facilities, from hatching to deployment (Imsland *et al.*, 2014; Powell *et al.*, 2018), but information about the species biology in captivity is limited (Powell *et al.*, 2018).

The commercial production is currently dependent on wild brood stock which is believed to be non-sustainable with regard to preservation of wild broodstock and risk of disease transfer between wild lumpfish and farmed salmon (Powell *et al.*, 2018), however, developing brood stocks for future production is under investigation. In nature, adults spawn in shallow waters water close to the shore and performs external fertilisation entailing the female releasing the eggs freely into water masses to a bottom substrate where they are immediately fertilised by a male (Davenport, 1985). The demersal eggs adhere to each other forming a compact mass in contact with sea water, and the average egg size ranges from 2.0-2.6 mm diameter (Davenport, 1985; Benfey and Methven, 1986; Brown *et al.*, 1992). During the incubation period fertilized eggs receives paternal care and protection (Davenport, 1985). Hatchlings do not share the same morphological characteristics as adults aside from a ventral suction disc between their pelvic fins which is already present at hatching and fully functional (Benfey and Methven, 1986; Treasurer, 2018). Lumpfish hatchlings are averaged 5-6 mm length and 2-3 mg weight (wet weight) (Benfey and Methven, 1986; Brown *et al.*, 1992).

Start-feeding of lumpfish larvae in commercial production is a challenge because the nutritional requirements of lumpfish are not well known (Imsland *et al.*, 2018). Therefore, their assumed needs have so far been based on the requirements of other marine fish species used in aquaculture. Optimal nutrition is crucial for fish larvae in terms of growth, optimal performance, survival and reproduction. Current lumpfish production is also characterised by high variability in individual larval size (Dahle *et al.*, 2017), and high mortality is observed post weaning, which is believed to be caused by the transition from live to formulated feeds (Powell *et al.*, 2018). Increased mortality is also experienced about 30 dph at Tjeldbergodden rensefisk AS (personal comment), a commercial producer of lumpfish, which is believed to be connected to final resorption of the yolk sac and also to weaning to formulated diet.

The lumpfish starts to actively feed 3-7 dph, depending on water temperature (Benfey and Methven, 1986; Brown *et al.*, 1992), before they have completely resorbed their yolk sac (Ingólfsson and Kristjánsson, 2002). Full consumption of the yolk sac is completed around 34 dph when larvae are sized about 10 mm (Marthinsen, 2018). In nature, lumpfish larvae and juveniles mainly feed on crustaceans from the calanoid and harpacticoid orders, located on or close to seaweed (Ingólfsson and Kristjánsson, 2002). Ingólfsson and Kristjánsson (2002) found that lumpfish larvae consumed larger prey as they grew older and that juveniles performed selective feeding by ignoring prey that were small, sessile and moved slowly, like gastropods, ostracods, rotifers, bivalves and nematodes. Currently, the most common practice in commercial production is to feed the lumpfish larvae with formulated feed from start (2-5 days post hatch) (Dahle *et al.*, 2017). Feeding with natural plankton (copepods) and *Artemia* nauplii followed by weaning to formulate feed is also applied (Treasurer, 2018). This will be further described in paragraph 1.3 Commercial diets used for lumpfish.

1.2.2 Lumpfish handling, welfare and stress

Lumpfish are subjected to several different stress factors during their life span as cleaner fish in salmon aquaculture, such as handling and change in environments. However, the knowledge about physiological responses to stressful conditions is not well known (Remen and Jonassen, 2017), and optimizing the robustness of lumpfish is crucial in the progress of improving lumpfish welfare and optimizing their lice grazing efficiency.

Outbreaks of salmon lice and amoebic gill disease (AGD), which is caused by the parasitic marine amoeba *Paramoeba sp.* (Munday et al., 1990), in sea cages are most commonly treated by the use of freshwater or hydrogen peroxide (H_2O_2) directly in the sea cage or in well boats (Powell *et al.*, 2015). In recent years the salmon producers have initiated separation of the cleaner fish from the salmon before the salmon are treated for salmon lice, which improves cleaner fish welfare (Hjeltnes *et al.*, 2016). However, not all are sorted out and by an outbreak of AGD the cleaner fish are treated the same way as the salmon as they may also be infected (Karlsbakk *et al.*, 2013; Hjeltnes *et al.*, 2016). Increased cleaner fish mortality have been observed after handling and treatments of salmon lice, especially in freshwater (Hjeltnes *et al.*, 2016; Skiftesvik *et al.*, 2018). Skiftesvik *et al.* (2018) found that ballan wrasse, corkwing wrasse (*Symphodus melops*) and goldsinny wrasse did not tolerate transfer directly to freshwater as well as the lumpfish, which seemed unaffected after 2 hours of exposure in a pilot study. A lower osmotic flux caused by relatively small gills, thick skin and clumpy shape was believed to be connected to the higher tolerance observed in lumpfish compared to the labrid species along with geographical occurrence in areas with low salinity (Skiftesvik *et al.*, 2018). However, a following equivalent experiment of the same study showed a much lower lumpfish survival rate after freshwater exposure. The reason was unknown. Aside from signs of irritation and aversion, all the four species handled a 20-minute exposure to hydrogen peroxide (c = 1 500 ppm active substance, T ~ 12 °C) (Skiftesvik *et al.*, 2018). The results show that disease and lice treatment can have negative effects on lumpfish, however, death may also be related to rough handling together with the salmon (Skiftesvik *et al.*, 2018).

Remen and Jonassen (2017) found increased levels of the stress hormone cortisol in the plasma of lumpfish after handling associated with transport (e.g. car). Normally, stress responses in fish can be detected as elevated concentrations of the stress hormone cortisol and glucose in the blood plasma (King and Berlinsky, 2006). Crowding and pumping associated with transfer from tank to transport trigger stress responses prolonged and strengthened by following transportation, and deploying stressed lumpfish in sea cages increase mortality and the risk of developing chronic stress (Remen and Jonassen, 2017).

Stress tolerance of marine fish larvae and juveniles can be connected to the quality of the early larval diet. Dietary phospholipids (PL), which is a type of polar lipids, have shown to improve larval tolerance towards stress (Coutteau *et al.*, 1997). Increased levels of DHA in the diet increase the robustness of marine fish towards various stressful conditions, such as salinity and temperature changes, as observed in larval red sea bream (*Pagrus major*), yellow fin (*Seriola*

quinqueradiata) and juvenile marbled sole (*Limanda yokohamae*) (Watanabe and Kiron, 1994; Kanazawa, 1997). The presence of dietary arachidonic acid (ARA, 20:4*n*-6) has also shown to be beneficial with regard to larval stress from handling (Koven *et al.*, 2001), so has dietary soybean lecithin, which contain phospholipids (Kanazawa, 1997), vitamin C (Noori *et al.*, 2011) and 1,2-di-20:5-phosphatidylcholine (PC) (Tago *et al.*, 1999). However, there are currently no known studies concerning dietary effects on robustness of lumpfish larvae.

1.3 Commercial diets used for lumpfish

1.3.1 Formulated feed

Formulated feeds (microdiets) are more simple and cost-effective to produce compared to live feed organisms (Hamre *et al.*, 2013). The feed is available in a wide range of shapes and sizes, and possess diverse nutrient profiles, suitable for many different fish species. Formulated diets must satisfy several requirements in terms of structure and biochemical content, attractiveness (need to be identified as food) and digestibility in order to function as a sufficient start feed for fish larvae (Hamre *et al.*, 2013). However, these are not always met. Formulated larval feeds are subjected to loss of hydrosoluble nutrients, like amino acids and certain vitamins, due to leaching when rehydrated (Hamre, 2006; Hamre *et al.*, 2013).This changes the biochemical profile of the diet before it is ingested by the larvae in the rearing tanks, which can cause dietary deficiencies. In terms of digestibility, the fish larvae's ability to digest formulated feed and assimilate nutrients is believed to be less efficient than in adult fish as most fish larvae lack a functional stomach (Govoni *et al.*, 1986). The lack of a functional stomach is considered one of the main challenges of start-feeding in larval rearing and the reason why development of formulated diets for marine pelagic larvae have been challenging (Kjørsvik *et al.*, 2004). Additional disadvantages with formulated diets are variable sinking rates in the water column and accumulation of uneaten pellets on the tank bottom causing increased bacterial growth and suboptimal water conditions (Dahle *et al.*, 2017).

Lumpfish larvae are relatively large and advanced at the point of hatching (Brown, 1986), compared to marine pelagic larvae (Kjørsvik *et al.*, 2004), and are able to feed on formulated diets from start (Benfey and Methven, 1986). Until now, formulated feed originally developed for other marine species, such as cod, have been used in commercial lumpfish larva rearing as there have not been any formulated feeds available specially adapted for lumpfish due to unknown nutritional requirements (Treasurer, 2018). Recent studies with lumpfish larva have shown that the lumpfish larvae do not have a functional stomach until 21-34 dph when they are sized about 8-10 mm (Marthinsen, 2018) and that feeding with formulated feed can negatively affect gut epithelium and energy storage in the liver (Dahle *et al.*, 2017).

1.3.2 Copepods

Copepods are small crustaceans (most species are about 0.5-2.0 mm in size), which serves as important, natural prey organisms for many marine fish larvae (Evjemo *et al.*, 2003; Rafferty, 2019). Copepods are slow-sinking, and their bursting zigzag-movements followed by gliding are attractive for the fish larvae as they initiate fundamental foraging instincts and provide visual stimulus (Lavens and Sorgeloos, 1996). Disadvantages with copepods are high production costs, labour-demanding cultivation procedures and challenging infrastructures compared to *Artemia* and formulated feeds (Lavens and Sorgeloos, 1996).

Marine fish larvae usually have high requirement of the *n*-3 highly unsaturated fatty acids (HUFAs) docosahexaenoic acid (DHA, 22:6*n*-3) and eicosapentaenoic acid (EPA, 20:5*n*-3) for normal growth and development (Sargent *et al.*, 1997). DHA and EPA are essential fatty acids which needs to be supplied trough the diet as many marine fish are not able to synthesise enough of these fatty acids on their own (Watanabe, 1982). Copepods have a high DHA/EPA ratio (van der Meeren *et al.*, 2008), and the naturally high content of DHA and EPA incorporated into PLs, compared to enriched rotifers and *Artemia* is an essential advantage with copepods (Evjemo *et al.*, 2003; van der Meeren *et al.*, 2008). HUFAs stored in phospholipids have resulted in significantly better growth, development, bone ossification and survival in marine fish larvae, such as European seabass (*Dicentrarchus labrax*) and Atlantic cod (*Gadus morhua*), than HUFAs stored in neutral lipids (NLs), such as triacylglycerols (TAG) (Gisbert et al., 2005; Kjørsvik et al., 2009; Wold et al., 2009).

Higher survival, superior eye migration and pigmentation have been observed in halibut (*Hippoglossus hippoglossus*) larvae fed copepods compared to larvae fed enriched *Artemia* (Shields *et al.*, 1999; Evjemo *et al.*, 2003). The better results with copepods was connected to the higher levels of DHA than in the enriched *Artemia* (Evjemo *et al.*, 2003). Atlantic cod larvae have shown significantly higher growth after three weeks of hatching when fed copepods than enriched rotifers followed by *Artemia* (Karlsen *et al.*, 2015), and Romundstad (2015) found better growth and liver and gut development in ballan wrasse. Higher stress tolerance has also been observed in larvae of ballan wrasse and Atlantic cod fed with copepods (*A. tonsa*) compared to larvae fed rotifers (*Brachionus* sp.) and *Artemia* (Øie *et al.*, 2017). This was believed to be caused by the live feed's different nutritional lipid qualities. Feeding lumpfish larvae with copepods have also proven beneficial on lumpfish larvae growth compared to use of formulated feed (Dahle *et al.*, 2017).

1.3.3 *Artemia* sp.

Artemia, brine shrimp, is a crustacean with wide size ranges (0.4-15 mm) (Léger *et al.*, 1987). Encysted eggs are harvested from salt lakes world-wide and the nauplii is one of the most commonly used live prey organisms in commercial production of marine fish larvae, despite not begin a "natural" prey organism for marine predators (Léger *et al.*, 1987). *Artemia* are easy and cheap to cultivate (Lavens and Sorgeloos, 1996). They are orange-red, moves continuously and easy for the fish larvae to catch as they lack efficient escape responses (Léger *et al.*, 1987).

Variable nutritional quality and naturally low content of DHA and EPA in *Artemia* are substantial disadvantages (Lavens and Sorgeloos, 1996). Despite this *Artemia* has proven to be one of the most common and successful prey organisms for aquaculture purposes (Léger *et al.*, 1987). Fatty-acid enrichment of the *Artemia* nauplii with lipid emulsions increase their nutritional value which satisfy the nutritional requirements of marine fish larvae to a higher degree (Léger *et al.*, 1987; Sorgeloos *et al.*, 2001) and is believed to have played a substantial part of this success. Fish larvae fed enriched *Artemia* have shown less malformations as well as improved growth, pigmentation, survival and resistance towards infections and stress (Léger *et al.*, 1987; Sorgeloos *et al.*, 2001), but the larger *Artemia*-size can be a disadvantage for the early life stages of some predator species (Léger *et al.*, 1987). Hanssen (2018) and Marthinsen (2018) found higher growth and survival of lumpfish larvae fed enriched *Artemia* nauplii compared to copepods, but the difference could have been caused by insufficient amounts of copepods as the larvae fed copepods were starved. However, n-3 HUFAs in enriched *Artemia* are less available for fish larvae growth and advancement as they are mainly stored in the NLs and not in the PLs (Coutteau *et al.*, 1997; Gisbert *et al.*, 2005). Of the total lipid content in *Artemia* about 30 % is found in PLs and 60 % in the NLs (Hamre *et al.*, 2002; van der Meeren *et al.*, 2008). Enriched *Artemia* do also selectively metabolize DHA (Rainuzzo *et al.*, 1997; Navarro *et al.*, 1999), which is unfavourable for the DHA/EPA ratio. *Artemia* have a naturally low DHA/EPA ratio, often below the recommended ratio for marine fish larvae which is about 2:1 (Sargent *et al.*, 1999; Conceição *et al.*, 2010). DHA is believed to have a superior function than EPA as an EFA during the stages of larvae development (Watanabe, 1993) as it possesses an important role in brain and retina neural tissue development (Mourente *et al.*, 1991; Bell *et al.*, 1995). While increased ratios of dietary DHA to EPA can improve survival, growth and pigmentation of marine fish larvae (Rainuzzo *et al.*, 1994; Reitan *et al.*, 1994; Rodrıguez *et al.*, 1998), a too low DHA/EPA ratio can cause imbalance in the PLs and the structural composition of cell membranes, which is unfavourable for larvae growth and quality (Watanabe, 1993).

1.4 Aim of study and hypotheses

The purpose of this study was to optimize start-feeding regime of lumpfish for use in commercial cultivation with basis on commonly used feed types; enriched *Artemia* nauplii, *A. tonsa* and formulated feed. Use of copepods in start-feeding of several marine fish species have shown improved larval growth and survival compared to use of enriched *Artemia* and/or rotifers (Shields *et al.*, 1999; Rajkumar, 2006; Dahle *et al.*, 2014; Karlsen *et al.*, 2015; Romundstad, 2015; Øie *et al.*, 2017). However, a recent experiment with lumpfish larvae has shown higher growth and survival with use of enriched *Artemia* compared to *Acartia tonsa* and/or formulated feed (Hanssen, 2018; Marthinsen, 2018). As these larvae fed copepods were starved in Hanssen and Marthinsen's study, it was an aim in the present study to increase the biomass of copepods in order to evaluate if the copepods then would have superior effects on growth and survival. However, higher growth and survival was still expected in larvae fed *Artemia*. These were the aims of the master project:

Aims

1. Compare the use of *Artemia* and *A. tonsa* at equal biomass with respect to larval growth and survival of *C. lumpus*
2. Characterize the *C. lumpus* larvae preference of the two live feed organisms
3. Compare effects of *Artemia* and *A. tonsa* at equal biomass with respect to larval quality (robustness) of *C. lumpus*

This was studied by conducting a start-feeding experiment (2-35 dph) with two groups of larvae fed live prey for 12 days either with enriched *Artemia* nauplii or copepods, and a third group fed enriched *Artemia* nauplii for 25 days. The last 4-5 days with use of live feed, the larvae were weaned to a formulated diet. The diet effects were measured in growth, survival and robustness. These were the hypotheses:

Hypothesis

1. Use of *Artemia* (12 days) will give better start-feeding success compared to use of copepods (12 days)
2. Use of live feed (*Artemia*) for 25 days will give better start-feeding success than use of live feed (*Artemia*) for 12 days
3. Lumpfish will select *Artemia* as they are bigger in size than copepods
4. Lumpfish fed copepods (12 days) will have better robustness than larvae fed *Artemia* (12 days), because copepods are assumed to have a better general nutritional value
5. Lumpfish fed live feed (*Artemia*) for 25 days will show higher robustness than those fed live feed (*Artemia*) for 12 days

The start-feeding experiment was conducted in collaboration with Håvard Rekdal Dybvik, who investigated the dietary effects of *Artemia* and *A. tonsa* on the lipid content of lumpfish larvae.

2. Materials and methods

2.1 Laboratory facility and water treatment

The start-feeding experiment with the lumpfish larvae was conducted in the CodTech-laboratory at NTNU SeaLab (Centre of Fisheries and Aquaculture, Trondheim) in a temperature regulated room with 16 tanks (Kunststoff-Spranger GmbH, Germany) (Figure 2.1). The experiment was run in 12 experimental units (fish tanks). The remaining 4 tanks were used as storage tanks for the copepods supplied during the experiment. Water volume in the fish tanks was set to 100 L during the experiment. The inlet sea water (34 ppt) was pumped from 70 m depth of Trondheimsfjorden. Before entering the fish tanks the seawater was filtered through a sand filter and a 1µm filter, heated, microbially matured (Skjermo *et al.*, 1997), and finally degassed to avoid N_2 supersaturation. The temperature of the sea water was 10 °C.

The tanks were coloured light beige, had a cylindrical shape and a horizontal bottom. The water outlet (hollow tube with big oval shaped holes) positioned in the centre of the fish tanks was supplied with a filter (mesh size 700 µm) to prevent fish larvae from escaping, though still allowing sufficient water exchange and outlet of live prey over time. The filters were cleaned with a brush when required. The water exchange was increased from twice a day at start to 20 times/day at day 30 (Table 2.1), depending on maintaining the water quality with increasing amount of formulated feed fed to the tanks. Each tank was equipped with a magnet driven cleaning arm (Kunststoff-Spranger GmbH, Germany), which cleaned the bottom and side walls, and peristaltic pumps (SEKO Kronos 50 Dosing Pump, Italy) for feeding of live prey to the lumpfish. In addition, dark grey silicone sheets were placed in the tanks for increasing surface area for the lumpfish larvae and skimmers were used to collect organic compounds at the water surface. The light regime was constant light during the 24 hours, and the lumpfish were fed continuously throughout both day and night. Led lights (Flex Tube SE SC, colour temp. 4000K, 10.6W/m, 439 Lumen/m) located above each fish tank simulated daylight. The lights across the roof was fluorescent.

