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Lipids and fatty acids development in lumpfish (*Cyclopterus lumpus*) larvae fed with different start-feeding diets (*Artemia*, cirripedia, copepod (*Acartia tonsa*) and formulated diet)

Master's thesis

NTNU
Norwegian University of Science and Technology
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Department of Biology

Master's thesis in Ocean Resources
Supervisor: Elin Kjørsvik
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Preface

This master thesis was carried out at the Department of Biology at the Norwegian University of Science and Technology (NTNU). The experiment took place in the laboratories at Sealab NTNU (Trondheim). The experiment was in collaboration with SINTEF Ocean AS and was part of a bigger project called “STARTRENS” (project nr. 901561). The Norwegian Seafood Research Fund (FHF) provided the main funds. The thesis was written under supervision of Professor Elin Kjørsvik and co-supervisor Arne Malzahn & Andreas Hagemannat the department of Biology.

First, I would like to thank my supervisor and co-supervisor for valuable feedback and guidance when writing this thesis and for helping with both the writing and statistics. I would also like to thank the people from SINTEF Ocean AS for good teamwork and discussions. A big thank you for the brilliant guidance in the experimental period; Tu Anh Vo, Luciana Alves Musialak and Frank Thomas Mlingi. Tora Bardal I am very grateful for your assistance and for teaching me the laboratory work. Additionally, thank you to Iurgi Imanol Salaverria-Zabalegui, Arne Kjølsnes and Dag Altin for help with technical problems.

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Saba Akbar

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Abstract

Salmon lice (*Lepeophtheirus salmonis*) infestations on Salmonids (*Salmo salar)* in cages has brought Norwegian aquaculture to a stable production since 2012. Many chemotherapeutic , chemical, and mechanical approaches have been used to control the sea lice attack. Unfortunately, sea lice have developed resistance against chemotherapeutic treatments and other approaches are either not considered ecofriendly or effect fish health. Biological control is deploying cleaner fish in cages to combat the sea lice. Lumpfish (*Cyclopterus lumpus*) is of commercial interest as a cleaner fish since it can be deployed at age of 4 months. The complicated first feeding phase and a lack of functional feeding protocols are the major barriers to large-scale lumpfish cultivation.

The current study aimed to contribute to the optimization of start-feeding regimes for lumpfish larvae in commercial farming. This was accomplished by comparing the effects of various start-feeding diets on the growth and survival of fish larvae. In addition to this development of lipids and fatty acids in lumpsucker larvae in relation to different feeding regimes was studied. Five feeding regimes were used in the experiment where lumpsucker larvae were fed from 2days post hatch to 35 dph. One group of larvae were fed with enriched artemia which were then weaned to formulated diet (FD). Second group were initially fed with copepods and then ultimately weaned to FD. Third group had Cirripedia initially and then weaned to FD. Fourth group had three types of feed initially copepods , then Cirripedia and then FD. Fifth group were fed with formulated diet firstly with small pellets(150nm) and then with large pellets (300nm).

Larvae fed with Artemia had best growth and survival as compared to other feeding regimes. Total lipid content and fatty acid content was highest in Art larvae initially and decreased on weaning to formulated diet but still significant. Artemia had significant amount of omega 3 and omega 6 fatty acids contributing to good growth and survival. Until 35 dph lipid content went steady in Art larvae. Lipidomics revealed that several lipid and fatty acid species are correlated either negatively or positively. The results clearly show the importance and responses of certain fatty acids in starting feed and can be helpful in optimizing start-feed of lumpsucker larvae.

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List of Abbreviations

Art	Artemia nauplii
Cir	Cirripedia nauplii diet
Cop/FD	Copepod nauplii and Formulated diet
FD	Formulated diet
Cop/Cir	Copepod and Cirripedia nauplii diet
ARA	Arachidonic acid (C20:4n6)
Art larvae	Larvae fed with Artemia and Formulated diet
Cir larvae	Larvae fed with Cirripedia and Formulated diet
Cop/Cir larvae	Larvae fed with copepod, Cirripedia and Formulated diet
Cop/FD larva	Larvae feed with Copepod and Formulated diet
DHA	Docosahexaenoic acid (22:6*n*-3)
DPH	Days post hatch
DW	Dry weight
DWI	Daily weight increase %
EPA	Eicosapentaenoic acid (20:5n-3)

FA	Fatty acid
FD larvae	Larvae feed with formulated diet
FHF	Fiskeri- og havbruksnæringens forskningsfond (Norwegian Seafood Research Fund)
MUFA	Mono-unsaturated fatty acids
NL	Neutral lipids
PL	Phospholipids
PUFA	Poly unsaturated fatty acids
SD	Standard error
SE	Standard deviation
SFA	Saturated fatty acids
SGR	Specific growth rate
SL	Standard length
TAG	Triacylglycerol.
TFA	Total fatty acids
TL	Total lipids

Chapter 1: Introduction

1.1 Norwegian aquaculture and cleaner fish

According to current Norwegian aquaculture statistics, Norway is among the world's leading producers in terms of sea cage salmon production, with about 986 locations in operation till date along with 91 land based production plants (Norwegian Directorate of Fisheries, 2020). Norway is exporting Salmon to more than 150 countries mainly European. In year 2018-19, 93% of the total production (Statistisk Sentralbyrå, Statistics Norway, 29 october, 2020) was exported and this production was raised by 2.7% in year 2021(Rolland, 2021). Huge production of Salmon is frequently accompanied by certain environmental issues(Klinger & Naylor, 2012), like attraction of ectoparasites .

The incidence of the ectoparasite, sea lice (*Lepeophtheirus salmonis*) has grown due to greater salmon production in sea cages *Lepoptheirus salmonis* and *Caligus elongatus* are the two major species in Norwegian waters with adverse effects on Salmon(Jansen et al., 2012). Due to sea lice being one of the key issues, yearly output has remained relatively steady after hitting 1.2 million tonnes in 2012(*Risikorapport norsk fiskeoppdrett* 2021). Therefore, it is among the most significant challenges currently confronting Norwegian salmon aquaculture, as it affects primarily fish wellbeing, as well as the producer's financial profit (Costello, 2009).

Lice has its natural existence in marine environment and is attracted to salmon sea cages. By devouring mucous, skin, and blood, the sea lice(Liu & Bjelland, 2014) poses a danger to the host fish. This increases the fish's susceptibility to additional infestations such as bacteria, viruses, and fungus, as well as affecting the fish's osmoregulation(Carvalho et al., 2020; Mustafa & Piasecki, 2005; Ørjan Karlsen et al.). Control on sea lice infestation is vital to not only maintain a sustained increase of Salmon production, but also to improve fish welfare and achieve a long-term, financially beneficial development(Anne Berit Skiftesvik et al., 2018; Costello, 2009; Overton et al., 2019). So, the ultimate way is to reduce the control sea lice. Sea lice infestations in Norwegian Aquaculture is monitored by traffic light system to avoid adverse effects on cage fish as well as wild population(Saue, 2017).

To remove salmon lice, there are mechanical, chemical, and pharmacological remedies, as well as other approaches and technical aspects. Unfortunately, sea lice have acquired resistance against most commonly deployed chemotherapeutic treatments over time(Denholm et al., 2002; Treasurer et al., 2000). Chemical treatments impacted local biodiversity of other wild species , and

mechanical treatments such as thermolicers and hydrolicers brought the fish under physical stress, causing impairment and, in some cases, death(Overton et al., 2019; A. Skiftesvik et al., 2014). The demand for more sustainable alternatives is required like use of cleaner fish such as ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*). Use of cleaner fish as a biological, non-pharmaceutical, and amicable delousing solution in marine cages accommodating salmon(Anne Berit Skiftesvik et al., 2018; Treasurer, 2002) is now one of the preventatives and ecologically friendly therapies (Anne Berit Skiftesvik et al., 2018; Treasurer, 2002). Deploying cleaner fish is advantageous to both producers and fish because it is both cost effective and favorable to fish welfare. In the Norwegian salmon aquaculture, the lumpfish (*Cyclopterus lumpus*) and four different wrasse species—the rock cook wrasse (*Centrolabrus exoletus*), ballan wrasse (*Labrus bergylta),* corkwing wrasse (*Symphodus melops*), and goldsinny wrasse *(Ctenolabrus rupestris*) have been primarily deployed into the net pens(Powell, Treasurer, et al., 2018; Treasurer, 2002). Cleaner fish deployment grew popular in the 1980s, and the first species to be deployed was the goldsinny wrasse *(Ctenolabrus rupestris)(Bjordal, 1991; A. B. Skiftesvik et al., 2014).* Labridae are convenient; however, their temperature sensitivity makes them less effective and inactive when exposed to temperature below 6 °C or 8 °C, they go into a hypometabolic condition, drastically reducing their ability to graze on lice (Blanco Gonzalez & de Boer, 2017; Sayer & Reader, 1996). Alternatively, lumpfish are active at 4°C being cold water specie (Bjordal, 1991; Kelly et al., 2014; Powell, Treasurer, et al., 2018).

Other factors making lumpfish a good candidate is that it is ready to be deployed at its age of 4 months,(Kelly et al., 2014) while Ballan Wrasse takes longer about 18 months which makes it less cost effective(Brooker et al., 2018; Muncaster et al., 2010; Powell, Treasurer, et al., 2018). Additionally, only the ballan wrasse is currently grown, and the great majority of lumpfish utilized today come from commercial farming, whilst the majority of wrasse source is still wild catch (Blanco Gonzalez & de Boer, 2017; *Risikorapport norsk fiskeoppdrett* 2021) (Figure 1.1). This is because multiple research ((Dahle et al., 2017; Marthinsen, 2018; Rian, 2019b; Romundstad, 2015) have found lumpfish to be faster to culture and have a typically far greater survival rate than ballan wrasse. Lumpfish commercial production has increased from 431,000 in 2012 to over 36 million in 2020 (Fig1.1) and is now the most often utilized cleaner fish but ballan wrasse output is comparatively less (Norwegian Directorate of Fisheries, 2020).

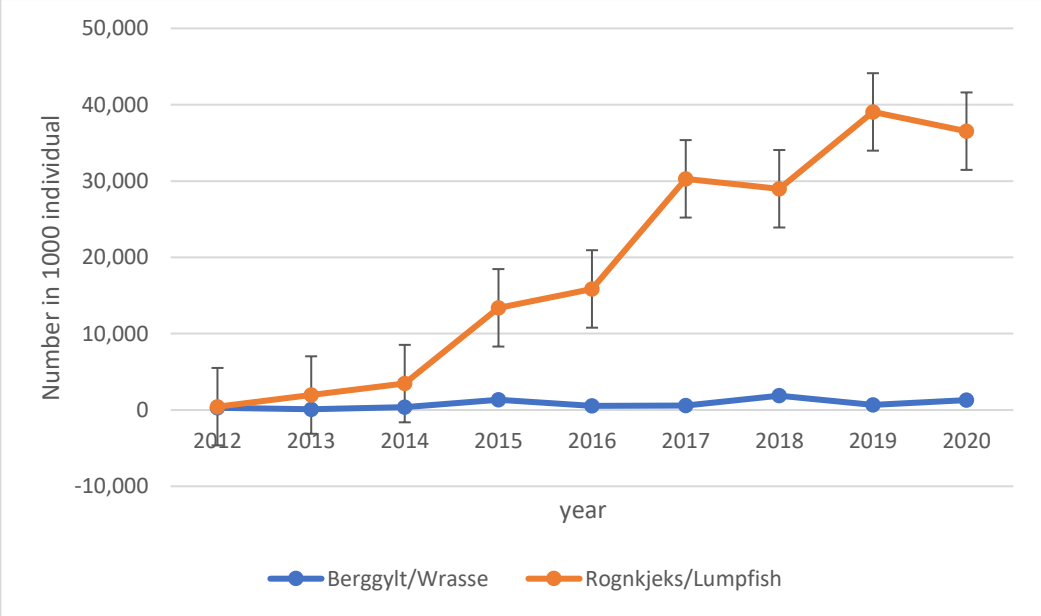


Figure 1.1 Sale of farmed lumpfish (Cyclopterus lumpus) and ballan wrasse (Labrus bergylta) to producers of Atlantic salmon and Rainbow trout in millions from year 2012-2020 in Norway. Source: Norwegian Directorate of Fisheries (2020)

1.2 Challenges of lumpfish production in Norwegian aquaculture

Larviculture, is one of the key stages in the production cycle of lumpfish often known as the method of raising fish larvae from hatching to ongrowth, ((Marthinsen, 2018). The larval is defined by fast growth and development, making it a critical life phase if mass rearing individuals with high quality is desired (Kjørsvik et al., 2004; Powell, Treasurer, et al., 2018). After all, one of the most crucial stages in a fish's existence is when it switches from an endogenous energy source given by the yolk sac to an external energy source from the diet (De Silva Sena S. et al., 1994). Currently, the survival, quality, and size of the larvae produced by lumpfish larval rearing can be regarded as variable(Anne Berit Skiftesvik et al., 2018; Dahle et al., 2017; Garcia de Leaniz et al., 2022; Powell, Pooley, et al., 2018). The transition from a live to a manufactured diet is thought to be the reason of the high post-weaning mortality that has also been observed (Powell et al., 2018). Being a relatively new species in aquaculture, the lumpfish's variable rearing performance has frequently been attributed to the lack of information on the proper feeding schedules and nutritional needs for the species. (Imsland et al., 2018; Powell, Treasurer, et al., 2018). Start-feeding regimens have currently been based on such techniques whereas other marine fish species have been produced in aquaculture. (Benfey & Methven, 1986; Powell, Treasurer, et al., 2018) feeding regimes that are uniquely suited to lumpfish is therefore essential to overcoming the uneven rearing success. First ever industrial lumpfish production pilot trials began in 2011 (Imsland et al., 2016; Towers, 2013).

Lumpfish hatch from a demersal egg (Marthinsen, 2018) (Rian, 2019a) of 5-6mm in size It has suction disc and mostly like to stay attached to any substrate. Although it has abundant of prey types nearby but due to sessile nature makes its only feeds on crustaceans (Benfey & Methven, 1986; Dahle et al., 2017; Marthinsen, 2018)., and other potential prey is ignored (Ingólfsson et al., 2002). Having suction disc it mostly attaches itself to seaweeds and feed passively which helps in using less energy that costs less for foraging, but when food is scarce it behaves oppositely (Killen et al., 2007).

According to (Dahle et al., 2017; Imsland et al., 2019) (Jobling, 2018), most producers now use FD directly, administered at 2 to 5 dph. Different live feeds, such as Artemia nauplii, copepods, and cirripeds, have also been investigated before weaning to formulated diets. (FHF, 2019; Jonassen et al., 2018)However, little is yet understood about how the various feeds and feeding regimens now being employed impact the fish's continued development and survival as well as their main objective of lice-grazing (FHF, 2019)It is crucial to understand the overall nutritional needs of fish larvae and how they are met by the industry standard (formulated diet) and various live prey species to establish an effective feeding regime.

Current production still relies on the acquisition of wild brood stock, which has already been suggested unsustainable(Committee & Butterworth, 2014). The species must be extensively raised in captivity to equip the salmon farming business with the number of lumpfish required for louse control(Brooker et al., 2018).. Utilization of cleaner fish to delouse farmed salmon has become considerably more well-known in recent years, however above 80% of studies mention wrasse and >13% percent mention lumpfish(Bailey, 2014; Powell et al., 2017; Powell, Treasurer, et al., 2018). As per current Salmon production, Lumpfish production must expand to around 50 million fish per year to accommodate global industry demands, and this can only be achieved through aquaculture. These information gaps must be overcome through research to improve animal welfare and ensure integrated and sustainable lumpfish production. This project will emphasize issues such as adequate feeding regimes, larval rearing in terms of growth, fatality, and stress.

1.3 Commercial diets for lumpfish production

1.3.1 Formulated diet

The industry has usually preferred prepared diets since they need less labor and are cost effective than deploying live prey (Hamre et al., 2013). Additionally, prepared diets are available in a variety of forms, sizes, and nutritional compositions, making them appropriate for a wide range of fish species.(Hamre et al., 2013) Microdiets are cost effective and need to comply with biochemical and structural characteristics for making it suitable as first feed specially in terms of

digestibility of pelagic larvae like lumpfish(Kjørsvik et al., 2004). Formulated feed must be substantial to dissolution along with leaching of hydrosluble components. Addition of some attractants to the diet like amino acids is helpful in digestion(Hughes, 1989; Langdon, 2003; Yúfera et al., 2003). Trials are in progress to replace live feed with formulated diet as that can be used as early as possible in larval phase, but underdeveloped stomach of marine pelagic larvae makes it further challenging (Cahu & Zambonino Infante, 2001; Kjørsvik et al., 2004).

Additionally, the granules of the formulated diet must be recognized by the larvae as food source to be consumed (Hamre et al., 2013). Since lumpfish larvae have functioning eyes from the moment they hatch, it is probable that most fish larva utilize their eyesight to seek for food (Hunter, 1981). (Brown, 1986). However, the feed particles can only travel in the directions determined by the water currents in the tanks, and if the currents are weak, the feed particles may end up settling and collecting at the bottom of the tank (D'Abramo, 2019). Despite the lumpfish's size and ability to consume formulated diets from the beginning (Kjørsvik et al., 2004) .It should be fed with live feed due to several reasons. It has been demonstrated that feeding lumpfish larvae a prepared diet can have a deleterious impact on the gut epithelium and energy storage in the liver, and that the lumpfish larval's stomach is not fully functional until 21–34 days after hatching (Dahle et al., 2014; Marthinsen, 2018).

1.3.2 Live feed

Despite improvements in the manufacturing of inert feeds for larvae, several aquaculture species still rely upon live feeds throughout the early phases of their lives.(Govoni et al., 1986) Live feeds are the predominant source of nutrition for cultured larvae and are especially important when producing altricial(development relies on yolk sac resorption) marine fish larvae. Because of rudimentary digestive system in early-stage larvae can’t process formulated diet so it relies on live feed(Cahu & Zambonino Infante, 2001). Another reason of live feed being priority is that live feed keeps on swimming in water column while formulated one aggregates and sinks to the bottom. The most used live feeds in aquaculture of marine larvae are rotifers (*Brachionus sp.),* planktons, copepods(*Acartia tonsa*), and brine shrimp (Artemia sp.), because of their cost-effective mass production(Conceicao et al., 2010).

Artemia Smaller shrimp-like crustaceans called Artemia, commonly known as "brine shrimp," are mostly utilized live prey in marine aquaculture due to their economic availability and usefulness (Dhont et al., 2013; Van Stappen & G., 1996). Cysts, or latent embryos, are used by artemia to reproduce, which is perhaps the key characteristic that makes it such a useful live feed to use.

Saltwater incubation of 24 hours is used to rehydrate the cyst which emerges f ree-swimming nauplii. After hatching, Nauplii instar I (400–500 m long) can be utilized as live prey (Van Stappen & G., 1996). Artemia are simple and inexpensive to grow. They are orange-red, move constantly, and are simple to catch for larvae because they lack effective escape reflexes(Prusińska et al., 2015). Harvesting, processing, and storing determine retention of their nutritional value(Baert et al., 1996). It has been questioned if Artemia is sufficient in providing the nutritional demands of marine fish larvae, despite the fact that it is easily grown and frequently employed as a live feed.

Artemia spp. are divided into two groups based on their lipid and fatty acid composition: marine and freshwater. Marine-type strains contain substantially greater lipid and triacylglycerol levels, and higher EPA and ARA levels and lower linolenic acid (LNA; 18:3n-3) levels compared to freshwater-type strains.(Harel et al., 2002) Over the years, a wide range of lipid enrichment techniques, both commercial products and improvised, have been used for enhancing its nutritional content since artemia is among preferred feed of lumpfish.(Navarro et al., 1999) Alternative enrichment products have been developed as knowledge of the lipid requirements of marine fish larvae, as well as advances in marine biotechnology and industrial feed processing technologies(Conceicao et al., 2010; Navarro et al., 1999).

Copepods are tiny crustaceans that serve as the natural food of larval marine fish (Støttrup & McEvoy., 2003). Lumpfish larvae have also been observed to eat copepods (Ingólfsson et al., 2002). Copepods descend slowly, and their darting zigzag movements preceded by drifting glides are appealing to fish larvae because they stimulate basic foraging instincts and give visual stimulation (FAO, 1996). Despite massive cultivation of copepods, implementing cost-effective mass-production processes of copepods remains a difficulty(von Vaupel, 2008). When rotifers and/or Artemia were used as live feed for marine fish larvae, copepods produced significantly better outcomes in terms of larval efficiency . Current findings on the physicochemical characteristics of copepods, which includes macronutrients (lipids and amino acids) as well as micronutrients (pigments and vitamins), revealed that neritic calanoid species like Acartia tonsa seemed to have a high level of stability(van der Meeren et al., 2008).

In comparison to Artemia and Rotifers, it has a higher EPA:DHA ratio and their inclusion in PLs, making it a more nutritious diet. HUFA is significantly more readily accessible, digested, and retained in tissue phospholipids in copepods as compared to HUFA in NLs, which makes copepods effective for marine fish larvae(Cahu et al., 2003; van der Meeren et al., 2008). In marine fish larvae like European seabass *(Dicentrarchus labrax)* and Atlantic cod *(Gadus morhua)*, HUFAs stored in

phospholipids outperformed such as triacylglycerols(TAG), in terms of growth, proliferation, maturation, calcification of the bones, and long-term survival, then HUFAs stored in neutral lipids(NL's)(Cahu et al., 2003; Küllenberg et al., 2012).Copepods also have a high protein content, particularly free amino acids, which aids protein utilization and growth, particularly when the stomach of larvae is immature(Kjørsvik et al., 2009), which makes it good option as prey.

Cirripedes Sessile crustaceans belonging to the subclass Cirripedia, or barnacles spend the most of their life permanently attached to substrates like coral and rock (López, 2012). The larval development of all of them begins with a series of free-swimming naupliar instars and ends with a cypris larva. A barnacle is a crustacean that looks like a shrimp. Cirripedes , typically cryopreserved zooplankton nauplii also called barnacles are thought to be a good replacement for both artemia and rotifers. Cirripedia nauplii contains PL's in high amount specially DHA and EPA which is the natural diet for marine fish(Dhont et al., 2013; Moon et al., 2016) in PL's without any enrichment. This nauplii stage was used by the firm Planktonic AS, who sold it as a preserved and effective substitute for conventional live feeds (Plankton, 2022). The cirripeds are still alive and capable of swimming after the rejuvenation time, according to the producer, as the cryopreservation method has just paused their metabolism(Plankton, 2022). As the cirripeds are claimed to have an ideal nutritional profile for marine fish larvae, they may then be given directly to the fish (Planktonic AS, 2022). This is because the cirripeds' phospholipids contain significant amounts of the fatty acids DHA and EPA (Plankton, 2022). As per fact there are no research articles which can prove cirripedes to be the good option but can still be tested because of its good nutritional value.

1.4 Why study larval lipids and fatty acids in early larval stages of lumpsucker larvae

Norway has invested significantly in aquaculture research, focused on factors influencing the survival and development of larvae of commercial cleaner fish species (Norwegian Directorate of Fisheries, 2020; Liu & Bjelland, 2014). The limited effectiveness of intensive marine fish culture may well be explained in part by ignorance or inadequate knowledge on larval feeding demands (Kjørsvik et al., 2009). Presumably, having a thorough understanding of the dietary needs of larvae throughout their growth would help to optimize feeds and feeding regimes as well as juvenile quality. Lipid, typically n-3 fatty acids nutrition is thought to be optimal solution to these problems.

Quality of larvae is seen to be affected by lipid nutrition. For the quality of the larvae, lipids in the nutrition of the broodstock are thought to be crucial. Lack of (n-3) highly unsaturated fatty acids (HUFA) in broodstock adversely affects the fecundity, fertilization rate, and spawning rate of fish

larvae. Prior to first feeding, lipids are main reserve of energy during the embryonic and larval stages along with protein and carbohydrates (Rainuzzo et al., 1997).

Phospholipids (PL) make up most of the structural polar lipids that make up fish cell and intracellular membranes (Parrish, 2013; Sargent, Bell, et al., 1999). The physico-chemical characteristics of cellular membranes are known to be influenced by the fatty acid content of dietary phospholipids (Bell et al., 2003; Lund et al., 2018; Sargent, McEvoy, et al., 1999a) As a result, PL are thought to have conservative fatty acid profiles that slightly correspond to diet. Triacylglycerols (TAG) are the main component of fish reserve lipids, which predominantly comprise fatty acids obtained from dietary sources. Since begning of 1980s, research has shown that membrane phospholipid is vital for fish larval feeding,(Kanazawa et al., 1981; Kanazawa et al., 1983) found that PL increased life expectancy and growth.

The potential of dietary phospholipid to promote the growth and development of fish larvae is now well documented (L. A. Copeman et al., 2002; Geurden et al., 1995; Kanazawa, 1997; Kanazawa et al., 1983; Şen Özdemir et al., 2019) et al., 1995), which could also help to explain why copepod nauplii do well in comparison to other live prey (McEvoy et al., 1998). The effectiveness of dietary phospholipids tends to decline with aging and may be due to the rudimentary structure of the digestive system in marine fish larvae, which do not have a completely functioning digestive tract until they have undergone metamorphosis and digestive enzymes (Bisbal & Bengtson, 1995; Munilla-Moran & Stark, 1989). There is also considerable evidence that dietary phospholipids can improve feed intake (Koven et al., 1998), function as gut emulsifiers (Koven et al., 1993)prevent lipid peroxidation in the intestines and during enrichment (Gatlin III & Bai, 1993; Kanazawa et al., 1981; McEvoy et al., 1995) and induce the production of lipoprotein in intestinal enterocytes (Fontagné et al., 1998; Geurden et al., 1998) inconsistency of DHA:EPA leading to eyes impairment in terms of rods and cones (Shields et al., 1999).Combining all of these will considerably increase dietary lipid utilization, which will help growth and development. Due to their inability to synthesize PL de novo in sufficient quantities to fulfill needs, early developing marine fish larvae are known to have a strict demand for pre-formed phospholipids (Teshima Shin-ichi et al., 1987).

The proportion of DHA and EPA in the larval diet is another key factor that is of interest. However, it appears that the larvae are unable to transform and retain these essential fatty acids(EFAs) from the dietary triacylglycerides (TAG) in their tissues,(Evjemo & Olsen, 1997) as evidenced by the fact that their growth and development are less favorable than if the fatty acids were present in the phospholipids of the prey (i.e. in the tissue membranes and nerve tissues)(Bell et al., 2003; Sargent,

McEvoy, et al., 1999b). According to research using halibut larvae that was fed by enriched prey, phospholipids from rotifers and copepods can be incorporated directly into the cellular membrane of quickly growing larvae (Evjemo & Olsen, 1997) and will display a profile of biologically active HUFA, particularly 22:6n-3, that will be advantageous for larval development and progression.

Copepod nauplii is rich in EFAs but rotifers and artemia requires enrichment. Copepods are nutritionally beneficial due to their naturally high levels of the essential highly unsaturated fatty acids (HUFA), 20:5n-3 (Eicosapentaenoic acid; EPA) and 22:6n-3 (docosahexaenoic acid; DHA) (Evjemo & Olsen, 1997), which are predominantly in the form of phospholipids. We will focus over development of fatty acids in larvae over time also the relation with growth and survival. It should not be overlooked, though, that lipids provide the "fuel" for development in the form of saturated and, particularly, MUFA. Also, it is crucial to keep a healthy balance between these acids and the less unsaturated fatty acids as energy reserves.

1.5 Aims and objectives

The expansion of lumpfish commercial production must be complemented by deeper understanding of suitable start-feeding regimes for lumpfish larviculture to be viable, which is the driving force for the current work. Therefore, the study's goals were to help optimize feeding regimes in lumpfish farming and aims were

Aim 1: Assess the effects of various start-feeding diets on the survival and growth of lumpfish larvae.

Aim 2: Assess the impact of various start-feeding diets on the production of lipids and fatty acids in larvae and their potential utility for lumpfish larvae growth and survival.

In previous studies of start feed larvae showed good growth with Artemia(Marthinsen, 2018; Rian, 2019a) while on commercial scale cryoplanktons can be a good live prey (Malzahn et al., 2022; Plankton, 2022). Based on its lipid and fatty acid profile(Malzahn et al., 2022) it can be tested and might give good results. In our study we used five feeding regimes composed of four diets. Artemia and copepods have possess significant quantities of Phospholipids, which is deemed essential for fish nutrition(Tocher, 2010). The low levels of omega 3 fatty acids in Artemia, on the other hand, are insufficient for early life stages, and must be enhanced before being deployed as prey (Conceicao et al., 2010)(ie et al., 2011). Copepods, on the other hand, are rich in phospholipids and EFA in the wild. (Drillet & Lombard, 2015). In rotifers and Artemia, the fatty acid composition of these animals

can be altered via enrichments(Castell et al., 2003; Hawkyard et al., 2015; Óscar Monroig 1 et al., 2003). We enriched our diet as explained in methodology to enhance nutritional quality of prey.

Based on pilot study following hypothesis was formulated.

Hypothesis:

> **Different start feeding diets (Art, Cir, Cop/FD, Cop/Cir, and FD) containing different lipid profile will have differential effect on larvae development.**

Larval growth was analyzed by dry weight(DW), standard length (SL) and Daily weight increase(DWI). While lipid was evaluated by analyzing the lipid and fatty acid profiles of feeding regimes and larvae.