Figure 2.1. *Illustration of the tank setup in the Cod-Tech laboratory. 12 tanks contained lumpfish, 4 contained live feed (copepods). Each of the three treatments (Cop, Art 1, Art 2) was allocated four tanks. "Cop" represents the lumpfish fed copepods for 12 days (tank 3, 6, 11, 16), "Art 1" represents the lumpfish fed Artemia for 12 days (tank 2, 8, 9, 15) and "Art 2" represents the lumpfish fed Artemia for 25 days (tank 1, 7, 10, 12) and. Tank 4, 5, 13 and 14 was used as storage for live copepods.*

2.2 Larval rearing

2.2.1 Egg incubation

The eggs for the start-feeding experiment were obtained from "Skjerneset fisk" (Averøya). Eggs and sperm were collected from wild caught *Cyclopterus lumpus* females and males. The eggs were fertilized and incubated at temperatures ranging from 2.2 to 4.2 °C from day of fertilizing until day 52 and then transported to NTNU in Trondheim by boat. Six egg "cakes" weighing 2 kg in total (egg volume = 2.65 L, ca 50 000 eggs/L, 132 500 eggs in total, mean number of eggs fertilized was 84 %) from six different females were transported dry kept on ice in Styrofoam boxes. At arrival, the eggs were incubated in four Sterner family hatching incubators supplied with matured seawater (34 ppt) at high exchange rate, covered with black plastic to create darkness. At NTNU SeaLab the eggs were incubated for 18 days before hatching at 290 degree-days (d°). The starting temperature was 5.6 °C and was increased with 1 °C every second day until reaching 10 °C, in order to control the hatching date.

In addition to these egg groups, one egg batch obtained from Akvaplan-niva in Tromsø containing 0.6 L lumpfish eggs fertilised 24.01.19 (63 % fertilized) was shipped by air to NTNU SeaLab 16.02.19. In Tromsø the lumpfish eggs were incubated for 25 days in temperatures varying from 4.3-8.1 °C. At arrival to SeaLab the egg cake was divided and incubated in two incubators and treated similarly to the eggs from the previous egg batches described in the previous paragraph. The temperature was gradually increased from 5.0-9.7 from start until hatching, which was 20 days after incubation. This batch of lumpfish larvae was used in prey preference experiment 2.7.1 (experiment 1 – unfed yolk-sac lumpfish larvae (4 dph))

2.2.2 Transferring eggs to fish tanks

The eggs were transferred to the fish tanks five days prior to hatching. Before transfer, each egg cake was divided into 12 equal sized pieces and placed in 12 measuring cups filled with an equal amount seawater (500 mL) in order to obtain the same volume of mixed eggs in each tank. The eggs from all the egg cakes were combined to obtain as homogenous egg groups as possible in each fish tank, taking into account that the six egg batches delivered could have varying hatching rate and quality. Further, the measuring cups with eggs and water was weighed to disclose potential significant differences which was eliminated by adding or removing eggs in order to obtain similar weight in all cups. Ultimately, the eggs were transferred to sieves submerged in the tanks below the sea water inlet. After hatching, the sieves with the egg residues were removed to avoid potential bacterial growth. The total lumpfish density in the tanks after complete hatching varied from 16-22 larvae/L.

2.2.3 Maintenance of fish tanks

The lumpfish tanks were cleaned daily, occasionally twice a day when required. A siphon was used to remove and transfer any excess feed, dead fish, bacterial growth (slime) and other organic load from the tanks into a plastic bucket. Any live lumpfish larvae collected by the siphon was removed from the waste bucket and transferred back to their respective tanks. The number of dead larvae obtained from siphoning was counted and registered every day.

2.3 Feeding regimes

Three different feeding regimes were used in the start-feeding experiment. One of the regimes consisted of copepods and formulated feed, and two regimes of *Artemia* and formulated feed (Table 2.1). The three treatments were:

1. **Copepods for 12 days (Cop)**
   Lumpfish larvae were fed copepods for 7 days (2-8 dph) before weaned to formulated feed for 5 days (9-13 dph). Ultimately, the larvae were fed formulated feed exclusively for 22 days (14-35 dph).

2. ***Artemia* for 12 days (Art 1)**
   Lumpfish larvae were fed enriched *Artemia* for 7 days (2-8 dph) before weaned to formulated feed for 5 days (9-13 dph). Ultimately, the larvae were fed formulated feed exclusively for 22 days (14-35 dph).

3. ***Artemia* for 25 days (Art 2)**
   Lumpfish larvae were fed enriched *Artemia* for 21 days (2-22 dph) before weaned to formulated feed for 4 days (23-26 dph). The larvae were fed formulated feed exclusively for the last 9 days (27-35 dph).

Each of the three treatment groups were run in 4 fish tanks (representing 4 replicates). The allocation of the replicates in the lab facility was randomized to prevent experimental errors (variation between individuals not considered in the statistical analysis, Figure 2.1). The feeding regimes were based on earlier experiences and experiments concerning start-feeding of lumpfish and mainly from a study conducted by Julie Hanssen and Joachim Marthinsen in 2018 (Hanssen, 2018; Marthinsen, 2018). Their different regimes were based on the aim of comparing effects of the two types of live feed used and disclose if the live feed availability over different time intervals could result in significant differences in terms of growth and survival. Hanssen and Marthinsen used the same density (600 000-1 800 000 prey/tank/day from 2-22 dph) regardless of live prey type, while the present experiment used the same biomass, not the same density, in order to compensate for the size difference between *Artemia* and copepods. Enriched *Artemia* (size 800 µm, 0.77 µg C, enriched 24 hours) are about 1.4 times larger and have about 1.2 times higher carbon content than copepods (size 580 µm, 0.62

µm C, life stage C3) based on live feed characteristics presented by Evjemo and Olsen (1999) and Hagemann (2013). Therefore, the density of copepods fed to the lumpfish larvae was higher than for *Artemia*, but the overall biomass was equal for both species.

In the present study, the enriched *Artemia* and copepods were kept in live feed reservoirs (25 L containers) located right beside each larval tank and were fed continuously to the lumpfish larvae by use of peristaltic pumps. The reservoirs were refilled with live feed daily. Feeding periods was estimated to ± 24 hours and the pumping speed and volume (with varying live prey concentrations) fed per hour was set depending on the total volume in the 25 L containers. The density of copepods and *Artemia* in the reservoirs did not exceed 100/mL and 500/mL, respectively, in order to maintain an optimal environment for the live feed (levels above this could lead to oxygen depletion). This was taken into account when deciding the volume in both the storage tanks and feeding reservoirs. The amount of live prey fed to the lumpfish was dependent on the feeding regime and feeding amounts increased as the larvae grew bigger and was based on estimated larval growth and larval densities in the tanks.

Table 2.1. *The start-feeding time schedule of the experiment with dates, days post hatch (dph), degree-days (d°), water exchange rate (tank volumes/day), feeding regimes, samplings performed and number of larvae sampled from each tank for photos, standard length (SL), dry weight (DW), C:N-analysis, and experiments conducted to test larval prey preference and stress tolerance.*

Date	**August 2018**											**September 2018**																									
	21.08	22.08	23.08	24.08	25.08	26.08	27.08	28.08	29.08	30.08	31.08	01.09	02.09	03.09	04.09	05.09	06.09	07.09	08.09	09.09	10.09	11.09	12.09	13.09	14.09	15.09	16.09	17.09	18.09	19.09	20.09	21.09	22.09	23.09	24.09	25.09
Dph	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
d°	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640
Water exchange			2 times/day							5 times/day					8 times/day				10 times/day			15 times/day										20 times/day				
Regime 1 (Cop)			*Acartia tonsa (fed Rhodomonas baltica*)																																	
										Formulated feed (Clean start 200)																										
																								Formulated feed (Clean start 300)												
Regime 2 (Art 1)			*Artemia* (enriched with Multigain)*																																	
										Formulated feed (Clean start 200)																										
																								Formulated feed (Clean start 300)												
Regime 3 (Art 2)			*Artemia* (enriched with Multigain)*																																	
																								Formulated feed (Clean start 300)												
Sampling																																				
Photo SL DW C:N			4							12														12												20
Experiments																																				
Prey selection					X**							X		X																						
Stress tests																														X					X	

* From 4-7 dph the lumpfish got a mix of newly hatched and enriched *Artemia* due to prey loss (Appendix 1, Table A2). This was not originally planned as a part of the feeding regime

** This prey selection experiment was performed on another batch of lumpfish 13.03.19 (from Akvaplan-niva, Tromsø, 2019)

2.4 Feed types used

2.4.1 Copepods

Acartia tonsa (Clone DFH.AT1) was supplied in 25 L containers by C-Feed (Vanvikan, Norway) in a total of three shipments; 1, 7 and 10 dph. The first batch (mean size 180 µm, life stage N3-N5) was used from 2-6 dph, the second batch (mean size 400 µm, life stage C1-C2) from 7-9 dph and the third batch (mean size 700 µm, life stage C4) from 10-11 dph. The nauplii (N) and copepod (C) stages are retrieved from Hagemann (2013). Each container (25 L) contained about 5 million *A. tonsa*. The *A. tonsa* were stored in four storage tanks (4, 5, 13, 14) identical to the fish tanks, prepared and filled with seawater in advance. The water outlet of the tanks was covered with a filter (mesh size 100 µm) enabling sufficient water exchange without the copepods being flushed out. The light regime was the same as for the lumpfish larvae, 24 hours light. The copepod density in the storage tanks were also measured daily in order to estimate the feeding requirements of the larval tanks. Water samples for counting copepods were obtained by situating a hollow glass rod vertically in the water column and place a finger on top to create a vacuum. The samples were transferred to a plastic cup and the density measured by counting the number of copepods in a drop of 100 µL added 1-2 drops of fytofix (Lugol's iodine solution) in a 6-well Nunc cell plate under a stereomicroscope. Lowest and highest counts were eliminated, and density estimated based on the remaining counts. The desired volume of copepods was harvested from the storage tanks, transferred to the live feed reservoirs (25 L containers) and fed to the lumpfish using the peristaltic pumps. The densities of copepods fed to the lumpfish is listed in Appendix 1, Table A1.

The copepods were fed with microalgae before they were fed to the lumpfish. The microalga *Rhodomonas baltica* (Clone NIVA-5/91, Cryptophyceae: Pyrenomonadales) was obtained from stock cultures at NTNU SeaLab. The microalgae were cultivated semi-continuously in round bottom flasks (5 L) added autoclaved seawater (4 L) and inorganic nutrients (Conway-medium (4 mL; 1 mL Conway per L seawater)) supplied with air, CO_2 and light from one side only. The inoculation started six days prior to the first supply of copepods for the cultures to reach maximum growth before used as feed. 1.5-2 L of the cultures were harvested daily from each bottle and replaced with the equivalent amount of autoclaved seawater and Conway medium.

2.4.2 *Artemia*

The production of *Artemia* were conducted according to a protocol by Jan Ove Evjemo based on FAO's "Manual on the production and use of live food for aquaculture" by Lavens and Sorgeloos (1996). The production comprised of decapsulation, hatching and lipid enrichment at water temperatures ranging from 25-28 °C and is described in Appendix 2. The densities of *Artemia* fed to the lumpfish larvae are listed in Appendix 1 (Table A2) and the *Artemia* were at life stages instar I-III (500-1000 µm, mean 750 µm).

2.4.3 Formulated feed

Formulated feed from Skretting AS (CLEAN start, Norway) was used for weaning and feed after the live prey stages. Clean start 200 was used from 9-23 dph and Clean start 300 from 23-35 dph (Table 2.1), based on the increasing size of the lumpfish larvae. The feeding amounts to the tanks were based on expected larvae biomass increase. The formulated dry fed was fed to the lumpfish using automated feeding dispensers (Sterner Aquatech, UK) hanging right above each tank. A controller program (Normatic WebServer) was used to manage the desired feeding times, amounts and time intervals for each feeding. The feeding amounts and feeding frequencies increased as the larvae grew bigger (Appendix 1, Table A3), based on the same factors as for the live feed.

2.5 Sampling

Sampling of lumpfish larvae for development and growth was performed at 2, 9, 23 and 35 dph in association with changes of feed types fed to the larvae during the experiment. 4, 12, 12 and 20 larvae were sampled from each tank at 2, 9, 23 and 35 dph, respectively (Table 2.1). All larvae were sampled randomly from the fish tank's side walls, bottom, cleaning arm, silicone sheets and free water column (from bottom to surface) into plastic cups by a spatula. The obtained larvae were euthanized with an overdose of tricaine methane sulfonate (MS-222 Finquel vet., Agent Chemical Laboratories Inc., USA; 2 g/L sea water) and rinsed in distilled water to remove salt particles before further analysis. The larvae were analysed for developmental stage, standard length (SL), dry weight (DW), and carbon and nitrogen content.

2.5.1 Developmental stage and standard length

The euthanized and rinsed larvae was photographed to assess the developmental stage and standard length. The larvae were photographed at magnification 1.0 and 0.63 using a stereomicroscope (Leica MX 7.5, Germany) connected to a camera (Axiocam ERc 5s, Carl Zeiss microscopy GmbH, Germany) and computer software (Zen imaging software by Zeiss). By adding a ruler to the pictures and selecting "burn in annotations" when saving the pictures, the standard lengths was measured subsequently by using Windows Software Image J (version 1.8.0_112). The larval standard length was measured from the tip of the snout (and mouth opening) to the end of the notochord (Figure 2.2) until the notochord was no longer visible, then the caudal peduncle was used as the posterior measuring mark (Figure 2.3).

Figure 2.2. *Standard length measurement of lumpfish larvae, 2 dph (Art 1).*

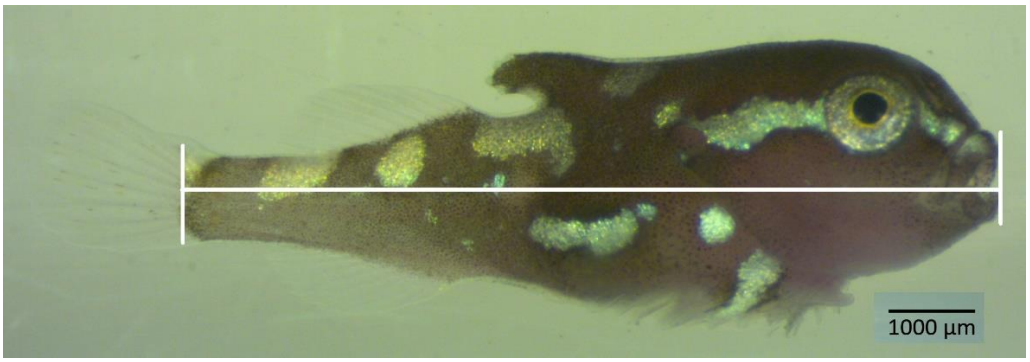

Figure 2.3. *Standard length measurement of lumpfish larvae, 35 dph (Art 1).*

2.5.2 Dry weight and growth rate

After being photographed, the larvae were transferred to pre-weighed tin capsules (size 4x6 mm and 5x9 mm, Säntis, Switzerland) placed in a 96-Nunc well cell plate and set in a drying cabinet (60 °C) for at least 48 hours before weighed on an ultra-microbalance weight (Mettler-Toledo UMX2, USA) to estimate dry weight.

Fish growth was estimated as Specific Growth Rates (SGR) in specific sampling intervals. SGR was calculated according to equation 2.1 (Houde, 1981):

$$SGR = \frac{lnDW_2 - lnDW_1}{t_2 - t_1} \quad (2.1)$$

where DW_1 represents the initial mean dry weight and DW_2 the final mean dry weight of the interval, t_1 the time of DW_1 and t_2 the time of DW_2. The SGR was further used to calculate the percentage daily weight increase (% DWI) (Houde, 1981) according to equation 2.2:

$$\%\, DWI = (e^{SGR} - 1) \cdot 100\% \quad (2.2)$$

2.5.3 Carbon and nitrogen content

The carbon and nitrogen analysis of the lumpfish larvae was performed after the tin capsules with the lumpfish larvae had been weighed to estimate dry weight. The tin capsules were tightly packed into compact balls before they were placed into a 96-Nunc well cell plate. Kjersti Andresen (situated at Trondheim Biological station) analysed the samples using a Vario EL CUBE, Elementar (Germany). The protein content per lumpfish larva was calculated by multiplying the estimates of nitrogen per larva with 6.25, a commonly used nitrogen-to-protein conversion factor (Mariotti *et al.*, 2008; Diniz *et al.*, 2013).

2.6 Survival

When the start-feeding experiment ended (35 dph) all the fish were collected from each fish tank, euthanized with an overdose of MS-222 (2 g/L sea water) and transferred to 12 containers filled with 70 % ethanol where they were preserved until counting. The initial number of lumpfishes in each tank was estimated based on the number of dead larvae added to the final number of lumpfishes remaining in the tank at the final day. The survival (S_t) of lumpfish larvae in each tank was calculated according to equation 2.3:

$$S_t = \frac{N_t}{N_0} \cdot 100\ \% \quad (2.3)$$

where N_t is the number of lumpfish alive at time t (dph) and N_0 is the number of lumpfish alive at start (dph 0). The sampled larvae were excluded in the estimation of total survival as the number of larvae sampled was low compared to the total larval density in the tanks and was not expected to create significant differences.

2.7 Prey selection experiments

Three live feed selection experiments were conducted during the start-feeding experiment to assess the lumpfish larvae's preference for either *Artemia* or copepods. All larvae used in the three prey preference experiments were sampled randomly from the fish tanks in similar ways as described for larvae sampled for development and growth. White, cylindrical experimental containers (5 L buckets) filled with 4 L of sea water was used. The containers were placed on a table supplied with air bubbles created by battery driven pumps from C-Feed or air pumps (HiBlow HP 40) to create stirring and even distribution of the live feed. Total experimental time was 3 hours for all experiments. Water samples were obtained from each experimental bucket before experiment start (0 hours) and then every 1 + ½ hour to document ratio between the *Artemia* and *A. tonsa* in the water. Visual observations of general swimming behaviour were also conducted. The prey density was measured by counting the number of copepods and *Artemia* as described previously in paragraph 2.4.1 in water drops of 1 mL. There was a planned time difference between every action to assure collection of water samples at the same time intervals for each container as time-delays between the first and last container could have interfered with the results. At every sampling time, eight samples from the vertical water column of each container were obtained with a hollow glass rod. The water column was stirred before every sampling to create an even distribution of *Artemia* and copepods in the water column.

The desired amount of live prey in each container for all three prey preference experiments was 5 *Artemia*/mL and 5 copepods/mL (10 prey/mL in total) in the 4 L of sea water. The volume of live feed required from the live feed storage tanks in order to achieve this prey density was calculated according to equation 2.4:

$$C_1V_1 = C_2V_2 \quad (2.4)$$

Where C_1 represents the initial concentration of the solution (live feed density in storage tanks), V_1 the initial volume of the solution (the volume of live feed added to the 4 L in the experimental containers), C_2 the final concentration of solution (5 prey/mL), and V_2 the final volume of the solution (4 L).

2.7.1 Experiment 1 – unfed yolk-sac lumpfish larvae (4 dph)

The experiment was performed on lumpfish yolk-sac larvae from a different batch (Akvaplan-niva, Tromsø, 2019) than in the start-feeding experiment (2018). A total of 96 larvae were sampled divided on the 4 experimental containers (24 larvae in each container). The larvae had not received any type of feed prior to the start of the experiment. There was a total of eight experimental containers of which four contained lumpfish and live prey. The remaining four containers represented controls without lumpfish, but the live prey concentration was the same as for the containers with lumpfish.

2.7.2 Experiment 2 – fed lumpfish larvae (11 dph)

The experiment was performed on lumpfish larvae from the start-feeding experiment (2018). There was a total of 4 experimental containers with fish. 12 larvae were used per container, half the number of larvae used in experiment 1, because all were assumed to have started exogenous feeding and therefore had higher consumption rates than yolk-sac larvae. All larvae were sampled from tank 1, 7, 10 and 12 representing the treatment group fed *Artemia* for 25 days (Art 2), which was not yet weaned to formulated feed. The lumpfish was fed with live prey until start of experiment.

2.7.3 Experiment 3 – starved lumpfish larvae (13 dph)

The experiment was performed on lumpfish larvae from the start-feeding experiment (2018). The number of experimental containers and lumpfish larvae used was the same as for in experiment 2, and all larvae were sampled from Art 2. The larvae were starved for 3 hours prior to start of the experiment.

2.8 Tests of robustness

Two stress tests were performed during the start-feeding experiment in order to assess the larval robustness (quality), and to evaluate the possible effect of feeding regime on the performance of lumpfish larvae. The first experiment was exposing the lumpfish to a combination of brackish water and shaking conditions, and the second experiment was exposing the lumpfish to only freshwater. The different exposure conditions were chosen to simulate environmental conditions that the lumpfish can experience as cleaner fish and during disease treatments and delousing procedures in sea cages. For both experiments 15 lumpfish larvae were sampled randomly as described earlier from the 12 fish tanks. The larvae were then transferred to 12 white, cylindrical experimental containers (5 L buckets) marked with the respective tank numbers (1, 2, 3, 6, 7, 8, 9, 10, 11, 12, 15, 16) representing each of the four treatments. There was no addition of feed to the containers nor any CO_2 or O_2-supply during the experiments. Temperature and O_2 were measured at the start and end of exposure periods and at the start and end of the recovery periods. The general behaviour of the larvae was assessed by visual observation and a larva was defined dead when it had flipped over to the side, did not gasp with their mouth, and did not respond to external stimuli when touching it with a spatula. Different lumpfish larvae were used in all the experiments (no reuse), and all larvae were euthanized with an overdose of MS-222 (2 g/L sea water) after completion of the tests. The experiments were approved by the Food Safety Authority (Mattilsynet, FOTS ID 15601).

2.8.1 Experiment 1 – Brackish water combined with shaking

A test on exposing lumpfish larvae to brackish water combined with shaking conditions was conducted at 29 dph. The experimental containers were filled with 1 L brackish water (10 ppt) and placed on a shaking table (Orbitron, Infors HT, Switzerland) set to 90 revolutions per minute (rpm). The lumpfish were exposed to this combination of brackish water and shaking for 3 hours before the containers were removed from the shaking table and the lumpfish was left in the brackish water for another 6 hours followed by a recovery period in sea water of about 12 hours. At the start of the recovering period the brackish water was exchanged with normal sea water (34 ppt) by using a siphon with a filter at the front to avoid siphoning the lumpfish. The number of dead larvae was observed and documented every 30^{th} minute during the active experiment (0-3 hours), according to time schedule (Table 2.2).

Table 2.2. *Time schedule lumpfish larval stress test with brackish water combined with shaking with time and associated action. Total time of the active experiment was 3 hours plus an extension period of 6 hours. Total recovery period was about 12 hours.*

Time (hours:minutes)	**Action**
00:00	Start of experiment – lumpfish added to the experimental containers
00:15-02:45	Observation of behaviour and documentation of dead lumpfish larvae every 30th minute
03:00	End of shaking, larvae was left in brackish water (10 ppt)
09:00	End of brackish water experiment – lumpfish netted and water exchanged from brackish water (10 ppt) to SW (34 ppt)
09:15	Start of recovery period in normal seawater
21:00	End of recovery period

2.8.2 Experiment 2 – Only freshwater

A test on exposing the lumpfish larvae exclusively to freshwater was conducted at 34 dph. The experimental containers were filled with 2 L of freshwater (salinity < 0.5 ppt) and placed on a table. All the lumpfish were transferred to the experimental containers at the same time, marking the start of the experiment. The exposure time was 3 hours in total. After 3 hours the water in the containers was exchanged with normal seawater for larval recovery in which the fish was netted, freshwater was poured out, and 4 L of sea water was added the containers. The recovery period was about 20 hours. The number of dead larvae was observed and documented every 15th minute during the active experiment (0-3 hours), according to schedule (Table 2.3).

Table 2.3. *Time schedule lumpfish larval stress test with only freshwater with time and associated action. Total time of active experiment was 3 hours and total recovery period was about 20 hours.*

Time (hours:minutes)	**Action**
00:00	Start of experiment – lumpfish added to the experimental containers
00:15-02:45	Observation of behaviour and documentation of dead lumpfish larvae every 15th minute
03:00	End of exposure with freshwater – lumpfish netted and water exchanged from FW (< 0.5 ppt) to SW (34 ppt)
03:15	Start of recovery period with normal seawater
23:00	End of recovery period

2.9 Statistics

All statistical analysis was performed with the computer programme IBM SPSS Statistics 25 (Statistical Package for the Social Sciences) and all graphs were created in Microsoft Excel (version 2016). All statistical test applied had a significance level of $\alpha = 0.05$, and differences were considered statistically significant if $p < 0.05$. Correlations and regressions had a significance level of $\alpha = 0.01$, and where considered statistically significant if $p < 0.01$. All datasets with percentage values were arsin-transformed in SPSS before used in statistical tests. Means and standard errors (SE) were obtained from the descriptive table of one-way ANOVA tests, of both normally and not normally distributed data.

The Shapiro-Wilk test was used to test if data was normally distributed. Homogeneity of variance (similarity between population variances) was estimated by Levene's test. If the variance between groups with normally distributed data was homogenous one-way analysis of covariance (ANOVA) followed by Student-Newman-Keul's (S-N-K) test was used to test for significant differences. If the variance between groups with normally distributed data was non-homogenous one-way ANOVA with Welch test was used compare the means of the groups and a Dunnett's T3 post hoc test was conducted for pairwise comparisons to determine which groups were significantly different from each other. The null hypothesis was "no significant difference between groups", and alternative hypothesis was "significant differences between groups". The non-parametric Kruskal-Wallis one-way ANOVA (2 sided, asymptotic differences) test was used to estimate significant differences between the independent groups of not normally distributed data. To disclose which groups were significantly different from each other the view was set to pairwise comparisons. A two-way ANOVA was applied when significant differences within groups or experiments were to be investigated. A Pearson's correlation test was used to test for linear correlation between continuous variables. Linear slopes of regression were estimated as well as coefficients of determination (r^2) if there were linear correlations between two variables. For non-linear data between two variables exponential slopes of regression was performed to explain their relationship.

3. Results

3.1 Larval development, colour and behaviour

The external morphology of the lumpfish larvae changed significantly from 2-35 dph (Figure 3.1). The development of distinctive fins with fin rays, pigmentation of the epidermal tissue and transparency, nostrils, ventral suction disc and overall shape was assessed by visual examination. There were differences in the characterisations between the individuals within each treatment group as well as between each treatment group, but the overall external morphological development was similar between all larvae investigated. Lumpfish larvae fed *Artemia* (Art 1 and Art 2) seemed to develop faster than larvae fed copepods (Cop). Table 3.1 describes the larval development more thoroughly and is based on the external morphology of larvae from Art 2, as the best growth was observed in this treatment group. Table 3.1 is in accordance with Figure 3.1.

Lumpfish larvae from all three treatment groups differed in colours (Figure 3.1). Larvae colours ranged from red, green, yellow, and dark brown, almost black, to dark spots. The majority of the lumpfish fed *Artemia* seemed to obtain a reddish colour, the rest were light green and dark brown. By visual observation the dark and light green larvae seemed smaller in size than the red ones. The majority of lumpfish fed copepods had light and dark green and light-yellow colours, but some were also reddish and dark brown. As many of the larvae grew bigger, they also possessed small, white shiny spots on their head, trunk and tail region.

Surface attachment by the ventral suction disc was the general behaviour for the majority of the lumpfish larvae, but their behaviour changed as they developed. Behavioural differences between the fish from the three different treatment groups and between larvae with different colours were also observed. Young larvae (2-9 dph) was not easy to remove from surfaces and were quick to attach themselves again if they were released. When the lumpfish larvae were scared by shadows or loud noises, they released themselves from the surfaces and started swimming before they settled down again. This response was observed more often in the older larvae (23-35 dph) than in the young larvae. Small larvae seemed to be more dependent on the capability of attaching to surfaces than larger larvae.

Table 3.1. *External morphology development of C. lumpus (2-35 dph). Overview for all sampling days (2, 9, 23, 35) from the present feeding experiment with associated external morphology descriptions. The table is based on the external morphology of larvae from Art 2. Morphology descriptions are based on Figure 3.1.*

Dph	External morphology
2	The larvae had a large head compared to the stream-lined trunk. Head and body were lightly pigmented to the point at which they were still transparent, except for the most posterior end by the end of the notochord which was not pigmented. The mouth was lightly pigmented. Vertical light bands across the eyes was present. Fin folds were developed with some few visible fin rays. A small first dorsal fin without fin fold, just overgrowth of epidermal tissue, had begun to develop. The suction disc was not pigmented, but fully functional.
9	The larvae had reached the state of notochord flexion, where the posterior end of the notochord had begun to bend upwards in the transparent fin fold. The head and trunk were fully pigmented except for some transparent patches dorsally in the tail region. The trunk and tail region were more pigmented and less transparent. Nostrils at the snout right above the mouth were clearly visible. The suction disc was lightly pigmented and fully functional. The consumed *Artemia* (orange colour) was visible in the stomachs of the fish fed *Artemia*.
23	The flexed notochord was no longer visible in the posterior caudal fin fold. Distinctive and solid fin rays had developed in all transparent fin folds, creating a caudal fin, second dorsal fin, and an anal fin. The larvae were highly pigmented and nearly no longer transparent. A clear distinction between the caudal fin and the tail region of the trunk was observed. The suction disc was highly pigmented and had developed a white, shiny band from the ventral side of the larvae and outwards along with the fin rays.
35	The entire larvae, expect for the fins, were highly pigmented and no longer transparent. All fins with fin rays were developed. The suction disc was highly pigmented. The lumpfish began to look similar to adult lumpfish, with a compact and more oval body shape, not as streamlined.

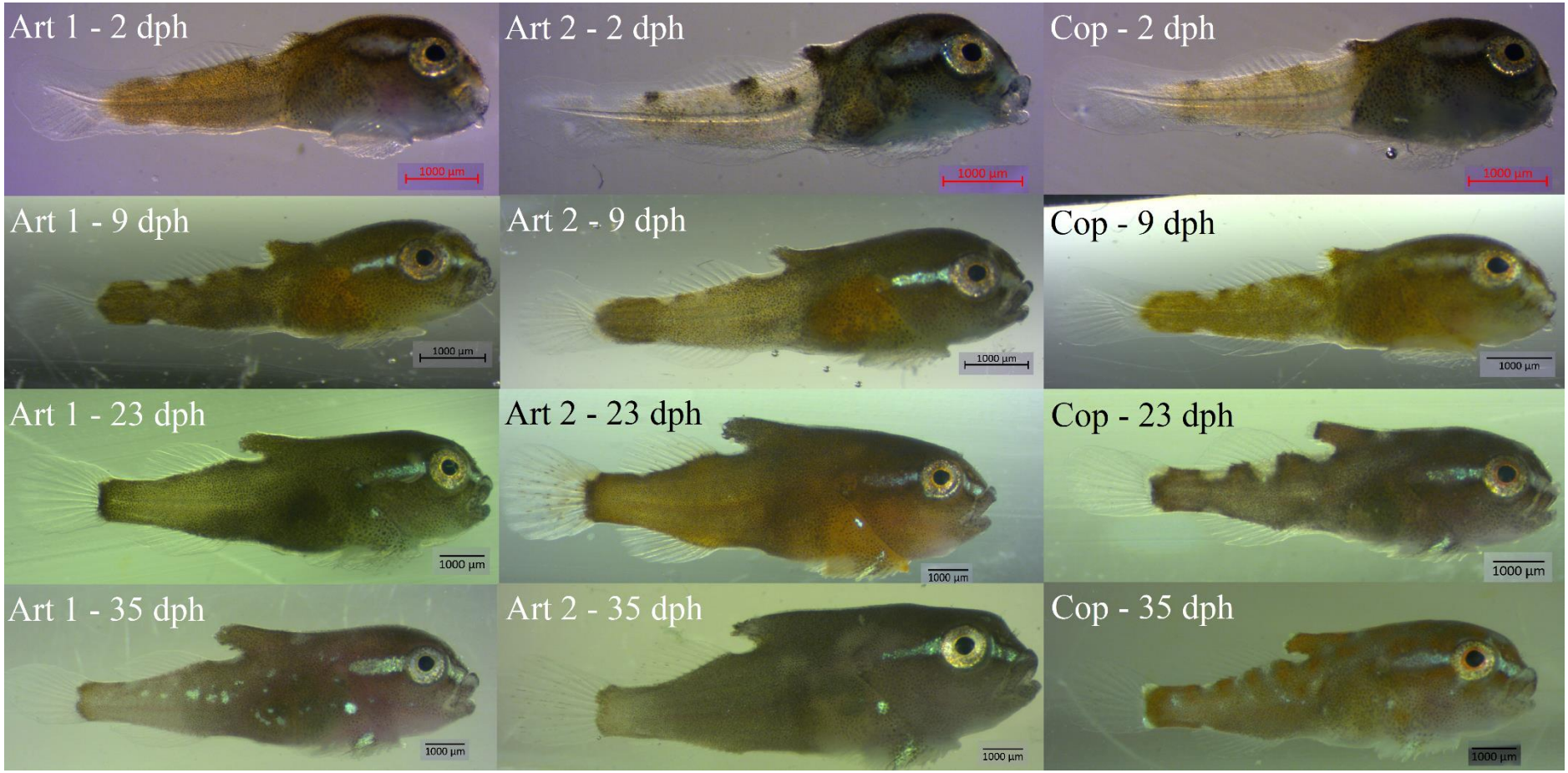

Figure 3.1. External morphology of *C. lumpus*, 2-35 dph. *The developmental stages are represented by one fish from each treatment (Art 1, Art 2, Cop) each sampling day (2, 9, 23, 35) with marked treatment and day post hatch (dph). "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed. Each picture is presented with respective scale bars. Magnification 1.0 and 0.63.*

3.2 Standard length

At the first sampling day (2 dph) the mean lumpfish larval standard length (SL) were about 6 mm (Figure 3.2). Larvae fed *Artemia* the longest period (Art 2) had significantly higher SLs at 23 and 35 dph than larvae fed *Artemia* and copepods for short periods (Art 1 and Cop). The larvae fed *Artemia* for short periods (Art 1) was significantly longer than Cop-larvae at 23 dph, but their size was no longer significantly different by the end of the experiment (35 dph). No considerable changes in size increase in neither of the treatment groups were observed after weaning of the larvae to formulated diet. From 23-35 dph the size increase was similar for all larvae regardless of feeding regime, and at the end of the experiment (35 dph), the Art 2-larvae had the highest mean SL of 11.6 ± 0.4 mm and Art 1 and Cop the smallest with 10.2 ± 0.2 mm and 9.6 ± 0.2 mm, respectively. The mean standard length per larva per tank each sampling day is listed in Appendix 3, Table A4.

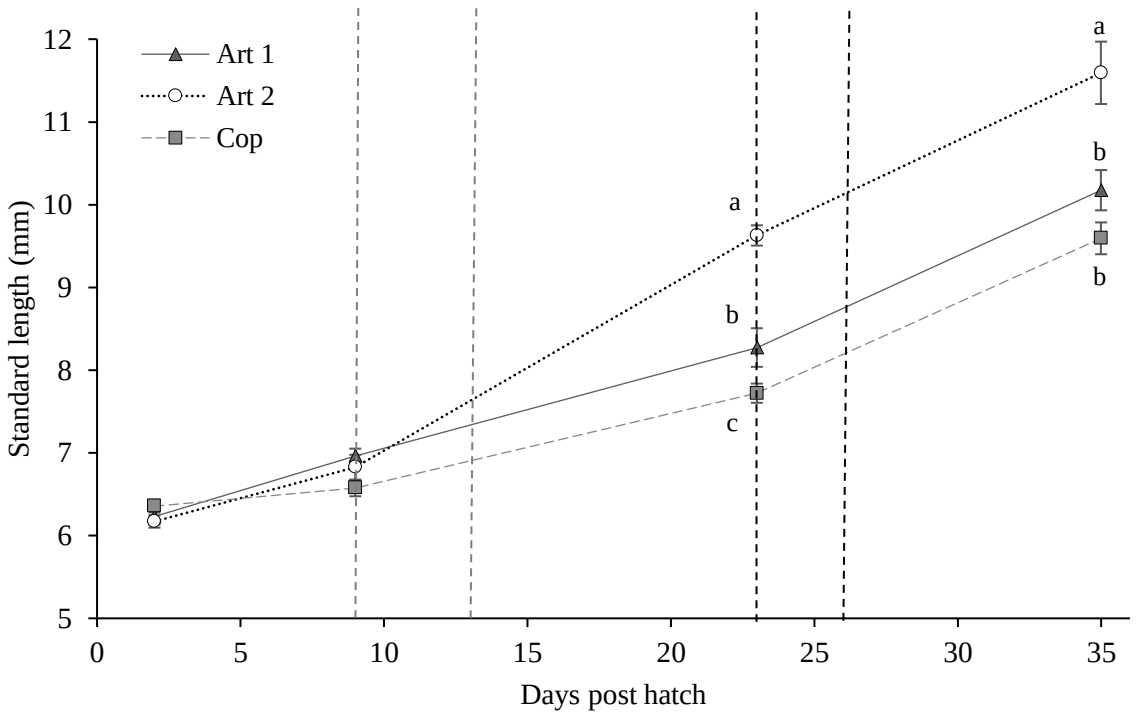

Figure 3.2. Mean standard length (SL) of *C. lumpus*, 2-35 dph. Mean *SL (mm) per larva as a function of days post hatch (dph); 2, 9, 23 and 35 dph. Each point is based on the average SL of each fish tank (n = 4) for each treatment each sampling day. Weaning to formulated feed for Art 1 and Cop (9-13 dph) and Art 2 (23-26 dph) is illustrated by vertical stapled lines in light grey and black, respectively. Standard errors (SE) are represented by error bars (±). Different letters represent significant differences (p < 0.05) between treatments. No letters represent no significant differences between treatment groups. "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed.*

3.3 Dry weight

At 2 dph lumpfish larvae from all treatment groups had the same mean dry weight (DW) of 0.7 mg (Figure 3.3). From 9-23 dph the larvae fed *Artemia* the longest period (Art 2) had a higher weight increase than larvae from both Art 1 and Cop and at day 23 the weight difference was statistically significant. From 23 dph and throughout the experiment the Art 2-larvae weighed almost the double of that of Art 1 and Cop-larvae. The weight increase of Art 1 and Cop-larvae slowed down from 9 dph, coinciding with the two groups being weaned to formulated feed. The same decrease was observed post weaning of Art 2-larvae. However, the weight increase picked up again for both Art 1 and Cop-larvae from 23-35 dph. After 35 days larvae from Art 2 had the highest DW of 7 ± 1 mg and were significantly heavier than the larvae from the Cop-group weighing 3.8 ± 0.3 mg, although Art 2 was no longer significantly heavier than Art 1, weighing 4.6 ± 0.2 mg, due to large variation in the Art-2 group. Art 1-larvae was not significantly heavier than the Cop-larvae. The mean dry weight per larva per tank each sampling day is listed in Appendix 4, Table A5.