This study is part of FHF project named STARTRENS ("Optimalisert startfôring av rensefisk)(https://www.fhf.no/prosjekter/prosjektbasen/901561/) lasted for 35 days(2-35dph). SINTEF Ocean AS and NTNU collaborated on the project, which was primarily sponsored by FHF (Fiskeri- og havbruksnringens forskningsfinansiering). The goal of "STARTRENS" was to improve cleaner fish start feeding regimes (Cyclopterus lumpus and Labrus Bergylta). Two other Masters students Sunniva Brevik Kværnø Growth, survival and liver histology in lumpfish (Cyclopterus lumpus) larvae fed different start-feeding diets (Artemia, copepods, cirripeds and formulated diet) and Marte Solli Lindskog (Muscle growth and development in lumpfish (*Cyclopterus lumpus*) larvae in relation to start-feeding diets (*Artemia*, cirripedia, copepod (*Acartia tonsa*) and formulated diet) were part of the study group.

Chapter 2: Materials and methodology

2.1: Experimental layout and start feeding of lumpfish larvae

2.1.1 Lab facility

The start-feeding experiment was done in the COD-Tech laboratory NTNU/SINTEF in Trondheim. It lasted for 36 days, from September 9 to October 14, 2020. Unfertilized lumpfish eggs from seven different females were taken from Akvaplan-niva in Tromso. Eggs were pooled together in a bucket after receiving at NTNU (Sealab) in Trondheim, and milt of males was used for fertilization. Fertilized eggs were divided into 15 groups and then incubated separately with continuous flow of water and gradual increase of temperature from and 5°C to 10 °C in incubators holding 300ml saltwater. The larvae were moved from the egg incubator to their designated tank through an outlet as the eggs hatched. Hatching rate was not uniform based on mixing different eggs. The average density was intended to be 100 larvae L^{-1} per tank however after calculating based on survival, it was variable.

2.1.2 Tank setup and larval rearing

Total 15 tanks were part of experimental setup(fig 2.1). Each cylindrical tank was having 100L of seawater. Seawater was pumped from Trondheim fjord at depth of 77 meters. Membrane filters of 1µm and sand filters were used to filter the water. Aerated tubes were placed close to the bottom of the tank to keep oxygen level up to the mark. Temperature of the tanks along with oxygen saturation was measured on daily basis, on average 10.3°C and 7.8 mg L-1, respectively.

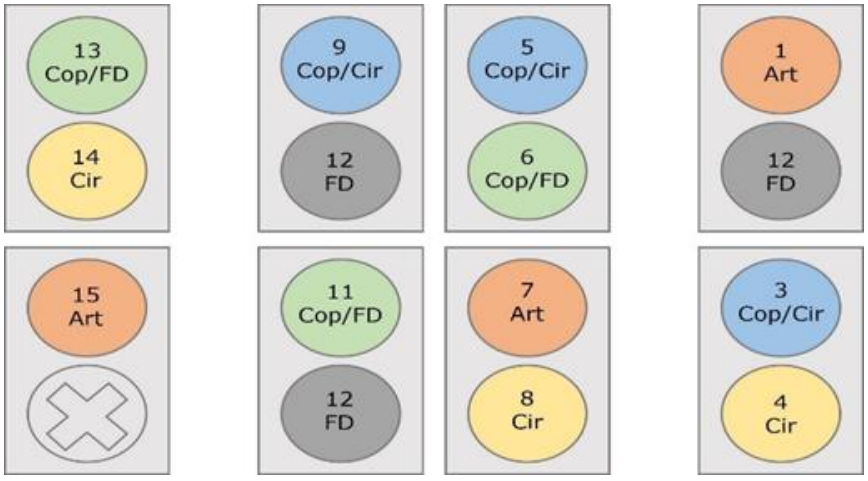


Figure 2.1 Experimental setup of five different feeding regimes in multiple of three each distributed randomly

Silicon plates were hanging down in the tanks for providing some site to the lumpsucker for attachment. Each tank was supplied with LED, placed above tank throughout the experiment, 24 hours a day, having 30% intensity at its maximum. The outlet of the tanks was covered with a mesh of different size depending on larval size as well as feed type. Rate of water exchange of the tanks was different depending on type of feed provided to the larvae in particular tanks A live prey feeding reservoir (KeyKeg, 20 L) was kept adjacent to each tank, and the live preys were consistently transported from the reservoir to the tank using a peristaltic transducer that was included in the tank configuration. Each tank was fitted with a feeding automat (Sterner Fish Tech AS, Norway), which was managed via the central operating facility (Normatic AS) at Sealab NTNU. The dry feed of two sizes was delivered to the tanks by an automated pump.

2.1.3 Tank maintenance

From 8 dph cleaning arm was used to gather the dead larvae and excess feed. A glass siphon was used to remove and count dead larvae on daily basis. Along with dead larvae all type of debris was removed twice a day. Alive larvae were taken out during cleaning and placed back into the tank. Dead larvae were registered on daily basis. Mesh used in tanks outlet was to flush out the live feed overtime. Mesh was changed and cleaned depending on its clogging due to feed and also on changing feed type. (Fig2.2)

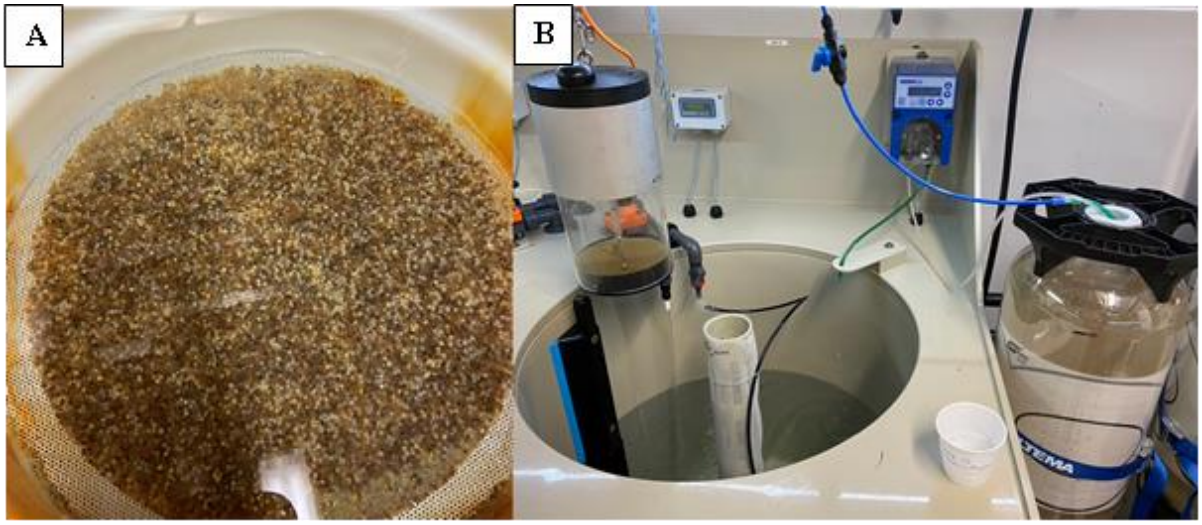


Figure 2.1 Incubation tank of C lumpus eggs and experimental setup supplied with oxygen, automated feed , mesh or seive, cleaning arm by Marte Lindskog

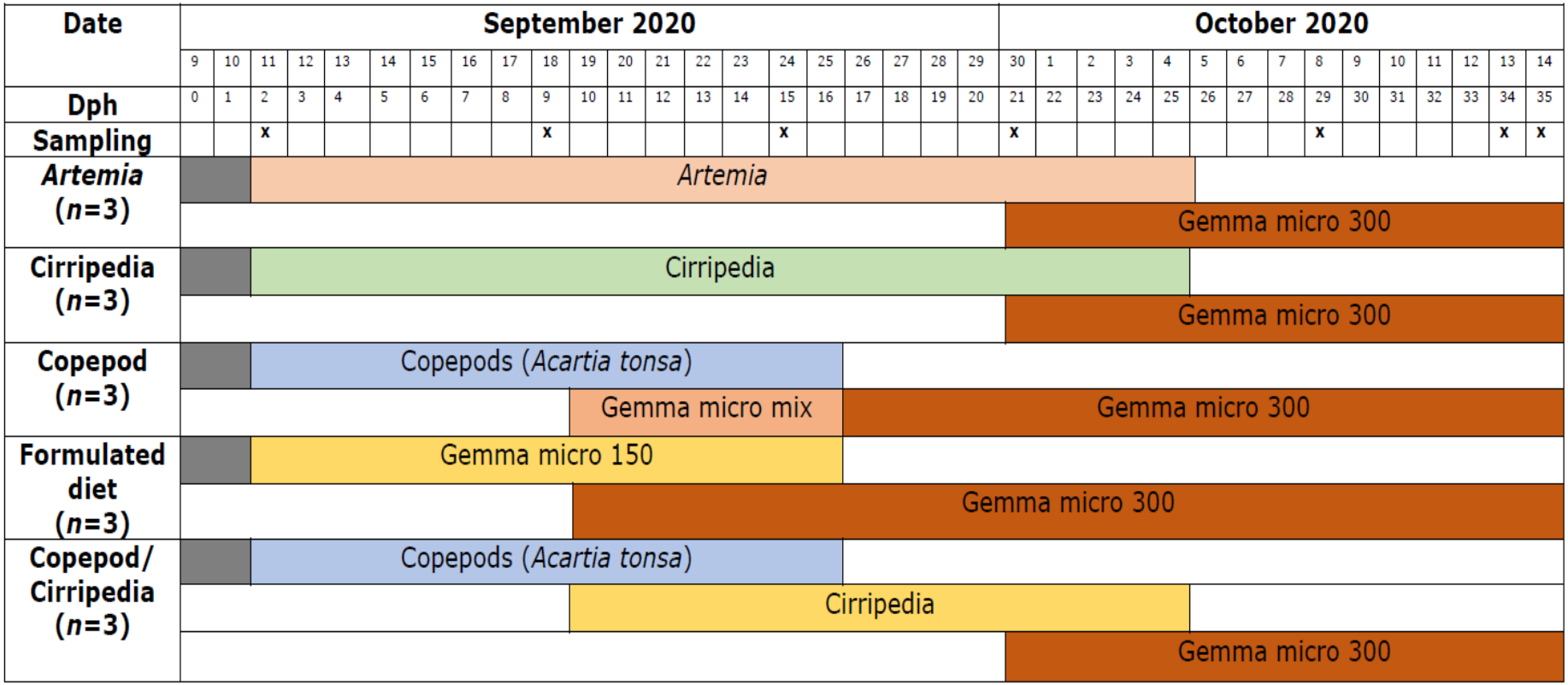
2.2: Five feeding regimes

Three types of live prey (Cirripedia, copepods and Artemia) and one formulated diet(FD) were used to create five start feeds. They were divided into 5 groups (table 1) All five regimes were having 3 replicates each making 15 tanks in total (fig 2.1)

Table 1. Feeding regimes

Type of feeding regimes	Size and feeding schedule	Specie and Source
1. Artemia nauplii (Art)	a. (Nauplii of 800µm used after 24 hour enrichment) b. Enriched artemia were fed for 18days(20dph).Larvae were then weaned to formulated feed until 34 dph	(EG ® INVE Aquaculture, Thailand)
2. Cirripedia (Cir) **.**	a. Nauplii were 150 µm and 350µm long and wide respectively b. 6 hours revitalization and thawing of Cirripedia were done before feeding. Larvae were fed with Cirripedia for 18days(20dph).Larvae were then weaned to formulated feed until 34 dph	Cryoplanktons: (Planktonic AS, Norway)
3. Copepod and formulated feed (Cop/FD)	a. Copepod size: (185-394 µm in stage n5/n6) b. Formulated feed: Pellets size was 150µm and 300µm c. Larvae were first fed with copepod for 7 days followed by small pellets of formulated feed for 7 days and rest with large pelletss until day 34.	A.tonsa (C-feed AS, Norway) Formulated feed :GEMMA Micro 150 and 300 µm (Skretting AS, Norway)
4. Formulated feed (FD)	a. Formulated diet :GEMMA Micro 150 and 300 µm larvae were fed with small pelletss from 2 to 9 DPH and then with big pelletss of formulated feed until 34 Dph.	Skretting AS, Norway)(Skretting, 2021)
5. Copepod and Cirripedia (Cop/Cir)	a. Cirripedia: Nauplii were 150 µm and 350µm long and wide respectively b. Copepod size: (185-394 µm in stage n5/n6 Larvae were fed with copepod till 9 dph then from 10 to 17 with Cirripedia and until 34 dph they were weaned to formulated feed.	

Table 3 Experimental setup*:* Feeding regimes for start feeding of lumpfish *(C.lumpus)* from Sep-Oct 2020 (2 to 35 dph). Triplicates of five feeding regimes were used (n=3). Sampling times for lipid and fatty acid analysis and growth is marked with an X. Weaning periods are indicated with the overlapping in each feeding regime.



2.3 Types of feed for lumpfish larvae rearing and their distribution

2.3.1 Artemia (Art):

A German scale was used to weigh amount of artemia cyst (EG ® INVE Aquaculture, Thailand).After weighing it was placed in 60L cylindrical tank with sea water stabilized at 25-30°C by using electronic aquarium heater (EHEIM thermo control e300, Germany). The rightful amount of cyst to be weighed was figured out based on hatching potency of 260 000 nauplii g-1 dry weight of cysts given by producer. After 24 hours of aeration cysts, newly hatched Artemia were separated by using magnetic separator (SEP-ArtMagnetic Artemia SEPARATOR, Australia).(fig 3)Newly hatched artemia were placed into another conical cylinder and enriched with 10 g Larviva Multigain (Biomar AS, Norway) twice in 24 hours as recommended by producer. Enrichment was done twice within 24 hours. 60µm planktonic mesh was used to harvest artemia and was fed to the larvae through feeding reservoirs. Amount fed was based on tank as per Appendix 2 and protocol are attached. (Appendix 1)

2.3.2 Copepods (Cop/FD):

$1m^3$ plastic container was used to store Acartia tonsa (A. tonsa) in a temperature control chamber at 10 degrees Celsius. *A.Tonsa* was fed with microalgae (*Rhodomonas baltica).* Both microalgae and copepod were heavily aerated and delivered by C-feed AS, Norway. *Acartia tonsa* was harvested by using a sieve of 53µm. The A. tonsa fed to the fish ranged in stage from N3 to N6, and it measured between 185 and 394 m (C FEED AS, 2014). (table 1 (Appendix 5)

2.3.3 Cirripedia: (Cir)

Cryoplankton stored in liquid nitrogen at -196 °C were received from Planktonic AS Norway twice Cryoplankton are the frozen pellets of cirripedia protected by cryoprotectant Agent (CPA). The frozen pellets were thawed in sea water to remove CPA via rinsing and then transferred to 100 µm planktonic net, to rinse Cirripedia. They were then transferred into 55L tank until use. About 6 hours were mandatory for rejuvenation and then transferred to feeding reservoir. Cirripedia supplied to the larvae were 350 µm long and 150 µm wide (table 1). Protocol is attached (Appendix 3)

2.3.4 Dry/formulated feed: (FD)

Two sizes of dry feed (Gemma Microns) were used depending on larval size, GM150µm and 300µm. Feed was supplied by Skretting AS, Norway. Feed automats (Sterner 905, Fish Tech AS, Norway; Figure 2.2B) were used to drop the prepared feed straight into the rearing tanks four to

twenty-four times per day (Sterner Fish Tech13 AS, Norway) which were placed on the top of tank. Same diet was used for all the tanks from day 21dph till 35dph (table 1)

2.4 Larval sampling

All the larvae were enesthesized in tricaine methane sulfonate (MS-222 Finquel®, Agent Chemical Laboratories Inc., USA) immediately after sampling. Larval sampling for standard length(SL) and dry weight(DW) were first subjected to distilled water to remove salt particles. Immediately after rinsing SL and dry weight was measured. Sampling days are according to the days mentioned in table. Initially on 2dph(days post hatch) and 9dph 5 larvae per tank were pooled and on later sampling days 15 larvae per tank were collected for DW and SL.

Samples collected for Lipids and fatty acid after enesthesizing were deionized by distilled water, dried, moved to cryotubes, flushed with Nitrogen, and then stored at -80 °C until analyzed. While feed samples were taken in three replicates on four consecutive feeding days.

On last day(35dph) of sampling at least 250 larvae per tank were sampled to check for possible sampling bias. Total number of larvae in each tank were also calculated to compare growth, survival as well as mortality among different feeding regimes (table 4).

Table 4 Number of sampled larvae for growth, total lipid, and Fatty acid(FA) analysis. On 2 dph the number represents the total sample size as the treatments had not been distinguished yet. From 9 to 25dph the sample size is from each tank for growth and from each treatment for lipids and FA analysis

Dph(days post hatch)	0	2	9	15	21	29	34	35
Growth samples	0	15	30	30	30	45	45	250
Lipids and FA samples	15	45	45	45	45	45	45	0

2.5 Survival and growth of lumpfish larvae

2.5.1 Growth (Standard length and dry weight)

Growth was measured in terms of standard length, dry weight, and daily weight increase. Standard length SL of fish larvae is measured from the apex of snout till the end of notochord. For measuring SL *software ImageJ* was used. It was measured from 2dph till 35dph. The images were captured with a 0.63x magnification and 10x objective stereo microscope (Leica MZ75, Leica Microsystems, Germany; Zeiss Axiocam ERc 5c, Zeiss Inc., Germany). External morphology was also assessed using these images.

For dry weight (DW) larvae had been dried for two days (48 hours) at 60°C. Dry weight was measured in pre-weighed tin capsules using an ultra-microbalance weight (UMX2

Ultramicrobalance, Mettler-Toledo, USA). The following equation(Houde et al., 1981) was used to compute specific growth rates (SGR) for particular sampling intervals:

$$SGR = \frac{lnW_2 - lnW_1}{t_2 - t_1}$$

W2 and W1 are the average larval dry weight for each tank, while T2 and T1 are the time (dph). The percentage daily weight increase (DWI) was determined based on the calculated SGR-values and equation used by

$$DWI = (e^{SGR} - 1) * 100\%$$

2.5.2 Survival

Larvae were registered on every sampling day as mentioned in table 3 throughout the experiment. Mortality was also noted by counting dead larvae each day during cleaning of tanks. On last day 250 larvae were pooled , together . All of these were used to estimate Survival (S_t), which was calculated based on total alive and dead larvae in each tank by using following equation.

$$S_t = \frac{N_t}{N_0} * 100\%$$

Where,

S_t = Survival

N_t = Total number of larvae at given time (dph)

N_0= Number of larvae at the time 0 i.e., beginning of experiment

2.6 Lipids and fatty acid analysis

2.6.1 Lipid analysis

Lipid extraction was done at SINTEF by using Methyl-tert-Butyl Ether (MTBE) extraction method introduced in 2008 by (Matyash et al., 2008). MTBE/methanol/water solvent system was done in volumetric ratios of 10:3:2.5 (v/v/v). *m1* was the weight of kimax tube in which sample was transferred for MTBE extraction. After weighing larval sample(m_{sample}) it was initially diluted with a mixture of MTBE and methanol (10:3; v/v) which brought small metabolites into solution, whereas other macromolecules were precipitated for instance proteins (Liquid Extraction: Folch). To induce phase separation, water was then added to the crude extract. Top phase was carefully removed and

evaporated with Nitrogen to avoid any of the other metabolite. Removed phase of sample was reweighed (m2) and lipid content was measured by using following equation.

$$\text{Lipid content} = (100\% \times \frac{m2-m1}{m_{sample}})$$

Left over sample was re-dissolved in 500µl of $CHCl_3$ for lipid class and lipidomics and again stored at -80°C, protocol attached as Appendix 6

2.6.2 Fatty acids analysis

Total fatty acid and lipid profile was evaluated by Folch method (Folch et al., 1957)appendix . There were two types of samples larval samples as well as feed samples. Samples from freezer stored at -80degree Celsius were taken and transferred to pre weighed kimax tubes (W_1). Sample was dissolved in iso-octane, dried with Nitrogen evaporator, and placed in desiccator for 1 hour. Tube was again weighed(W_2) to get dry weight of lipid ($W=W_2-W_1$). Stock solution (10mg/ml) was added to samples and divided into 2 parallels for reducing error. After adding Internal Standard and CH_3OH-H_2SO_4(mixure of methanol and diluted sulphuric acid) samples were placed on heating block for 18-20 hours.

Samples were removed from heating block cooled down and then subjected to three times centrifugation along with addition of sodium chloride (NaCl) and iso-octane. Again, sample was subjected to Nitrogen evaporator and mixed in 200µl of isooctane. Small viles specialized for Gas chromatography were filled with 40µl of prepared samples and run-on GC for fatty acid analysis. A 7890B GC system equipped with a flame ionization detector (FID) and a mass spectrometry detector (5977B MSD), all from Agilent Technologies, was used for fatty acid analysis. Detailed protocol is attached Appendix 7

2.7 Statistical analysis

IBM SPSS Statistics 27 and SigmaPlot v.14 (Systat Software Inc., USA), graphing application with built-in facilities for data analysis, were used for all statistical studies. All statistical tests had a significance threshold of 0.05. Prior to statistical analysis, percentage data underwent arcsine transformation. In addition to Microsoft Excel v.2109, SigmaPlot and was mostly used to create the graphs (Microsoft, USA). All tables were created using Microsoft Word v.2109 (Microsoft, USA).

Difference between total lipids and fatty acids between treatments were determined through one way ANOVA test. Levene test was applied for checking homogeneity of variance. For non-normal distribution Pairwise comparison Mann Whitney test was applied.Pearson Correlation among different FAs was done to check dependency of variables on each other. Results are presented as

mean ± standard deviation (SD) and the statistical significance was established at $p < 0.05$ and 95% confidence interval was taken. All statistical analyses were performed using IBM® SPSS Statistics 27.0 software package for Windows.

In order to rule out sampling bias, a larger sample of SL was taken at 35 dph (n = 750 larvae per treatment group). The SGR between 29-34 dph was used to estimate how long the larvae sampled at 34 dph (n=45 larvae per treatment group) would have been at 35 dph. Welch's t-test was used to determine whether the sample of lengths measured within treatment groups differed significantly from the predicted length at 35 dph.

Chapter 3: Results

3.1 Larval general features and developmental morphology

From the very beginning of the feeding experiment, it was observed that the external features of lumpfish were changing considerably overtime. Eventually all the larvae from 5 treatments had similar characteristics like skin color, fin development, body shape etc. therefore following general description is based on Artemia-larvae. Larvae of lumpfish is found in various colours ranging from shades of green, pale yellow, and brown, however, Art-larvae was mostly found in dark red colour as shown in **Fig 3.1**.

(2 days post hatch): The head of larvae was round and larger than the body having compressed long tail comprising 2/3rd of the body length and giving larvae a tadpole-like shape. There were separate bands of light pigments on the sides of each eye ranges from mouth till operculum, however, the head of larvae was dominated by the pigment of yellow colour with brown and black dots that goes up to its trunk. Despite the pigmentation, the visibility of the yolk sac across the skin was clear. The tail was covered with light yellow pigment with visible notochord; however, the median fins folds were observed to be transparent. At this stage, fin rays were seen on the finfold on the dorsal side within the pectoral fins. In the centre of the trunk area, the ventrally located functional disc was found.

(9 days post hatch): The pigmented bands around the eyes were more reflective making the eyes more visible with respect to the brown and dull yellow colour of the rest of the body. However, the pigmentation in the tail of larvae was increased and it became less compressed laterally with visible notochord. The finfold had begun to resorb, forming two dorsal fins and one ventral fin. In all of these, fin rays had formed. Epidermal tissue has somewhat overrun the anterior dorsal fin. The fin rays in the suction disk were more noticeable since it had darkened and pigmented.

(21 days post hatch): It was observed at that the larval body was covered with brown and opaque red pigmentation. With a thick grown tail and oval head and trunk, the larvae body looked more seamless and streamlined. Also, with the complete reabsorption of the finfold, the fin rays become more visible in all fins. The first dorsal fin overgrew with epidermal tissues and the caudal fin was in a round shape.

(34 days post hatch): At 34dph the body shape of larvae looked more streamlined than instead of the tadpole. The overall colour of the body became red tinted with brown speckles and the pigmentation continued through the suction disk also. However, the reflective band with

pigmentation around the eyes remained noticeable. It was also observed that the caudal fin, dorsal fin, and posterior ventral fin were light yellows pigmented.

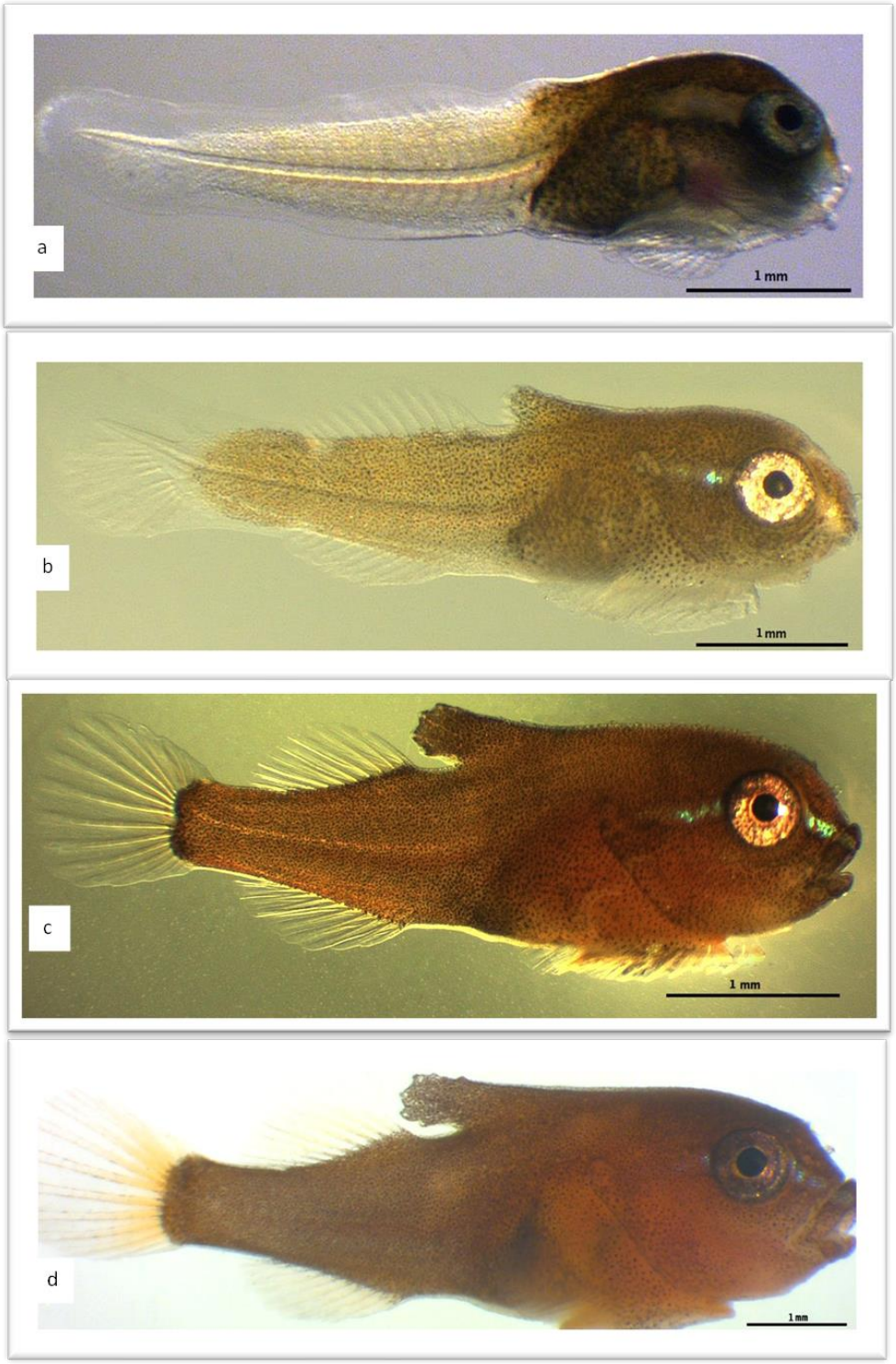


Fig3.1 a)2dph, b)9dph ,c)21dph , and d)33dph. 1mm Scale bar. 2dph unfed . 9,21 and 33 are fed with Artemia showing best growth in comparison to other feeding regimes picture credits (Kværnø, 2022)

3.2. Observations for growth and survival

3.2.1 Dry weight

The mean dry weight of the larvae at 2 dph was 0.910.05 mg (Figure 3.2). The Art- larvae were substantially larger than the Cir-larvae by 9 dph, but not the other larval groups. At 15 and 21 dph, the Art-larvae grew steadily and had a much greater DW as compared to other larval groups. between 15 to 21Dph Cir-, Cop/Cir-, and FD-larvae also grew but not as much as the Art-larvae. The Cop/FD-DW, larvae on the other hand, had only remained steady on 21 dph, much lower than the other larval groups. During 29 and 34 dph, the Cir-DW larvae's and Art-larvae grew to similar extent and were no longer statistically different. At 29 and 34 dph, Art- and Cir- larvae were substantially heavier than larvae from the other three larval groups. The three other larval groups, Cir-, Cop/Cir-, and FD-larvae, all experienced an increase in DW between 30 and 34 dph, although not to the same extent as the Art- and Cir-larvae. The dry weights of Cir-, Cop/Cir-, and FD-larvae were not substantially different on any of the final two sampling days. Appendix 8, Table A3 shows the mean DW per tank for each treatment group.