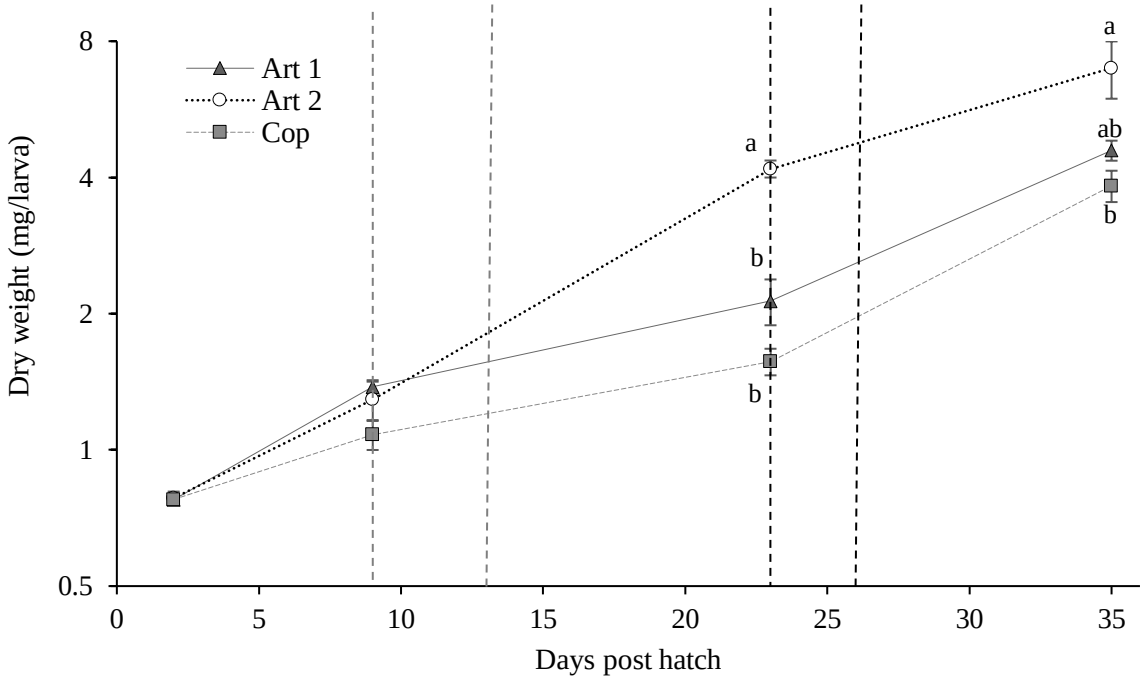

Figure 3.3. Mean dry weight (DW) of *C. lumpus*, 2-35 dph. *Mean DW (mg) per larva as a function of days post hatch (dph); 2, 9, 23 and 35 dph. Each point is based on the average DW of each fish tank (n = 4) for each treatment each sampling day. The vertical axis (y-axis) is in logarithmic scale. Weaning to formulated feed for Art 1 and Cop (9-13 dph) and Art 2 (23-26 dph) is illustrated by vertical stapled lines in light grey and black, respectively. Standard errors (SE) are represented by error bars (±). Different letters represent significant differences (p < 0.05) between treatments. No letters represent no significant differences between treatment groups. "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed.*

3.4 Correlation between dry weight and standard length

There was a strong exponential correlation between larval SL and DW for all three treatment groups; Art 1 (r = 0.932), Art 2 (r = 0.938) and Cop (r = 0.949) (Figure 3.4). All the exponential regression lines were statistically significant ($p < 0.01$) and more than 89 % of the variation observed in larval DW could be explained by larval SL as r^2 ranged from 0.89-0.92. The points for Art 2-larvae reached values considerably greater than both Art 1 and Cop which showed higher DWs and SLs for larvae fed *Artemia* for 25 days.

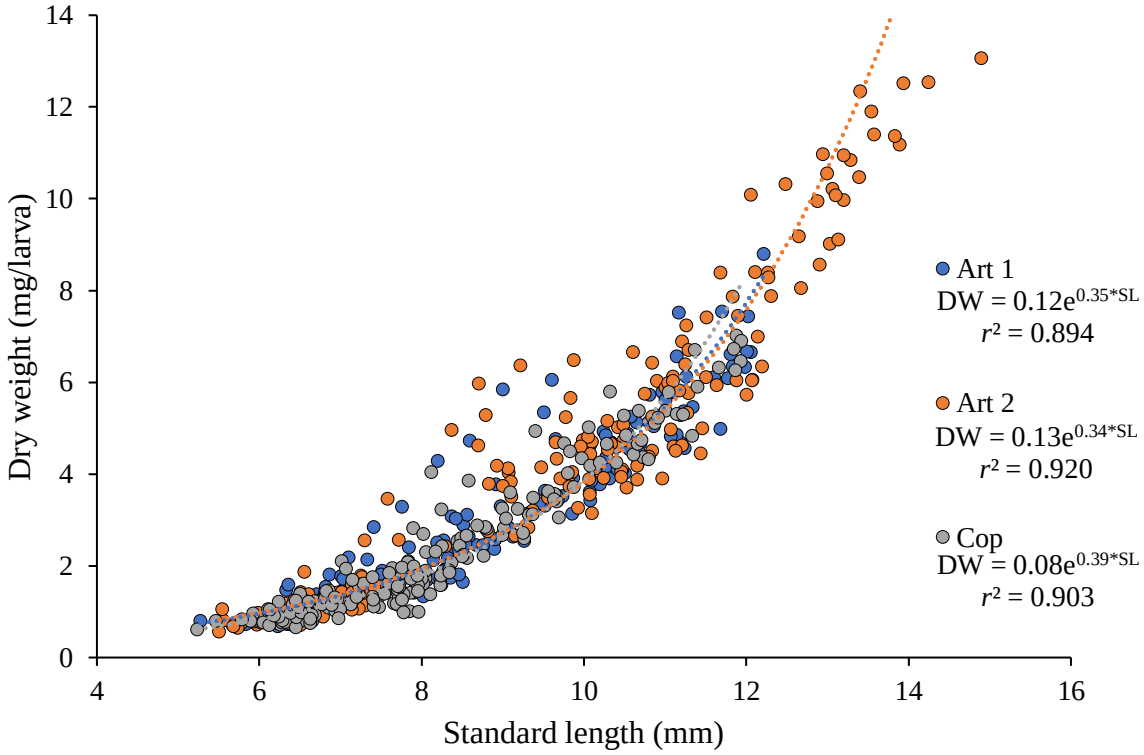

Figure 3.4. Correlation between dry weight (DW) and standard length (SL) of *C. lumpus*, 2-35 dph. *DW (mg/larva) as a function of SL. The DW and the corresponding SL estimates represents each point in the plot and are based on n = 184 (Art 1), n = 189 (Art 2), n = 185 (Cop) (incorrect estimates removed). Three exponential regression lines represents the data from the three different treatment groups. "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed.*

3.5 Daily weight increase

The mean daily weight increase (% DWI) from 2-9 dph was higher for the lumpfish larvae fed *Artemia* (Art 1 and Art 2) than of the larvae fed copepods (Figure 3.5), however, the difference was not statistically significant due to large variation in the Art 2 and Cop group. From 9-23 dph the mean % DWI of Art 2 was significantly higher than both Art 1 and Cop, whereas the larvae from Art 1 and Cop did not grow as fast as a result of weaning to formulated diet. A similar slowdown in mean daily weight gain was observed in larvae fed live prey the longest period (Art 2) during the period of transition from live prey to formulated feed (23-35 dph), but it did not create any significant differences between the treatment groups. The overall mean % DWI during the whole start-feeding experiment (2-35 dph) was 5.5 ± 0.2 %, 6.7 ± 0.5 % and 4.9 ± 0.3 % for Art 1, Art 2 and Cop, respectively, and the difference between Art 2 and the two other groups (Art 1 and Cop) was of statistical significance. There were no significant differences found between larvae fed live prey for short periods (Art 1 and Cop) during this period. The mean daily weight increase per larva per tank is listed in Appendix 5, Table A6.

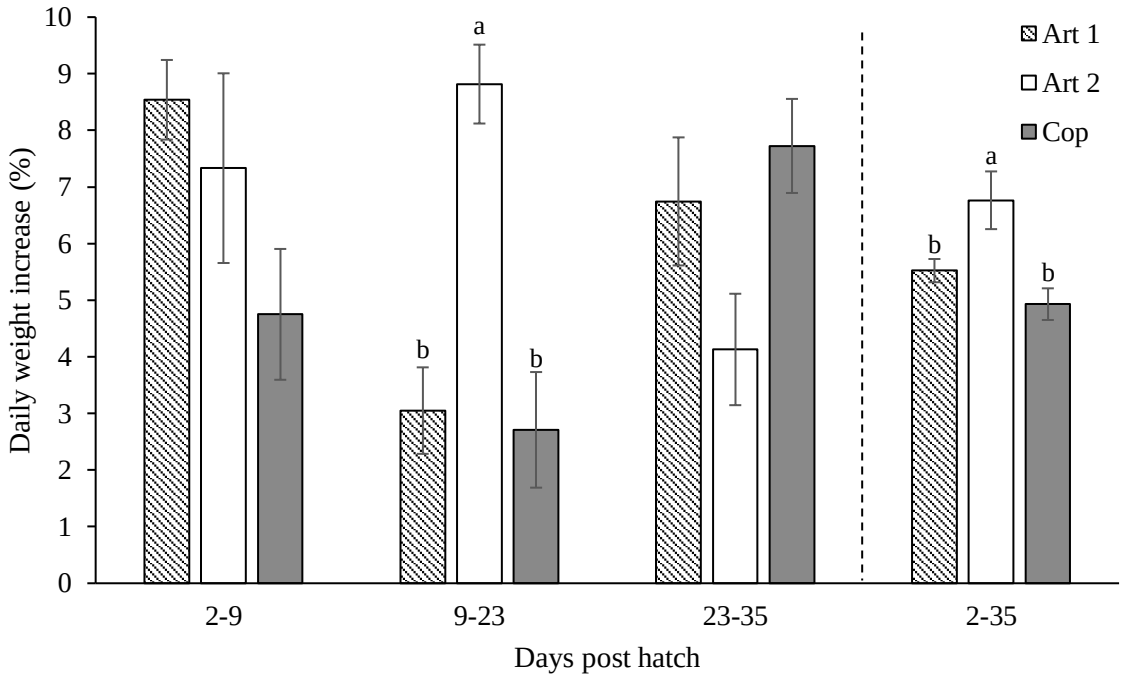

Figure 3.5. Mean daily weight increase (% DWI) of *C. lumpus*. Mean *% DWI as a function of four short time-intervals; 2-9 dph, 9-23 dph, 23-35 dph and overall interval for the whole period of the experiment; 2-35 dph. The estimates are based on the mean % DWI for each fish tank (n = 4). Standard errors (SE) are represented by error bars (±). Different letters indicate statistically significant differences (p < 0.05) between treatments. No letters represent no significant differences between treatment groups. "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed.*

3.6 Carbon content

At 2 dph the mean carbon content was similar for larvae in all treatment groups; 0.41 ± 0.01 mg (Art 1), 0.42 ± 0.01 mg (Art 2), 0.41 ± 0.005 mg (Cop) (Figure 3.6). The increase in carbon content of larvae from Art 1 and Cop slowed down from 9-23 dph as a result of weaning to formulated diet, whereas the carbon content of Art 2-larvae increased steadily until 23 dph by which the carbon content was significantly higher than in Art 1 and Cop-larvae. At this point the larvae from Art 1 and Cop were not significantly different. All groups had a further increase in carbon content from day 23-35, but the rate was higher for Art 1 and Cop compared to Art 2-larvae which was weaned to formulated feed during this period. At the last sampling day (35 dph) the mean carbon content of the Art 2, Art 1 and Cop-larvae was 2.87 ± 0.48 mg, 2.03 ± 0.11 mg and 1.70 ± 0.13 mg, respectively, and none of the groups were significantly different from each other. The mean carbon content per larva per tank each sampling day is listed in Appendix 6, Table A7.

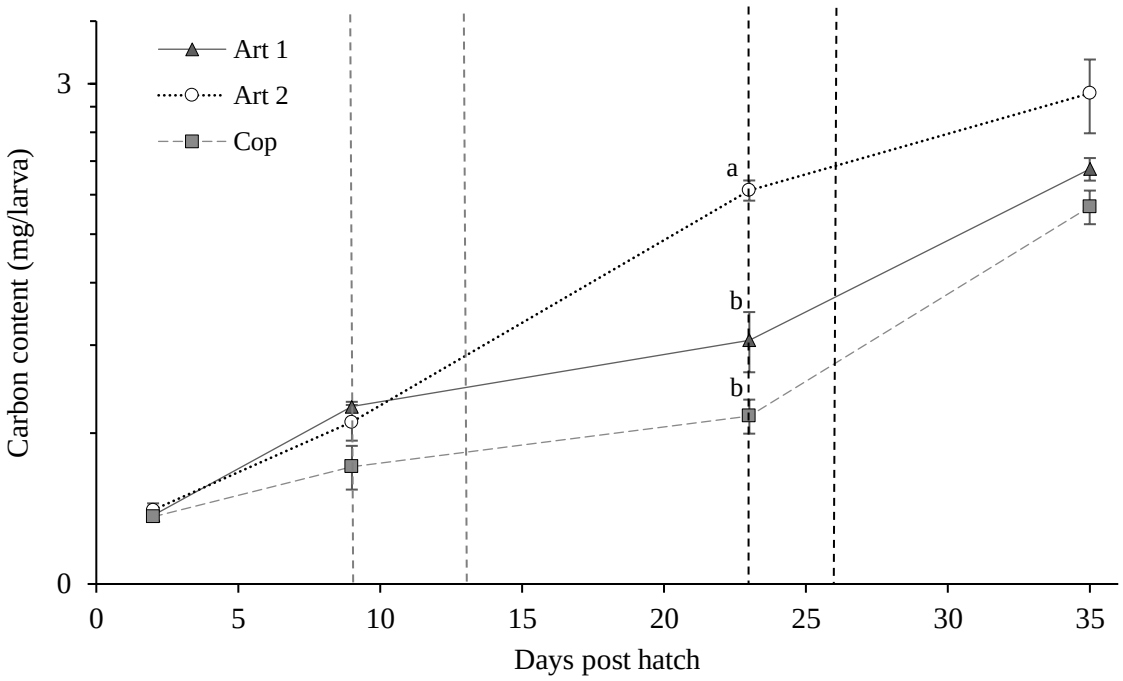

Figure 3.6. Mean carbon content (mg) per *C. lumpus*, 2-35 dph. Mean *carbon content per larva as a function of days post hatch (dph); 2, 9, 23 and 35 dph. Each point is based on the average carbon content/larva of each fish tank (n = 4) for each treatment each sampling day. The vertical axis (y-axis) is in logarithmic scale. Weaning to formulated feed for Art 1 and Cop (9-13 dph) and Art 2 (23-26 dph) is illustrated by vertical stapled lines in light grey and black, respectively. Standard errors (SE) are represented by error bars (±). Different letters represent significant differences (p < 0.05) between treatments. No letters represent no significant differences between treatment groups. "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed.*

The DW and carbon content per lumpfish larva had a positive linear correlation for all three treatment groups (Figure 3.7), meaning that the larval DW increased linearly with increasing carbon content. All linear regression lines were statistically significant with $p < 0.01$. Pearson-correlation values were $r = 0.995$, $r = 0.997$, $r = 0.994$ for Art 1, Art 2 and Cop, respectively, showing a strong correlation between DW and carbon content for larvae from all treatments. About 99 % of the variation observed in larval DW could be explained by the amount of carbon as r^2 was about 0.99 for all treatments. The points for Art 2-larvae reached values greater than both Art 1 and Cop representing higher DW and carbon content of the larvae fed *Artemia* for 25 days.

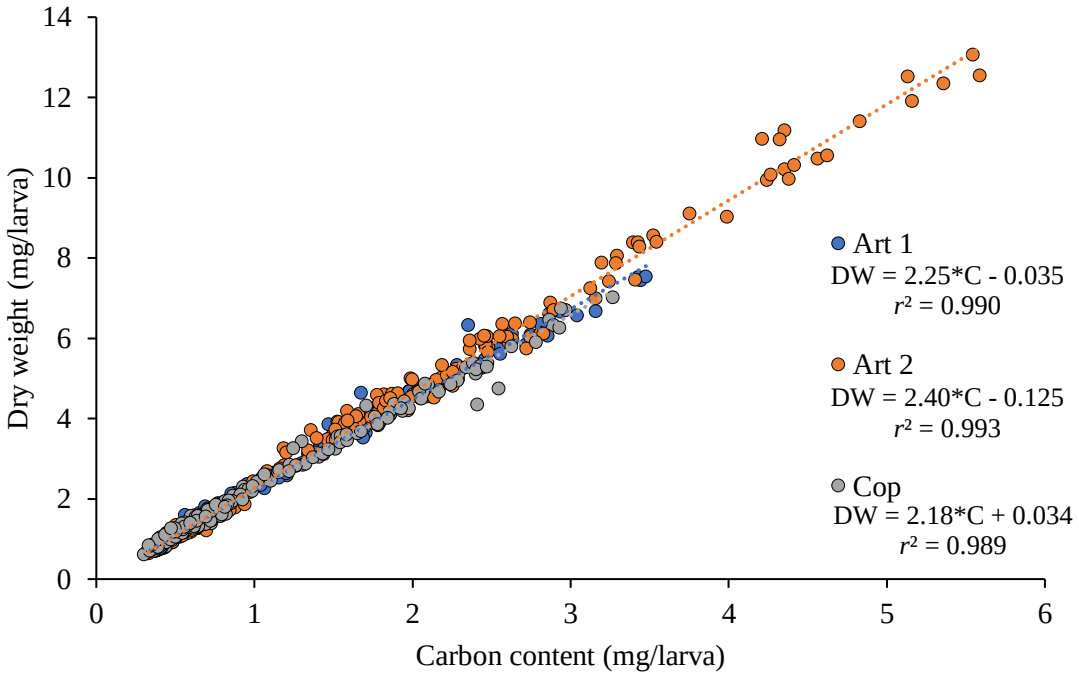

Figure 3.7. Correlation between dry weight (mg) and carbon content (mg) per *C. lumpus*. DW (mg/larva) as a function of carbon content (mg/larva). *The DW and the corresponding carbon estimates represents each point in the plot and is based on n = 180 (Art 1), n = 177 (Art 2), n = 178 (Cop) (incorrect estimates removed). Three positive linear regression lines represents the data from the three different treatment groups. Pearson correlation: r = 0.995 (Art 1, p < 0.01), r = 0.997 (Art 2, p < 0.01), r = 0.994 (Cop, p < 0.01). "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed.*

The carbon/dry weight (C/DW) ratio and DW per lumpfish larva had a negative linear correlation for all three treatment groups (Figure 3.8), meaning that the C/DW decreased linearly with increasing DW. The linear regression lines were statistically significant with $p < 0.01$ for all three treatment groups. Pearson-correlation values were $r = -0.380$, $r = -0.549$, $r = -0.153$ for Art 1, Art 2 and Cop, respectively, showing a somewhat weak correlation between C/DW ratio and DW for Art 1 and Cop-larvae, as they were close to 0. The strongest correlation was observed in Art 2-larvae. The slopes had a gradient of -0.008 for Art 1 and Art 2 and -0.004 for Cop, showing a steeper decrease in C/DW for larvae from Art 1 and Art 2 as the DW increased than for Cop-larvae. 30 % of the variation observed in C/DW ratio per larva for Art 2 could be explained by the larval DW as $r^2 = 0.301$. Only 2.3 % of the variation in C/DW ratio for Cop-larvae could be explained by the larval DW.

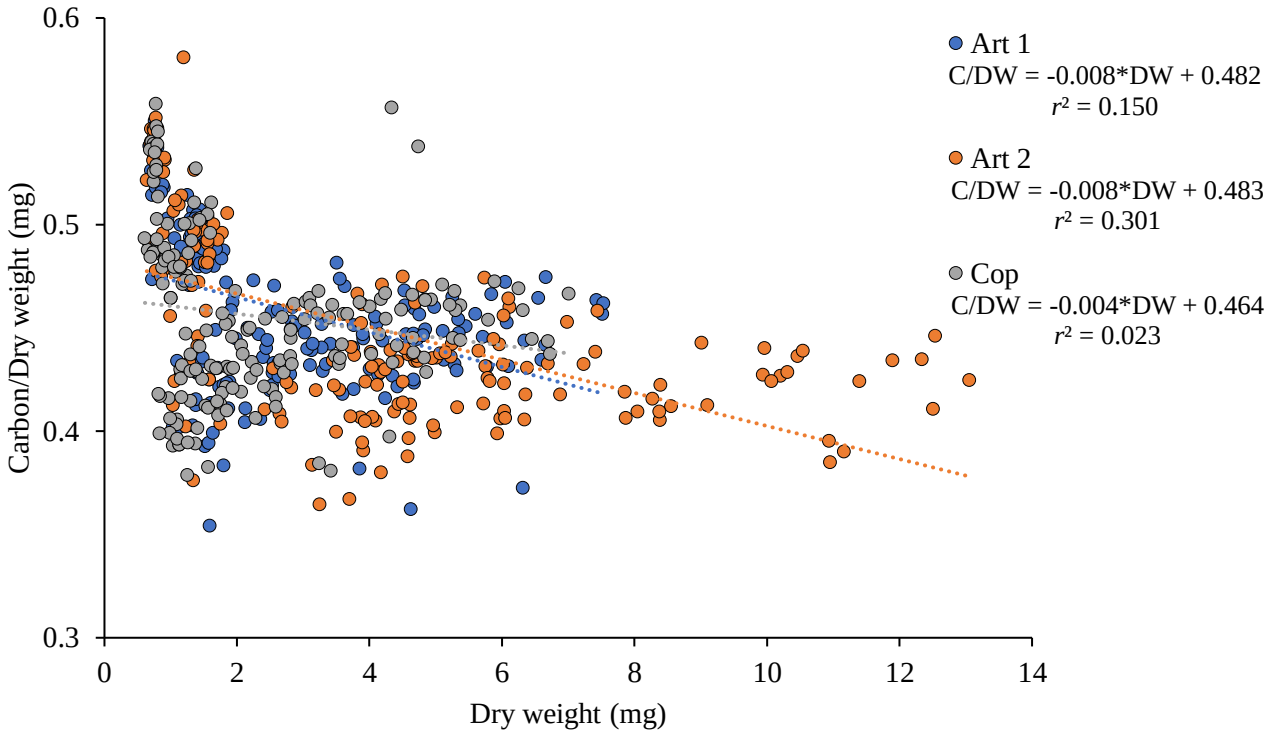

Figure 3.8. Correlation between C/DW ratio (mg) and dry weight (mg) of *C. lumpus* larvae. *The C/DW ratio and the corresponding dry weight estimates represents each point in the plot and is based on n = 180 (Art 1), n = 177 (Art 2), n = 178 (Cop) (incorrect estimates removed). Three negative linear regression lines represents the data from the three different treatment groups. Pearson correlation: r = -0.38 (Art 1, p < 0.01), r = -0.549 (Art 2, p < 0.01), r = -0.153 (Cop, p < 0.01). "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed.*

3.7 Nitrogen content

The mean nitrogen content per lumpfish larva per sampling day (2, 9, 23 and 35 dph) is presented in Table 3.2. At 2 dph the nitrogen content per larva were about 7.5 µg, regardless of feeding regime. By 9 dph the larvae fed *Artemia* (Art 1 and Art 2) had a higher content of nitrogen than larvae fed copepods, and this pattern was observed until the last day of the experiment (35 dph). The difference in nitrogen content between Art 1 and Art 2-larvae increased after the larvae from Art 1 were weaned to formulated diet. Larvae from the Art 2-group had a significantly higher carbon content than both Art 1 and Cop-larvae at 23 dph, however, the difference was no longer significant by the end of the experiment (35 dph) most likely because the Art 2-larvae had weaned to formulated feed, but maybe also due to high variation in the Art 2-group. The mean nitrogen content per larva per tank each sampling day is listed in Appendix 7, Table A8.

The mean protein content per lumpfish larva was estimated based on the mean nitrogen content per larva (Table 3.2). Therefore, the larval protein content had a similar pattern as described for the nitrogen content. Late weaning to formulated feed resulted in higher mean larval protein content from 9-35 dph.

The mean protein content per DW per larva at 2 dph by which the larvae had not eaten much yet was about 6 %, while it was around 6.5 % for all larvae from 9-35 dph, regardless of feeding regime (Table 3.2). No significant differences were observed. This indicated that the protein content per larval DW was not dependent on the feeding regime and quality of the feed.

Table 3.2. Mean nitrogen and protein content of *C. lumpus*, 2-35 dph. *The nitrogen estimates per larva is based on the mean nitrogen content of the 4 tanks representing each treatment (n = 4). n = 4 (2 dph), n = 12 (9 dph), n = 12 (23 dph) and n = 20 (23 dph) for each of the 4 tanks. Protein per dry weight (protein/DW (%)) is based on the protein estimates per larva (µg) and the corresponding DW-estimates (µg) per larva. Standard errors (SE) are represented by ± SE. Different letters represent significant differences (p < 0.05) between treatments. No letters represent no significant differences between treatment groups. "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed.*

Dph	Treatment	N/larva (µg) ± SE	protein/larva (µg) ± SE	protein/DW (%) ± SE
2	Art 1	7.68 ± 0.19	48.00 ± 1.19	6.18 ± 0.03
	Art 2	7.79 ± 0.21	48.67 ± 1.28	6.23 ± 0.06
	Cop	7.54 ± 0.03	47.11 ± 0.18	6.07 ± 0.14
9	Art 1	13.99 ± 0.39	87.43 ± 2.42	6.35 ± 0.13
	Art 2	13.41 ± 1.14	83.84 ± 7.15	6.49 ± 0.11
	Cop	11.49 ± 0.81	71.84 ± 5.07	6.65 ± 0.10
23	Art 1	22.29 ± 2.77^{b}	139.34 ± 17.28^{b}	6.52 ± 0.07
	Art 2	42.25 ± 1.75^{a}	264.07 ± 10.92^{a}	6.32 ± 0.06
	Cop	15.93 ± 1.30^{b}	99.58 ± 8.12^{b}	6.34 ± 0.14
35	Art 1	47.48 ± 2.20	296.74 ± 13.73	6.47 ± 0.11
	Art 2	70.87 ± 11.27	442.92 ± 70.44	6.31 ± 0.20
	Cop	38.63 ± 2.84	241.46 ± 17.72	6.31 ± 0.19

3.8 Survival

The first increased mortality observed during the start-feeding experiment was in the larvae fed copepods (Cop) at 9 dph (Figure 3.9), the same day they started to be weaned to formulated diet, and bacterial growth occurred in the fish tanks. Larvae from all treatment groups had a higher mortality from about 14 dph until 23 dph, however it quickly stagnated for the larvae fed *Artemia* while it continued to decrease for the Cop-larvae until the end of the experiment (35 dph). The larvae fed *Artemia* the longest period (Art 2) did not have high mortality after weaning to formulated feed, but a slightly higher mortality was observed for the larvae fed *Artemia* for a shorter period (Art 1). From 18 dph and throughout the experiment larvae fed *Artemia* (Art 1 and Art 2) had a higher survival than larvae fed copepods (Cop) and the difference was statistically significant from 27 dph. Total survival rate at the last day (35 dph) was 77 ± 4.3 %, 71 ± 1.5 % and 25 ± 6.8 % for the Art 2, Art 1 and Cop-group, respectively. The number of larvae alive per tank each sampling day is listed in Appendix 8, Table A9.

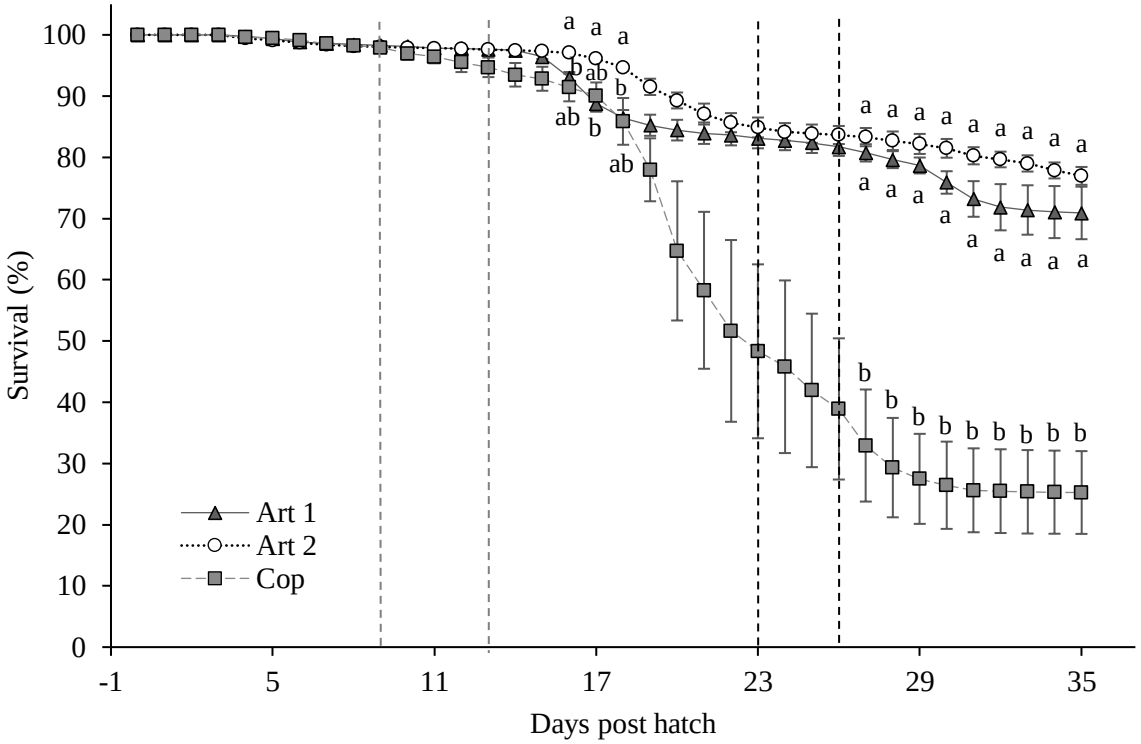

Figure 3.9. Mean total survival (%) of *C. lumpus* per treatment (Art 1, Art 2, Cop), 2-35 dph. *Each point is based on the mean survival of the 4 tanks (n = 4) representing each treatment group. Weaning to formulated feed for Art 1 and Cop (9-13 dph) and Art 2 (23-26 dph) is illustrated by vertical stapled lines in light grey and black, respectively. Standard errors (SE) are represented by error bars (±). Different letters represent significant differences (p < 0.05) between treatments. No letters represent no significant differences between treatment groups. "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed.*

3.9 Prey selection

By visual observation it was observed that a majority of the larvae used their suction disc to attach to the bottom and side walls of the experimental buckets during all prey selection experiments and did not have high swimming activity. The results from the three prey preference experiments showed that the lumpfish larvae had a variation in preference of *Artemia* and copepods during the 3 hours of feeding, as shown in Table 3.3. Although there was a variation in the density of the two prey species until 1.5 hours there was observed an overall lower density of *Artemia* than copepods for all three experiments by 3 hours. The highest larval consumption rate of live prey was observed in the experiment with starved lumpfish larvae. In experiment 2 at 1.5 hours the density of *Artemia* was significantly lower than the density of copepods. None of the other observations from the other experiments were statistically significant. The concentrations of *Artemia* and copepods per experimental bucket for each experiment is listed in Appendix 9, Table A10.

Table 3.3. Mean percent change in prey density for all prey selection experiments after 0, 1.5 and 3 hours. *Each value represents the average density change in percent of Artemia and copepods in the water samples of the 4 experimental containers (n =4) at time 0, 1.5 and 3 hours. The original density values of Artemia and copepods are listed in Appendix 9. The experiments were run in experimental buckets with unfed, fed and starved lumpfish larvae and control-buckets without fish. 100 % density represents approximately 5 copepods/mL and 5 Artemia/mL (10 prey/mL in total). Standard errors (SE) are represented by ± SE. Different letters represent significant differences ($p < 0.05$) between treatments. No letters represent no significant differences between treatment groups.*

Treatment	Prey type	Mean prey density ± SE (%)		
		Time (hours)		
		0	1.5	3
Control Without larvae (4 dph)	*Artemia* Copepods	100 100	126 ± 8 113 ± 11	117 ± 7 103 ± 12
Experiment 1 Unfed yolk sac larvae (4 dph)	*Artemia* Copepods	100 100	95 ± 9 84 ± 10	87 ± 3 91 ± 4
Experiment 2 Fed larvae (11 dph)	*Artemia* Copepods	100 100	82 ± 8[a] 113 ± 5[b]	85 ± 7 96 ± 5
Experiment 3 Starved larvae (13 dph)	*Artemia* Copepods	100 100	70 ± 12 69 ± 8	68 ± 4 74 ± 9

The *Artemia*/copepod ratios in the lumpfish larva preference experiments is shown in Figure 3.10 and are based on the concentrations presented in Appendix 9. In experiment 1 with unfed yolk-sac larvae the ratio of copepods was lower than that of *Artemia* after 1.5 hours, while the number of *Artemia* was lower than that of copepods after 3 hours, compared to the control group which was nearly stable throughout the experiment. The opposite pattern was observed in experiment 2 with fed lumpfish larvae. The results from experiment 3, using starved lumpfish, showed a nearly unchanged ratio between *Artemia* and copepods during the hours of larval feeding. None of the changes in ratios from 0-3 hours within each experiment was statistically significant. These results suggest that during the 3 hours period of the experiment there was no clearly specific larval preference for *Artemia* or copepods in neither of the three experiments.

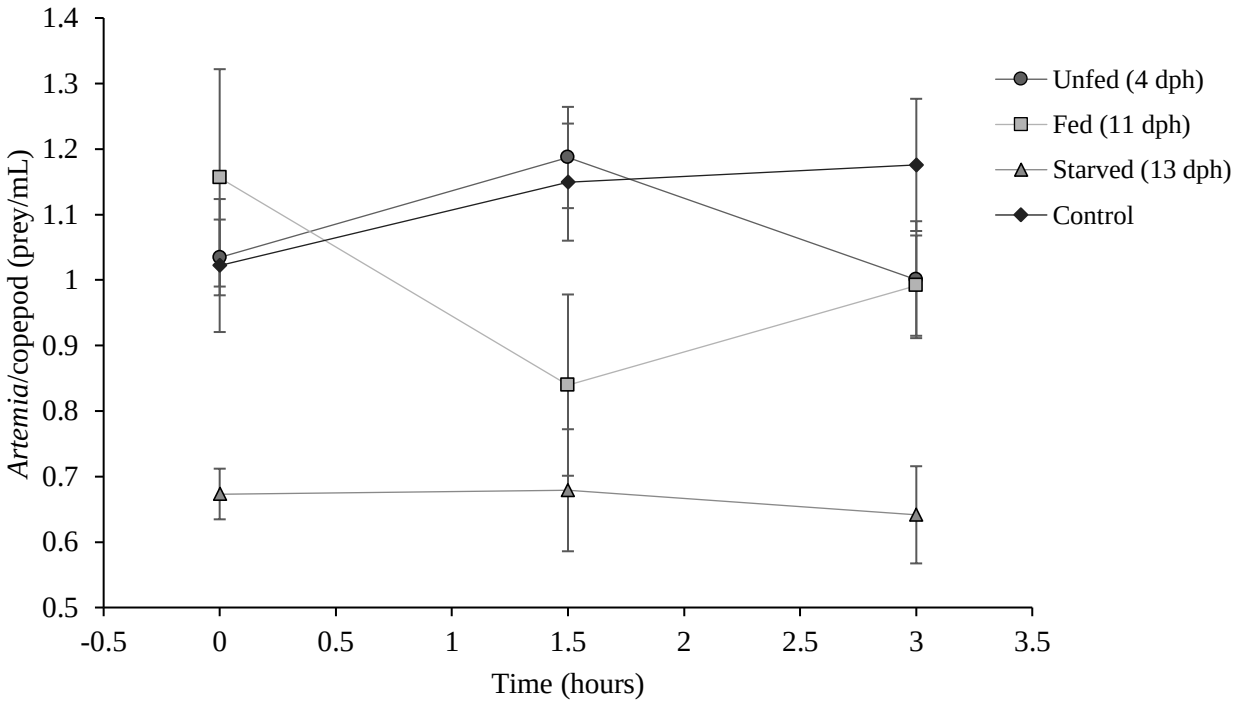

Figure 3.10. Mean *Artemia*/copepod ratio for all prey selection experiments after 0, 1.5 and 3 hours. *The curves represent the mean ratio values of Artemia/copepods as prey/mL for unfed, fed and starved lumpfish larvae and control without fish. Each point represents the average density ratio values of Artemia and copepods based on the mean densities of the 4 experimental containers (n =4). The experiments were run in experimental buckets with unfed yolk-sac larvae (experiment 1, 4 dph), fed lumpfish larvae (experiment 2, 11 dph) and starved larvae (experiment 3, 13 dph) and control-buckets without fish. Standard errors (SE) are represented by error bars (±). No letters represent no significant differences within the experiments.*

3.10 Robustness

3.10.1 Experiment 1 – Brackish water and shaking

During the exposure to shaking the majority of the lumpfish larvae attached themselves in the centre of the buckets, where the hydrostatic forces were less strong. Most larvae had their mouth wide open and gasped a lot during the active experiment. The lumpfish larvae fed *Artemia* (Art 1 and Art 2) tolerated exposure to a combination of brackish water and shaking conditions better than the larvae fed copepods (Figure 3.11). The first mortality was observed in lumpfish larvae fed copepods (Cop) after 1 hour. No mortality was observed in neither Art 1 or Art 2-larvae during the exposure to both brackish water and shaking, nor during the additionally 6 hours with exposure to only brackish water. By the end of the recovery period the survival rate had decreased further for Cop-larvae and the first mortality was observed in Art 1, while no mortalities were recorded in the Art 2-group. However, no significant differences in survival were recorded between any of the treatment groups.