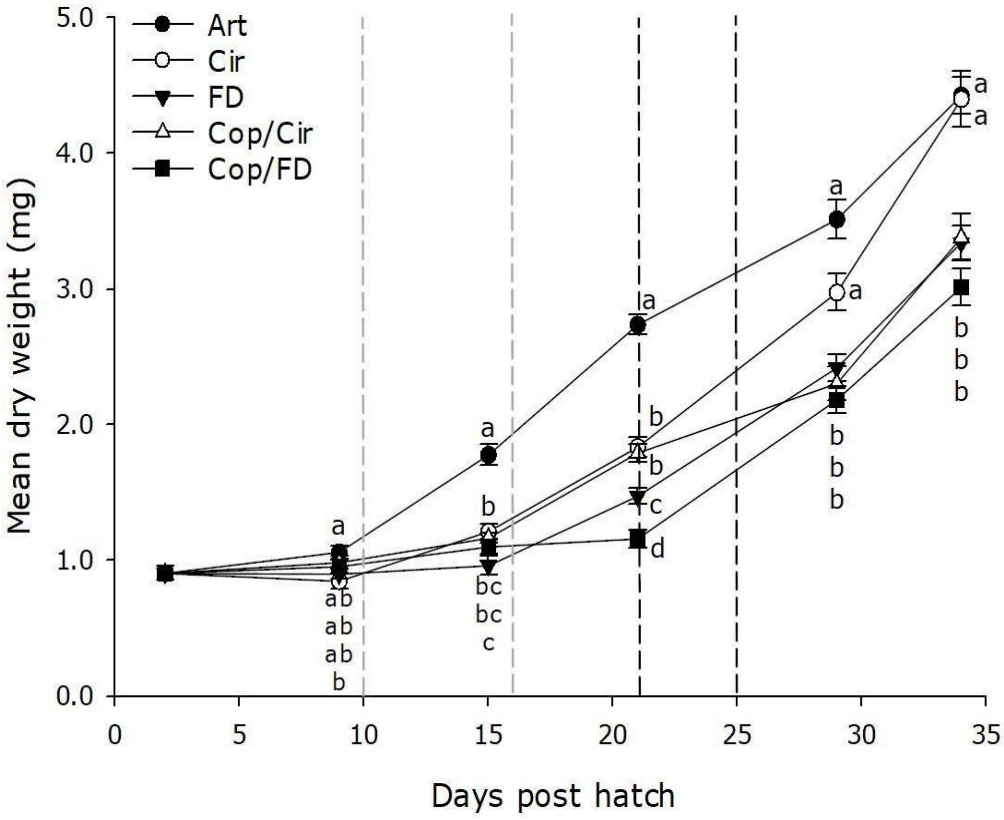


Fig 3.2: Mean dry weight of lumpsucker larvae fed with 5 different feeding regimes Dry weight is given in mg n=15 larvae for 2dph, 9dph and 15dph and n= 30, 45 and 75 larvae pooled on 21, 34 and 35 dph respectively. Grey line shows change of feed from copepod to formulated diet for Cop/FD while from copepod to Cirripedia for Cop/Cir .Dark line shows weaning to formulated diet for all the treatments.

Significant differences ($p<0.05$) letters indicate significance of treatments. Bar indicates ± standard error (SE).

3.2.2 Standard length

Larvae were 5.94±0.10 mm length at 2 dph (Figure 3.3). Art-larvae were already much longer than Cir-, Cop/FD-, and FD-larvae by 15 dph. At 21 dph, the Art-larvae were also substantially longer than any other larvae. However, weaning to a formulated diet between 21-25 dph for the Art-, Cir-, and Cop/Cir- larvae, the Cir-length larvae enhanced to the same range as the Art-at larvae's 29 and 34 dph. Cop/Cir-larvae SL, on the other hand, did not increase promptly in length after weaning. At 29 and 34 dph after weaning, Art- and Cir-larvae were substantially longer than all other larval groups and statistically significant. On 35 dph there were 750 samples per treatment group (250/tank), and results were different than 34dph. At 35 dph, Art-larvae were significantly longer than all other larvae, including Cir-larvae . Furthermore, at 35 dph, FD- and Cop/Cir- larvae were substantially longer than Cop/FD-larvae but not statistically different between the two groups. However, no significant difference was identified among treatments between the projected length at 35 dph (based on 34 dph larvae and SGR) and the substantial sample of SL at the end of the trial. Appendix 10, Table A5 shows the mean SL per tank for each treatment group.

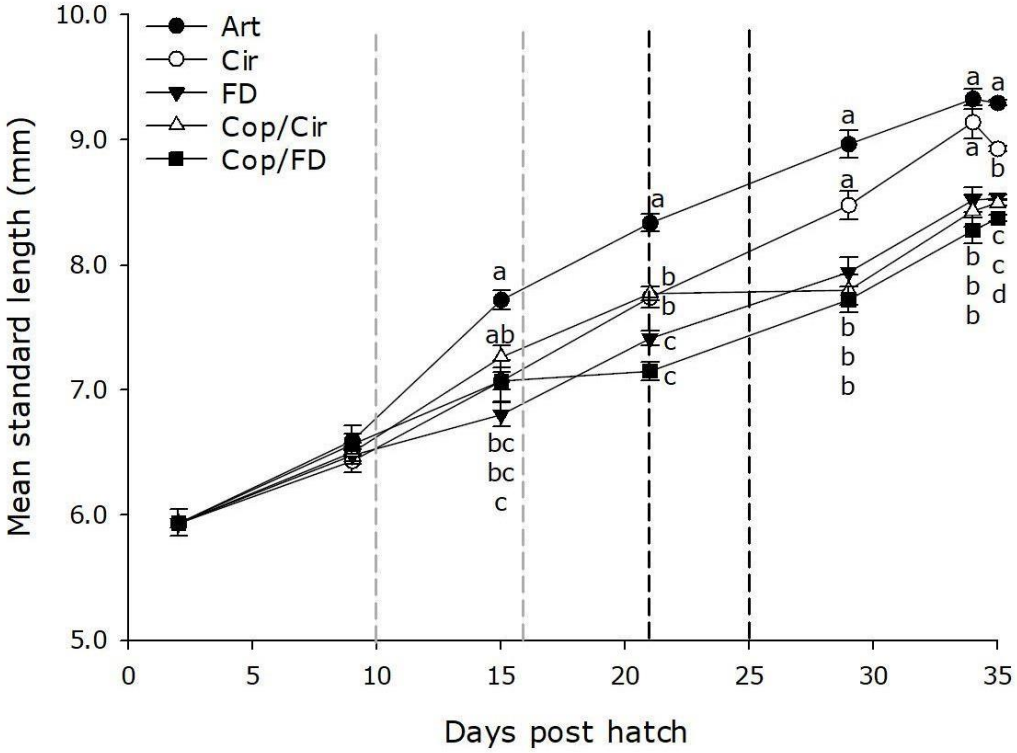


Fig 3.3 Mean standard length(2 to 35dph):length is given in mm. Five treatments. 15 larvae per treatment were pooled on 2dph, 9dph and 15dph. 30, 45 and 75 larvae were pooled on 21, 34 and 35 dph respectively. Grey line shows change of feed from copepod to formulated diet for Cop/FD while from copepod to Cirripedia for Cop/Cir .Dark line shows weaning to formulated diet for all the treatments. Significant differences ($p<0.05$) letters indicate significance of treatments Error bars indicate ± standard error (SE).

3.2.3 Correlation between standard length(mm) and mean dry weight (mg)

The pooled data of five treatment groups showed strong positive polynomial correlation between the DW and SL (Figure 3.4), showing that the DW of the larvae rose exponentially as SL increased. The growth rate of larvae was slow initially that is the DW was 1mg approx. when larvae had SL of 5 - 7mm. A visible increase was observed both in DW and SL after larvae achieved the SL of 7mm Regardless of treatment, the pooled data's Pearson-correlation value was r=0.970, demonstrating a significant association between DW and SL for all larvae.

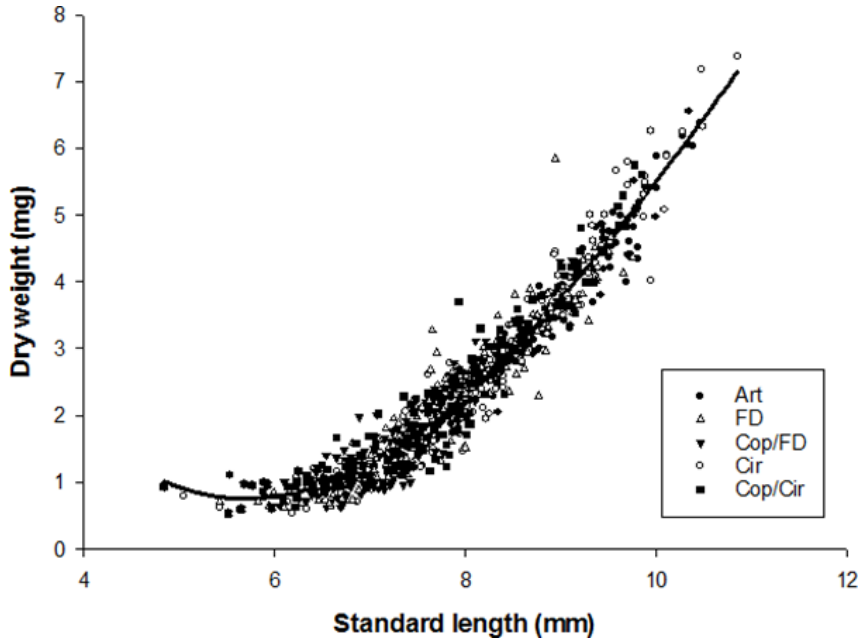


Fig 3.4 Correlation among standard length and dry weight for lumpsucker larvae 2-34dph. Artemia n=166, Cirripedia n=165 for copepod, cop/cir and FD-group. (r2=0.970, P<0.0001). Each point indicates DW in correspondence to SL per larvae made from pooled data of all treatments.

3.2.4 Survival of lumpsucker larvae

Initially from 2dph to 10dph Cop/Fd larvae and Cir larvae had little less survival rate but not statistically different from other three treatment groups (Figure 3.5). Cir larvae stabilized after 10dph with decrease of about 1% while copepod larvae dropped until 35dph after weaning to formulated diet. Art- Larvae were having highest survival rate of 95±0 % throughout, only significantly different from Cop/FD not other treatment groups. At 35dph the Cop/FD larvae showed 10% less survival

than the best one which was Artemia larvae. Number of larvae per tank is given in Appendix 12 (Table A7)

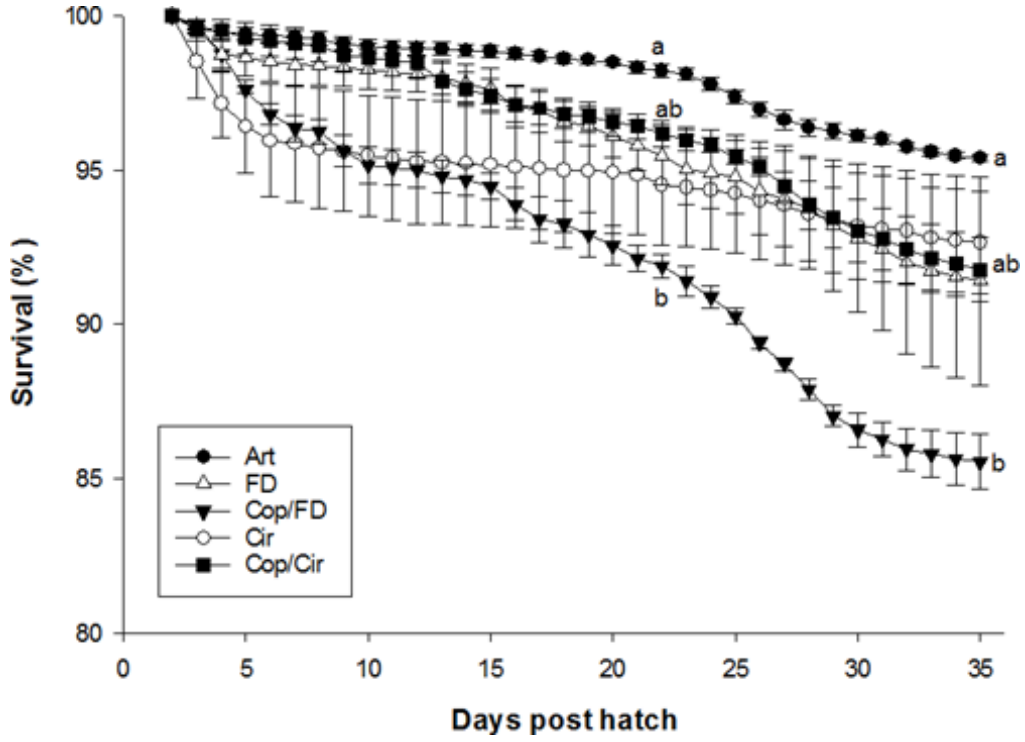


Fig 3.5 Mean survival rate of 5 different feeding regimes with three replicates each (n=3) with corresponding error bars ± SE. letters indicate significance at ($p<0.05$) tested statically at 2, 9,15,21 ,29, 34 and 35 dph. Note vertical axis begin from 80%.

3.2.5 Daily weight increase (DWI)

The DWI in the Cirripedia-fed larvae remain negative from 2dph to 9 dph but a significant increase was seen after 9dph till the end of the experiment. From 9dph to 21dph the increase in daily weight (DWI) of larvae with Cirripedia feeding regime is like the trend seen from dph 21 – 34. The larvae fed on Cop/FD showed reduced DWI between 2dph to 9dph and from 9 to 21dph which is significantly less than that calculated for Artemia-fed larvae and Cirripedia-fed larvae in the duration of 9 to 21dph. For the DWI between the duration of 21dph to 34dph, larvae fed with Cop/FD showed enhanced values and were evidently higher than that of larvae fed with Artemia with a value of $p=0.007$. The DWI measured for Cop/Cir fed larvae was the same between duration of 9dph to 21dph and from 21 to 34dph. However, FD larvae, the significant increase was observed in DWI from duration of 21dph to 34dph. From the experiment, it was evident that Artemia-fed larvae and Cirripedia-fed larvae showed higher DWI that is 5% in comparison to the larvae following other feeding regimes where DWI was calculated as 4% with $p<0.05$.

The larvae from groups with feeding regimes of Cop/FD, Art and Cop/Cir showed significant increase within 2-9dph interval, while FD and Cirripedia exhibited a negative tendency (Figure 3.6 Appendix 9, Table A4) However, no other significant differences were found when larvae from different feeding regimes were compared during this time interval. During 9-21dph interval larvae fed with Artemia remained significantly highest in DWI. On weaning to formulated diet Art larvae and Cir larvae showed decrease in DWI while FD and Cop/FD were significantly lower than Art larvae during 21-34 dph interval ($p<0.05$).

DWI in interval 2-34 indicates is the mean from beginning till the end of experiment which were significantly similar higher in Cir and Art larvae. Cop/FD, FD and Cop/Cir larvae had similar DWI during this interval.

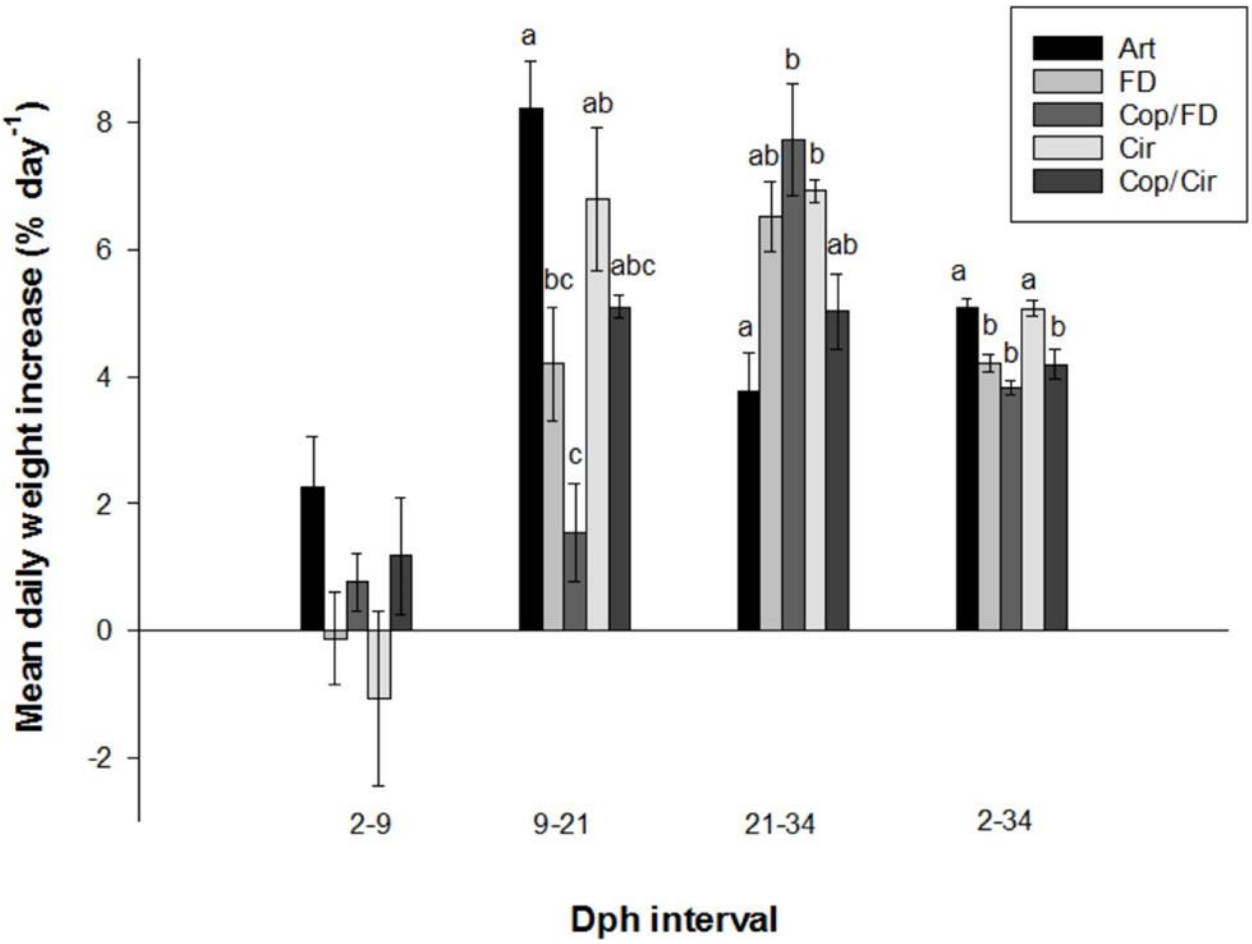


Fig 3.6 Mean daily weight increase (%/day-1) in C.lumpus from 2-34 dph. X-axis shows days post hatch intervals and y axis have mean daily weight increase $\%day^{-1}$. Bars show standard error(±SE). Letters indicates significance of DWI in particular interval larvae group.

3.3 Lipids and fatty acids in lumpsucker larvae and feed

3.3.1 Total lipid and fatty acid content of prey

Total lipid content had significant differences among feed types (Fig.3.7, Appendix 13). Artemia has shown the highest amount of lipid(% of dry weight) and formulated diet stands second both were statistically significant (P>0.05). Lipid content of both feed pellets Gemma micro 150nm and 300nm were similar so placed as one. Cirripedia and copepod had similar % of lipid and there was no significant difference between them.

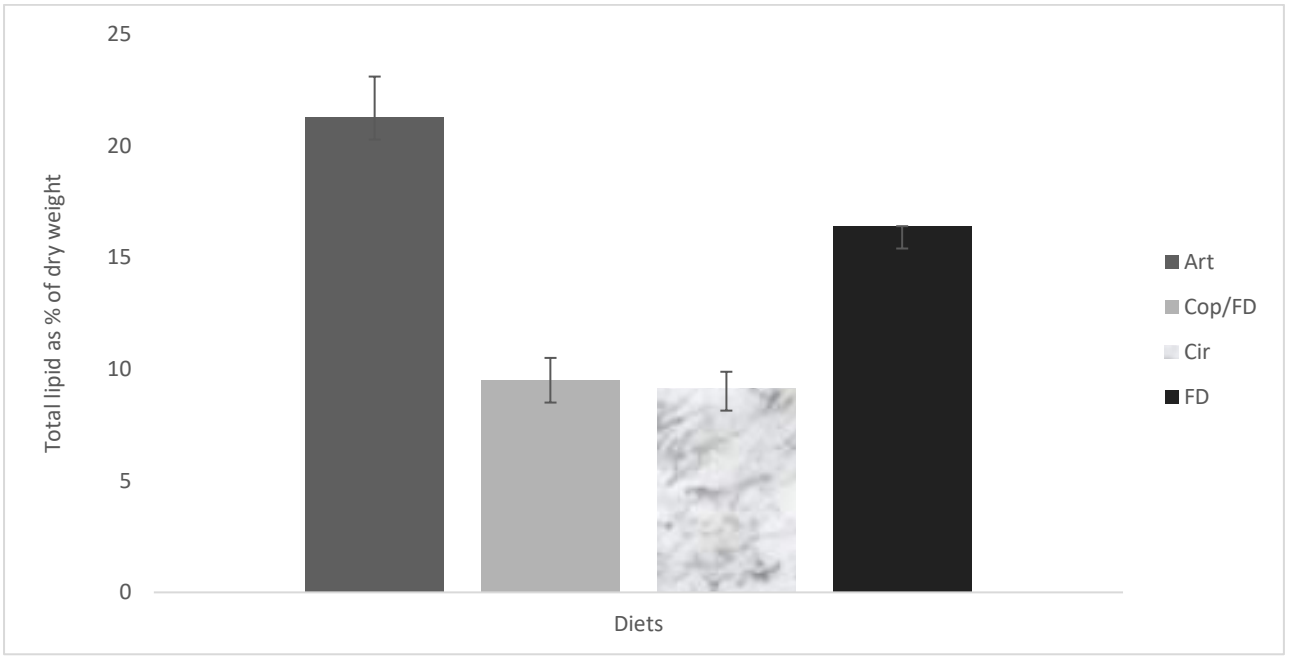


Fig3.7 Total lipid content of feed significance P>0.05. Lipid content is % of dry weight

The enriched diet (Artemia) had higher lipid levels than the other 2 live prey (cirripeds and copepods) used in 4 feeding regimes. Artemia was quite higher in lipids than the other groups, and no significant differences were observed between cirripeds and copepods (ANOVA p 0.05, followed by Holm Sidak post hoc test).

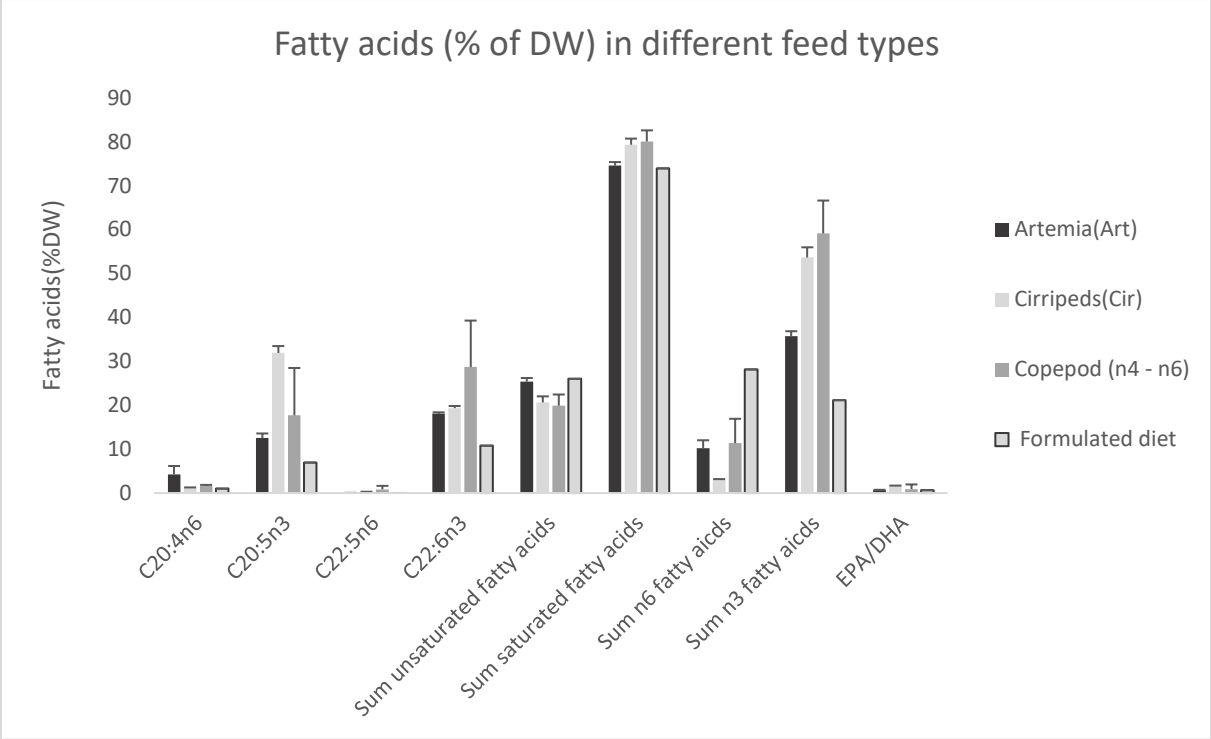


Fig 3.8 Selected fatty acids of four types of feeds (Copepods, Artemia, formulated diet and Cirripedia). Bars shows ± standard deviation.

Fig 3.8 shows some selected fatty acids percentage in four types of feeds from Appendix. Arachidonic Acid (ARA) was highest in Artemia and significantly similar in copepods and cirripedes, EPA and DHA were significantly high in Artemia. Sum of saturated fatty acids in artemia and formulated diet. In general, Eicosapentaenoic acid (EPA) were higher in cirripedes than artemia but statistically no significant difference between both. Docosahexaenoic acid (DHA) was significantly high in Copepods. Saturated fatty acids were highest in copepods but had similar significance as in copepods. Artemia and FD had no significant difference in saturated fatty acids. n-3 fatty acids were significantly highest in copepods than artemia and cirripedes and FD. n-6 fatty acids were significantly high in FD and similar in copepods and artemia with no significance difference between them.

3.3.2 Total lipid content in *C. lumpus* (lumpsucker) larvae (2-34dph)

Total lipid was highest in Cop/Cir larvae from beginning till end. Art/Fd and Cir had similar trend of total lipids initially the content was high but then dropped on weaning to formulated diet on 21dph and gets stabilized on 34 dph in both larvae. Cop/Fd lipid content dropped from 2dph to 21 dph and then increased on weaning to FD on 21dph. Total lipid content in FD larvae remain steady throughout the trial with a little change.(Figure 3.9, Appendix 14)

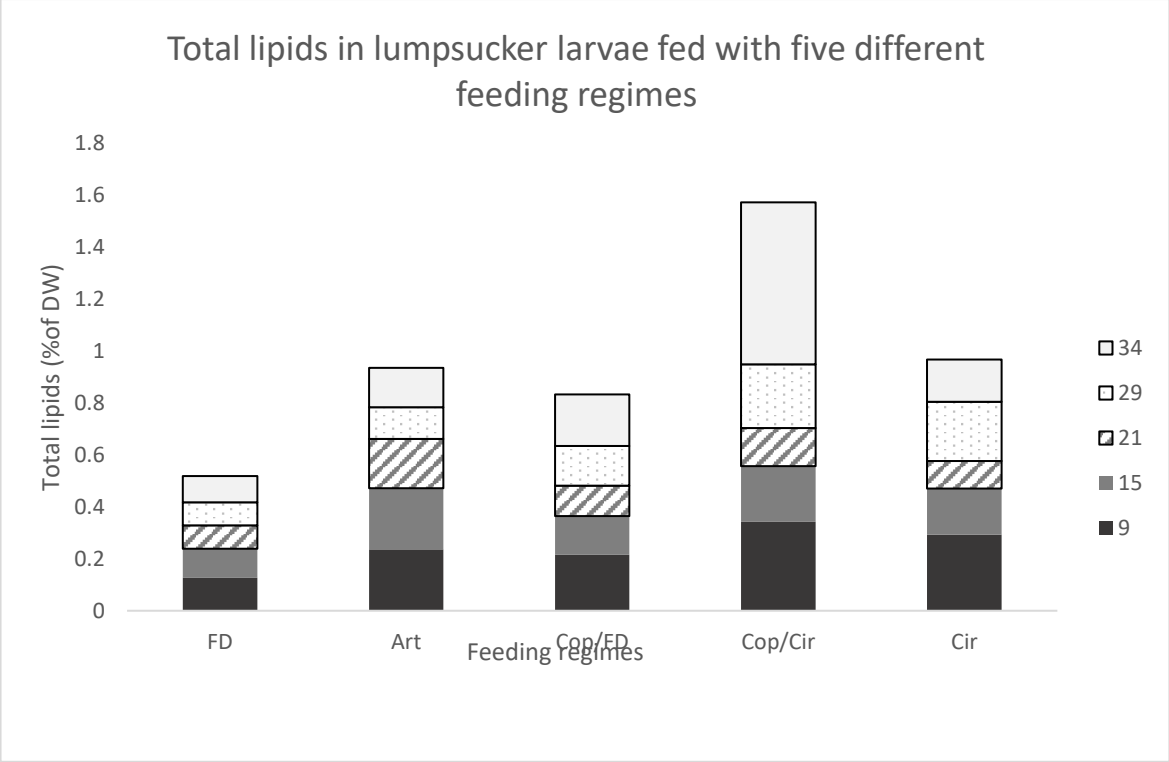


Figure 3.9 Development of total lipids (mg/g)over time in larvae fed with five different feeding regimes. (Values are mean n=5). Bars indicate development of lipid in each group of larvae from 2dph to 34 dph. Every tile indicates dph.