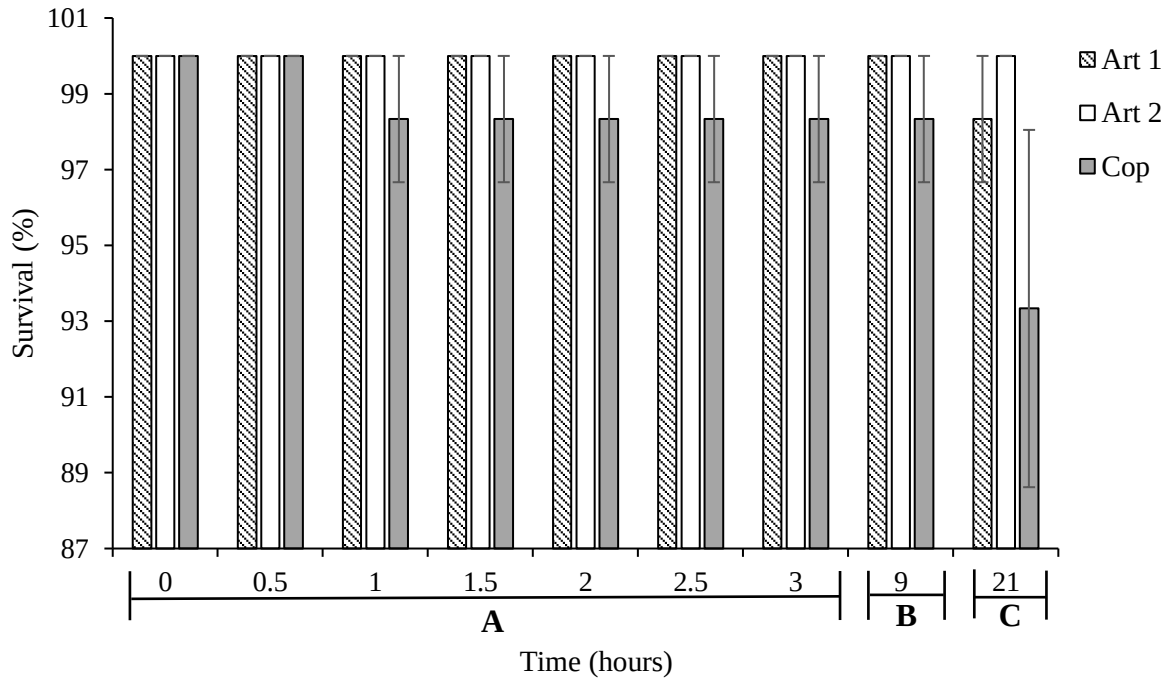

Figure 3.11. Stress test brackish water (10 ppt) + shaking (90 rpm) of *C. lumpus*, 29 dph. *Survival rates in percent as a function of time in hours. Each bar is based on the mean survival rates of 4 tanks representing each treatment (n =4). Exposure time to brackish water combined with shaking was 3 hours (0-3 hours, interval A), prolonged exposure time to brackish water was 6 hours (3-9 h, interval B) and recovery period was 12 hours (9-21 h, interval C) (Table 2.2). Standard errors (SE) are represented by error bars (±). Different letters represent significant differences ($p < 0.05$) between treatments. No letters represent no significant differences between treatment groups. "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed.*

3.10.2 Experiment 2 – Freshwater

A majority of the lumpfish larvae obtained elevated swimming activity, as in a state of panic, before they settled down and attached themselves to the sidewalls and bottom of the experimental buckets when transferred to freshwater. Most larvae had their mouth wide open, gasped a lot and began to swim in circles with their head down towards the bottom before they flipped over to the side. The lumpfish larvae fed *Artemia* the longest period (Art 2) tolerated exposure to freshwater better than the larvae fed live prey for a shorter period (Art 1 and Cop) (Figure 3.12). After 2 hours in freshwater mortality was observed in all treatment groups, however the Art 2-larvae performed better than both the Cop and Art 1-larvae from this point and throughout the experiment. Their difference in viability was statistically significant from 2.75-3 hours in freshwater as well as after 20 hours in recovery.

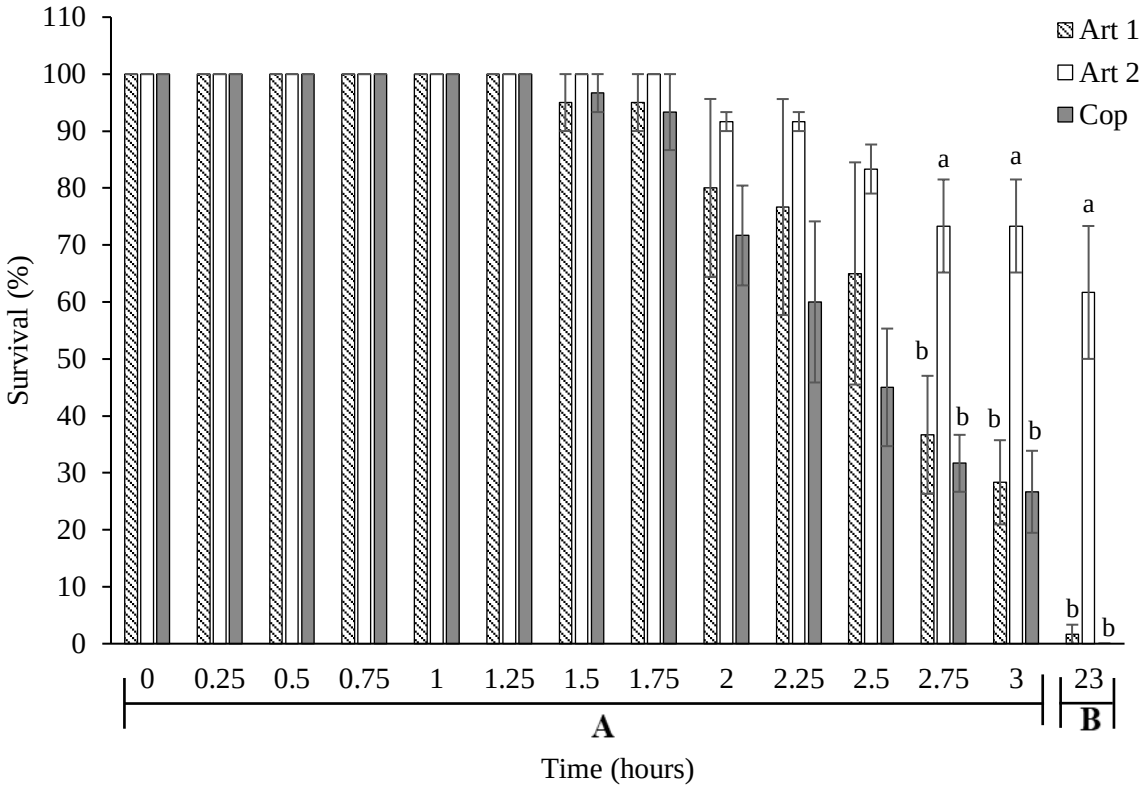

Figure 3.12. Stress test freshwater of *C. lumpus*, 34 dph. *Survival rates in percent as a function of time in hours after freshwater exposure. Each bar is based on the mean survival rates of 4 tanks representing each treatment (n =4). Exposure time to freshwater was 3 hours (0-3 h, interval A) and recovery period was 20 hours (3-23 h, interval B) (Table 2.3). Standard errors (SE) are represented by error bars (±). Different letters represent significant differences (p < 0.05) between treatments. No letters represent no significant differences between treatment groups. "Art 1" were fed Artemia for 7 days, "Art 2" were fed Artemia for 21 days, and "Cop" were fed copepods for 7 days, before weaned to formulated feed.*

4. Discussion

4.1 Feeding regime effects on larval growth and survival

Use of enriched *Artemia* nauplii (12 days) gave higher larval growth and survival than use of copepods (12 days). Later weaning from live prey (*Artemia*) to formulated feed did also result in higher larval growth and survival, already from about 10 dph and throughout the experiment. By the end of the experiment the larvae fed *Artemia* was almost twice as heavy than the larvae fed copepods and *Artemia* for shorter periods.

Higher survival in halibut larvae (Shields *et al.*, 1999), better growth and survival in seabass larvae (Rajkumar, 2006), and better growth in Atlantic cod and ballan wrasse (Dahle *et al.*, 2014; Karlsen *et al.*, 2015; Romundstad, 2015; Øie *et al.*, 2017) have been observed when fed copepods compared to when fed enriched *Artemia* and/or rotifers during early larval stages. Despite the clear superior effects of using copepods as start feed for small pelagic marine fish larvae, this pattern was not observed for the lumpfish larvae in the present study which supports hypothesis nr. 1 stating that the use of *Artemia* (12 days) will give better start-feeding success compared to use of copepods (12 days). The results suggest that the nutritional content of enriched *Artemia* nauplii satisfied the nutritional requirements of lumpfish larvae to a higher degree than the content of copepods. This might be because the lumpfish larvae, with demersal eggs, are relatively large and advanced at the point of hatching (Brown, 1986), and do not require the same amount of essential nutrients and lipids, like DHA, compared to marine pelagic larvae, which is much smaller and less developed at hatching and by the time of start-feeding (Kjørsvik *et al.*, 2004).

Artemia have a higher total lipid content than copepods (van der Meeren *et al.*, 2008). This is in accordance with lipid analysis of the live feed from the present study which revealed an average total lipid content of 13 % (n = 3) and 19 % (n = 3) per DW for copepods and *Artemia,* respectively (Håvard Dybvik's master thesis). However, most of these lipids are in *Artemia* stored in NLs, and HUFAs stored in NLs is not beneficial for development, as seen in cod (Kjørsvik *et al.*, 2009; Wold *et al.*, 2009). Copepods do also have a naturally higher DHA/EPA ratio than enriched *Artemia* (van der Meeren *et al.*, 2008). As low DHA/EPA ratios may reduce fish growth (Watanabe, 1993; Hamre *et al.*, 2002) one would assume improved growth in the larvae fed copepods compared to *Artemia*. Despite this, better growth and development was

observed in larvae fed *Artemia* than copepods, which implies that the higher total lipid content in *Artemia* may be more beneficial for lumpfish larvae than the content of DHA and amount of HUFAs stored in the PL. Small marine fish larvae, like cod, are larger than the lumpfish larvae before they develop a functional stomach (Pedersen and Falk-Petersen, 1992), and seem to be dependent on EFAs supplied in PLs as they are easier to digest than NLs (Olsen *et al.*, 1991; Coutteau *et al.*, 1997). As lumpfish larvae develop a functional stomach at a smaller size (Marthinsen, 2018) it is likely that they are able to digest and utilize the n-3 HUFAs stored in the NLs of the enriched *Artemia* for growth and development in a higher degree than small pelagic marine fish species.

The results from the present study corresponds with the results from the similar study conducted by Hanssen (2018) and (Marthinsen, 2018) which showed higher growth and survival as well as earlier bone ossification and faster development of the stomach in larvae fed *Artemia* compared to copepods. There was not found any more skeletal deformities in larvae fed *Artemia* than copepods which implies that using *Artemia* is just as beneficial as using copepods.

Despite increasing the biomass of copepods, the growth and survival did not improve in the larvae fed copepods compared to using the same density, as applied in Hanssen and Marthinsen's study. This implies that lumpfish larvae may not necessarily consume more copepods even though they were able to, and that they ingested more *Artemia* than copepods. Visual observations of the copepods showed that the copepods were less active than the *Artemia*, which can have made them less attractive as a food organism. *Artemia* may also be easier to locate and catch as they are orange-red and lack escape responses compared to copepods which are more transparent and have zigzag movements followed by gliding motions (Léger *et al.*, 1987; Lavens and Sorgeloos, 1996). This somewhat corresponds with the occasionally slightly higher selection of *Artemia* in the prey selection experiments conducted in the present study, however, the results did not show significant lower selection for copepods than *Artemia*. This will be discussed further in paragraph 4.2.

Reduced growth in terms of DW during the days post weaning was observed for all lumpfish larvae regardless of early or late weaning and live prey used. This was not observed for larvae fed *Artemia* in the study of Hanssen (2018) and Marthinsen (2018), which only found lowered growth in the days after weaning in larvae fed copepods. The reason why it was difference between the studies is not known, but the transition from live feed to formulated feed can be characterised by larval mortalities in lumpfish (Powell *et al.*, 2018). It has been shown that weaning can be challenging as the larvae needs to accept formulated feed as a food item and

may not able to efficiently digest it because of an undeveloped stomach (Hamre *et al.*, 2013). The live feed might also have been suspended in the water column longer than the formulated feed, leaving more time for the larvae to catch them. However, late weaning to formulated feed gave higher growth and survival in lumpfish larvae than early weaning in the present study, which supports hypothesis nr. 2 stating that the use of live fed (*Artemia*) for 25 days will give better start-feeding success than use of live feed (*Artemia*) for only 12 days. This is assumed to be because the larvae had not yet developed a functional stomach and digestive system during the early weaning periods. Marthinsen (2018) found that lumpfish larvae do not have a functional stomach until 21-34 dph, when they are about 8-10 mm in size. The larvae in present study was weaned to formulated feed already at day 9 and was most likely not able to efficiently digest and assimilate nutrients from the formulated diet as efficiently as when fed live feed.

The formulated feed used during the experiment was especially developed for lumpfish larvae (Skretting, 2019). Despite this, it did not seem to satisfy the nutritional requirements of the lumpfish larvae to any greater extent than the live feed which is likely due to the nutritional requirements of lumpfish larvae not yet being concluded. The formulated diet may also have been subjected to nutrient loss due leaching before consumed by the fish larvae (Hamre, 2006; Hamre *et al.*, 2013). Therefore, the nutritional content of the live feed was likely more adequate to the nutritional requirements of the lumpfish compared to the formulated feed, explaining the better start-feeding success of lumpfish larvae fed live feed (*Artemia*) the longest period before weaned to formulated feed. The behaviour and appearance of the live feed is beneficial compared to formulated feed (Tandler, 1985), and is believed to have caused enhanced feeding rates and in turn higher larval growth. However, it seemed like the larvae fed *Artemia* for shorter periods handled the weaning to formulated feed better than larvae fed copepods for shorter periods as the survival rate was much higher for *Artemia*-larvae post weaning. The reason for this is believed to be because the larvae fed *Artemia* had a more rapid development of a functional stomach than larvae fed copepods and formulated feed, as observed by Marthinsen (2018), and was able to more efficiently digest the formulated feed.

The mean survival rate was much lower after weaning and throughout the experiment for the larvae fed copepods than *Artemia*, and the variations between the fish tanks were considerably higher in the copepod tanks. A majority of the death is believed to be caused by bacterial growth appearing as gelatinous matter (slime) on the cleaning arm, water inlet, aeration tubes, and the silicone sheets. The lumpfish larvae died as they swam into the slime, got stuck and ingested it. The bacteria did also create suboptimal water quality for the lumpfish larvae, which in turn may

have affected the larval growth rate in a negative manner. It was first observed in the fish tanks fed copepods at the start of weaning to formulated feed (9 dph). The combination of organic loads from the copepods and the formulated feed seemed to trigger an earlier bloom of bacteria, as it was not observed in the tanks with *Artemia* before until 17 dph. The *Artemia* cultures were rinsed and filtered several times before fed to the lumpfish to remove organic waste and particles, while the copepod-cultures were never washed. The larvae fed *Artemia* seemed to handle the bacterial exposure better than the larvae fed copepods as they never reached the same mortality rates as the copepod-larvae.

Larvae fed *Artemia* the shortest period experienced higher mortality around dph 30 and could be connected to final absorption of the yolk sac, which according to Marthinsen (2018) is fully resorbed by 34 dph in lumpfish larvae. From this point the larvae had to depend solely on exogenous feeding. Such mortality was only detected to a small degree in the larvae fed *Artemia* the longest but was not detected in the larvae fed copepods with early weaning to formulated feed. The explanation for this is not known.

The results from the present study shows that carbon content per lumpfish larva can be used to estimate larval biomass since the there was a strong relationship between larval DW and carbon content. Furthermore, the mean carbon content per lumpfish larva was higher for larvae fed *Artemia* the longest (Art 2) than larvae fed live feed (*Artemia* and copepods) for shorter periods (Art 1 and Cop). However, the mean carbon content per mean DW of the lumpfish larvae was about 53-54 % at 2 dph for all treatment groups. At 35 dph the carbon content had decreased to 44-45 % for both Art 1 and Cop and to 41 % for Art 2. The C/DW ratio decreased as the DW increased. This implies that the C/DW ratio is somewhat dependent on size and larger lumpfish larvae tended to have a lower C/DW ratio than smaller larvae. The reason for this is unclear. The overall carbon content in the lumpfish larvae of the present study corresponded with previous carbon estimates obtained from 23-day-old turbot larvae (Reitan *et al.*, 1993) and walleye pollock larvae (Harris *et al.*, 1986) which was about 43 % per DW.