3.3.3 Development of fatty acids in Cir larvae

Certain fatty acids of Cirripedia larvae over time are plotted in Figure 3.10 (Appendix 15). ∑PUFA(n-6) dropped from 2dph to 9dph and remained similar till 21dph with no significance difference. From 21dph to 29dph ∑PUFA(n-6) increased. ∑PUFA(n-3) increased from 2 to 9dph and then dropped on 15dph. It again rises on 21dph significantly and then dropped. ∑PUFA(n-3) and ∑MUFA had similar trend with different content and are significantly correlated($p < 0.05$). ∑SFA had decreasing trend from 2 to 21dph and an increasing trend till 34dph. ∑SFA is highly correlated with ∑MUFA ($p < 0.05$), ∑PUFA(n-3) ($p<0.05$), and ∑FA($p<0.01$).

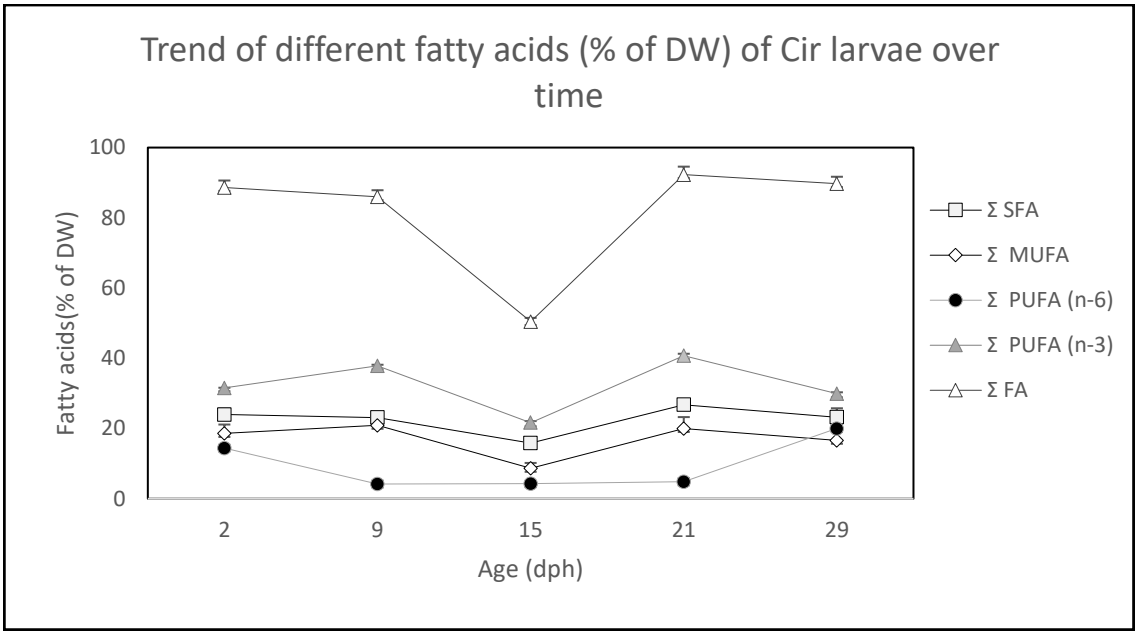


Fig 3.10 Trend of selected fatty acids(% of DW) over time in Cirripedia larvae from 2dph to 34dph. Bars indicate ±SE. Significance is taken at $P<0.05$

Fig 3.11 shows percentage of DHA, EPA and ARA in lumpsucker larvae fed with Cirripedia (Cir) on selected days and their respective ratios. DHA content increased from 2dph to 9dph. DHA had its least percentage on 15dph. DHA was strongly correlated with ∑MUFA and ∑PUFA(n-3). Its percentage again increased until end of the trial. Percentage of EPA was less than DHA but followed similar trend. It was also significantly correlated with ∑PUFA(n-3). ARA remained very low throughout the experiment and was negatively correlated with ∑MUFA and ∑PUFA(n-6). DHA/EPA was non-significant while EPA/ARA was significant and positively correlated to ∑MUFA and PUFA(n-3) (Appendix 15).

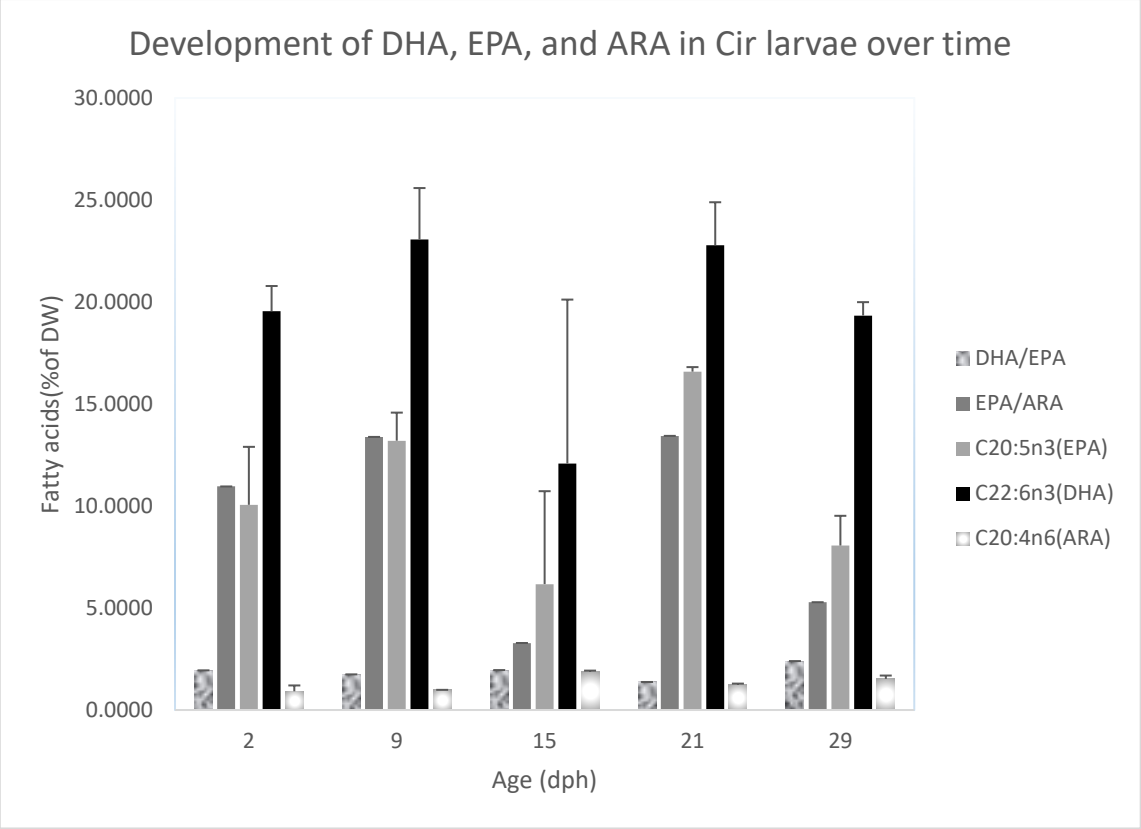


Fig 3.11 Development of DHA, EPA, and ARA in selected days post hatch of Cirripedia fed lumpsucker larvae. Bars indicate ±SE. Significance P<0.05.

3.3.4 Development of fatty acids in FD larvae

∑MUFA from 2dph till 34dph remained almost same with a slight decrease from 9dph to 15dph (Figure 3.12 Appendix 16). It was significantly positively correlated with ∑PUFA(n-3) and EPA/ARA. ∑SFA increased from 2dph to 9dph and then remained same. It was non-significant. ∑PUFA(n-6) dropped from 2dph to 9dph and then increased until 21dph and remained same till the end of trial. ∑PUFA(n-6) was positively correlated to ARA and DHA/EPA and negatively correlated to EPA/ARA. ∑PUFA(n-3) increased until 9dph then dropped till 21dph and remained same until 34dph. ∑PUFA(n-3) is positively correlated with ∑MUFA and negatively correlated to EPA/DHA but positively correlated to EPA/ARA.

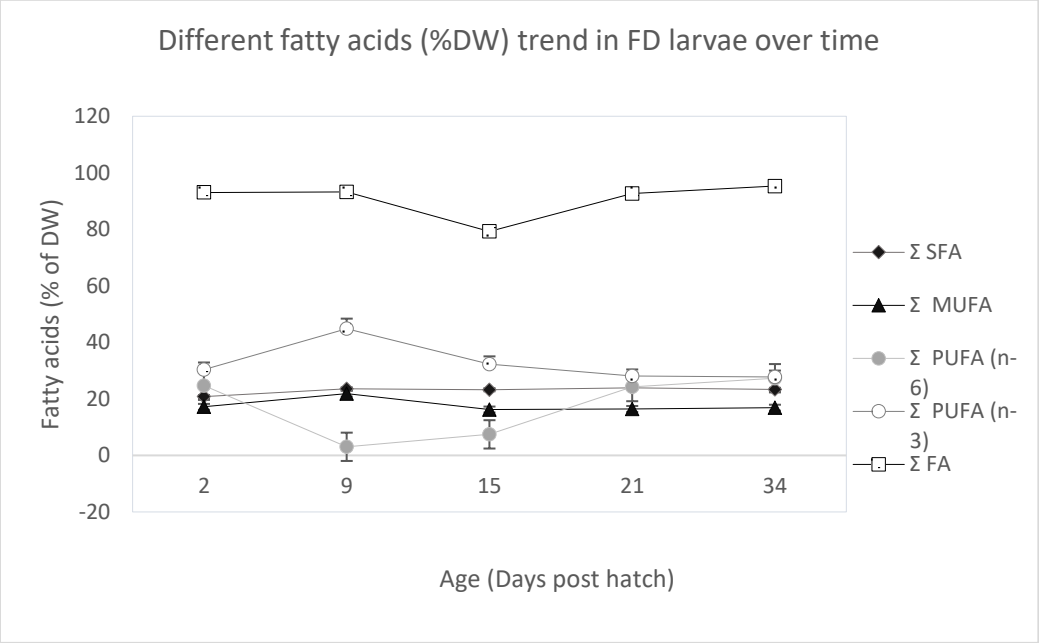


Fig 3.12 Trend of selected fatty acids in lumpsucker larvae fed with FD. Bars indicate ±SE. N=5 Significance P<0.05.

DHA increased from 2 to 9dph and decreased until 21dph and remained same until 34dph (Fig 3.13 Appendix 16). DHA is positively correlated with ∑MUFA, ∑PUFA(n-3) and EPA/ARA while negatively correlated to ∑PUFA(n-6), ARA and DHA/EPA. EPA is increased from 2 to 9dph and then decreased till 34dph. EPA is positively correlated to ∑MUFA, ∑PUFA(n-3) and EPA/ARA and negatively correlated to DHA/EPA. ARA percentage remained low, decreased from 2 to 9dph and increased until end of the trial. EPA is negatively correlated to ∑PUFA(n-3) and EPA/ARA and positively correlated to DHA/EPA ($p<0.05$).

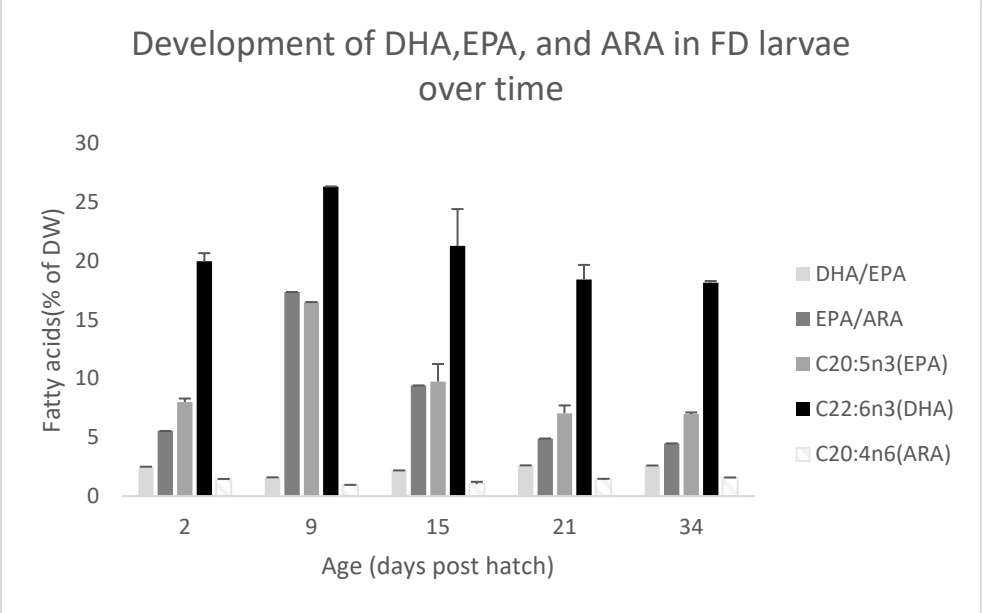


Fig 3.13 Development of EPA, ARA, and DHA from 2-34 dph in lumpsucker larvae fed with formulated diet. (N=5) $p<0.05$ Bars indicates ±SE

3.3.5 Development of fatty acids in Art larvae

On 2dph ∑SFA, ∑MUFA, and ∑PUFA(n-3) were almost same. ∑MUFA was almost same throughout the trial and non-significant. (Fig 3.14 Appendix 16)∑SFA increased slightly from 2 to 9dph and then remained same until 34dph. It was only significantly related to EPA/ARA. ∑PUFA(n-3) increased from 2 to 9dph and dropped until 21dph while remained same until 34dph.

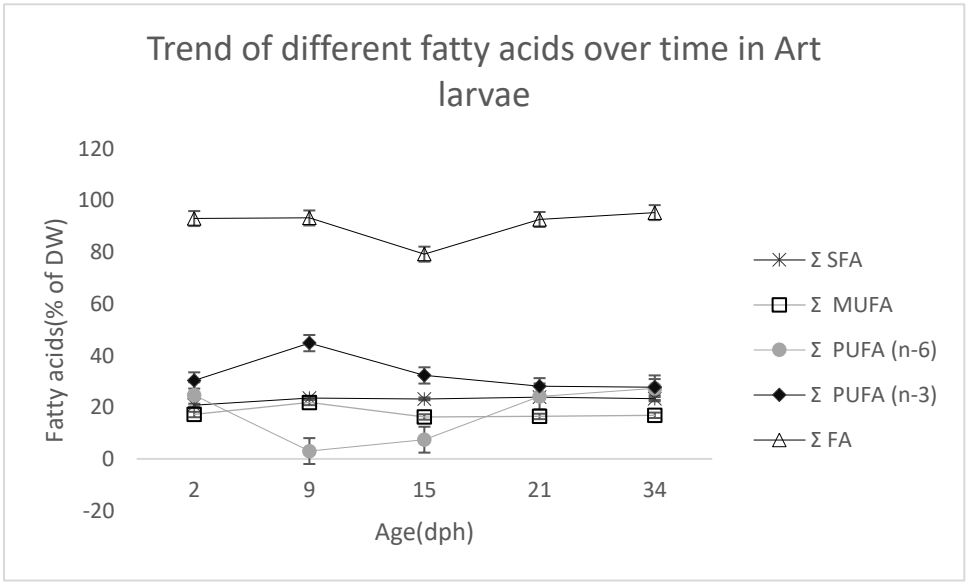


Fig 3.14 trend of selected fatty acids over time in larvae of lumpsucker fed with Artemia from 2 to 34 dph. Bars indicate ±SE (N=5 and $p<0.05$)

∑PUFA(n-3) is only positively and significantly ($p<0.05$) correlated to DHA.∑PUFA(n-6) decreased from 2dph to 9dph and remained same until 34 dph with a slight change over time. It is negatively and significantly correlated with (n-3)/(n-6) and EPA.

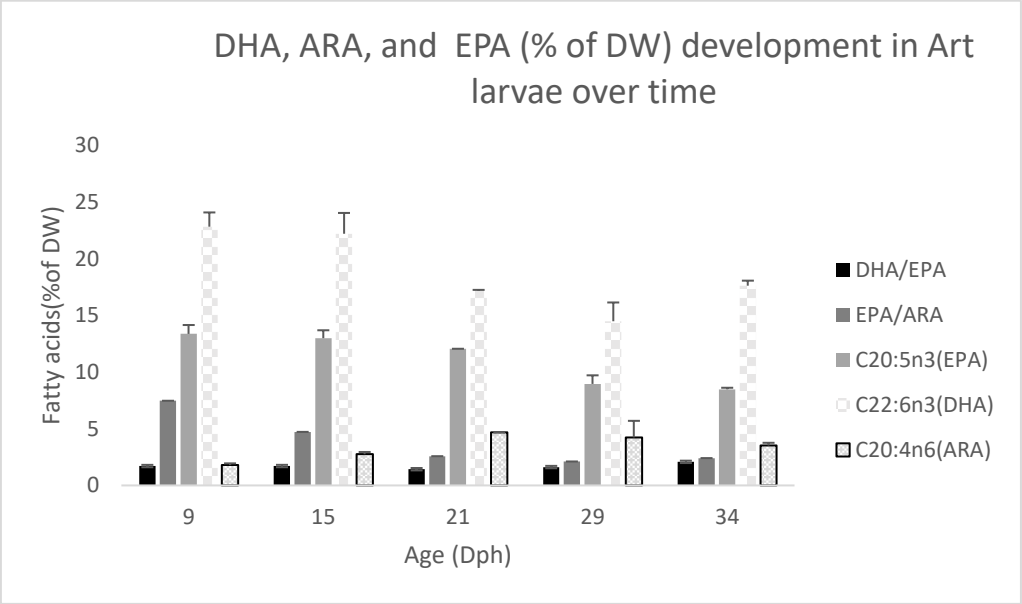


Fig 3.15 Development of EPA, DHA, and ARA over time in lumpsucker larvae fed with Artemia. (n=5, $p<0.05$) Bars indicates ±SE

EPA was highest in the beginning and decreased slightly from 9dph till 34 dph. It is negatively correlated to PUFA(n-6) and positively related to PUFA (n-3). Percentage of DHA was highest in the beginning on 2dph and 9dph and then dropped until 21dph while on 34dph it seemed increasing again. DHA was negatively correlated with ARA but positively correlated with ∑PUFA(n-3). EPA/ARA decreased from beginning and remained same on 21 dph and 34 dph and was negatively corr3elated with ARA. DHA/EPA was almost same with slight increase on 34dph. It is significantly and positively correlated to ∑SFA. (Fig 3.15, Appendix 17)

3.3.6 Development of fatty acids in Cop/Cir larvae

∑SFA and ∑MUFA increased slightly from 2 to 9 and then remained almost constant till 34 dph. ∑MUFA was significantly correlated with DHA, ARA, and ∑PUFA(n-3) but ∑SFA was non-significant. ∑PUFA(n-3) remains quite similar throughout experiment. It was negatively correlated with DHA/EPA and ∑PUFA(n-6) while positively correlated with DHA, ∑MUFA, and EPA/ARA. ∑PUFA(n-6) showed fluctuating trend. It increased from 9 to 15dph dropped on dph and again

increased until 34dph. PUFA(n-6) is negatively correlated with ∑MUFA and DHA.($p<0.05$)(Fig 3.16)

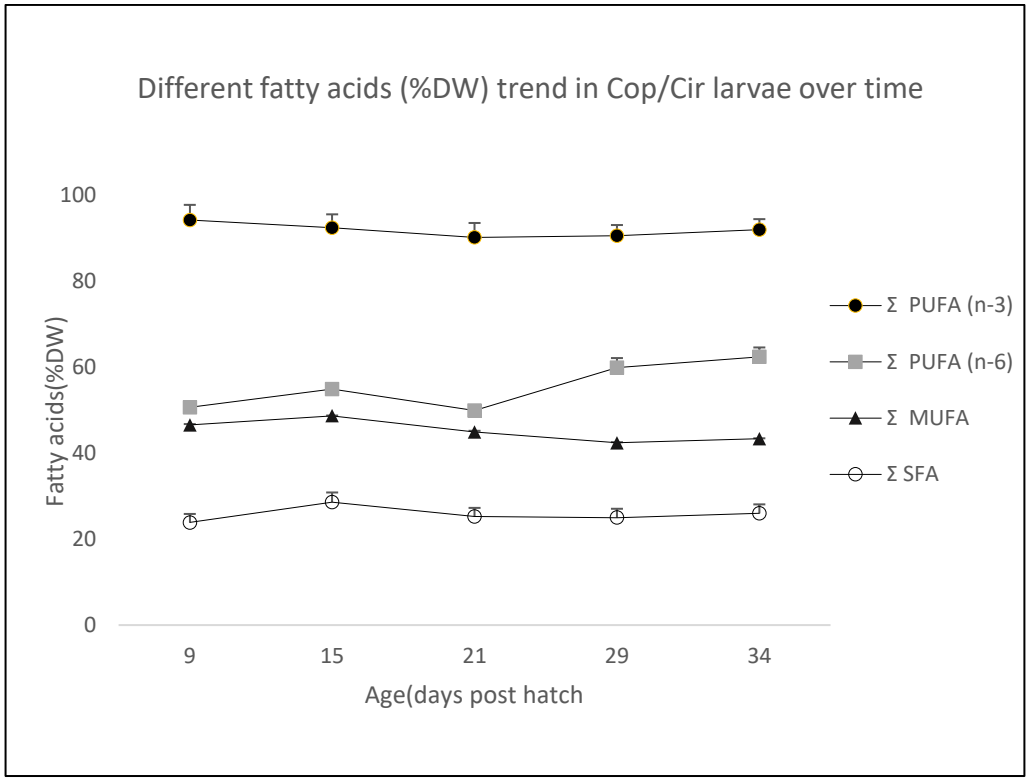


Fig 3.17 Trend of selected fatty acids (% of DW) over time in lumpsucker larvae fed with Cop/Cir. Bars indicate ±SE (n=5, $P<0.05$)

DHA content was high in beginning till 9dph dropped little on 15dph and again enhanced on 21 dph. While it remained same on 29 and 34 dph. EPA was high from 9 to 21dph but dropped on 29 and 34dph. DHA and EPA were negatively correlated with ∑PUFA(n-6) and DHA/EPA and, positively correlated with ∑PUFA(n-3) and ∑MUFA. ARA remained same throughout the experiment EPA/ARA has decreasing trend from 9dph to 34 dph while DHA/EPA remained constant. DHA/EPA were negatively correlated with ∑MUFA, ∑PUFA(n-3), EPA/ARA, and DHA. while EPA/ARA were negatively correlated with ARA and PUFA(n-6). (Appendix 18, Fig 3.18)

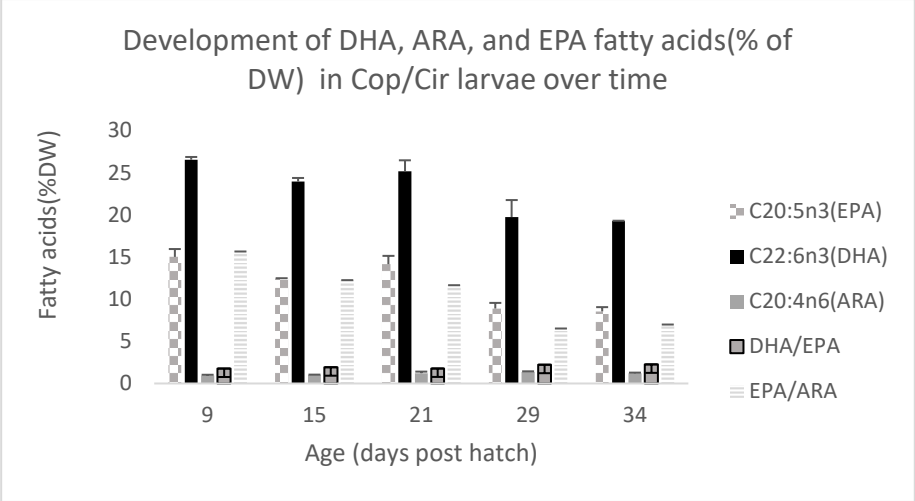


Fig 3.18 Development of ARA, EPA, and DHA in relation to time (2-34dph) in lumpsucker larvae fed with Cop/Cir. Bars indicates ±SE. (N=5. P<0.05)

3.3.7: Development of fatty acids in Cop/FD larvae

∑PUFA(n-3) increased slightly from 2dph till 15 dph and then dropped suddenly on 21dph which remained same until 34dph. ∑PUFA was significant and positively correlated with DHA and EPA while negatively correlated to ∑SFA had quite stable value throughout the trial. ∑SFA was non-significant and had no correlation. ∑PUFA (n-6) was significant and negatively correlated to DHA and ∑PUFA(n-3). Its percentage remained static from 2 to15 dph and then increased on 21dph with further slight rise until 34dph.∑MUFA had almost stable value throughout the trial with a little drop on 21and 34dph. It was positively correlated to EPA/ARA and negatively correlated to DHA/EPA. (Appendix 19, Fig 3.19)

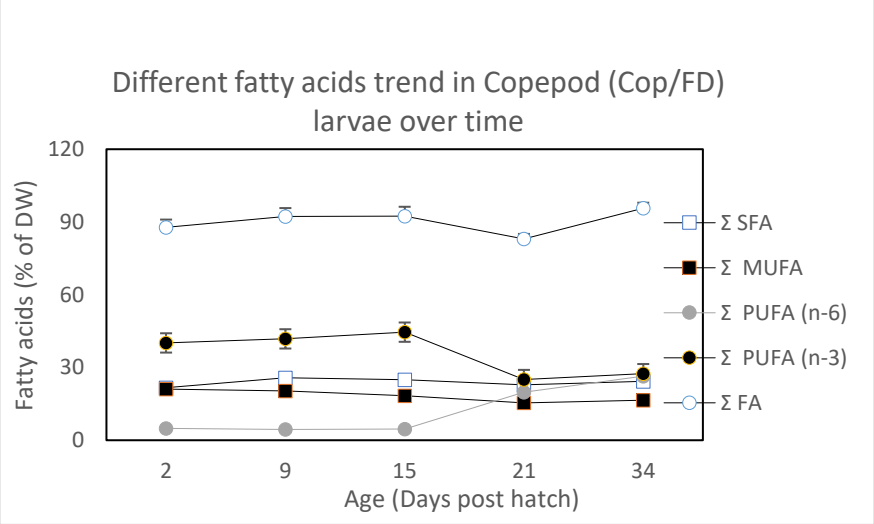


Fig 3.19 Trend of different fatty acids in lumpsucker larvae fed with Cop/Fd in relation to time. Bars indicates ±SE. (N=5, P<0.05)

DHA content increased from 2 to 15dph and then dropped on 21dph but again started rising on 34dph. EPA decreased continuously from 2dph to 21dph and remained same on 34dph. It was statistically significant had a positive correlation with DHA, ∑MUFA, ∑PUFA(n-6 and DHA/EPA. ARA was less but increased very minutely on 15 and 21dph. DHA/EPA remained constant over time while EPA/ARA decreased continuously. Both are negatively correlated with each other. DHA/EPA is significantly related to ARA and negatively correlated to ∑MUFA and is opposite for EPA-ARA (Fig 3.20).

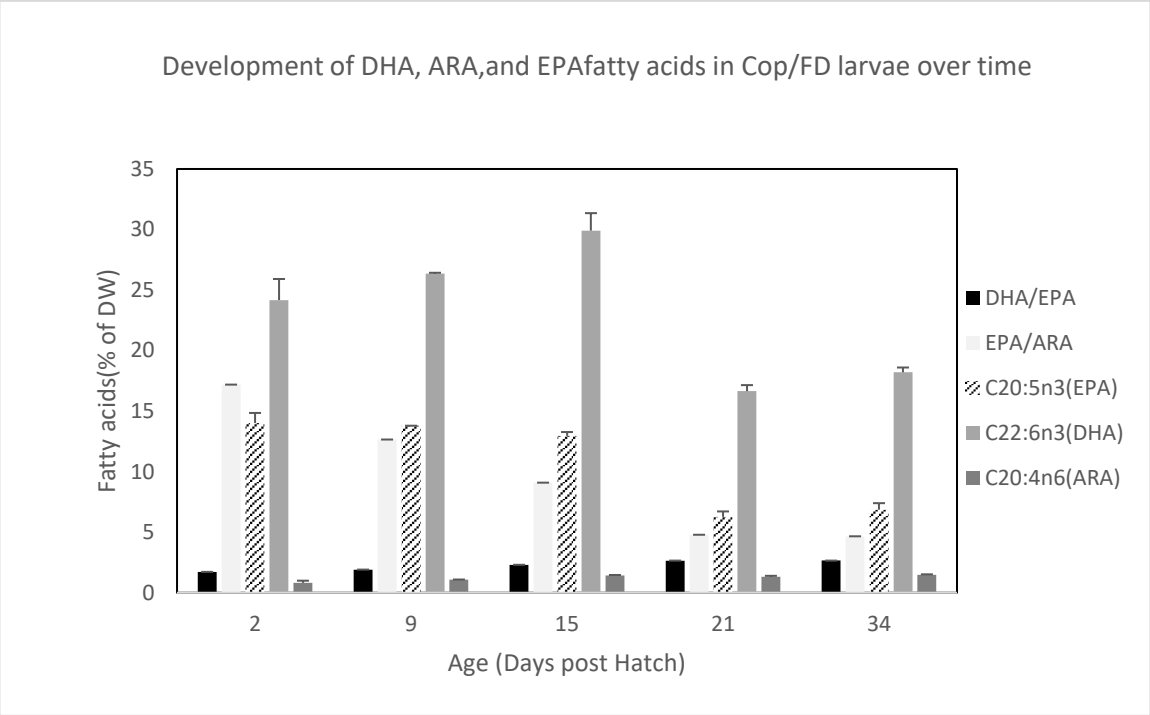


Fig 3.20 Development of EPA, DHA, and ARA (%of Fatty acids) in larvae fed with COP/FD. (N05, $p<0.05$) Bars indicate ±SE.

Chapter 4 : Discussion

4.1 Effects of start-feeding regimes on larval development and survival

The feeding-regime using enriched Artemia nauplii generally gave the highest growth and survival in the experiment. However, Cir-larvae increased to a similar DW and SL to Art- larvae after weaning to formulated diet towards the experiments end. Both start-feeding regimes initiating with copepods, Cop/Cir and Cop/FD, were not as successful, with the latter treatment group having the highest mortality by the experiments end. Similarly, the larval diet consisting solely of formulated diet for FD-larvae also yielded a lower growth and survival than the diets using Artemia and cirripeds. It should however be noted that all larval groups had a high survival rate at the experiments end, ranging from 86-95 %, with only the Cop/FD-larvae having a significantly lower survival rate than the Art-larvae.

At 15 dph and up to the completion of the trial, growth and survival were both noticeably higher (35 dph) among larvae fed with Artemia nauplii. Other research have also shown that feeding lumpfish larvae Artemia has a positive impact on growth (Hanssen, 2018; Marthinsen, 2018; Rian, 2019a) Furthermore, at 21 dph, prior to weaning with formulated feed, Art-larvae were substantially heavier and longer than larvae from the other treatment groups. Due to its larger size as compared to the other feed types employed in the current study, the Artemia nauplii may be favored (Baert et al., 1996)

Greater accessible biomass and quicker detection of larger prey allow for increased consumption with less energy input (Killen et al., 2007). Swimming speed of Artemia nauplii may be optimal for the lumpfish larvae to conserve its energy. Due to low aerobic energy for foraging and swimming lumpfish prefers passive foraging (Hvas et al., 2018) The Artemia-fed larvae originally exhibited a greater DWI than the other regimes during the live prey phase till weaning (21 dph) started. Weaning itself may be stressful, which would explain why development is slower at that point, but overall survival is still good in the Artemia-fed larvae at that point. Realizing that the larvae typically take larger feed as they develop, the switch to a much smaller feed (Gemma micro 300nm) might be difficult and stressful (Yúfera, 2011). FD may not elicit predatory behavior to the same degree as Artemia nauplii since they flow with water currents (D'Abramo, 2019). But still predatory behavior can’t be considered primary cause for the decline in DWI as Cirripedia fed with formulated diet after 21 dph showed higher DWI in comparison to others.

During the trial, the larvae fed Cirripedia nauplii had the second greatest growth rate, and the largest development spurt took place at weaning to FD at 21 dph. Larvae fed with Cirripedia nauplii

stands second highest to larvae fed with Artemia but still better than larvae fed with copepods and FD smallest Initially just after hatching entire energy of larvae fed with Cirripedia was utilized in growth resulting in lowest dry weight and negative DWI. However, this may not have been the case for all of the larvae. The growth of the larvae was much worse when fed Cop/FD compared to the Artemia and Cirripedia group. However in certain other pelagic species like cod and ballan wrasse copepods and rotifer fed larvae (Berg, 2012; Rajkumar & Kumaraguru vasagam, 2006)has shown better growth and survival rates while in case of lumpfish (Marthinsen, 2018; Rian, 2019b)and (Hanssen, 2018) has similar results as us.

Pelagic fish has a smaller egg size as compared to lumpfish which hatch from demersal egg and takes more time in development i.e. from fertilization to hatching (Kjørsvik et al., 2004). Large egg contains a larger yolk and hence produces big larvae like lumpfish having better digestive system and big mouth and eyes (Kjørsvik et al., 2004). Therefore, artemia and Cirripedia are suitable for lumpfish , providing higher biomass by utilizing less energy (Plankton, 2022). Greater biomass is made accessible by larger prey, and if larvae are fed with diet in accordance with their mouth size, they might consume more food with less energy (Killen et al., 2007). On the other hand, copepods move swiftly as compared to artemia and Cirripedia and requires more energy to catch but lumpfish has low aerobic scope and swimming speed, (Hvas et al., 2018) so it's not a preferred diet for lumpsucker in our trial. Hence confirming that larvae fed with artemia will have better growth and survival as compared to larvae fed with copepods, formulated diet (Hanssen, 2018; Marthinsen, 2018).

High growth potential of fish not only relies on quality but on location and quantity as well, which demands a high concentration of polar lipids (Brown, 2005; Moksness et al., 2008; Navarro et al., 1999) rather than NL for proper development and growth of several marine fish species larvae. (Cahu et al., 2003; Evjemo & Olsen, 1997; Fontagné et al., 1998; Watanabe, 1982). Not only their concentration but their location also matters (Kjørsvik et al., 2009). After enrichment, artemia do have more lipid content than copepods do(van der Meeren et al., 2008). It's possible that cirripeds also do as well because they are believed to have a comparable nutrient profile to copepods (Plankton, 2022). Proteins and amino acids are another part of dietary quality that can impact both development and survival (Holt et al., 2011; Karlsen et al., 2015). The protein content of Cirripedia and copepod nauplii is roughly equal (67g/100g DW), and it is nearly twice as high as that of Artemia nauplii (35g/100g DW) (Karlsen et al., 2015; Plankton, 2022) . Nevertheless, the Artemia-fed larvae had the best growth and survival rates at the end of the trial. Therefore, the position of the

FA in the Particular lipid class like polar lipid may not be as important for optimal use in the lumpfish larvae and the protein may not hinder the larval development.

In Cop/Cir group, primary shift of larvae from copepod to Cir feed enhanced the development rate but the second shift to GM300 resulted in delayed growth. Study by (Hansen et al., 2018; Marthinsen, 2018) gave negative survival initially but enhanced on larger FD pellets. Cod larvae also demonstrated a favorable shift from rotifers to copepods, but not the other way around (Koedijk et al., 2010). Because they were both fed Copepods, the Cop/Cir and Cop/FD groups both progressed equally in terms of SL, DW, and DWI in the first 10dph. Weaning Cop/FD to formulated diets has been associated with decreased survival at secondary diet change, whereas Cop/Cir had significantly higher SL and DW. This impact may be brought on by stress factors, such as pellet size and mouth size, etc. Despite the fact that lumpfish already have effective eyes, smaller feed pellets may be more difficult to discover (Hunter 1981) and their size may not be compatible with the mouths of the fish (Brown 1986). . The copepod and the smallest diet (150 m) may have appeared too tiny for the lumpfish larvae during hatching. Since the FD-pellets float with the water currents rather than actively swimming, the rate of ingestion in the FD group and the Cop/Cir group (after weaning to FD) may be reduced. As a result, it might not stimulate the larvae's predatory behavior to the same degree as live prey (D'Abramo, 2019). Considering the fact that the functional stomach of lumpfish does not fully develop until 34 dph, (Marthinsen, 2018) the aggregation of proteins utilized to avoid leaching (Nordgrreen et al., 2009) may have hindered the digestion of the formulated diet. This suggests that using prepared pellets at the beginning of exogenous feeding may not be ideal since lumpfish larvae lack a fully developed and functional digestive system.

The average survival rate across all larvae fed with different feeding regimes was above 85%, and other research also indicate that cultured lumpfish larvae have great survival rate. (Dahle et al., 2017; Hanssen, 2018; Marthinsen, 2018)All the larvae showed peak in mortality at around 21 dph, with the Cop/FD larvae exhibiting the greatest mortality. Knowing that the Cop/FD larvae were smaller compared to the other larvae at that moment but were fed the same quantity of FD, indicating excessive intake the cause of high mortality rate. The highest decrease(85-92%) in survival was noticed in the Cop/FD-fed larvae, which was also noted by (Hanssen, 2018; Marthinsen, 2018; Rian, 2019a) Hanssen et al. (2018), and (Dahle et al., 2017). However, compared to pelagic species such as ballan wrasse (Berg, 2012; Romundstad, 2015) and Atlantic cod (Øie et al., 2017), lumpfish larviculture has a substantially better overall survival rate.

The nutritional needs of lumpfish larvae at the commencement of feeding are still not fully understood based on the current studies. Better development and survivability of the lumpfish larvae

were obtained in the current study and a few other start feeding trials by feeding them with Artemia(Hanssen, 2018; Marthinsen, 2018; Rian, 2019a).

According to (Conceicao et al., 2010), smaller species of pelagic fish seem to benefit more from feeding on copepods. Even though prepared diets may be preferable for larger fish species because lumpfish larvae's stomachs don't fully separate until 34 days after hatching (dph)(Marthinsen, 2018). The best course of action for this medium-sized fish may be to begin feeding on Artemia nauplii and gradually switch to a bigger FD size, maybe after the stomach has fully differentiated (34 dph), since that can boost the ability for digesting protein in the larvae(Marthinsen, 2018). Weaning to FD was place in the current study at two distinct dates (10 and 21 dph), and although the growth suggests that a later weaning is preferable, the weaning at 10 dph may have benefited from utilizing a bigger FD. Due to the lumpfish's apparent acceptance of and preference for huge prey even right after hatching.

Because of the rapid larval development in the current study, and the fact that it is a relatively novel prey, future research on start-feeding lumpfish should examine feeding regimes using cirripedia nauplii. Another intriguing feeding regime would consist entirely of FD, but with a bigger FD size from the beginning than in the current study, since lumpfish has functional eyes from hatching.

4.2 Larval lipids and fatty acids development in relation to feeding regimes

Lipids are the key contributors of metabolic fuel during the rapid development of early stages of the fish (Glencross, 2009; Sargent, 1995). and provide at least two thirds' times more energy per gram than that of provided by proteins or carbohydrates (Parrish, 2013). In our study lipid content is highest in Artemia nauplii (22%) since it was enriched using lipid emulsion. Technique of long-term and short-term enrichment as devised by (Olsen et al., 1993)was used that proved to build up 14-18% lipid content in Artemia which is also evident in our study . While formulated diet has about 18-19% lipid content and least was in found Cirripedia and copepods nauplii> 10%. However, Copepods are main source of food for fish in wild and are nutritionally superior to artemia and rotifers (Evjemo & Olsen, 1997; Støttrup & McEvoy., 2003). Though it has been observed that growth and survival of Artemia fed larvae was higher than Cop/Cir nauplii, however the total lipid content was higher in Larvae that was fed with Cop/Cir (35%) than that of artemia (15%).

With the variation in species (Cetta & Capuzzo, 1982; Finn et al., 1996; Fraser et al., 1988; Tocher, 2003) the lipid class preference and hydrolyzation also varies along with the ratio of phospholipids to neutral lipids(Hamre et al., 2013) (Hamre et al). Provided with the required content of PUFA, EPA and DHA the fish exhibit high potential for growth (Navarro et al., 1999). However all the lipid

classes are not preferred by marine fish species for growth and survival, but some fatty acids are considered vital and termed as essential fatty acids (EFAs)(Glencross, 2009; Parrish, 2013) while others are used as fuel for activities like swimming , preying etc. n-3 and n-6 are the two families of PUFA which are categorized as essential fatty acids. In different diets the amount of PUFA found varies as it makes up 50 % of the total fatty acid in FD, 47% in Cop/FD, 46% in Art group, 42% in Cir group and 47% in Cop/Cir fed larvae. These two families (n-3 and n-6) are very effective for contributing to fish growth and survival if they are part of phospholipid.

In early 1980s studies have shown significance of dietary Phospholipids for the growth and survival of a range of species (Izquierdo & Koven, 2011; Izquierdo et al., 2000; Tocher et al., 2008)However, it has also been observed that the capacity of production of phospholipids is limited in fish larvae and juveniles (Izquierdo & Koven, 2011). PL are structural constituents of bio-membranes and therefore a basic requirement of fast-growing larvae (Cahu & Zambonino Infante, 2001; Cahu et al., 2003; Geurden et al., 1995; Kanazawa et al., 1983; Kjørsvik et al., 2009). Along with growth PL also plays an important part in digestion, absorption, and transportation of lipids from the intestine to the rest of the body. It has been observed that the optimal level required for the integration of dietary PL depends on the species, the PL source and classes, and the criteria on which evaluation is based like growth, survival, stress resistance, malformations in larvae(Bell et al., 2003; T Takeuchi, 1990; Tocher et al., 2008).

The presence of DHA in phospholipids sustains the structure and function of cell membranes especially when it is a major component of polar lipids like in neural tissues such as brain and eye (Seelig & Seelig, 1974; Stillwell & Wassall, 2003) . It is expected that requirement of DHA is higher in different stages of development where growth is fast, and it is required to satisfy the demands of rapidly forming tissues that accumulate DHA. Likewise, EPA has a major role as a precursor of highly bioactive compounds such as eicosanoids, and it can also partly satisfy requirements of DHA in species with adequate elongate and desaturase activities to convert EPA to DHA (Castro et al., 2016). In addition, previous studies have shown decrease in absolute requirement for n-3 LC-PUFA when there is increase in DHA/EPA dietary ratio (Rodríguez et al., 1998; Rodriguez et al., 1994). Thus, the relative proportion of DHA and EPA is also an imperative aspect to be considered during formulation of feeds along with absolute dietary level of both, during the fast-growing stages of the fish (Rodríguez et al., 1998). As per NRC the requirement of dietary DHA/EPA ratios range for marine fish has been reported from 0.5 to 2.0(*National Research Council (2011) Committee on the Nutrient Requirements of Fish and Shrimp Nutrient requirements of fish and shrimp. Washington, DC: National Academies Press.*). In our study larvae fed with Art and Cir

had almost equal DHA/EPA ratio that is 1.71 and 1.88 respectively while cop/cir had 1.989. Other feeding regimes had DHA/EPA ratio above 2. Previous studies have indicated that the high level of PUFA induces oxidative stress in juvenile of sea bream and lipid peroxidation in liver. Similarly in our study formulated diet (FD) and Cop/FD has comparatively less rate of growth and high mortality rate that might be due to increased levels of fatty acids also reported in a study by (Qian et al., 2015). DHA/EPA ratio was also higher than recommended (optimum between 0.5 to 2 for marine species) in larvae fed with formulated diet It might be due to high concentrated feed that had induced stress and mortality as the content of Omega 3 fatty acids is not significantly high from other diets.

The essential fatty acids DHA and EPA must be found in phospholipids (polar lipids) rather than neutral lipids for appropriate development of several marine fish species, as they have shown to be more effectively utilized by the larvae (Cahu et al., 2003; Evjemo et al., 2003; Fontagné et al., 1998; Gisbert et al., 2008) (Kjørsvik et al., 2009). Artemia does show higher lipid content than copepods after enrichment (van der Meeren et al., 2008), and likely also cirripeds, as they are said to have a similar nutrient composition as copepods (Plankton, 2022).(However, the lipids found in Artemia are approximately 60-70 % neutral lipids, meaning the majority of DHA and EPA are not found in the polar lipids(Hamre et al., 2013)(Øie et al., 2017).It would therefore be natural to assume that Artemia would result in reduced growth compared to the copepod A. tonsa, which in contrast have a polar lipid fraction at approximately 70 % (Øie et al., 2017).This was however not the case in the present study. It could therefore be reasonable to assume that the higher lipid content in the Artemia in general are of greater importance than whether the PUFAs are in the neutral or polar lipid fraction. Furthermore, lumpfish hatch from demersal eggs and at a more developed stage than many of the larvae that require the PUFAs in the polar lipid fraction.

Compared to the studies of n-3 LC-PUFA, ARA nutrition studies in marine organisms are scarce. Recently few studies on arachidonic acid (20:4n–6, ARA) revealed that it has role in skeleteogenesis (Boglino et al., 2014) growth performance (Araújo et al., 2020; Nayak et al., 2017; Torrecillas et al., 2018)survival, and stress resistance (Araújo et al., 2021; Boglino et al., 2014)disease resistance (Furuita et al., 2003) and reproduction (Yuan et al., 2015). It has been suggested that increased dietary ARA positively affects cortisol levels and stress response in marine fishes (Koven et al., 2001; Lund et al., 2007; Martins et al., 2013). However, excess in ARA levels can induce adverse effects in development (L. Copeman et al., 2002; Lund et al., 2007). Another important functional role of the LC-PUFA, especially EPA and ARA, is the substrate to the eicosanoid synthesis by the action of some specific enzymes (Araújo et al., 2021; Bell et al., 2003).Thus an adequate ARA/EPA ratio in diets to marine fish is essential for the proper functioning of the immune system (Bell et al.,

2003). Our study revealed that level of EPA/ARA ratio was lowest in artemia 3.86 as compared to other diets. However, the content of ARA in fatty acids were not high enough but still correlated with statistical significance P>0.001. Still, we need to work on its responses that how it effects fish larvae in different stages of life like larval and juvenile and its exact amount required needs to be configured.

Overall, our study was designed to formulate the first feeding for lumpsucker larvae. The results obtained from the study has supported proved that artemia is the best diet for Lumpsucker larvae. During the studies it has been observed that fish larvae has preferred enriched artemia diet due to compatibility of its size, and behavior of fish. Copepods are found to be least preferred diet that can be explained in terms of size of nauplii and swimming capacity of both larvae and nauplii. Formulated diet had also shown significant results however, there is a need to adjust the concentration of diet for avoiding stress response and over-crowding. It has been proved that only the highest amount of omega 3 fatty acids does not give better survival and growth rate rather it is optimum content of the components which must be in ratio of DHA/EPA ≤ 2.

4.3 Limitations and challenges

The experiment went well and gave useful information for formulating start feed of lumpsucker fish. Due to some technical fault, I lost some samples and because of limited time I could not repeat my extraction and analysis which decreased my sample size, and I could not get more accurate and significant values for fatty acids of larvae. In the beginning of the experiment when eggs were incubated, we mixed the eggs of 5 females. Every individual has its own strength and certain weakness as well and most important in larval development is the egg quality. In my opinion it might be a good idea use eggs of one female to avoid ambiguities in results.

For further study on role of DHA, EPA, and ARA in phospholipids and neutral lipids , we can use any labelling technique at molecular level to analyze exact amount in neutral lipids and polar lipids. Secondly there must be a comparison of how much individual fatty acid were in feed and how many are incorporated in larvae that can give us exact value needed for larvae based on its age.

Chapter 5: Conclusion

The results of the current study showed that during the trial, lumpfish larvae grew and survived the best when enriched Artemia nauplii were used. It also showed that the recently developed live feed cryopreserved cirripeds may be a choice for lumpfish starter feeding. When weaned to a formulated diet, the cirripeds-fed larvae and Art larvae resulted in increase in standard length than the larvae of other feeding regimes and both had equal sizes at the experiment's conclusion. The cirripeds-fed larvae initially developed poorly. It is logical to suggest that a feeding regime that integrates both enriched Artemia and cirriped nauplii before the fish are weaned to formulated diet might be viable alternative in start-feeding regimes for lumpfish larvae because the cirripeds clearly had a positive carry-over effect when weaned to formulated diet. Regardless of whether the fish were weaned to cirripeds later or immediately started on a prepared diet, feeding regimens beginning with copepods had a deleterious impact on the lumpfish's development. According to the study's findings, copepods are probably not a good live food source for lumpfish larvae. However, It can be good for other marine fish larvae. The feeding regime that introduced the lumpfish to the formulated diet immediately without a live feed phase prior also appears to be inappropriate because it led to less development. Furthermore, development of total lipid and total fatty acids content in larvae is correlated with type of feed, preference by larvae, and the nutritional value of the feed as well. Although total lipid content was high in Cop/FD and formulated diet FD but still the larvae fed with artemia and Cirripedia gave highest survival and development of adequate phospholipids as well. Total fatty acids profile declined significantly when fish larvae were weaned to formulated diet and then raised again till the end of experiment. So, there is a chance if the experiment duration was enhanced the fish might have adapted itself completely to formulated diet and gave further better results.

Future studies on lumpfish start-feeding should focus on determining the nutritional needs of the lumpfish and how various live feeds satisfy these needs. As there is little literature on cirripeds as live feed organisms, there is also a clear need for additional study of their nutritional makeup. Additionally, the long-term implications of feeding regimens are still unknown. To develop a good start-feed which can cope with industrial standards one must know accurate required content of individual fatty acids like DHA , EPA ad ARA based on their location in PLs or NLs, we must study them on molecular level using markers to identify their exact location, development, and responses in physiological development on fish larvae.

References

Anne Berit Skiftesvik, Reidun Bjelland, Caroline Durif, Lene Moltumyr, og, R. B. H., & Halvorsen, K. T. (2018). *Cleanerfish program: Behaviour and species interactions in the seacages. Final FHF report project 900978* (900978) https://www.fhf.no/prosjekter/prosjektbasen/900978/

Araújo, B. C., Flores-Galvez, K., Honji, R. M., Barbosa, V. M., Viana, M. T., Tinajero, A., & Mata-Sotres, J. A. (2020). Arachidonic acid effects on the overall performance, fatty acid profile, hepatopancreas morphology and lipid-relevant genes in Litopenaeus vannamei juveniles. *Aquaculture*, *523*, 735207. https://doi.org/https://doi.org/10.1016/j.aquaculture.2020.735207

Araújo, B. C., Rodriguez, M., Honji, R. M., Rombenso, A. N., del Rio-Zaragoza, O. B., Cano, A., Tinajero, A., Mata-Sotres, J. A., & Viana, M. T. (2021). Arachidonic acid modulated lipid metabolism and improved productive performance of striped bass (Morone saxatilis) juvenile under sub- to optimal temperatures. *Aquaculture*, *530*, 735939. https://doi.org/https://doi.org/10.1016/j.aquaculture.2020.735939

Baert, P., Bosteels, T., & Sorgeloos, P. (1996). Manual on the Production and Use of Live Food for Aquaculture. *Pond production*, 196-251.

Bailey, J. (2014). Looking for sustainable solutions in salmon aquaculture. *Etikk i praksis - Nordic Journal of Applied Ethics*, *8*(1), 22-40. https://doi.org/10.5324/eip.v8i1.1801

Bell, J. G., McEvoy, L. A., Estévez, A., Shields, R. J., & Sargent, J. R. (2003). Optimising lipid nutrition in first-feeding flatfish larvae. *Aquaculture*, *227*, 211-220.

Benfey, T. J., & Methven, D. A. (1986). Pilot-scale rearing of larval and juvenile lumpfish (Cyclopterus lumpus L.), with some notes on early development. *Aquaculture*, *56*(3), 301-306. https://doi.org/10.1016/0044-8486(86)90344-3

Berg, M. (2012). The effect of different live prey types on growth and muscle development, in ballan wrasse (Labrus bergylta, Ascanius 1767) larvae. In E. Kjørsvik, G. Øie, T. Galloway, & F. f. n. o. t. I. f. b. Norges teknisk-naturvitenskapelige universitet (Eds.): Institutt for biologi.

Bisbal, G. A., & Bengtson, D. A. (1995). Development of the digestive tract in larval summer flounder. *Journal of fish biology*, *47*(2), 277-291. https://doi.org/10.1111/j.1095-8649.1995.tb01895.x

Bjordal, Å. (1991). Wrasse as cleaner-fish for farmed salmon.

Blanco Gonzalez, E., & de Boer, F. (2017). The development of the Norwegian wrassefishery and the use of wrasses as cleaner fish in the salmon aquacultureindustry. *Fisheries Science*, *83*(5), 661-670. https://doi.org/10.1007/s12562-017-1110-4

Boglino, A., Darias, M., Andree, K., Estevez, A., & Gisbert, E. (2014). The effects of dietary arachidonic acid on bone in flatfish larvae: The last but not the least of the essential fatty acids. *Journal of Applied Ichthyology*, *30*. https://doi.org/10.1111/jai.12511

Brooker, A. J., Papadopoulou, A., Gutierrez, C., Rey, S., Davie, A., & Migaud, H. (2018). Sustainable production and use of cleaner fish for the biological control of sea lice: recent advances and current challenges. *Vet Rec*, *183*(12), 383. https://doi.org/10.1136/vr.104966

Brown, J. A. (2005). Book review: Culture of Cold-water Marine Fish. E. Moksness, E. Kjørsvik and Y. Olsen (eds). Fishing News Books (Blackwell Publishing), Oxford, 2004. 528 pp. ISBN 0-85238-276-6. Price £95 (HB). In (Vol. 13, pp. 383-385). Dordrecht: Dordrecht: Springer Nature B.V.

C FEED AS. (2014). Product specification. In.

Cahu, C., & Zambonino Infante, J. (2001). Substitution of live food by formulated diets in marine fish larvae. *Aquaculture*, *200*(1), 161-180. https://doi.org/10.1016/S0044-8486(01)00699-8

Cahu, C. L., Infante, J. L. Z., & Barbosa, V. (2003). Effect of dietary phospholipid level and phospholipid:neutral lipid value on the development of sea bass (Dicentrarchus labrax) larvae fed a compound diet. *Br J Nutr*, *90*(1), 21-28. https://doi.org/10.1079/BJN2003880

Carvalho, L. A., Whyte, S. K., Braden, L. M., Purcell, S. L., Manning, A. J., Muckle, A., & Fast, M. D. (2020). Impact of co-infection with Lepeophtheirus salmonis and Moritella viscosa on inflammatory and immune responses of Atlantic salmon (Salmo salar). *J Fish Dis*, *43*(4), 459-473. https://doi.org/10.1111/jfd.13144

Castell, J., Blair, T., Neil, S., Howes, K., Mercer, S., Reid, J., Young-Lai, W., Gullison, B., Dhert, P., & Sorgeloos, P. (2003). The effect of different HUFA enrichment emulsions on the nutritional value of rotifers (Brachionus plicatilis) fed to larval haddock (Melanogrammus aeglefinus). *Aquaculture International*, *11*, 109-117. https://doi.org/10.1023/A:1024154106656

Castro, L. F., Tocher, D. R., & Monroig, O. (2016). Long-chain polyunsaturated fatty acid biosynthesis in chordates: Insights into the evolution of Fads and Elovl gene repertoire. *Prog Lipid Res*, *62*, 25-40. https://doi.org/10.1016/j.plipres.2016.01.001

Cetta, C. M., & Capuzzo, J. M. (1982). Physiological and biochemical aspects of embryonic and larval development of the winter flounder Pseudopleuronectes americanus. *Marine Biology*, *71*(3), 327-337. https://doi.org/10.1007/BF00397049

Committee, F., & Butterworth, A. (2014). *Farm Animal Welfare Committee / Opinion on the Welfare of Farmed Fish at the time of Killing*.

Conceicao, L. E. C., Yufera, M., Makridis, P., Morais, S., & Dinis, M. T. (2010). Live feeds for early stages of fish rearing. *Aquaculture Research*, *41*(5), 613-640. https://doi.org/10.1111/j.1365-2109.2009.02242.x

Copeman, L., Parrish, C., Brown, J. A., & Harel, M. (2002). Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (Limanda ferruginea): A live food enrichment experiment. *Aquaculture*, *210*, 285-304. https://doi.org/10.1016/S0044-8486(01)00849-3

Copeman, L. A., Parrish, C. C., Brown, J. A., & Harel, M. (2002). Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (Limanda ferruginea): a live food enrichment experiment. *Aquaculture*, *210*(1), 285-304. https://doi.org/https://doi.org/10.1016/S0044-8486(01)00849-3

Costello, M. J. (2009). The global economic cost of sea lice to the salmonid farming industry. *J Fish Dis*, *32*(1), 115-118. https://doi.org/10.1111/j.1365-2761.2008.01011.x

D'Abramo, L. R. (2019). Challenges in Developing Successful Formulated Feed for Culture of Larval Fish and Crustaceans. *Avances en Nutrición Acuicola*, *0*(0). https://nutricionacuicola.uanl.mx/index.php/acu/article/view/233

Dahle, S., Hagemann, A., Attramadal, Y., Stavrakaki, M., Evjemo, J., & & Kjørsvik, E. (2014). Long term feeding with copepods https://www.sintef.no/contentassets/431f06e53bc448998fb856156c6799d5/final-report_-

production-of-ballan-wrasse_-science-and-practice.pdf

Dahle, S. V. W., Hagemann, A., Attramadal, K., Kjørsvik, E., & Bardal, T. (2017). OC2017 A-029 - Vannkvalitet og startfôring av rognkjeks - Effekt av fôring med levende copepoder i tidlige faser (FHF prosjekt nr 901174). In: SINTEF Ocean AS.

De Silva Sena S. , S.S. De Silva, and, & Anderson, T. A. (1994). *Fish nutrition in aquaculture*.