The protein content in the lumpfish larvae, which is based on the nitrogen values and describes larval muscle mass, was about 6 % in all lumpfish larvae in the present study, regardless of feeding regime. This was very low compared to reports for other marine fish species which is about 50-70% of the DW (Harris *et al.*, 1986; Yúfera *et al.*, 1999). In addition, the commonly used nitrogen-to-protein conversion factor of 6.25 used in the present study have been experienced to provide too high protein estimates (Mariotti *et al.*, 2008; Diniz *et al.*, 2013).

Therefore, it is believed to be an error with the N-analysis and the nitrogen estimates (see paragraph 2.5.3). If not, the explanation for the low protein content is unclear.

The water outlet filters in the fish tanks was 700 µm in mesh size, the same as in Hanssen and Marthinsen's study. As the size of the copepods ranged from 180-700 µm and the mean *Artemia* size was above 700 µm there is a strong possibility that many more copepods than *Artemia* may have escaped through the filter and resulted in lower copepod biomass in the fish tanks fed this type of live feed. This could explain some of the lower growth rates observed in larvae fed copepods. More frequent density measures of the live feed in the fish tanks should have been conducted to ensure the live prey density was as aimed. In addition, using larger copepods more similar in size to the *Artemia* would prevent this potential difference in density and should be considered if future experiments with similar live prey were to be conducted.

4.2 Larvae live prey preference

The results from the three experiments assessing live prey selection in lumpfish larvae showed no clear preference for neither *Artemia* nor copepods. The larvae seemed to be more generalists and utilized the resources they had available. However, the density of *Artemia* were somewhat more reduced than the copepod-density after three hours of grazing for all experiments, which indicated that the lumpfish larvae occasionally could seem to prefer *Artemia*.

That larvae seemed to have a slightly higher preference for *Artemia* and did not select copepods to a greater extent, does somewhat support hypothesis nr. 3, which implied that lumpfish will select *Artemia* as they are bigger in size than copepods. It could be reasonable to assume that lumpfish larvae might have preferred copepods as they are a natural prey organism to lumpfish larvae and juveniles (Ingólfsson and Kristjánsson, 2002) while *Artemia* is not (Léger *et al.*, 1987). However, as the lumpfish larvae grow bigger, they tend to choose the largest prey available as long as it is not sessile or slow-moving (Ingólfsson and Kristjánsson, 2002). In this experiment, the *Artemia* was the largest prey available. The attractiveness of the prey type could also have caused additional differences. Copepods are relatively transparent, lightly green coloured and employs zigzag movements closely followed by gliding motions (Lavens and Sorgeloos, 1996). *Artemia* nauplii are coloured lightly orange, moves continuously and do not have efficient escape responses, which makes them easy to locate and catch for predators (Léger *et al.*, 1987). It is important to remember that the difference in larval prey selection in the

present experiments was very small and that both species of live feed seemed to be an attractive food organism for lumpfish larvae, regardless of size, colour and behaviour. The reason why the difference in prey preference was not significant can be because the larvae did not actively chase and catch the live prey. Most larvae were attached to surfaces by the use of their ventral suction disc during the experiments and is therefore believed to have grazed more passively and maybe less selectively. Similar feeding behaviour have also been reported by (Brown, 1986).

The smallest changes in *Artemia*/Copepod ratio and highest decreases in density, regardless of prey type, was observed in experiment 3, with the starved lumpfish larvae, compared to the experiments with the unfed yolk-sac larvae (experiment 1) and fed larvae (experiment 2). The results from experiment 3 showed that the larvae did not feed selectively and were more opportunistic, probably because they were hungry. The larvae from experiment 1 had not yet started exogenous feeding as this was initiated at the start of the experiment. Due to this the lumpfish larvae might have used some time to comprehend the exogenous feeding before effective grazing and decrease in prey density started. The larvae in experiment 2 were already fed prior to the experiment which may explain why their decrease in densities was not as great.

The 12 larvae used per experimental bucket in experiment 2 and 3 were obtained from Art 2 because they were not yet weaned to formulated feed as Art 1 and Cop-larvae and might have developed a preference for *Artemia* over copepods prior to the conduction of the experiment. This could have potentially provided an unintentional impact on the final results which indicated that there was a slightly higher selection for *Artemia*. To avoid potential unexpected variation interfering with the results it would have been more optimal to have obtained one lumpfish larva from each of the twelve fish tanks.

To avoid uneven distribution of live prey in the water column the buckets were supplied with continuous bubbling and the water in the experimental buckets was stirred thoroughly before the water samples were obtained. However, when interpreting the results, it seems like the prey distribution might still might have been quite uneven as the prey densities sometimes increased, which is not possible. Nevertheless, the *Artemia* had overall steadier, less variating declines, while the copepods had more variating densities, which can be interpreted as the *Artemia*-densities declining to a greater extent. Counting uncertainties of live prey in the water samples must also be taken into account as a source of methodological errors. For potentially upcoming experiments a higher number of fish larvae per experimental tank could increase the grazing effects and provide higher and more significant declines in the densities of the *Artemia* and copepods. Higher starting densities of live prey could also be an alternative.

In order to get more accurate results, it would have been more optimal to count the stomach content of the lumpfish larvae as well as collecting water samples to see if they correlated. It is difficult to get accurate estimates with water samples, much because the distribution of prey in the water column can be uneven even though the bucket is supplied with air and bubbles. Stomach content provides more reliable results but was not performed because *A. tonsa* is experienced to deteriorate more quickly in the gut than *Artemia* (Pedersen, 1984; Støttrup *et al.*, 1986). One could also have observed larval feeding behaviour directly by visually observing the fish in an aquarium (Brown, 1986) or used a camera to record it. However, this would may be very time consuming.

There should have been applied control buckets without lumpfish for experiment 2 and 3 as applied in experiment 1, but logistical reasons made this difficult. Therefore, the control group of experiment 1 was used as a base for the possible control groups for the buckets with lumpfish.

Because of methodological challenges in the present experiments it is difficult to draw clear conclusions of whether the lumpfish larvae prefer copepods or *Artemia*. However, the present study demonstrate that the method applied can be used to asses prey selection of lumpfish larvae. Further studies investigating prey preference in lumpfish larvae should be conducted as the number of studies concerning this topic is scarce.

4.3 Feeding regime effects on larval robustness

Lumpfish larvae from all treatment groups tolerated 3-hour exposure to brackish water and shaking better than 3-hour exposure to freshwater, however the highest survival was observed in larvae fed *Artemia*. The larvae fed live feed (*Artemia*) the longest period had a significantly higher robustness towards freshwater than larvae fed live feed (*Artemia* and copepods) for shorter periods, and the survival rate for larvae fed *Artemia* the shortest was slightly better than for larvae fed copepods for a similar period. These results show that robustness in lumpfish larvae is dependent on start-feeding regime and quality of the live feed.

Hypothesis nr. 4 stated that one expected to observe better robustness in the larvae fed copepods for 12 days (Cop) compared to the lumpfish fed *Artemia* for 12 days (Art 1), as higher stress tolerance have been observed in larval red sea bream (*Pagrus major*), yellow fin (*Seriola quinqueradiata*) and juvenile marbled sole (*Limanda yokohamae*) fed high levels of dietary DHA (Watanabe and Kiron, 1994; Kanazawa, 1997). The results from the robustness tests did

not support this statement as the highest robustness was observed in larvae fed enriched *Artemia* nauplii, which selectively metabolize DHA (Rainuzzo *et al.*, 1997; Navarro *et al.*, 1999) and thereby probably contained less DHA than copepods. The difference in larval robustness observed in the lumpfish larvae from the present study is therefore most likely not dependent on the content of DHA in the live feed.

The difference in survival in the robustness test may be connected to the larval size. Results from the present study showed that larvae fed copepods were smaller in size than larvae fed *Artemia* already from 9 dph and throughout the experiment. It is therefore believed that the development of gills, which are crucial for osmoregulation in adult teleost fish (Evans *et al.*, 1999), may have been less developed in the larvae fed copepods. The skin has also proven important for ion exchange in marine fish larvae (Schreiber, 2001; Kjørsvik *et al.*, 2004). Thicker skin, bulkier shape and smaller gills compared to body size may be an additional reason to why a higher robustness was observed in larvae fed *Artemia* than in copepod-larvae as it may have lowered the osmotic influx and in turn increased tolerance toward environmental changes in salinity. This characteristic appearance also seems to be the reason why adult lumpfish is less affected by freshwater exposure than labrid species (Skiftesvik *et al.*, 2018).

The results from both of the robustness tests confirmed hypothesis nr. 5 stated, which stated that when larvae were fed *Artemia* it could be expected that use of live feed for 25 days gave higher robustness than larvae fed live feed (*Artemia*) for 12 days. This can be explained by the fact that lumpfish larvae do not have a functional stomach until 21 dph (Marthinsen, 2018) and early feeding to formulated feed can negatively affect growth and survival. The difference can also be explained by the larger size of Art 2-larvae than Art 1-larvae and likely more advanced gills and thicker skin, similar to the difference in robustness observed between larvae fed *Artemia* and copepods for short periods. It is therefore most likely that the size of the lumpfish larvae, which was determined by the live feed used, is the overall factor determining larval robustness and tolerance to changes in salinity.

A methodological limitation of the present experiments was how to assess the robustness. The lumpfish larvae stress response was measured as number of survivors after stress exposure for both robustness tests. The stress response could also have been detected as elevated levels of the stress hormone cortisol or glucose in the blood plasma, which is normal stress indicators for fish. However, as the lumpfish larvae was very small and had a small blood volume it would have been difficult to obtain adequate blood samples for analysis. In addition, despite having recorded increased plasma cortisol in lumpfish in other studies (Remen and Jonassen, 2017),

lumpfish have also shown to respond with weak elevations in cortisol levels when stressed (Jørgensen *et al.*, 2017; Remen and Jonassen, 2017). Stress could also be detected in behavioural studies of the lumpfish, like swimming activity and attachment to surfaces but scarce information on how stressed lumpfish behave made this suboptimal. Viability was therefore considered the most optimal alternative for the present study.

The results from the present study demonstrate that stress responses in lumpfish larvae caused by suboptimal environmental conditions can be used to determine larval quality. In addition, it is reasonable to believe that the quality of the live feed fed during early stages of lumpfish larvae most likely will have an effect on the performance of stages older than juveniles as well. However, this needs to be more thoroughly investigated, as so does the general physiological responses of lumpfish larvae in terms of stress. Currently there is not much information available on this topic and knowledge is crucial in order to improve lumpfish welfare in aquaculture.

5. Conclusions

Use of enriched *Artemia* nauplii (12 days) resulted in higher growth and survival rate of lumpfish larvae than use of copepods (12 days). Feeding lumpfish larvae with live feed (*Artemia*) for 25 days gave better start-feeding success than use of live feed (*Artemia*) for only 12 days and demonstrated that later weaning to formulated feed increased larval growth and survival rates. Overall, the present study showed that use of *Artemia* for at least 25 days (including 4 days of weaning) gave more effective and reliable production of lumpfish larvae than with use of copepods (12 days).

The difference in selection between *Artemia* and copepods was small, however the larvae seemed to prefer *Artemia* slightly better than copepods. To increase feeding rates and in turn increase and optimize production efficiency one should apply *Artemia* in the commercial production of lumpfish larva.

Lumpfish larvae fed live feed (*Artemia*) for 25 days showed higher robustness than those fed live feed for 12 days, where larvae fed *Artemia* (12 days) had higher robustness than larvae fed copepods (12 days). Feeding the lumpfish with *Artemia* for at least 25 days (including 4 days of weaning) will provide higher larvae robustness and increase the lumpfish welfare.

It should be taken into consideration that the results from the present study might have been affected by methodological challenges related to live prey biomass and that one should interpret the results and conclusions with a critical view. Nevertheless, the findings are a solid contribution to the characterisation of optimized start-feeding of lumpfish larvae.

There is still need to characterise the dietary effects on the quality and robustness of the lumpfish when deployed in sea cages. Therefore, it could be important to follow how the lumpfish larvae from the different feeding regimes performed later in the sea cages and observe if the survival remained consistent with the results from the present study. Further studies on use of lumpfish in aquaculture should by of high priority in order to provide a sustainable production of lumpfish and in turn a sustainable production of Norwegian salmon.

6. References

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7. Appendices

Appendix 1. Feeding amounts of copepods, *Artemia* and formulated feed

Table A1. Feeding amounts of *A. tonsa* from 0-35 dph. *Density of copepods (prey/tank/day) fed to the lumpfish larvae of Cop from 2-13 dph including weaning period to formulated feed from 9-13 dph (5 days). Considering approximately 5000 lumpfish larvae per tank from 0 dph.*

Feeding amounts of copepods (size 200-800 µm)		
Dph	**Copepod density (# copepods/tank/day)**	**Comments**
	Copepods 12 days (Cop)	
0	0	
1		**First** shipment of copepods (mean size 180 µm), 12 containers. This amount of prey was batch added (to reach starting concentration) as a starting concentration directly into the fish tanks on 2 dph.
2	627 000 + 220 500	The 672 000 was batch fed to get the aimed initiating density and the 220 500 was fed continuously to the next day after batch feeding
3	840 000	
4	1 680 000	
5	1 680 000	
6	2 800 000	Almost all of the copepods in the samples had low swimming activity
7	3 750 000	**Second** shipment of copepods (mean size 400 µm), 12 containers. Due to low Cop-density 1 mill Cop was added immediately to the fish tanks, and 2.75 mill was set to normal continuous feeding.
8	2 800 000	
9	2 150 000	Started weaning to formulated feed by adding dry feed manually (hand feeding)
10	1 400 000	**Third** shipment of copepods (mean size 700 µm), 6 containers.
11	750 000	
12	675 000	
13	425 000	
14-35	0	End of weaning, only fed dry feed

Table A2. Feeding amounts of *Artemia* from 0-35 dph. *Density of Artemia (prey/tank/day) fed to the lumpfish larvae of Art 1 and 2 from 2-13 dph and 2-26 dph, respectively, including weaning periods to formulated feed of 4-5 days. Considering approximately 5000 lumpfish larvae per tank from 0 dph.*

Feeding amounts of *Artemia* (size 500-700 µm)			
Dph	***Artemia* density (# *Artemia*/tank/day)**		**Comments**
	***Artemia* 12 days (Art 1)**	***Artemia* 25 days (Art 2)**	
0-1	0	0	
2	200 000 + 157 500	200 000 + 157 500	The 200 000 was batch fed to get the aimed initiating density of 2000 prey/L and the 157 700 was fed continuously to the next day after batch feeding
3	525 000	525 000	
4	1 200 000	1 200 000	The lumpfish fed was a mix of newly hatched and enriched *Artemia* (1:3) due to prey loss
5	1 200 000	1 200 000	The lumpfish fed was a mix of newly hatched and enriched Artemia (2:3) due to prey loss
6	2 000 000	2 000 000	The lumpfish fed was a mix of newly hatched and enriched Artemia (ca 1:4) due to prey loss
7	2 000 000	2 000 000	The lumpfish fed was a mix of newly hatched and enriched Artemia (ca 1:6) due to prey loss
8	2 000 000	2 000 000	
9	1 540 000	2 400 000	Art 1 started weaning to formulated feed (cs 200, hand fed)
10	1 000 000	2 400 000	
11	750 000	2 800 00	
12	675 000	3 200 000	
13	425 000	3 200 000	
14	0	3 600 000	End of weaning for Art 1, only fed dry feed
15	0	4 000 000	
16-19	0	3 600 000	Too much *Artemia* on the tank bottom, decreased the feeding amount
20	0	4 600 000	
21	0	4 800 000	
22	0	3 600 000	
23	0	3 600 000	Art 2 started weaning to formulated feed (cs 300) and Art 1 changed from cs 200 to cs 300
24	0	2 700 000	
25	0	1 800 000	
26	0	900 000	
27-35	0	0	End of weaning for Art 2, only fed dry feed

Table A3. Feeding amounts of formulated feed from 9-35 dph. *Feeding amounts of formulated feed for Art 1, Art 2 and Cop estimated for approximately 5000 larvae per tank.*

Formulated feed – Art 1, Art 2 and Cop			
Dph	**Total feeding amount (g)/day**	**Feeding time (sec)**	**Feeding frequency (times/day)**
9	3	Hand fed	2
10-11	5	Hand fed	2
12	5	Hand fed	5
13	8.3	1	5
14	9	1	-
15	10	1	11.8
16	10.5	1	-
17	12	1	-
18	12.3	1	-
19	13.2	1	-
20	14.3	1	-
21	15.4	1	-
22	16.7	1	23.9
23	18	1	16.5
24	19.5	1	-
25	21	1	-
26	22.7	1	-
27	24.5	1	-
28	26.5	1	-
29	29.1	1	-
30	31.5	1	-
31	34.1	1	-
32	36.9	1	-
33	39.9	1	-
34	43.2	1	-
35	46.7	1	-

Appendix 2. *Artemia* production

The production consisted of three main processes; decapsulation, hatching and enrichment. Initially, 500 g dry *Artemia*-cysts (1 g dry cysts: 180 000 – 200 000 nauplii) were decapsulated. The dry cysts were hydrated (1-2 hours) in a conic tank with freshwater (4.9 L, 10-25 °C) supplied with aeration to ensure sufficient O_2-concentration. To break the chorion coating, sodium hydroxide (NaOH) solution (150 mL) (59.4 g NaOH (solid) dissolved in clean water (150-200 mL) in a glass beaker placed on ice (exothermic reaction releasing heat)) and sodium hypochlorite (NaOCl) (1.44 L) was added to the hydrated cysts. Ice was added if temperature exceeded 30 °C. The process was terminated when cysts changed colour from white to orange (4-5 minutes). The cysts were then flushed through the bottom valve into a plankton net (mesh size 64 µm) and rinsed in freshwater (15-25 °C, 5-7 minutes) until the water colour changed from brown to clear (chlorine removed). Next, the net with the *Artemia* cysts was placed in a bucket with freshwater (15 L) added 0.1 % sodium thiosulfate ($Na_2S_2O_3$) (15 g, concentration 1 g/L) to deactivate the chlorine (5-7 minutes). Ultimately, cysts were rinsed in freshwater (5-7 minutes), excess water was removed by tightening the plankton net, and cysts transferred to a beaker and stored in a fridge (4 °C) for maximum 7 days.

For hatching decapsulated *Artemia* cysts (based on the factor of dry cysts to wet cysts and hatching rate) was added to a conic tank with sea water (60 L, 25-28 °C), a heat bulb, and heavy aeration from the bottom ensuring sufficient O_2-concentration and avoiding cyst sedimentation. After hatching (24 hours incubation), aeration was turned off (10 min) and sedimented unhatched cysts was flushed out through the bottom valve (2-3 seconds).

The hatched *Artemia* nauplii were flushed into a plankton net, rinsed with sea water and transferred to an enrichment culture tank with similar conditions as for hatching. The enrichment diet (10 g Multigain, Larviva Multigain, Biomar AS, Norway, per 60 L of water) were mixed in warm water by a hand mixer, foam was removed, and the remains added to the enrichment tank. The enrichment diet was added twice a day (early and late) to the enrichment culture. Culture density was estimated after hatching and after enrichment as described for copepods. The enriched *Artemia* was harvested into a plankton net (mesh size 64 µm), rinsed with sea water and added to live feed reservoirs (25 L containers) where they were fed to the lumpfish by the peristaltic pumps.