Denholm, I., Devine, G. J., Horsberg, T. E., Sevatdal, S., Fallang, A., Nolan, D. V., & Powell, R. (2002). Analysis and management of resistance to chemotherapeutants in salmon lice, Lepeophtheirus salmonis (Copepoda: Caligidae). *Pest Management Science*, *58*(6), 528-536. https://doi.org/https://doi.org/10.1002/ps.482

Dhont, J., Dierckens, K., Støttrup, J. G., Stappen, G., Wille, M., & Sorgeloos, P. (2013). Rotifers, Artemia and copepods as live feeds for fish larvae in aquaculture. In (pp. 157-202). https://doi.org/10.1533/9780857097460.1.157

Drillet, G., & Lombard, F. (2015). A first step towards improving copepod cultivation using modelling: the effects of density, crowding, cannibalism, tank design and strain selection on copepod egg production yields. *Aquaculture Research*, *46*(7), 1638-1647. https://doi.org/10.1111/are.12317

Evjemo, J. O., & Olsen, Y. (1997). Lipid and fatty acid content in cultivated live feed organisms compared to marine copepods. *Hydrobiologia*, *358*(1), 159-162. https://doi.org/10.1023/A:1003196829109

Evjemo, J. O., Reitan, K. I., & Olsen, Y. (2003). Copepods as live food organisms in the larval rearing of halibut larvae (Hippoglossus hippoglossus L.) with special emphasis on the nutritional value. *Aquaculture*, *227*(1), 191-210. https://doi.org/10.1016/S0044-8486(03)00503-9

FAO. (1996). *Manual on the Production and Use of Live Food for Aquaculture. (FAO Fisheries Technical Paper 361). Rome, Italy: FAO*

FHF. (2019). *Optimalisert startfôring av rensefisk (STARTRENS).* https://www.fhf.no/prosjekter/prosjektbasen/901561/

Finn, R. N., Fyhn, H. J., Henderson, R. J., & Evjen, M. S. (1996). The sequence of catabolic substrate oxidation and enthalpy balance of developing embryos and yolk-sac larvae of turbot (Scophthalmus maximus L.). *Comparative biochemistry and physiology. A, Comparative physiology*, *115*(2), 133-151. https://doi.org/10.1016/0300-9629(96)00026-6

Fisheries, N. D. o. (2020). *Sale of farmed cleaner fish(lumpfish and Wrasse)*. Retrieved from https://www.fiskeridir.no/English/Aquaculture/Statistics/Cleanerfish-Lumpfish-and-Wrasse

Fisheries, N. D. o. (2020). *Sale of farmed lumpfish (Cyclopterus lumpus) and ballan wrasse (Labrus bergylta) to producers of Atlantic salmon and Rainbow trout in millions from year 2012-2020 in Norway* https://www.fiskeridir.no/English/Aquaculture/Statistics/Cleanerfish-Lumpfish-and-Wrasse

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem*, *226*(1), 497-509.

Fontagné, S., Geurden, I., Escaffre, A.-M., & Bergot, P. (1998). Histological changes induced by dietary phospholipids in intestine and liver of common carp (Cyprinus carpio L.) larvae. *Aquaculture*, *161*(1), 213-223. https://doi.org/https://doi.org/10.1016/S0044-8486(97)00271-8

Fraser, A. J., Gamble, J. C., & Sargent, J. R. (1988). Changes in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (Gadus morhua). *Marine Biology*, *99*(3), 307-313. https://doi.org/10.1007/BF02112122

Furuita, H., Ohta, H., Unuma, T., Tanaka, H., Kagawa, H., Suzuki, N., & Yamamoto, T. (2003). Biochemical composition of eggsin relation to egg quality in the Japanese eel, Anguilla japonica. *Fish Physiology and Biochemistry*, *29*(1), 37-46. https://doi.org/10.1023/B:FISH.0000035897.58924.9d

Garcia de Leaniz, C., Gutierrez Rabadan, C., Barrento, S. I., Stringwell, R., Howes, P. N., Whittaker, B. A., Minett, J. F., Smith, R. G., Pooley, C. L., Overland, B. J., Biddiscombe, L., Lloyd, R., Consuegra, S., Maddocks, J. K., Deacon, P. T. J., Jennings, B. T., Rey Planellas, S., Deakin, A., Moore, A. I., . . . Pavlidis, M. (2022). Addressing the welfare needs of farmed lumpfish: Knowledge gaps, challenges and solutions [https://doi.org/10.1111/raq.12589]. *Reviews in aquaculture*, *14*(1), 139-155. https://doi.org/https://doi.org/10.1111/raq.12589

Gatlin III, D. M., & Bai, S. C. (1993). Effects of dietary lipid and reduced glutathione on composition and storage quality of channel catfish, Ictalurus punctatus (Rafinesque). *Aquaculture Research*, *24*(4), 457-463. https://doi.org/https://doi.org/10.1111/j.1365-2109.1993.tb00620.x

Geurden, I., Bergot, P., Schwarz, L., & Sorgeloos, P. (1998). Relationship between dietary phospholipid classes and neutral lipid absorption in newly-weaned turbot, shape Scophthalmus maximus. *Fish Physiology and Biochemistry*, *19*(3), 217-228. https://doi.org/10.1023/A:1007723515204

Geurden, I., Radünz-Neto, J., & Bergot, P. (1995). Essentiality of dietary phospholipids for carp (Cyprinus carpio L.) larvae. *Aquaculture*, *131*(3), 303-314. https://doi.org/10.1016/0044-8486(94)00344-N

Gisbert, E., Ortiz-Delgado, J. B., & Sarasquete, C. (2008). Nutritional cellular biomarkers in early life stages of fish. *Histol Histopathol*, *23*(12), 1525-1539. https://doi.org/10.14670/hh-23.1525

Glencross, B. D. (2009). Exploring the nutritional demand for essential fatty acids by aquaculture species. *Reviews in aquaculture*, *1*(2), 71-124. https://doi.org/https://doi.org/10.1111/j.1753-5131.2009.01006.x

Govoni, J. J., Boehlert, G. W., & Watanabe, Y. (1986). The physiology of digestion in fish larvae. *Environmental biology of fishes*, *16*(1-3), 59-77. https://doi.org/10.1007/BF00005160

Hamre, K., Yúfera, M., Rønnestad, I., Boglione, C., Conceição, L. E. C., & Izquierdo, M. (2013). Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. *Reviews in aquaculture*, *5*(s1), S26-S58. https://doi.org/10.1111/j.1753-5131.2012.01086.x

Hansen, B. W., Boesen, E., Brodnicke, O. B., Corfixen, N. L., Jepsen, Per M., Larsen, S. M., Læssøe, C. D., Munch, P. S., Nielsen, P. K. F., Olesen, J., Vismann, B., & Nilsson, B. (2018). Interactions between populations of the calanoid copepod Acartia tonsa Dana and the harpacticoid copepod Tisbe holothuriae Humes in mixed cultures of live feed for fish larvae. *Aquaculture Research*, *49*(3), 1274-1283. https://doi.org/10.1111/are.13581

Hanssen, J. T. (2018). Effects on growth, survival and bone development from start feeding lumpsucker (Cyclopterus lumpus) larvae with Artemia, copepods and fomulated feed.

Harel, M., Koven, W., Lein, I., Bar, Y., Behrens, P., Stubblefield, J., Zohar, Y., & Place, A. R. (2002). Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. *Aquaculture*, *213*(1), 347-362. https://doi.org/10.1016/S0044-8486(02)00047-9

Hawkyard, M., Laurel, B., Barr, Y., Hamre, K., & Langdon, C. (2015). Evaluation of Liposomes for the Enrichment of Rotifers (brachionus Sp.) with Taurine and Their Subsequent Effects On the Growth and Development of Northern Rock Sole (lepidopsetta Polyxystra) Larvae. *Elsevier*. https://ir.library.oregonstate.edu/concern/articles/8k71nj77h

Holt, G., Webb, K., & Rust, M. (2011). Larval Fish Nutrition. In (pp. 353-372). https://doi.org/10.1002/9780470959862.ch13

Houde, D., E., & & Schekter, R. C. (1981). Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations, . pp. 441-453.

Hughes, S. G. (1989). Effects of aqueous amino acid solutions on the feed intake of juvenile Atlantic salmon. *Salmonid*, *13*, 13-14. http://pubs.er.usgs.gov/publication/1014587

Hvas, M., Folkedal, O., Imsland, A., & Oppedal, F. (2018). Metabolic rates, swimming capabilities, thermal niche and stress response of the lumpfish, Cyclopterus lumpus. *Biology open*, *7*(9). https://doi.org/10.1242/bio.036079

Imsland, A., Reynolds, P., Eliassen, G., Hangstad, T., Jónsdóttir, Ó., Elvegård, T., Lemmens, S., Rydland, R., & Nytrø, A. (2016). Investigation of behavioural interactions between lumpfish (Cyclopterus lumpus) and goldsinny wrasse (Ctenolabrus rupestris) under controlled conditions. *Aquaculture International*, *24*. https://doi.org/10.1007/s10499-016-0008-y

Imsland, A. K. D., Hanssen, A., Nytrø, A. V., Reynolds, P., Jonassen, T. M., Hangstad, T. A., Elvegård, T. A., Urskog, T. C., & Mikalsen, B. (2018). It works! Lumpfish can significantly lower sea lice infestation in large-scale salmon farming. *Biology open*, *7*(9), bio036301. https://doi.org/10.1242/bio.036301

Imsland, A. K. D., Reynolds, P., Jonassen, T. M., Hangstad, T. A., Elvegård, T. A., Urskog, T. C., Hanssen, A., & Mikalsen, B. (2019). Effects of different feeding frequencies on growth, cataract development and histopathology of lumpfish (Cyclopterus lumpus L.). *Aquaculture*, *501*, 161-168. https://doi.org/https://doi.org/10.1016/j.aquaculture.2018.11.026

Ingólfsson, A., Kristjánsson, B., & Schaefer, S. (2002). Diet of Juvenile Lumpsucker Cyclopterus lumpus (Cyclopteridae) in Floating Seaweed: Effects of Ontogeny and Prey Availability. *Copeia*, *2002*, 472-476. https://doi.org/10.1643/0045-8511(2002)002[0472:DOJLCL]2.0.CO;2

Izquierdo, M., & Koven, W. (2011). Lipids. In (pp. 47-81). https://doi.org/10.1002/9780470959862.ch2

Izquierdo, M. S., Socorro, J., Arantzamendi, L., & Hernández-Cruz, C. M. (2000). Recent advances in lipid nutrition in fish larvae. *Fish Physiology and Biochemistry*, *22*(2), 97-107. https://doi.org/10.1023/A:1007810506259

Jansen, P. A., Kristoffersen, A. B., Viljugrein, H., Jimenez, D., Aldrin, M., & Stien, A. (2012). Sea lice as a density-dependent constraint to salmonid farming. *Proc Biol Sci*, *279*(1737), 2330-2338. https://doi.org/10.1098/rspb.2012.0084

Jobling, M. (2018). J. Treasurer (editor): Cleaner fish biology and aquaculture applications. *Aquaculture International*, *26*(5), 1207-1209. https://doi.org/10.1007/s10499-018-0279-6

Jonassen, T. M., Lein, &, I., & Nytrø, A. V. (2018). Hatchery management of lumpfish, in Treasurer, . *Cleaner Fish Biology and Aquaculture Applications. Sheffield, UK*, 114-136.

Kanazawa, A. (1997). Effects of docosahexaenoic acid and phospholipids on stress tolerance of fish. *Aquaculture*, *155*(1), 129-134. https://doi.org/https://doi.org/10.1016/S0044-8486(97)00123-3

Kanazawa, A., Teshima, S.-i., Inamori, S., Iwashita, T., & Nagao, A. (1981). Effects of Phospholipids on Growth, Survival Rate, and Incidence of Malformation in the Larval Ayu. *鹿児島大学水産学部紀要=Memoirs of Faculty of Fisheries Kagoshima University*, *30*, 301-309.

Kanazawa, A., Teshima, S.-i., Kobayashi, T., Takae, M., Iwashita, T., & Uehara, R. (1983). Necessity of Dietary Phospholipids for Growth of the Larval Ayu. *鹿児島大学水産学部紀要=Memoirs of Faculty of Fisheries Kagoshima University*, *32*, 115-120.

Karlsen, Ø., van der Meeren, T., Rønnestad, I., Mangor-Jensen, A., Galloway, T. F., Kjørsvik, E., & Hamre, K. (2015). Copepods enhance nutritional status, growth and development in Atlantic cod (Gadus morhua L.) larvae - can we identify the underlying factors? *PeerJ*, *3*, e902. https://doi.org/10.7717/peerj.902

Kelly, N., Alzaid, A., Nash, G., & Gamperl, A. (2014). Metabolic Depression in Cunner (Tautogolabrus adspersus) Is Influenced by Ontogeny, and Enhances Thermal Tolerance. *PloS one*, *9*, e114765. https://doi.org/10.1371/journal.pone.0114765

Killen, S. S., BROWN, J. A., & GAMPERL, A. K. (2007). The effect of prey density on foraging mode selection in juvenile lumpfish: balancing food intake with the metabolic cost of foraging. *Journal of Animal Ecology*, *76*(4), 814-825. https://doi.org/https://doi.org/10.1111/j.1365-2656.2007.01237.x

Kjørsvik, E., Olsen, C., Wold, P.-A., Hoehne-Reitan, K., Cahu, C. L., Rainuzzo, J., Olsen, A. I., Øie, G., & Olsen, Y. (2009). Comparison of dietary phospholipids and neutral lipids on skeletal development and fatty acid composition in Atlantic cod (Gadus morhua). *Aquaculture*, *294*(3), 246-255. https://doi.org/10.1016/j.aquaculture.2009.06.012

Kjørsvik, E., Pittman, K., & Pavlov, D. (2004). From Fertilisation to the End of Metamorphosis—Functional Development. In (pp. 204-278). Oxford, UK: Oxford, UK: Blackwell Publishing Ltd.

Klinger, D., & Naylor, R. (2012). Searching for Solutions in Aquaculture: Charting a Sustainable Course. *Annual Review of Environment and Resources*, *37*, 247-276. https://doi.org/10.1146/annurev-environ-021111-161531

Koedijk, R. M., Folkvord, A., Foss, A., Pittman, K., Stefansson, S. O., Handeland, S., & Imsland, A. K. (2010). The influence of first-feeding diet on the Atlantic cod Gadus morhua phenotype: survival, development and long-term consequences for growth. *J Fish Biol*, *77*(1), 1-19. https://doi.org/10.1111/j.1095-8649.2010.02652.x

Koven, W., Barr, Y., Lutzky, S., Ben-Atia, I., Weiss, R., Harel, M., Behrens, P., & Tandler, A. (2001). The effect of dietary arachidonic acid (20:4n−6) on growth, survival and resistance to handling stress in gilthead seabream (Sparus aurata) larvae. *Aquaculture*, *193*(1), 107-122. https://doi.org/https://doi.org/10.1016/S0044-8486(00)00479-8

Koven, W. M., Kolkovski, S., Tandler, A., Kissil, G. W., & Sklan, D. (1993). The effect of dietary lecithin and lipase, as a function of age, on n-9 fatty acid incorporation in the tissue lipids of Sparus aurata larvae. *Fish Physiol Biochem*, *10*(5), 357-364. https://doi.org/10.1007/bf00004502

Koven, W. M., Parra, G., Kolkovski, S., & Tandler, A. (1998). The effect of dietary phosphatidylcholine and its constituent fatty acids on microdiet ingestion and fatty acid absorption rate in gilthead sea bream, Sparus auratus, larvae. *Aquaculture Nutrition*, *4*(1), 39-45. https://doi.org/10.1046/j.1365-2095.1998.00101.x

Küllenberg, D., Taylor, L. A., Schneider, M., & Massing, U. (2012). Health effects of dietary phospholipids. *Lipids Health Dis*, *11*(1), 3-3. https://doi.org/10.1186/1476-511X-11-3

Kværnø, S. B. (2022). Growth, survival and liver histology in lumpfish (Cyclopterus lumpus) larvae fed different start-feeding diets (Artemia, copepods, cirripeds and formulated diet). In E. Kjørsvik, T. A. Vo, A. Malzahn, & A. Hagemann (Eds.): NTNU.

Langdon, C. (2003). Microparticle types for delivering nutrients to marine fish larvae. *Aquaculture*, *227*(1), 259-275. https://doi.org/10.1016/S0044-8486(03)00508-8

Liu, Y., & Bjelland, H. V. (2014). Estimating costs of sea lice control strategy in Norway. *Prev Vet Med*, *117*(3-4), 469-477. https://doi.org/10.1016/j.prevetmed.2014.08.018

López, D. A. (2012). Potency of Barnacle in Aquaculture Industry. In. IntechOpen. https://doi.org/10.5772/30396

Lund, I., El Kertaoui, N., Izquierdo, M. S., Dominguez, D., Hansen, B. W., & Kestemont, P. (2018). The importance of phospholipids combined with long-chain PUFA in formulated diets for pikeperch (Sander lucioperca) larvae. *British Journal of Nutrition*, *120*(6), 628-644. https://doi.org/10.1017/S0007114518001794

Lund, I., Steenfeldt, S. J., & Hansen, B. W. (2007). Effect of dietary arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid on survival, growth and pigmentation in larvae of common sole (Solea solea L.). *Aquaculture*, *273*(4), 532-544. https://doi.org/https://doi.org/10.1016/j.aquaculture.2007.10.047

Malzahn, A. M., Ribičić, D., Hansen, B. H., Sarno, A., Kjørsvik, E., Aase, A. S. N., Musialak, L. A., García-Calvo, L., & Hagemann, A. (2022). First feed matters: The first diet of larval fish programmes growth, survival, and metabolism of larval ballan wrasse (Labrus bergylta). *Aquaculture*, *561*, 738586. https://doi.org/https://doi.org/10.1016/j.aquaculture.2022.738586

Marthinsen, J. L. (2018). *Ontogeny of the digestive system in lumpfish (Cyclopterus lumpus L.) larvae in relation to growth and start feeding diet - A histological and stereological approach* Norwegian University of Science and technology , Norway]. NTNU, Norway. http://hdl.handle.net/11250/2504221

Martins, D. A., Rocha, F., Castanheira, F., Mendes, A., Pousão-Ferreira, P., Bandarra, N., Coutinho, J., Morais, S., Yúfera, M., Conceição, L. E., & Martínez-Rodríguez, G. (2013). Effects of dietary arachidonic acid on cortisol production and gene expression in stress response in Senegalese sole (Solea senegalensis) post-larvae. *Fish Physiol Biochem*, *39*(5), 1223-1238. https://doi.org/10.1007/s10695-013-9778-6

Matyash, V., Liebisch, G., Kurzchalia, T. V., Shevchenko, A., & Schwudke, D. (2008). Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. *J Lipid Res*, *49*(5), 1137-1146. https://doi.org/10.1194/jlr.D700041-JLR200

McEvoy, L. A., Naess, T., Bell, J. G., & Lie, Ø. (1998). Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (Hippoglossus hippoglossus) fed enriched Artemia: a comparison with fry fed wild copepods. *Aquaculture*, *163*(3), 237-250. https://doi.org/10.1016/S0044-8486(98)00237-3

McEvoy, L. A., Navarro, J. C., Bell, J. G., & Sargent, J. R. (1995). Autoxidation of oil emulsions during the Artemia enrichment process. *Aquaculture*, *134*(1), 101-112. https://doi.org/https://doi.org/10.1016/0044-8486(95)00048-7

Moksness, E., Kjørsvik, E., & Olsen, Y. (2008). *Culture of cold-water marine fish*. New York, NY: Blackwell Publishing. https://doi.org/10.1002/9780470995617

Moon, S.-K., Kim, I.-S., & Jeong, B.-Y. (2016). Characteristics of Food Nutrition Components of Common Stalked Barnacle Pollicipes mitella. *Korean Journal of Fisheries and Aquatic Sciences*, *49*, 862-866. https://doi.org/10.5657/KFAS.2016.0862

Muncaster, S., Andersson, E., Kjesbu, O., Taranger, G., Skiftesvik, A., & Norberg, B. (2010). The reproductive cycle of female Ballan wrasse Labrus bergylta in high latitude, temperate waters. *Journal of fish biology*, *77*, 494-511. https://doi.org/10.1111/j.1095-8649.2010.02691.x

Munilla-Moran, R., & Stark, J. R. (1989). Protein digestion in early turbot larvae, Scophthalmus maximus (L.). *Aquaculture*, *81*(3), 315-327. https://doi.org/10.1016/0044-8486(89)90156-7

Mustafa, A., & Piasecki, W. (2005). Factors affecting the susceptibility of sea lice infection in Atlantic salmon. *Wiad Parazytol*, *51*(4), 281-285.

National Research Council (2011) Committee on the Nutrient Requirements of Fish and Shrimp Nutrient requirements of fish and shrimp. Washington, DC: National Academies Press. . https://nap.nationalacademies.org/catalog/13039/nutrient-requirements-of-fish-and-shrimp

Navarro, J. C., Henderson, R. J., McEvoy, L. A., Bell, M. V., & Amat, F. (1999). Lipid conversions during enrichment of Artemia. *Aquaculture*, *174*(1), 155-166. https://doi.org/10.1016/S0044-8486(99)00004-6

Nayak, S., Koven, W., Meiri, I., Khozin-Goldberg, I., Isakov, N., Zibdeh, M., & Zilberg, D. (2017). Dietary arachidonic acid affects immune function and fatty acid composition in cultured rabbitfish Siganus rivulatus. *Fish Shellfish Immunol*, *68*, 46-53. https://doi.org/10.1016/j.fsi.2017.07.003

Nordgrreen, A., TONHEIM, S., & HAMRE, K. (2009). Protein quality of larval feed with increased concentration of hydrolysed protein: effects of heat treatment and leaching. *Aquaculture Nutrition*, *15*(5), 525-536. https://doi.org/https://doi.org/10.1111/j.1365-2095.2008.00619.x

Norwegian Directorate of Fisheries. (2020). *Atlantic salmon and rainbow trout, Number of sites in sea water 2006-2020* https://www.fiskeridir.no/English/Aquaculture/Statistics/Atlantic-salmon-and-rainbow-trout

Øie, G., Galloway, T., Sørøy, M., Holmvaag Hansen, M., Norheim, I. A., Halseth, C. K., Almli, M., Berg, M., Gagnat, M. R., Wold, P.-A., Attramadal, K., Hagemann, A., Evjemo, J. O., & Kjørsvik, E. (2017). Effect of cultivated copepods (Acartia tonsa) in first-feeding of Atlantic cod (Gadus morhua) and ballan wrasse (Labrus bergylta) larvae. *Aquaculture Nutrition*, *23*(1), 3-17. https://doi.org/https://doi.org/10.1111/anu.12352

Olsen, Y., Rodriguez Rainuzzo, J., Reitan, K. I., & Vadstein, O. (1993). *Manipulation of lipids and ω3 fatty acids in Brachionus plicatilis*. Balkema.

Ørjan Karlsen , Jon Albretsen , Lars Asplin , Pål Arne Bjørn , Thomas Bøhn , Ingrid Askeland Johnsen (IMR) , Gunnar B. Lehmann (NORCE LFI) , Mari Skuggedal Myksvoll , Rune Nilsen , Anne Dagrun Sandvik ,

Rosa Maria Serra-Llinares , Skardhamar, J., & (IMR), a. B. Å. *Effect of salmon lice on wild salmonids (2010-2019)* (ISSN: 1893-4536). (Report from Marine research, Issue. https://www.hi.no/en/hi/nettrapporter/rapport-fra-havforskningen-2020-23

Óscar Monroig 1, JUAN CARLOS NAVARRO , ISABEL AMAT2, PEDRO GONZÁLEZ2, FRANCISCO AMAT1, & FRANCISCOHONTORIA. (2003). Enrichment of Artemia nauplii in PUFA,phospholipids, and water-soluble nutrients usingliposomes.

Aquaculture International, *11*, 151-161. https://www.academia.edu/13538916/Enrichment_of_Artemia_nauplii_in_PUFA_phospholipids_and_water_soluble_nutrients_using_liposomes

Overton, K., Dempster, T., Oppedal, F., Kristiansen, T. S., Gismervik, K., & Stien, L. H. (2019). Salmon lice treatments and salmon mortality in Norwegian aquaculture: a review. *Reviews in aquaculture*, *11*(4), 1398-1417. https://doi.org/10.1111/raq.12299

Parrish, C. C. (2013). Lipids in Marine Ecosystems. *ISRN Oceanography*, *2013*, 604045. https://doi.org/10.5402/2013/604045

Plankton, A. (2022). *Cryoplankton, optimal feed for optimal growth*. https://planktonic.no/

Powell, A., Pooley, C., Scolamacchia, M., & Garcia de Leaniz, C. (2018). *Cleaner fish biology and aquaculture applications (Ed. Jim Treasurer) - Chapter 6. Review of lumpfish biology*.

Powell, A., Treasurer, J., Pooley, C., Keay, A., lloyd, R., Imsland, A., & Garcia de Leaniz, C. (2017). Use of lumpfish for sea-lice control in salmon farming: Challenges and opportunities. *Reviews in aquaculture*, *10*. https://doi.org/10.1111/raq.12194

Powell, A., Treasurer, J. W., Pooley, C. L., Keay, A. J., Lloyd, R., Imsland, A. K., & Garcia de Leaniz, C. (2018). Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. *Reviews in aquaculture*, *10*(3), 683-702. https://doi.org/https://doi.org/10.1111/raq.12194

Prusińska, M., Kushniry, O., Khudyi, O., Khuda, L., & Kolman, R. (2015). Impact of enriching larval brine shrimp (Artemia sp.) with a supplement containing polyunsaturated fatty acids on their growth and mortality. *Archives of Polish Fisheries*, *23*(3), 149-154. https://doi.org/10.1515/aopf-2015-0017

Qian, Y., Li, X.-F., Zhang, D.-D., Cai, D.-S., Tian, H.-Y., & Liu, W.-B. (2015). Effects of Dietary Pantothenic Acid on Growth, Intestinal Function, Anti-Oxidative Status and Fatty Acids Synthesis of Juvenile Blunt Snout Bream Megalobrama amblycephala. *PloS one*, *10*(3), e0119518. https://doi.org/10.1371/journal.pone.0119518

Rainuzzo, J. R., Reitan, K. I., & Olsen, Y. (1997). The significance of lipids at early stages of marine fish: a review. *Aquaculture*, *155*(1), 103-115. https://doi.org/https://doi.org/10.1016/S0044-8486(97)00121-X

Rajkumar, M., & Kumaraguru vasagam, K. P. (2006). Suitability of the copepod, Acartia clausi as a live feed for Seabass larvae (Lates calcarifer Bloch): Compared to traditional live-food organisms with special emphasis on the nutritional value. *Aquaculture*, *261*(2), 649-658. https://doi.org/10.1016/j.aquaculture.2006.08.043

Rian, S. T. (2019a). *Start-feeding of lumpfish (Cyclopterus lumpus L.) larvae with Artemia and copepods, focusing on growth effects, survival, live prey selection and larval robustness*

Rian, S. T. (2019b). Start-feeding of lumpfish (Cyclopterus lumpus L.) larvae with Artemia and copepods, focusing on growth effects, survival, live prey selection and larval robustness. In K. I. Reitan & E. Kjørsvik (Eds.): NTNU.

Risikorapport norsk fiskeoppdrett (2021). https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-2021-8.

Rodríguez, C., Cejas, J. R., Martín, M. V., Badía, P., Samper, M., & Lorenzo, A. (1998). Influence of n-3 highly unsaturated fatty acid deficiency on the lipid composition of broodstock gilthead seabream (Sparus aurata L.) and on egg quality. *Fish Physiology and Biochemistry*, *18*(2), 177-187. https://doi.org/10.1023/A:1007750218840

Rodriguez, C., PEREZ, J. A., IZQUIERDO, M. S., MORA, J., LORENZO, A., & FERNANDEZ-PALACIOS, H. (1994). Essential fatty acid requirements of larval gilthead sea bream, Sparus aurata (L.). *Aquaculture Research*, *25*(3), 295-304. https://doi.org/https://doi.org/10.1111/j.1365-2109.1994.tb00692.x

Rolland, K. H. (2021). *The Salmon Farming Industry in Norway 2021 Report* (KONTALI, Issue. https://www.kontali.no/b/the-salmon-farming-industry-in-norway-2021-report

Romundstad, M. (2015). Effects of different live feed on ballan wrasse (Labrus bergylta) larval hepatocyte and enterocyte development. In E. Kjørsvik & J.-O. Evjemo (Eds.): NTNU.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., & Estevez, A. (1999). Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*, *177*(1), 191-199. https://doi.org/https://doi.org/10.1016/S0044-8486(99)00083-6

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., & Tocher, D. (1999a). Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture*, *179*(1), 217-229. https://doi.org/10.1016/S0044-8486(99)00191-X

Sargent, J., McEvoy, L., Estevez, A., Bell, J. G. B., Bell, M., Henderson, J., & Tocher, D. (1999b). Lipid nutrition of marine fish during early development: Current status and future directions. *Aquaculture*, *179*, 217-229. https://doi.org/10.1016/S0044-8486(99)00191-X

Sargent, J. R. (1995). Origins and functions of egg lipids : nutritional implications.

Saue, O. A. (2017, 24 oct , 2017). New 'traffic lights' system will regulate Norwegian salmon production.

Sayer, M. D. J., & Reader, J. P. (1996). Exposure of goldsinny, rock cook and corkwing wrasse to low temperature and low salinity: survival, blood physiology and seasonal variation. *Journal of fish biology*, *49*(1), 41-63. https://doi.org/https://doi.org/10.1111/j.1095-8649.1996.tb00004.x

Seelig, A., & Seelig, J. (1974). The dynamic structure of fatty acyl chains in a phospholipid bilayer measured by deuterium magnetic resonance. *Biochemistry*, *13*(23), 4839-4845. https://doi.org/10.1021/bi00720a024

Şen Özdemir, N., Parrish, C. C., Parzanini, C., & Mercier, A. (2019). Neutral and polar lipid fatty acids in five families of demersal and pelagic fish from the deep Northwest Atlantic. *ICES journal of marine science*, *76*(6), 1807-1815. https://doi.org/10.1093/icesjms/fsz054

Shields, R. J., Bell, J. G., Luizi, F. S., Gara, B., Bromage, N. R., & Sargent, J. R. (1999). Natural copepods are superior to enriched artemia nauplii as feed for halibut larvae (Hippoglossus hippoglossus) in terms of survival, pigmentation and retinal morphology: relation to dietary essential fatty acids. *J Nutr*, *129*(6), 1186-1194. https://doi.org/10.1093/jn/129.6.1186

Skiftesvik, A., Bjelland, R., Durif, C., Johansen, I., & Browman, H. (2014). Delousing of Atlantic salmon (Salmo salar) by cultured vs. wild ballan wrasse (Labrus bergylta). *Aquaculture*, *s 402–403*, 113–118. https://doi.org/10.1016/j.aquaculture.2013.03.032

Skiftesvik, A. B., Blom, G., Agnalt, A.-L., Durif, C. M. F., Browman, H. I., Bjelland, R. M., Harkestad, L. S., Farestveit, E., Paulsen, O. I., Fauske, M., Havelin, T., Johnsen, K., & Mortensen, S. (2014). Wrasse (Labridae) as cleaner fish in salmonid aquaculture – The Hardangerfjord as a case study. *Marine Biology Research*, *10*(3), 289-300. https://doi.org/10.1080/17451000.2013.810760

Skretting. (2021). *Feed and Services for aquaculture* https://www.skretting.com/en-us/feed-and-services-for-aquaculture/gemma-micro-US65/

Statistisk Sentralbyrå, Statistics Norway. (29 october, 2020). https://www.ssb.no/en/fiskeoppdrett/

Stillwell, W., & Wassall, S. R. (2003). Docosahexaenoic acid: membrane properties of a unique fatty acid. *Chem Phys Lipids*, *126*(1), 1-27. https://doi.org/10.1016/s0009-3084(03)00101-4

Støttrup, J. G., & McEvoy., L. A. (2003). Production and nutritional value of Copepods. Live feeds in marine aquaculture. editor *Oxford : Blackwell Science Ltd*, *5*, pp. 145-206.

T Takeuchi, J. S., T Nomura, T Watanabe. (1990). Lipid contents and classes in gill of masu salmon Oncorhynchus masou during the parr-smolt transformation. *Nippon Suisan Gakkaishi,* (56), 1527-1536.

Teshima Shin-ichi , Akio Kanazawa, Koji Horinouchi, Shigehisa Yamasaki, & Hirata, H. (1987). Phospholipids of the Rotifer, Prawn, and Larval Fish*. *Nippon Suisan Gakkaishi 53(4)*, 609-615.

Tocher, D. R. (2003). Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. *Reviews in fisheries science*, *11*(2), 107-184. https://doi.org/10.1080/713610925

Tocher, D. R. (2010). Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research*, *41*(5), 717-732. https://doi.org/https://doi.org/10.1111/j.1365-2109.2008.02150.x

Tocher, D. R., Bendiksen, E. Å., Campbell, P. J., & Bell, J. G. (2008). The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture*, *280*(1-4), 21-34. https://doi.org/10.1016/j.aquaculture.2008.04.034

Torrecillas, S., Betancor, M. B., Caballero, M. J., Rivero, F., Robaina, L., Izquierdo, M., & Montero, D. (2018). Supplementation of arachidonic acid rich oil in European sea bass juveniles (Dicentrarchus labrax) diets: effects on growth performance, tissue fatty acid profile and lipid metabolism. *Fish Physiol Biochem*, *44*(1), 283-300. https://doi.org/10.1007/s10695-017-0433-5

Towers, L. (2013). *Lumpfish: The Latest Weapon in the Battle Against Sea Lice?* https://thefishsite.com/articles/lumpfish-the-latest-weapon-in-the-battle-against-sea-lice

Treasurer, J. W. (2002). A review of potential pathogens of sea lice and the application of cleaner fish in biological control. *Pest Manag Sci*, *58*(6), 546-558. https://doi.org/10.1002/ps.509

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

van der Meeren, T., Olsen, R. E., Hamre, K., & Fyhn, H. J. (2008). Biochemical composition of copepods for evaluation of feed quality in production of juvenile marine fish. *Aquaculture*, *274*(2-4), 375-397. https://doi.org/10.1016/j.aquaculture.2007.11.041

Van Stappen, & G. (1996). Introduction, biology and ecology of Artemia. In I. F. Rome (Ed.).

von Vaupel, K. (2008). C.-S. Lee, P. J. O'Bryen & N. H. Marcus (eds.), 2005. Copepods in aquaculture. *CR*, *81*(6), 765-766. https://doi.org/10.1163/156854008784513375

Watanabe, T. (1982). Lipid nutrition in fish. *Comparative Biochemistry and Physiology B*, *73*, 3-15.

Yuan, Y., Li, S., Mai, K., Xu, W., Zhang, Y., & Ai, Q. (2015). The effect of dietary arachidonic acid (ARA) on growth performance, fatty acid composition and expression of ARA metabolism-related genes in larval half-smooth tongue sole (Cynoglossus semilaevis). *Br J Nutr*, *113*(10), 1518-1530. https://doi.org/10.1017/s0007114515000781

Yúfera, M. (2011). Feeding behavior in Larval Fish. In *Larval Fish Nutrition* (pp. 283-305). https://doi.org/https://doi.org/10.1002/9780470959862.ch10

Yúfera, M., Kolkovski, S., Fernández, D., x, az, C., Rinchard, J., Lee, K. J., & Dabrowski, K. (2003). Delivering bioactive compounds to fish larvae using microencapsulated diets. *Aquaculture*, *227*(1), 277-291. https://doi.org/10.1016/S0044-8486(03)00509-X

Appendix 1. Outlet mesh size and water exchange rate

Table A1. Outlet mesh size (µm) and water exchange rate (%) in rearing tanks for the different treatments throughout the experimental period (dph).

	Treatments									
	Artemia		Formulateddiet		Copepods		Cirripedia		Cop/Cir	
Dph	Meshsize (µm)	Water exchange rate (%)	Meshsize (µm)	Water exchangerate (%)	Meshsize (µm)	Water exchangerate (%)	Meshsize (µm)	Water exchange rate (%)	Meshsize (µm)	Water exchange rate (%)
0	750	2400	750	2400	100	300	350	1200	100	300
1	750	2400	750	2400	100	300	350	1200	100	300
2	750	2400	750	2400	100	300	350	1200	100	300
3	750	2400	750	2400	100	300	350	1200	100	300
4	750	2400	750	2400	100	300	350	1200	100	300
5	750	2400	750	2400	100	300	350	1200	100	300
6	750	2400	750	2400	100	300	350	1200	100	300
7	750	2400	750	2400	100	300	350	1200	100	300
8	750	2400	750	2400	100	300	350	1200	100	300
9	750	2400	750	2400	100	300	350	1200	100	300
10	750	2400	750	2400	100	300	350	1200	100	300
11	750	2400	750	2400	100	300	350	1200	100	300
12	750	2400	750	2400	100	300	350	1200	100	300
13	750	2400	750	2400	100	300	350	1200	100	300
14	750	2400	750	2400	100	300	350	1200	100	300
15	750	2400	750	2400	100	600	350	1200	100	600
16	750	2400	750	2400	100	600	350	1200	100	600

17	750	2400	750	2400	750	2400	350	1200	350	1200
18	750	2400	750	2400	750	2400	350	1200	350	1200
19	750	2400	750	2400	750	2400	350	1200	350	1200
20	750	2400	750	2400	750	2400	350	1200	350	1200
21	750	2400	750	2400	750	2400	350	2400	350	2400
22	750	2400	750	2400	750	2400	350	2400	350	2400
23	750	2400	750	2400	750	2400	350	2400	350	2400
24	750	2400	750	2400	750	2400	350	2400	350	2400
25	750	2400	750	2400	750	2400	350	2400	350	2400
26	750	2400	750	2400	750	2400	750	2400	750	2400
27	750	2400	750	2400	750	2400	750	2400	750	2400
28	750	2400	750	2400	750	2400	750	2400	750	2400
29	750	2400	750	2400	750	2400	750	2400	750	2400
30	750	2400	750	2400	750	2400	750	2400	750	2400
31	750	2400	750	2400	750	2400	750	2400	750	2400
32	750	2400	750	2400	750	2400	750	2400	750	2400
33	750	2400	750	2400	750	2400	750	2400	750	2400
34	750	2400	750	2400	750	2400	750	2400	750	2400
35	750	2400	750	2400	750	2400	750	2400	750	2400

Appendix 2. Feeding amount

Table A2. The amount of *Artemia* (volume/ml), copepod (ind/ml), cirripedia (ind/ml) andformulated diet (g/day) provided to the tanks.

	Treatments									
	Artemia		FD	Copepods		Cirripedia		Cop/Cir		
Dph	Art(ml vol)	FD (g/day)	FD (g/day)	Cop (ind/ml)	FD (g/day)	Cir (ind/ml)	FD (g/day)	Cop (ind/ml)	Cir (ind/ml)	FD (g/day)
2	20	-	-	30	-	18	-	30	-	-
3	21	-	4g-1s	30	-	24	-	30	-	-
4	22	-	4g-1s	50	-	30	-	50	-	-
5	23	-	6g-1s	50	-	30	-	50	-	-
6	34	-	6g-1s	50	-	42	-	50	-	-
7	36	-	12g-1s	70	-	42	-	70	-	-
8	38	-	12g-1s	70	-	42	-	70	-	-
9	41	-	24g-2s	90	-	54	-	90	-	-
10	50	-	24g-2s	70	6g-1s	54	-	70	54	-
11	54	-	24g-2s	70	6g-1s	60	-	70	60	-
12	59	-	24g-2s	50	12g-1s	60	-	50	60	-
13	63	-	24g-2s	30	12g-1s	60	-	30	60	-
14	68	-	24g-2s	30	24g-2s	72	-	30	72	-
15	74	-	30g-2s	20	24g-2s	72	-	20	72	-
16	80	-	30g-2s	10	30g-2s	78	-	10	78	-
17	86	-	30g-2s	-	30g-2s	78	-	-	78	-
18	93	-	30g-2s	-	30g-2s	78	-	-	78	-
19	101	-	30g-2s	-	30g-2s	78	-	-	78	-
20	109	-	30g-2s	-	30g-2s	78	-	-	78	-

21	176	6g-1s	30g-2s	-	30g-2s	60	6g-1s	-	60	6g-1s
22	152	6g-1s	30g-2s	-	30g-2s	54	6g-1s	-	54	6g-1s
23	123	12g-1s	30g-2s	-	30g-2s	24	12g-1s	-	24	12g-1s
24	89	12g-1s	30g-2s	-	30g-2s	18	12g-1s	-	18	12g-1s
25	48	24g-2s	30g-2s	-	30g-2s	12	24g-2s	-	12	24g-2s
26		24g-2s	30g-2s	-	30g-2s	-	24g-2s	-	-	24g-2s
27		30g-2s	30g-2s	-	30g-2s	-	30g-2s	-	-	30g-2s
28		30g-2s	30g-2s	-	30g-2s	-	30g-2s	-	-	30g-2s
29		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
30		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
31		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
32		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
33		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
34		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s
35		24g-1s	24g-1s	-	24g-1s	-	24g-1s	-	-	24g-1s

Appendix 3. Artemia protocol

Working protocol for preparing and enriching *Artemia*. The protocol is made in accordance with producer recommendations and NTNU Sealab facilities.

PROTOCOL FOR Artemia HATCHING, HARVESTING AND ENRICHMENT – STARTRENS 2020

- *Prepare two incubation cones, screw aeration caps, leave valves open, fill them with **60 L of sea water, plug in heaters and start aerations** (Do this daily - the day before the incubation)*
- *Label the cones and note which tanks you use*
- *NB: Prepare the needed Artemia 2 days before they are going to be fed to the fish larvae*

INCUBATING *Artemia* CYSTS

1. Take out the pre-weighed bag containing Artemia-cysts from the fridge, **see to that you take the correct one**
2. Check water temperature in the cone first (25-29°C), make sure the aeration and heater are on. Then pour the cysts into the cone
3. Gently rinse the cysts into the water, they should not stick on the sides
4. It will take about 24h for the hatching to be complete

HARVESTING *Artemia* NAUPLII

1. **Unplug the heater**
2. Close the bottom valve, THEN close the aeration (which is at the top)
3. Let the system settle for about 5 min
4. Prepare the *Artemia* separator: on a nicely levelled surface, put strainers at the outlet
5. **Remove the heater**
6. Unscrew the aeration-cap on the bottom of the cone
7. **Open and close the valve on the tank quickly** to flush out unhatched cysts at the bottom, if there are any
8. Place a bucket underneath and slowly empty the cone water into the bucket, remember to close the valve when the bucket is full. Don't empty the tank too fast, as high pressure/speed will damage/kill the *Artemia*
9. Take the water+*Artemia* and pour gently into the separator to separate hatched nauplii from empty shells/unhatched cysts
10. Take the strainer containing the separated *Artemia* and rinse it with clean seawater into the second cone with clean seawater (60L, 25-29°C, aerated)

PROTOCOL FOR Artemia HATCHING, HARVESTING AND ENRICHMENT – STARTRENS 2020

11. Continue to empty the cone until it's about 5 cm of water left. This last water you can just flush out (mostly unhatched cysts and are not easily trapped by the magnets)
12. Tip the separator slightly to flush out the last *Artemia* in it and put them in the second cone as well
13. Clean the cone and other equipment (buckets, separator, floor) with warm water and get it ready for use again if needed (60L water, 25-29°C). The aeration-cap at the bottom needs to be rinsed as well before you screw it back on. Make sure the **gasket (round rubber band)** is in place!

1ST ENRICHMENT

1. Take the enrichment (Larviva Multigain) out from the fridge
2. Measure **10g** of the enrichment (Larviva Multigain) in a measuring cup with a spoon
3. Fill ¼ of the measuring cup (with the enrichment in) with luke warm water
4. Stir with a hand-mixer for 3 minutes
5. Remove as much foam as possible with a spoon
6. Rinse the foam which are still around the measuring cup
7. Take it to the other room and empty it into the cone with **the newly hatched *Artemia* nauplii,** rinse the rest with seawater

2ND ENRICHMENT

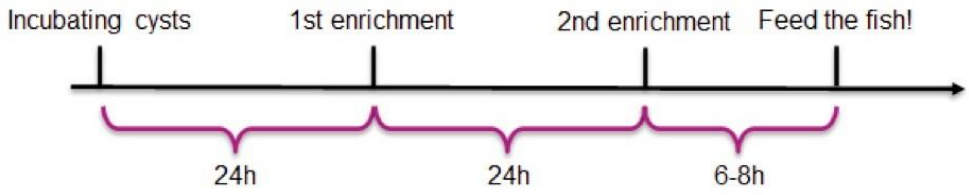
1. See 1st enrichment. Do this early in the morning.

HARVESTING FOR FEEDING THE LARVAE (AFTERNOON AFTER 2ND ENRICHMENT)

1. Prepare two buckets, **first bucket** be marked at a level which indicates for example 5 L
2. Add a small volume of clean seawater (ca 2 L) into the **first bucket** and put it on aeration
3. Add clean sea water to about half in the **second bucket**
4. In the cone with enriched *Artemia,* close the valve at the bottom, the aeration and unplug the heater
5. Wait for about 3 minutes for the heater to cool down, then take it out
6. Put a strainer in the second bucket and slide the bucket under the cone, open the cone to **gently** pour the enriched *Artemia* into the bucket while holding the strainer (the enriched *Atemia* will be soaked in the water but retained in the strainer)
7. Rinse the *Artemia* into the **first bucket**

8. Fill the bucket with clean seawater to the known volume (5 L), increase the aeration to distribute the *Artemia* and take 5 samples of 1 ml each to estimate the density under microscope
9. After estimating the density in for example 5 L **(average density in 1 ml x 5000 ml)**, you will be able to estimate the volume to add in each tank for feeding

TIMELINE



Appendix 4. Producer manual for Cirripedia (Planktonic AS)

Producer manual for thawing, washing and revitalization of Cirripedia nauplii (PlanktonicAS).

Procedure for thawing, washing andrevitalization of CryoPlankton

Versjon nr	Godkjent av
1	03.07.18 Maria Bergvik

Easy method for thawing, washing and revitalization			
Step	**To do**	**Description**	**Notice**
1	Prepare water for revitalization	Decide how much CryoPlankton is needed. Prepare the revitalization water. The water amount should be a minimum of 50 litres per kg CryoPlankton. Fill seawater in the tank and add ice. Use aeration to thaw the ice	Water temperature < 5°C, salinity 24-36 psu, aeration.
2	Prepare water for thawing	Fill the thawing tank with seawater. The water amount should be a minimum of 25 litres per kg CryoPlankton.	Water temperature 5- 12°C (no need for cooling), salinity of 24-36 psu, aeration.
3	Weigh out CryoPlankton	Find a scale, gloves, net, safety glasses and a container to transport the plankton in. Tare the container on the scale, collect the plankton and weigh out the wanted amount. Use safety equipment and protect arms and legs with clothes. Read the material safety data sheet before handling liquid nitrogen.	CryoPlankton that are collected from the thermos should not be transferred back to the thermos. CryoPlankton will not survive the extreme changes in temperature.
4	Thaw CryoPlankton	Add the plankton in the thawing tank with aeration.	The time between taking the plankton out of the thermos until it is transferred to the seawater should not exceed 5 minutes. The vitality of the nauplii could be affected.
5	Wash Cryoplankton	the plankton can be transferred to the net when plankton is thawed. Rinse with sea water for 5 minutes	The Cryo protection agent is important to remove because it is toxic for the fish
6	Revitalize cryoplankton	Transfer the planktonic to the revitalization tank with cold sea water and aeration. Cryoplankton is revitalized after 6 hours and ready to feed to the fish larvae	

Appendix 5. Protocol Copepods (*A.tonsa*)

Working protocol for handling, feeding and harvesting of Copepods (*A.tonsa*). Theprotocol is made in accordance with producer recommendations and NTNU Sealabfacilities.

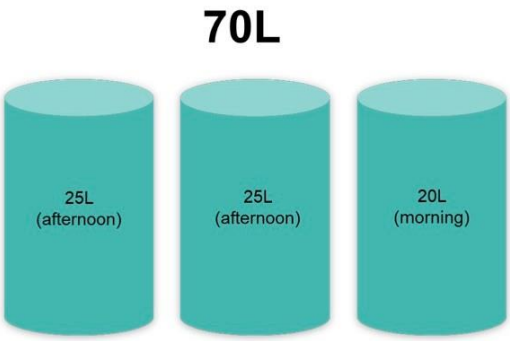
COPEPODS PROTOCOL

*The seawater is warm, to cool it, let it run for a few minutes before using.

AFTERNOON

Harvesting of *Acartia*

1. Check how many litres to filter into each of the 3 buckets (right number for each day will be posted on the wall).
2. Fill all the buckets with some seawater and put aeration before putting the copepods. Filter the right amount into the buckets.
3. When done with filtration, fill up the 3 buckets as show below:



Feeding of *Acartia*

4. Feed the big reservoir with around 20L of microalgae and the “morning” bucket with around 1.5 L.
5. Fill the big reservoir back to 900 L.
6. Around 15 min before feeding to the fish tanks (15:45), feed the “afternoon” buckets with around 2.5 L each. Transport them to CodTech lab with aeration.

MORNING

7. Feed the “morning” bucket with around 2 L of microalgae.
8. Put aeration in the tank to be transported (can use 2 of the microalgae containers with the attached aeration).
9. Transport the bucket to CodTech Lab.
10. Wait around 15 minutes from feeding microalgae, before filling the feeding reservoir to the fish tanks.

Filling the feeding reservoirs

	17h	7h
Normal treatment	8 L/tank	3.3 L/tank
Mix treatment	2.5 L/tank	1.0 L/tank

Appendix 6 Protocol: MTBE lipid extraction

Materials:

- Styrofoam box with ice
- 2 ml plastic tubes with lids
- Ceramic beads
- Precellys tissue homogenizer
- 1.5 ml microcentrifuge tubes
- 12 ml Kimax tubes (3 per sample)
- Plastic digital pipettes with tips
- High precision laboratory scale
- 0.15 M ammonium acetate
- Methanol, LCMS grade (MeOH)
- Methyl tert-butyl ether (MTBE)
- Chloroform ($CHCl_3$)
- SPLASH® II LIPIDOMIX® Mass Spec Standard (from Avanti, delivered by Sigma)

Experimental:

NB! Keep samples on ice between steps.

- Add 6-10 beads to 2 ml plastic tubes
- Tare scale with 2 ml bead-filled tube
- Transfer material to tubes and weigh, noting down weight as m_{sample}
    - For large eggs, roll over a glass surface to remove fluid surrounding eggs (*e.g.* for lumpsucker eggs)
    - For small eggs, transfer them to tubes as they are (*e.g.* Ballan wrasse)
- Add exactly 300 µl ice-cold MeOH
    - Add 5 µl mass spec standard
    - Standard can be mixed with MeOH solution and added together
- Homogenize with homogenizer 2x 30s at 6000 rpm
- Transfer homogenate to glass 12 ml Kimax tube
    - Centrifuge for 30-60 s if material is stuck to tube wall
- Rinse plastic tubes with 162 µl MeOH and transfer to Kimax tube
- Add 1540 µl MTBE to Kimax tube

    - This is 10:3 MTBE:MeOH
- Extract 1 h at room temperature
    - Vortex 30 s after 30 min
- Add 380 µl 0.15 M ammonium acetate
- Incubate 10 min at room temperature
- Centrifuge 10 min @ 1000 *g* 4 °C
- Weigh a new 12 ml Kimax tube, noting down weight as *m1*
- Carefully transfer the **top phase** of the samples to new tube using a digital plastic pipette
- Blow down sample with a gentle nitrogen stream until dry
- Weigh the dried tube, noting down weight as *m2*
- Lipid content is reported as: $Lipid\ content = 100\% \times \frac{m2-m1}{m_{sample}}$
- Re-dissolve in 500 µl $CHCl_3$
    - 250 µl for lipid class
    - 250 µl for lipidomics
- Store at -80 °C

Appendix 7. Analysis of total lipid and fatty acid profile by Folch Method

Folch methode

analysis of total lipid and fatty acid profile

1. Equipment and instruments

1.1 Equipments

- Kimax test-tube 100x15mm with PTFE screwcap (VWR, art.nr. KIML45066A-16100)
- GC-tubes 8mm, chromacol (VWR, art.nr. 548-1208)
- Pasteur pipettes 2mL, 150mm (VWR, art.nr. 612-1701)
- Eppendorf Research automatpipette (100-1000µL)
- Gloves Nitrile (VWR, art.nr. 112-2372)

1.2 Instruments

- Perkin Elmer AutoSystem XL Gasskromatograf (PSS-injektor, PPC FID- detektor, WCOT Fused Silica, coating CP-wax 52CB kapilærkolonne (Holger CP7713))
- Vortex mixer (Heidolph reax top)
- Centrifuge (Hettich universal 32R)
- Weight (Mettler toledo UMX2)
- Weight (Precisa 180A)
- Heating block
- Ultra-turrax T8 (homogenizer)
- Water purification system (Elix5)
- Pipetboy (VWR, art.no. 612-0927)
- Freeze-dryer (room 135)

2. Chemicals, solutions, and gasses

2.1 Chemicals

- Chloroform pro Analysis (VWR, art.nr. 1.02445.1000)
- Methanol pro Analysis (VWR, art.nr. 20847.295)
- Sulfuric Acid
- Isooctane pro Analysis (VWR, art.nr. 1.04727.1000)
- Sodium chloride, NaCl pro Analysis (VWR, art.nr. 1.06404.1000)
- Potassium chloride, KCl pro Analysis
- External standard 68D from NuChecPrep, 4 ampoules a 25mg (Chiron, art.nr:)
- Internal standard 23:0 / 19:0 from NuChecPrep (Chiron, art.nr.)

2.2 Solutions

2.2.1 Chloroform: methanol (2:1)

2 parts of chloroform is mixed with 1 part of methanol.

2.2.2 1% H_2SO_4: methanol (Methylation reagent)

1.0 mL H_2SO_4 is added **to** 99.0 mL methanol

NOTE: solution is made new every time, so adjust the quantity as needed!

2.2.3 Internal standard (IS 23:0)

IS 23: 0 is prepared so that IS added to the sample corresponds to 10% of total lipid (≤50ng / µl injected into GC)

2.2.4 External standard (68D)

Already prepared solutions, se appendix 1 for further details.

2.2.5 Saturated sodium chloride, NaCl

A minimum of 20.g NaCl (s) is mixed with 100mL dH_2O, which gives 20% NaCl (aq)

3. Preparation of equipment and samples

3.1 Equipment

All unclean equipment is soaked in a 5% deconex 15PF bath overnight before its washed in the dishwasher. Use program E, which is followed by a rinsing program with 1xdH_2O (preset).

After proper cleaning the equipment is heated at 400°C for 2 hours.

3.2 Samples

The samples used for lipid content and fatty acid profile should be flushed with nitrogen gas and stored in a -80°C freezer as soon as possible after sampling. All samples should be freeze-dried before analyzed.

4. Description of method

4.1 Measurement

Samples larger than 20 mg are weighed directly into test tubes on Precisa 180A weight, samples less then 20mg on the Mettler toledo UMX2 µg-weight. When using the Mettler toledo, remember to weigh the aluminum foil afterwards.

4.2 Extraction of total lipid

The tissue is homogenized with chloroform: methanol (2:1) to a final volume 20 times the volume of the tissue sample (0,5g sample in 10mL chloroform: methanol). The homogenization is performed by use of Ultra Turrax T8, which have knives of two different sizes.

Start by adding half the volume required. If the mixtures blend well, then use the rest of the solvent. If the mixture doesn't blend well, the sample needs filtration.

- **Filtrate** the solution you already have homogenized, then add more of the solutions to the test tube to clean out the rest of the sample. Use Whatman filter no.1 when you perform the filtration.

Next, add 0,88% KCl (aq) to the solution. The amount required is calculated from the total volume from the previous step. If 10.0 mL solvent is added, then 2.5mL 0,88% KCl should be added to the solution.

Use the vortex mixer to blend the solution properly before placing it on the centrifuge. Use program 4000rpm, 4min and 4°C. The organic phase will now be in the lower part of the tube. Use a glass pipette to collect and transfer the organic phase to the **pre-weight** 15mL test-tube. Make sure the test-tube doesn't have any damages on the top, and wear gloves when handling it. Blow dry the solution under nitrogen and place the tube in the desiccator for 1 hour to dry out further.

- **How to** operate the desiccator: Connect the desiccator to the water hose. Open the water suction on the bench, and **close** the desiccator tap when the vacuum reach 0.8-1.0 bar. Make sure the desiccator tap is closed properly before disconnecting the water hose. Perform in this manner to **avoid** any back splash of water into the desiccator.

Weigh the test-tube after further drying in the desiccator. Remember to wear gloves when handling the test tube.

- **How to** calculate the total lipid:
  Second weighting (mg) – pre-weight (mg) = the amount total lipid in the sample.

Prepare a stock solution of 10mg/ml by resolving the total lipid in chloroform: methanol (2:1).

4.3 Methylation

Add 4.0µl lipid from the stock solution to a new kimax-tubes.

Add 1.0 mL of $CHCl_3$ **w/ISTD** 23:0 and 2.0 mL 1% H_2SO_4: MeOH (methylation reagent) in the given order. Flush the tube with N_2-gas, close with screw cap and incubate on a 50°C heating block over night (16-18t). Tighten the screw cap after 10 minutes.

- **How to** prepare solutions of $CHCl_3$ w/ISTD C23:0:

The amount of internal standard added to the sample should be equal to 10% of the total lipid = Total lipid x 0.10% = the amount of C23:0 required for one sample in 1.0mL chloroform.

Then multiply the required amount of C23:0 with the number of samples you are preparing. Weigh in a bit more of C23:0 then the calculated amount. To find the volume of chloroform needed, divide the amount of C23:0 weighted with the amount required for a single sample.

4.4 After methylation

Take the samples off the heating block and cool them on the bench to room temperature. To wash out the lipid add a total of 5mL saturated NaCl and 6mL isooctane by the following method. Start by adding 5mL of saturated NaCl and 2mL isooctane to the sample. Vortex mix the samples, centrifuge at 4000rpm, 3 min and 4°C. The lipid will be in the upper layer, transfer the layer to a new kimax tube using a glass pipette. Avoid including the lower layer. Repeat the procedure by adding 2 more times the addition of 2.0 mL isooctane, and repeat the procedure with vortex, centrifuge, and separation.

Evaporate the lipid phase to dryness under N_2-gass and resolve it in 40µl isooctane. Transfer the solution to a GC glass including a insert.

Appendix 8. Mean dry weight per tank

Table A3. Mean dry weight in *C.lumpus* from 2-34 dph in each tank with corresponding standard error (± SE). *N* is the total number of larvae.