Appendix 3. Mean standard length per tank

Table A4. Mean standard length (SL) of *C. lumpus* per tank. Mean *standard length (mm) ± standard error (SE) per tank at sampling days 2, 9, 23 and 35 dph. Sampling size (total N) increase from 2-9 dph and 23-35 dph. Total N variation within groups is due to removal of inadequate standard length-measurements.*

Dph	Treatment Group	Tank	Total *N*	Mean SL ± SE (mm)
2	Art 1 (*Artemia* 12 days)	2	4	5.95 ± 0.20
		8	4	6.27 ± 0.13
		9	3	6.32 ± 0.08
		15	2	6.39 ± 0.17
	Art 2 (*Artemia* 25 days)	1	4	6.13 ± 0.17
		7	4	6.09 ± 0.14
		10	4	6.39 ± 0.07
		12	4	6.07 ± 0.18
	Cop (*Copepods* 12 days)	3	4	6.45 ± 0.08
		6	4	6.34 ± 0.04
		11	4	6.49 ± 0.06
		16	4	6.15 ± 0.08
9	Art 1 (*Artemia* 12 days)	2	12	7.18 ± 0.12
		8	12	6.92 ± 0.10
		9	11	6.76 ± 0.23
		15	12	7.00 ± 0.12
	Art 2 (*Artemia* 25 days)	1	11	6.91 ± 0.20
		7	12	7.05 ± 0.16
		10	12	6.97 ± 0.11
		12	12	6.39 ± 0.18
	Cop (*Copepods* 12 days)	3	10	6.56 ± 0.11
		6	10	6.35 ± 0.16
		11	12	6.83 ± 0.17
		16	12	6.56 ± 0.12
23	Art 1 (*Artemia* 12 days)	2	12	8.92 ± 0.23
		8	12	7.94 ± 0.26
		9	12	8.30 ± 0.13
		15	12	7.93 ± 0.21
	Art 2 (*Artemia* 25 days)	1	12	9.98 ± 0.25
		7	12	9.57 ± 0.25
		10	12	9.42 ± 0.43
		12	12	9.55 ± 0.35
	Cop (*Copepods* 12 days)	3	12	7.72 ± 0.19
		6	12	8.04 ± 0.16
		11	11	7.49 ± 0.28
		16	12	7.63 ± 0.13
35	Art 1 (*Artemia* 12 days)	2	19	10.73 ± 0.12
		8	19	10.07 ± 0.31
		9	19	9.57 ± 0.39
		15	19	10.33 ± 0.24

	Art 2 (*Artemia* 25 days)	1	20	12.27 ± 0.37
		7	20	10.64 ± 0.24
		10	20	12.12 ± 0.32
		12	18	11.34 ± 0.23
	Cop (*Copepods* 12 days)	3	20	9.16 ± 0.28
		6	19	10.08 ± 0.31
		11	20	9.66 ± 0.29
		16	19	9.48 ± 0.28

Appendix 4. Mean dry weight per tank

Table A5. Mean dry weight (DW) of *C. lumpus* per tank. *Values represents mean dry weight (mg) ± standard error (SE) per tank at sampling days 2, 9, 23 and 35 dph. Sampling size (total N) increase from 2-9 dph and 23-35 dph. Total N variation within groups is due to removal of inadequate dry weight-measurements.*

Dph	Treatment Group	Tank nr.	Total *N*	Mean DW ± SE (mg/larva)
2	Art 1 (*Artemia* 12 days)	2	4	0.73 ± 0.03
		8	4	0.79 ± 0.04
		9	4	0.79 ± 0.03
		15	4	0.79 ± 0.03
	Art 2 (*Artemia* 25 days)	1	4	0.72 ± 0.02
		7	4	0.76 ± 0.01
		10	4	0.84 ± 0.04
		12	4	0.81 ± 0.04
	Cop (*Copepods* 12 days)	3	4	0.75 ± 0.02
		6	4	0.76 ± 0.02
		11	4	0.76 ± 0.02
		16	4	0.84 ± 0.05
9	Art 1 (*Artemia* 12 days)	2	12	1.46 ± 0.05
		8	12	1.35 ± 0.05
		9	12	1.29 ± 0.09
		15	12	1.41 ± 0.06
	Art 2 (*Artemia* 25 days)	1	12	1.43 ± 0.10
		7	12	1.43 ± 0.06
		10	12	1.33 ± 0.06
		12	12	0.99 ± 0.08
	Cop (*Copepods* 12 days)	3	10	1.00 ± 0.05
		6	11	0.93 ± 0.07
		11	12	1.31 ± 0.08
		16	12	1.08 ± 0.05
23	Art 1 (*Artemia* 12 days)	2	12	2.82 ± 0.20
		8	12	1.83 ± 0.16
		9	12	2.16 ± 0.13
		15	12	1.72 ± 0.12
	Art 2 (*Artemia* 25 days)	1	12	4.68 ± 0.24
		7	12	3.82 ± 0.22
		10	12	4.14 ± 0.58
		12	12	4.07 ± 0.39
	Cop (*Copepods* 12 days)	3	12	1.64 ± 0.12
		6	12	1.82 ± 0.18
		11	12	1.33 ± 0.13
		16	12	1.48 ± 0.10
35	Art 1 (*Artemia* 12 days)	2	20	4.84 ± 0.19
		8	20	4.74 ± 0.37
		9	20	3.90 ± 0.40
		15	20	4.89 ± 0.26

	Art 2 (*Artemia* 25 days)	1 7 10 12	20 20 20 20	9.16 ± 0.56 5.22 ± 0.45 8.25 ± 0.57 5.31 ± 0.35
	Cop (*Copepods* 12 days)	3 6 11 16	20 20 20 20	3.14 ± 0.32 4.59 ± 0.35 3.99 ± 0.31 3.63 ± 0.27

Appendix 5. Mean daily weight increase per tank

Table A6. Mean daily weight increase (% DWI) of *C. lumpus* per tank. *The values represent mean daily weight increase for each tank in percent for each interval presented in days post hatch (dph) and are based on the average dry weight-estimates per tank.*

Dph interval	Treatment	Tank nr.	Mean DWI (%/day)
2-9	Art 1 (*Artemia* 12 days)	2 8 9 15	10.46 7.93 7.18 8.59
	Art 2 (*Artemia* 25 days)	1 7 10 12	10.33 9.41 6.75 2.85
	Cop (*Copepods* 12 days)	3 6 11 16	4.18 3.05 8.15 3.62
9-23	Art 1 (*Artemia* 12 days)	2 8 9 15	4.81 2.20 3.77 1.42
	Art 2 (*Artemia* 25 days)	1 7 10 12	8.84 7.29 8.48 10.66
	Cop (*Copepods* 12 days)	3 6 11 16	3.58 4.89 0.10 2.27
23-35	Art 1 (*Artemia* 12 days)	2 8 9 15	4.60 8.24 5.03 9.11
	Art 2 (*Artemia* 25 days)	1 7 10 12	5.75 2.63 5.90 2.24
	Cop (*Copepods* 12 days)	3 6 11 16	5.54 7.10 9.58 7.78

2-35	Art 1 (*Artemia* 12 days)	2 8 9 15	5.91 5.57 4.94 5.67
	Art 2 (*Artemia* 25 days)	1 7 10 12	8.02 6.01 7.17 5.86
	Cop (*Copepods* 12 days)	3 6 11 16	4.42 5.61 5.16 4.53

Appendix 6. Mean carbon content per tank

Table A7. Mean carbon content of *C. lumpus* per tank. *Values represents mean carbon content (µg) ± standard error (SE) per tank at sampling days 2, 9, 23 and 35 dph. Sampling size (total N) increase from 2-9 dph and 23-35 dph. Total N variation within groups is due to removal of incorrect carbon measurements.*

Dph	Treatment Group	Tank nr.	Total *N*	µg C/larva ± SE
2	Art 1 (*Artemia* 12 days)	2	4	385.09 ± 15.28
		8	4	423.63 ± 15.27
		9	4	417.71 ± 13.26
		15	4	419.15 ± 9.73
	Art 2 (*Artemia* 25 days)	1	4	388.35 ± 7.42
		7	4	414.57 ± 3.55
		10	4	446.93 ± 17.36
		12	4	437.23 ± 18.66
	Cop (*Copepods* 12 days)	3	4	407.17 ± 15.15
		6	4	402.28 ± 6.26
		11	4	403.22 ± 13.34
		16	3	422.13 ± 10.25
9	Art 1 (*Artemia* 12 days)	2	10	716.61 ± 25.00
		8	11	654.87 ± 22.94
		9	11	651.76 ± 40.45
		15	12	690.28 ± 26.95
	Art 2 (*Artemia* 25 days)	1	10	708.05 ± 57.03
		7	10	701.71 ± 25.11
		10	11	633.07 ± 27.06
		12	11	485.36 ± 32.1
	Cop (*Copepods* 12 days)	3	9	457.36 ± 20.60
		6	9	428.19 ± 32.37
		11	12	660.31 ± 43.47
		16	12	513.63 ± 20.40
23	Art 1 (*Artemia* 12 days)	2	12	1274.95 ± 96.23
		8	12	792.87 ± 75.23
		9	12	924.84 ± 60.10
		15	10	694.32 ± 55.41
	Art 2 (*Artemia* 25 days)	1	12	2063.12 ± 99.25
		7	12	1663.55 ± 105.88
		10	12	1856.98 ± 267.41
		12	12	1761.63 ± 171.96
	Cop (*Copepods* 12 days)	3	12	686.79 ± 53.06
		6	12	773.24 ± 76.63
		11	10	541.23 ± 65.58
		16	11	598.86 ± 44.76
35	Art 1 (*Artemia* 12 days)	2	18	2016.81 ± 96.96
		8	18	2088.21 ± 143.68
		9	18	1745.95 ± 188.61
		15	20	2249.88 ± 125.32

	Art 2 (*Artemia* 25 days)	1	17	3886.54 ± 254.66
		7	15	1901.28 ± 162.74
		10	19	3486.17 ± 263.61
		12	20	2205.17 ± 147.80
	Cop (*Copepods* 12 days)	3	20	1348.05 ± 146.33
		6	17	1947.34 ± 171.13
		11	20	1846.74 ± 147.82
		16	19	1668.67 ± 133.98

Appendix 7. Mean nitrogen content per tank

Table A8. Mean nitrogen content of *C. lumpus* larvae per tank. *Values represents mean nitrogen content (µg) ± standard error (SE) per tank at sampling days 2, 9, 23 and 35 dph. Sampling size (total N) increase from 2-9 dph and 23-35 dph. Total N variation within groups is due to removal of incorrect nitrogen measurements.*

Dph	Treatment Group	Tank nr.	Total *N*	µg N/larva ± SE
2	Art 1 (*Artemia* 12 days)	2	4	7.12 ± 0.28
		8	4	7.84 ± 0.34
		9	4	7.94 ± 0.31
		15	4	7.82 ± 0.34
	Art 2 (*Artemia* 25 days)	1	4	7.36 ± 0.15
		7	4	7.54 ± 0.07
		10	4	8.25 ± 0.40
		12	4	8.00 ± 0.41
	Cop (*Copepods* 12 days)	3	4	7.47 ± 0.16
		6	4	7.54 ± 0.10
		11	4	7.53 ± 0.18
		16	3	7.61 ± 0.02
9	Art 1 (*Artemia* 12 days)	2	12	14.59 ± 0.58
		8	12	13.08 ± 0.65
		9	12	13.60 ± 0.77
		15	12	14.68 ± 0.61
	Art 2 (*Artemia* 25 days)	1	12	14.38 ± 1.06
		7	12	15.17 ± 0.54
		10	12	12.78 ± 0.68
		12	10	11.05 ± 0.70
	Cop (*Copepods* 12 days)	3	10	11.10 ± 0.86
		6	10	9.69 ± 0.79
		11	12	13.61 ± 0.81
		16	12	11.57 ± 0.51
23	Art 1 (*Artemia* 12 days)	2	12	29.94 ± 2.14
		8	12	19.28 ± 1.77
		9	12	22.58 ± 1.36
		15	12	17.38 ± 1.32
	Art 2 (*Artemia* 25 days)	1	12	47.08 ± 2.08
		7	12	39.27 ± 2.28
		10	12	42.49 ± 5.94
		12	12	40.16 ± 3.71
	Cop (*Copepods* 12 days)	3	12	16.98 ± 1.21
		6	12	19.11 ± 1.82
		11	12	13.56 ± 1.39
		16	12	14.08 ± 1.12
35	Art 1 (*Artemia* 12 days)	2	19	47.70 ± 2.06
		8	18	49.24 ± 3.18
		9	18	41.35 ± 4.34
		15	20	51.62 ± 2.77

	Art 2 (*Artemia* 25 days)	1 7 10 12	17 18 19 20	94.87 ± 6.09 47.70 ± 3.92 84.78 ± 5.93 56.12 ± 3.51
	Cop (*Copepods* 12 days)	3 6 11 16	20 17 20 19	30.78 ± 3.15 43.06 ± 3.73 42.52 ± 3.34 38.17 ± 2.98

Appendix 8. Number of larvae per tank

Table A9. Number of C. lumpus larvae per tank. *Overview of the number of larvae estimated in each tank each day post hatch (0-35) for each of the three treatments. 4 tanks represented each treatment. Sampled larvae were excluded.*

Dph	Art 1 (*Artemia* 12 days)				Art 2 (*Artemia* 25 days)				Cop (Copepods 12 days)			
	Tank nr.				Tank nr.				Tank nr.			
	2	8	9	15	1	7	10	12	3	6	11	16
0	4028	3894	4267	4403	4463	3540	4339	3832	3866	3577	3620	3159
1	4028	3894	4267	4403	4463	3540	4339	3832	3866	3577	3620	3159
2	4028	3894	4267	4403	4463	3540	4339	3832	3866	3577	3620	3159
3	4028	3894	4267	4403	4463	3540	4339	3832	3866	3577	3620	3159
4	4017	3887	4248	4393	4436	3519	4317	3812	3830	3566	3614	3156
5	4013	3867	4228	4384	4420	3508	4300	3803	3818	3563	3609	3155
6	3993	3839	4201	4364	4402	3494	4274	3795	3793	3555	3601	3142
7	3984	3829	4189	4356	4378	3489	4259	3781	3768	3527	3590	3128
8	3977	3821	4181	4351	4370	3483	4250	3770	3750	3514	3579	3127
9	3973	3818	4170	4346	4366	3479	4241	3765	3741	3503	3549	3126
10	3964	3808	4164	4343	4358	3477	4238	3762	3636	3488	3525	3124
11	3958	3802	4148	4328	4352	3474	4234	3759	3608	3474	3513	3101
12	3952	3797	4148	4326	4347	3472	4231	3755	3513	3454	3498	3094
13	3948	3794	4137	4322	4339	3469	4222	3752	3483	3423	3460	3078
14	3940	3777	4135	4319	4334	3460	4215	3748	3406	3355	3435	3069
15	3901	3760	4070	4267	4330	3458	4211	3742	3384	3337	3385	3064
16	3766	3648	3854	4162	4303	3450	4204	3735	3308	3251	3358	3045
17	3661	3492	3624	3944	4271	3436	4172	3664	3275	3220	3262	3013
18	3591	3299	3567	3880	4179	3394	4106	3623	3082	2836	3248	2993
19	3568	3288	3464	3820	4112	3352	3946	3383	2806	2395	2972	2850
20	3542	3215	3444	3811	3988	3274	3870	3300	2033	1400	2866	2779
21	3531	3191	3419	3788	3939	3209	3768	3162	1788	1011	2706	2649
22	3504	3180	3414	3775	3884	3149	3689	3128	1471	595	2568	2558
23	3482	3167	3393	3743	3881	3118	3622	3104	1375	531	2373	2442
24	3475	3152	3383	3724	3843	3073	3607	3088	1225	482	2294	2358
25	3467	3142	3360	3692	3837	3064	3593	3070	1170	459	2037	2159
26	3414	3115	3348	3683	3827	3054	3585	3063	1083	432	1919	1975
27	3361	3087	3306	3642	3813	3043	3568	3049	939	410	1631	1608
28	3290	3049	3258	3617	3791	3025	3530	3030	810	382	1478	1416
29	3226	3012	3232	3588	3778	3016	3491	3006	787	366	1392	1292
30	2974	2868	3189	3582	3736	2988	3454	2995	763	343	1338	1247
31	2686	2814	3116	3559	3674	2938	3392	2971	744	335	1295	1203
32	2510	2794	3096	3556	3624	2918	3370	2962	739	331	1289	1198
33	2457	2792	3081	3553	3590	2903	3343	2932	739	327	1287	1190
34	2415	2791	3069	3552	3504	2877	3302	2895	739	326	1286	1184
35	2405	2788	3061	3550	3478	2853	3269	2836	739	326	1283	1180

Appendix 9. Prey density feed selection experiments

Table A10. Concentrations of *Artemia* and copepods for all three experiments with unfed, fed and starved *C. lumpus* larvae. *Each value is the mean result of density counts of Artemia and copepods in from a total of 24 mL (n = 24) at 0, 1.5 and 3 hours. Each experiment was run in 4 experimental buckets.*

Treatment	Bucket nr.	Prey type	Prey/mL		
			Time (hours)		
			0	1.5	3
Control (4 dph)	1	*Artemia* Copepods	4.88 4.88	5.88 5.63	5.88 6.50
	2	*Artemia* Copepods	4.75 5.38	7.00 6.63	6.25 5.00
	3	*Artemia* Copepods	6.25 4.75	6.75 6.25	6.13 5.25
	4	*Artemia* Copepods	5.00 5.63	6.38 4.50	5.88 4.25
Experiment 1 Unfed (4 dph)	1	*Artemia* Copepods	5.25 5.75	4.88 4.13	4.63 5.00
	2	*Artemia* Copepods	6.63 5.63	4.88 3.63	5.5 4.5
	3	*Artemia* Copepods	5.63 5.25	5.63 5.75	5.25 5.00
	4	*Artemia* Copepods	5.00 5.13	5.75 4.63	4.13 5.13
Experiment 2 Fed (11 dph)	1	*Artemia* Copepods	5.75 3.50	4.5 4.0	3.88 3.88
	2	*Artemia* Copepods	4.38 4.88	3.88 6.25	3.63 4.63
	3	*Artemia* Copepods	4.88 4.75	3.00 5.13	4.75 4.13
	4	*Artemia* Copepods	4.63 4.38	4.63 4.50	4.25 4.13
Experiment 3 Starved (13 dph)	1	*Artemia* Copepods	2.5 3.38	2.38 2.50	1.75 3.00
	2	*Artemia* Copepods	3.38 4.63	1.38 2.13	1.88 2.25
	3	*Artemia* Copepods	2.38 4.13	1.88 3.50	1.75 3.63
	4	*Artemia* Copepods	2.75 4.25	1.75 3.00	2.00 3.00

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