Dph	Group	Tank	Mean DW ± SE (mg/larva)	N	Dph	Group	Tank	Mean DW ± SE (mg/larva)	N
2	All	-	0.91 ± 0.05	15					
9	Art	1	1.04 ± 0.06	5	29	Art	1	2.92 ± 0.25	15
		7	1.17 ± 0.10	5			7	3.89 ± 0.20	15
		15	0.98 ± 0.04	5			15	3.66 ± 0.21	15
	FD	2	0.90 ± 0.06	5		FD	2	2.27 ± 0.21	15
		10	0.98 ± 0.03	5			10	2.42 ± 0.16	15
		12	0.82 ± 0.07	5			12	2.57 ± 0.11	15
	Cop/FD	6	0.92 ± 0.05	5		Cop/FD	6	2.27 ± 0.17	15
		11	1.02 ± 0.04	5			11	2.10 ± 0.13	15
		13	0.93 ± 0.11	5			13	2.18 ± 0.19	15
	Cir	4	0.80 ± 0.02	5		Cir	4	2.97 ± 0.24	15
		8	0.73 ± 0.11	5			8	3.11 ± 0.23	15
		14	1.01 ± 0.07	5			14	2.97 ± 0.21	15
	Cop/Cir	3	1.05 ± 0.08	5		Cop/Cir	3	2.12 ± 0.28	15
		5	1.04 ± 0.07	5			5	2.19 ± 0.15	15
		9	0.86 ± 0.06	5			9	2.60 ± 0.17	15
15	Art	1	1.73 ± 0.13	5	34	Art	1	4.35 ± 0.24	15
		7	1.68 ± 0.13	5			7	4.11 ± 0.17	15
		15	1.93 ± 0.09	5			15	4.82 ± 0.25	15
	FD	2	0.83 ± 0.06	5		FD	2	3.54 ± 0.23	15
		10	0.90 ± 0.09	5			10	2.96 ± 0.19	15
		12	1.16 ± 0.12	5			12	3.53 ± 0.17	15
	Cop/FD	6	1.07 ± 0.09	5		Cop/FD	6	2.85 ± 0.23	15
		11	1.14 ± 0.08	5			11	2.96 ± 0.24	15

		13	1.09 ± 0.09	5			13	3.23 ± 0.21	15
	Cir	4	1.17 ± 0.08	5		Cir	4	4.70 ± 0.26	15
		8	1.16 ± 0.09	5			8	4.38 ± 0.38	15
		14	1.29 ± 0.08	6			14	4.12 ± 0.38	15
	Cop/Cir	3	1.13 ± 0.07	5		Cop/Cir	3	3.86 ± 0.27	15
		5	1.24 ± 0.05	5			5	3.12 ± 0.29	15
		9	1.13 ± 0.06	5			9	3.16 ± 0.30	15
21	Art	1	3.03 ± 0.12	10					
		7	2.59 ± 0.12	10					
		15	2.59 ± 0.09	10					
	FD	2	1.36 ± 0.09	10					
		10	1.41 ± 0.07	10					
		12	1.65 ± 0.11	10					
	Cop/FD	6	0.93 ± 0.05	10					
		11	1.38 ± 0.09	10					
		13	1.18 ± 0.14	10					
	Cir	4	1.90 ± 0.12	10					
		8	1.90 ± 0.15	10					
		14	1.73 ± 0.09	10					
	Cop/Cir	3	1.96 ± 0.14	10					
		5	1.90 ± 0.07	10					
		9	1.51 ± 0.05	10					

Appendix 9. Daily weight increase (DWI)

Table A4. Daily weight increase in *C.lumpus* on different dph interval in each tank.

Dph interval	Group	Tank	DWI (%/day)	Dph interval	Group	Tank	DWI (&/day)
2-9	Art	1	1.97	21-34	Art	1	2.82
		7	3.73			7	3.63
		15	1.10			15	4.87
	FD	2	-0.10		FD	2	7.60
		10	1.12			10	5.87
		12	-1.40			12	6.05
	Cop/ FD	6	0.27		Cop/ FD	6	9.03
		11	1.66			11	6.06
		13	0.34			13	8.07
	Cir	4	-1.75		Cir	4	7.23
		8	-3.02			8	6.62
		14	1.59			14	6.90
	Cop/ Cir	3	2.10		Cop/ Cir	3	5.34
		5	2.05			5	3.87
		9	-0.65			9	5.86
9-21	Art	1	9.35	2-34	Art	1	5.03
		7	6.85			7	4.85
		15	8.48			15	5.36
	FD	2	3.54		FD	2	4.35
		10	3.10			10	3.94
		12	5.97			12	4.35
	Cop/	6	0.04		Cop/	6	3.65

	FD	11	2.56		FD	11	3.77
		13	2.01			13	4.05
	Cir	4	7.47		Cir	4	5.28
		8	8.31			8	5.05
		14	4.58			14	4.85
	Cop/	3	5.37		Cop/	3	4.64
	Cir	5	5.14		Cir	5	3.94
		9	4.75			9	3.99

Appendix 10. Mean standard length per tank

Table A5. Mean standard length in *C.lumpus* from 2-34 dph in each tank with corresponding standard error (± SE). *N* is the total number of larvae.

Dph	Group	Tank	Mean DW ± SE (mg/larva)	N	Dph	Group	Tank	Mean DW ± SE (mg/larva)	N
2	All	-	5.94 ± 0.10	15					
9	Art	1	6.39 ± 0.26	5	29	Art	1	8.51 ± 0.20	15
		7	6.78 ± 0.16	5			7	9.29 ± 0.13	15
		15	6.62 ± 0.07	5			15	9.04 ± 0.16	15
	FD	2	6.49 ± 0.11	5		FD	2	7.70 ± 0.26	15
		10	6.61 ± 0.07	5			10	8.01 ± 0.16	15
		12	6.34 ± 0.13	5			12	8.13 ± 0.13	15
	Cop/FD	6	6.65 ± 0.14	5		Cop/FD	6	7.80 ± 0.18	15
		11	6.44 ± 0.08	5			11	7.66 ± 0.15	15
		13	6.61 ± 0.15	5			13	7.71 ± 0.19	15
	Cir	4	6.60 ± 0.05	5		Cir	4	8.54 ± 0.20	15
		8	6.25 ± 0.21	5			8	8.51 ± 0.19	15
		14	6.46 ± 0.12	5			14	8.43 ± 0.19	15
	Cop/Cir	3	6.61 ± 0.10	5		Cop/Cir	3	7.62 ± 0.27	15
		5	6.40 ± 0.15	5			5	7.70 ± 0.15	15
		9	6.50 ± 0.08	5			9	8.08 ± 0.15	15
15	Art	1	7.87 ± 0.13	5	34	Art	1	9.26 ± 0.13	15
		7	7.51 ± 0.11	5			7	9.19 ± 0.11	15
		15	7.79 ± 0.07	5			15	9.54 ± 0.15	15
	FD	2	6.59 ± 0.16	5		FD	2	8.57 ± 0.17	15
		10	6.77 ± 0.12	5			10	8.23 ± 0.17	15
		12	7.05 ± 0.15	5			12	8.76 ± 0.14	15
	Cop/FD	6	7.04 ± 0.04	5		Cop/FD	6	8.09 ± 0.18	15
		11	7.04 ± 0.16	5			11	8.28 ± 0.19	15

		13	7.14 ± 0.12	5			13	8.46 ± 0.15	15
	Cir	4	7.08 ± 0.08	5		Cir	4	9.25 ± 0.15	15
		8	6.80 ± 0.39	5			8	9.14 ± 0.25	15
		14	7.37 ± 0.15	6			14	9.04 ± 0.26	15
	Cop/Cir	3	7.24 ± 0.19	5		Cop/Cir	3	8.78 ± 0.19	15
		5	7.40 ± 0.14	5			5	8.22 ± 0.21	15
		9	7.16 ± 0.07	5			9	8.29 ± 0.24	15
21	Art	1	8.62 ± 0.08	10					
		7	8.12 ± 0.11	10					
		15	8.28 ± 0.09	10					
	FD	2	7.37 ± 0.11	10					
		10	7.41 ± 0.10	10					
		12	7.46 ± 0.09	10					
	Cop/FD	6	6.94 ± 0.07	10					
		11	7.27 ± 0.11	10					
		13	7.24 ± 0.15	10					
	Cir	4	7.79 ± 0.16	10					
		8	7.80 ± 0.15	10					
		14	7.68 ± 0.10	10					
	Cop/Cir	3	7.85 ± 0.08	10					
		5	7.91 ± 0.08	10					
		9	7.57 ± 0.07	10					

Appendix 11. Mean standard length of 250 larvae per tank

Table A6. Mean standard length in *C.lumpus* on 35 dph in each tank with corresponding standard error (± SE). *N* is the total number of larvae.

Dph	Group	Tank	Mean DW ± SE (mg/larva)	N
35	Art	1	9.29 ± 0.04	250
		7	9.25 ± 0.04	250
		15	9.34 ± 0.04	250
	FD	2	8.67 ± 0.04	250
		10	8.39 ± 0.04	250
		12	8.55 ± 0.03	250
	Cop/FD	6	8.17 ± 0.04	250
		11	8.54 ± 0.04	250
		13	8.42 ± 0.04	250
	Cir	4	9.05 ± 0.04	250
		8	8.79 ± 0.05	250
		14	8.95 ± 0.04	250
	Cop/Cir	3	8.70 ± 0.05	250
		5	8.34 ± 0.05	250
		9	8.46 ± 0.04	250

Appendix 12. Number of lumpfish larvae per tank

Table A7. Estimated number of lumpfish larvae alive in each tank from 2-35 dph. The estimationis based on the remaining larvae left at 35 dph, sampled larvae and registered dead larvae.

	Treatments								
	Artemia			FD			Cop/FD		
	Tank								
Dph	1	7	15	2	10	12	6	11	13
2	6663	7017	4361	9314	5923	5680	6044	6982	3570
3	6610	7000	4361	9276	5897	5655	5993	6982	3570
4	6610	7000	4341	9267	5875	5544	5993	6831	3545
5	6594	6999	4341	9264	5863	5538	5911	6805	3483
6	6591	6995	4338	9258	5859	5524	5876	6777	3432
7	6590	6992	4335	9251	5851	5519	5863	6740	3411
8	6586	6990	4332	9249	5850	5517	5854	6732	3409
9	6572	6971	4330	9230	5848	5515	5813	6709	3378
10	6567	6971	4324	9224	5848	5512	5788	6686	3355
11	6565	6963	4324	9217	5842	5510	5781	6681	3353
12	6565	6958	4323	9209	5832	5509	5776	6678	3350
13	6564	6958	4323	9204	5819	5506	5768	6648	3347
14	6562	6953	4321	9199	5804	5489	5761	6630	3347
15	6562	6947	4321	9192	5793	5476	5752	6601	3347
16	6559	6940	4317	9165	5732	5459	5737	6537	3325
17	6557	6933	4312	9149	5732	5442	5728	6506	3294
18	6554	6927	4306	9109	5715	5423	5722	6498	3286
19	6552	6925	4304	9091	5701	5421	5692	6486	3273
20	6550	6923	4296	9082	5662	5409	5659	6466	3264

21	6528	6920	4290	9073	5632	5389	5606	6448	3260
22	6520	6920	4282	9066	5596	5370	5590	6427	3254
23	6516	6909	4278	9057	5543	5353	5569	6391	3232
24	6493	6888	4263	9047	5531	5352	5520	6364	3219
25	6465	6861	4243	9038	5514	5351	5473	6317	3204
26	6434	6831	4231	9030	5463	5326	5403	6268	3179
27	6401	6819	4215	9026	5424	5323	5347	6224	3161
28	6389	6791	4207	9019	5386	5313	5291	6182	3126
29	6383	6778	4204	9013	5295	5310	5231	6121	3103
30	6378	6764	4196	9004	5236	5299	5176	6112	3090
31	6378	6748	4193	8996	5184	5298	5156	6092	3080
32	6363	6725	4183	8983	5115	5295	5121	6080	3071
33	6357	6705	4178	8967	5079	5295	5103	6078	3068
34	6351	6695	4172	8961	5046	5295	5079	6075	3065
35	6347	6686	4171	8958	5028	5292	5070	6070	3064

Appendix 13. Total lipids(% of dry weight) and Fatty acids of the different feeds used in the start feeding experiment with Lump sucker

Fatty acids of the different feeds used in the start feeding experiment with Ballan wrasse. Fatty acids are expressed as % of total fatty acids +/− standard deviation in brackets. Total lipids (last row) are expressed as % of dry weight.

	Artemia(Art)	Cirripeds(Cir)	Copepod (n4 - n6)	Formulated diet
C14:0	1.45 (0.22)	1.59 (0.12)	1.97 (0.38)	2.48 (—)
C14:1n5	0 (0)	0.05 (0.01)	0.02 (0.03)	0.06 (—)
C15:0	0.12 (0.02)	0.28 (0.13)	0.2 (0.15)	0.08 (—)
C16:0	16.95 (0.32)	14.62 (0.8)	12.77 (2.39)	18.9 (—)
C16:1n5	0.26 (0.01)	0.04 (0.08)	0 (0)	0.33 (—)
C16:1n7	5.91 (0.46)	3.86 (0.24)	1.35 (1.7)	3.34 (—)
C16:1n9	0.21 (0.05)	0.17 (0.11)	0.39 (0.2)	0.36 (—)
C17:0	1.24 (0.08)	0.33 (0.02)	0.22 (0.09)	0.33 (—)
C17:1n7	0.2 (0.02)	0 (0)	0 (0)	0.18 (—)
C18:0	5.09 (0.32)	3.59 (0.5)	4.2 (0.43)	3.62 (—)
C18:1n7	8.02 (0.63)	9.14 (0.45)	4.17 (4.1)	3.38 (—)
C18:1n9	13.52 (0.46)	6.08 (0.25)	2.28 (2.91)	14.43 (—)
C18:2n6	5.46 (0.18)	0.9 (0.06)	7.01 (4.78)	26.85 (—)
C18:3n3	4.91 (0.4)	0.66 (0.04)	7.16 (4.38)	3.24 (—)
C18:3n6	0.33 (0.04)	0.15 (0.04)	0.36 (0.3)	0.09 (—)
C18:4n3	0.02 (0.03)	0.9 (0.61)	2.04 (1.76)	0 (—)
C20:0	0.19 (0.01)	0.12 (0.01)	0.17 (0.02)	0.35 (—)
C20:1n9	0.44 (0.09)	2.71 (0.13)	0.92 (1.49)	2.13 (—)
C20:2n6	0.15 (0)	0.62 (0.02)	1.54 (0.58)	0.26 (—)

C20:3n3	0.18 (0)	0.2 (0.01)	0.3 (0.08)	0.16 (—)
C20:4n3	0 (0)	0.28 (0.19)	2.73 (4.69)	0 (—)
C20:4n6 (ARA)	4.23 (1.93)	1.25 (0.03)	1.61 (0.25)	0.99 (—)
C20:5n3(EPA)	12.55 (1.03)	31.92 (1.56)	17.7 (10.79)	6.97 (—)
C22:0	0.36 (0.06)	0.11 (0.01)	0.38 (0.17)	0.26 (—)
C22:1n9	0.03 (0)	0.17 (0.02)	0.09 (0.09)	0.07 (—)
C22:5n3	0 (0)	0.36 (0.24)	0.48 (0.33)	0 (—)
C22:5n6	0.03 (0.02)	0.18 (0.12)	0.83 (0.81)	0 (—)
C22:6n3(DHA)	18.08 (0.31)	19.35 (0.49)	28.71 (10.58)	10.8 (—)
C24:1	0.08 (0.01)	0.37 (0.08)	0.38 (0.05)	0.35 (—)
Sum unsaturated fatty acids	25.4 (0.81)	20.64 (1.39)	19.91 (2.54)	26.02 (—)
Sum saturated fatty acids	74.6 (0.81)	79.36 (1.39)	80.09 (2.54)	73.98 (—)
Sum n3 fatty aicds	35.73 (1.14)	53.66 (2.33)	59.13 (7.51)	21.17 (—)
Sum n6 fatty aicds	10.21 (1.82)	3.11 (0.07)	11.35 (5.57)	28.19 (—)
EPA/DHA	0.69 (0.05)	1.65 (0.04)	0.9 (1.06)	0.65 (—)
TOTAL LIPIDS	21.28(1.82)	9.56(0.95)	9.14(0.74)	16.4

Appendix 14. Total lipids and dry weight of Lumpsucker larvae fed with 5 feeding regimes

Treatment	Rep	dph	Dry weight (mg)	Total lipid content (mg)	% Lipid content
FD	2	2	4.88	0.17	3.50%
FD	3	2	(-)	(-)	(-)
FD	1	9	5.94	0.82	13.80%
FD	2	9	7	0.97	13.90%
FD	3	9	5.1	0.53	10.40%
FD	1	15	5.02	0.31	6.20%
FD	2	15	7.49	0.92	12.30%
FD	3	15	6.97	1.05	15.10%
FD	1	21	12.15	1	8.20%
FD	2	21	9.36	0.69	7.40%
FD	3	21	11.9	1.38	11.60%
FD	1	29	15.78	1.43	9.10%
FD	2	29	16.74	1.78	10.60%
FD	3	29	16.02	1.13	7.10%
FD	1	34	27.16	2.67	9.80%
FD	2	34	33.38	4.26	12.80%
FD	3	34	28.03	2.15	7.70%
Art/FD	1	2	4.84	0.89	18.40%
Art/FD	2	2	(-)	(-)	(-)
Art/FD	3	2	(-)	(-)	(-)
Art/FD	1	9	7.54	1.98	26.30%
Art/FD	2	9	7.59	1.9	25.00%
Art/FD	3	9	7.77	1.54	19.80%
Art/FD	1	15	6.21	1.44	23.20%
Art/FD	2	15	11.22	2.53	22.50%
Art/FD	3	15	10.62	2.65	25.00%
Art/FD	1	21	27.74	4.41	15.90%
Art/FD	2	21	26.46	6.72	25.40%
Art/FD	3	21	17.17	2.64	15.40%
Art/FD	1	29	24.23	2.94	12.10%
Art/FD	2	29	20.04	2.52	12.60%
Art/FD	3	29	41.9	5.12	12.20%
Art/FD	1	34	52.69	5.87	11.10%
Art/FD	2	34	34.46	6.96	20.20%
Art/FD	3	34	36.51	5.14	14.10%
Cop/FD	1	2	5.61	1.58	28.20%
Cop/FD	2	2	(-)	(-)	(-)
Cop/FD	3	2	(-)	(-)	(-)
Cop/FD	1	9	5.98	1.41	23.60%
Cop/FD	2	9	7.49	1.45	19.40%
Cop/FD	3	9	8.37	1.82	21.70%

Cop/FD	1	15	6.62	1.29	19.50%
Cop/FD	2	15	7.37	0.87	11.80%
Cop/FD	3	15	7.67	1.04	13.60%
Cop/FD	1	21	9.67	1.23	12.70%
Cop/FD	2	21	8.17	0.88	10.80%
Cop/FD	3	21	10.59	1.23	11.60%
Cop/FD	1	29	15.6	1.7	10.90%
Cop/FD	2	29	13.26	3.07	23.20%
Cop/FD	3	29	18.88	2.23	11.80%
Cop/FD	1	34	27.33	9.71	35.50%
Cop/FD	2	34	34.37	4.64	13.50%
Cop/FD	3	34	20.45	2.15	10.50%
Cir	1	2	6.28	1.53	24.40%
Cir	2	2	16.16	2.32	14.40%
Cir	3	2	(-)	(-)	(-)
Cir	1	9	7.4	2.82	38.10%
Cir	2	9	7.43	1.66	22.30%
Cir	3	9	7.56	2.09	27.60%
Cir	1	15	6.77	0.95	14.00%
Cir	2	15	8	1.59	19.90%
Cir	3	15	7.59	1.45	19.10%
Cir	1	21	12.77	1.22	9.60%
Cir	2	21	15.11	1.8	11.90%
Cir	3	21	14.01	1.48	10.60%
Cir	1	29	24.79	10.03	40.50%
Cir	2	29	13.99	2.01	14.40%
Cir	3	29	10.17	1.39	13.70%
Cir	1	34	32.87	4.15	12.60%
Cir	2	34	19.38	2.56	13.20%
Cir	3	34	35.62	8.22	23.10%
Cop/Cir	1	2	6.69	1.94	29.00%
Cop/Cir	2	2	(-)	(-)	(-)
Cop/Cir	3	2	(-)	(-)	(-)
Cop/Cir	1	9	6.04	2.08	34.40%
Cop/Cir	2	9	4.16	1.95	46.90%
Cop/Cir	3	9	7.39	1.58	21.40%
Cop/Cir	1	15	9.94	2.07	20.80%
Cop/Cir	2	15	6.3	1.39	22.10%
Cop/Cir	3	15	4.46	0.97	21.70%
Cop/Cir	1	21	14.66	1.4	9.50%
Cop/Cir	2	21	5.88	0.95	16.20%
Cop/Cir	3	21	9.97	1.83	18.40%
Cop/Cir	1	29	18.23	4.28	23.50%
Cop/Cir	2	29	13.96	4.38	31.40%
Cop/Cir	3	29	13.37	2.5	18.70%
Cop/Cir	1	34	(-)	(-)	(-)

Cop/Cir	2	34	23.9	14.92	62.40%
Cop/Cir	3	34	9.63	0.81	8.40%

Appendix 15. Fatty acids of lumpsucker larvae fed with Cirripedia(Cir)

Fatty acids of lumpsucker larvae fed with Cirripedia (Cir). Fatty acids are in % of total fatty acids and SD is +/- standad deviation										
Age(days)	2	± SE	9	± SE	15	± SE	21	± SE	29	± SE
C14:0	0.9960	0.1765	0.8889	0.0902	0.5329	0.3239	0.9719	0.0963	0.7810	0.0429
C15:0	0.3341	0.0317	0.3471	0.0217	0.3852	0.2186	0.3260	0.0031	0.2788	0.0429
C16:0	15.9632	0.7220	14.9719	0.1417	8.8262	5.9100	18.4201	0.4294	15.8523	1.0007
C17:0	0.2665	0.0453	0.3393	0.0443	0.6069	0.0857	0.3273	0.0102	0.2614	0.0165
C18:0	5.5660	0.6582	5.3066	0.0349	3.7243	2.7056	5.8523	0.0401	5.1827	0.5679
C20:0	0.1149	0.0220	0.0773	0.0083	0.6721	0.3654	0.0575	0.0074	0.1410	0.0458
C21:0	0.0891	0.0000	0.1967	0.0000	0.4247	0.0292	0.0000	0.0000	0.1907	0.0000
C22:0	0.6682	0.1638	0.9615	0.4333	0.6694	0.5395	0.7929	0.1685	0.5032	0.0158
C14:1n5	0.0000	0.0000	0.0000	0.0000	0.1659	0.1332	0.0000	0.0000	0.0468	0.0000
C16:1n9	0.2625	0.0000	0.4265	0.0915	0.0334	0.0000	0.0000	0.0000	0.2711	0.0382
C16:1n7	1.1109	0.1649	1.0988	0.1155	0.4883	0.2274	1.6152	0.1464	1.1698	0.2089
C16:1n5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0862	0.0000
C17:1n7	0.1613	0.0122	0.2614	0.0485	0.0000	0.0000	0.1798	0.0308	0.2249	0.0195
C18:1n9	11.8478	1.3577	12.8419	1.6076	5.3054	3.6509	10.6198	0.2205	10.4635	0.2813
C18:1n7	2.5715	0.2332	3.1002	0.3455	1.6103	1.3178	6.1490	0.5229	2.6370	0.6824
C18:3n4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C20:1n9	1.4640	0.6498	2.1287	0.5810	0.6997	0.4128	1.0739	0.1181	1.1105	0.1467
C20:1n7	0.0000	0.0000	0.4161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C20:3n4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:1n11	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:1n9	0.2528	0.0747	0.3591	0.0176	0.1938	0.0000	0.1368	0.0157	0.1682	0.0070
C24:1	0.9253	0.5743	0.2879	0.1117	0.1723	0.0628	0.2330	0.0145	0.4769	0.0408
C16:4n3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C18:3n3	1.1107	0.6181	0.4294	0.1208	0.5263	0.0734	0.2339	0.0044	1.4798	0.0454
C18:4n3	0.4430	0.0669	0.5334	0.1898	1.8881	0.3700	0.3629	0.0008	0.5777	0.1537
C20:3n3	0.0599	0.0049	0.0928	0.0000	0.5331	0.2068	0.0000	0.0000	0.0728	0.0021
C20:4n3	0.3784	0.2166	0.5060	0.1638	0.4304	0.2125	0.7604	0.0284	0.3472	0.1031
C20:5n3 (EPA)	10.0582	2.8418	13.2007	1.3772	6.1708	4.5555	16.5871	0.2218	8.0634	1.4566
C22:5n3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:6n3 (DHA)	19.5531	1.2315	23.0626	2.5241	12.0811	8.0379	22.7881	2.1026	19.3363	0.6571
C18:2n6	10.5307	9.7259	1.0609	0.2863	0.4544	0.1889	0.6772	0.0473	16.0972	0.6349
C18:3n6	0.5926	0.0000	0.6974	0.2098	0.0440	0.0000	0.6444	0.0987	0.5247	0.0000
C20:2n6	0.2158	0.0321	0.2009	0.0554	0.1371	0.0000	0.1988	0.0161	0.2637	0.0315
C20:3n6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C20:4n6 (ARA)	0.9178	0.2865	0.9861	0.0024	1.8788	0.0516	1.2344	0.0599	1.5261	0.1635
C22:5n6	2.2052	0.5887	1.2461	0.9744	1.7953	1.3975	2.0697	0.3498	1.6127	0.0712
Σ SFA	23.9980	1.9615	23.0891	1.8307	15.8415	1.0546	26.7481	2.2595	23.1911	1.9457

Σ MUFA	18.5962	0.0000	20.9205	0.3330	8.6690	0.4798	20.0074	0.5531	16.6548	0.4310
Σ PUFA (n-6)	14.4620	1.6547	4.1914	0.2041	4.3097	0.3598	4.8244	0.3078	20.0244	2.5662
Σ PUFA (n-3)	31.6034	2.5362	37.8248	3.0707	21.6299	1.5228	40.7324	3.2398	29.8772	2.4274
Σ FA	88.6595	0.9002	86.0258	0.9627	50.4501	0.4961	92.3123	1.0387	89.7475	0.9316
(n-3) / (n-6)	2.1853	(-)	9.0244	(-)	5.0189	(-)	8.4430	(-)	1.4920	(-)
DHA/EPA	1.9440	(-)	1.7471	(-)	1.9578	(-)	1.3738	(-)	2.3980	(-)
EPA/ARA	10.9589	(-)	13.3868	(-)	3.2844	(-)	13.4373	(-)	5.2837	(-)

Appendix 16. Fatty acids of lumpsucker larvae fed with Formulated diet (FD)

Fatty acids of lumpsucker larvae fed with Formulated diet(FD) from 2dph to 34 dph. Fatty acids are in % of total fatty acids and SD is +/- standad deviation

	2	± SE	9	± SE	15	± SE	21	± SE	34	± SE
C14:0	0.46	-0.08	0.9517	0.1042	0.6883	0.0500	0.8658	0.0018	0.7801	0.0038
C15:0	0.21	0.01	0.3343	0.0011	0.4131	0.0971	0.3089	0.0512	0.2532	0.0211
C16:0	14.27	0.19	16.3680	0.2875	13.6047	0.1019	16.2010	0.2920	16.7397	0.0533
C17:0	0.26	0.03	0.4100	0.0288	0.2858	0.0000	0.2303	0.0001	0.2349	0.0197
C18:0	4.87	0.06	5.0305	0.0106	6.6622	0.6542	5.2476	0.1011	4.8667	0.1077
C20:0	0.12	0.01	0.0627	0.0040	0.1128	0.0000	0.1505	0.0318	0.1052	0.0016
C21:0	0.12	0.00	0.0000	0.0000	0.2075	0.0000	0.2178	0.0361	0.0664	0.0000
C22:0	0.51	0.11	0.3647	0.0088	1.2742	0.2126	0.6859	0.3429	0.3186	0.0153
C24:0		0.00		0		0.00		0.00		0
C14:1n5	0.00	0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C16:1n9	0.25	0.03	0.4438	0.0069	0.3261	0.0267	0.2433	0.0247	0.2826	0.0219
C16:1n7	1.13	0.02	1.1916	0.0367	0.7508	0.0843	1.1858	0.0732	1.3344	0.0493
C16:1n5	0.07	0.02	0.0000	0.0000	0.0000	0.0000	0.0899	0.0000	0.0000	0.0000
C17:1n7	0.18	0.02	0.2831	0.0060	0.1571	0.0395	0.1607	0.0035	0.2090	0.0290
C18:1n9	10.99	0.32	13.6435	0.0094	10.6061	1.4975	11.0394	1.0240	11.2461	0.2869
C18:1n7	2.62	0.08	3.0427	0.0041	2.1393	0.2596	2.0488	0.1515	2.0740	0.0248
C18:3n4	0.00	0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C20:1n9	1.10	0.04	2.3466	0.0024	1.6392	0.0940	1.0876	0.2083	1.0267	0.0638
C20:1n7	0.00	0.00	0.3001	0.0124	0.0000	0.0000	0.1082	0.0000	0.0734	0.0000
C20:3n4	0.00	0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:1n11	0.00	0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:1n9	0.23	0.02	0.3198	0.0084	0.3593	0.1014	0.1098	0.0235	0.1793	0.0617
C24:1	0.63	0.08	0.2901	0.0032	0.2538	0.0300	0.3661	0.0440	0.4608	0.1020
C16:4n3	0.00	0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C18:3n3	1.67	0.06	0.5261	0.0043	0.4210	0.1353	1.6418	0.0714	1.8948	0.0340
C18:4n3	0.43	0.02	0.7038	0.0026	0.4836	0.1280	0.5811	0.1346	0.4295	0.0037
C20:3n3	0.09	0.00	0.0942	0.0061	0.0000	0.0000	0.1035	0.0243	0.0750	0.0030
C20:4n3	0.21	0.02	0.6524	0.0042	0.3812	0.0392	0.3099	0.0254	0.2002	0.0059
C20:5n3(EPA)	7.99	0.31	16.5060	0.0088	9.7432	1.4962	7.0523	0.6639	6.9864	0.1327
C22:5n3	0.00	0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:6n3(DHA)	19.98	0.69	26.3396	0.0133	21.2857	3.1428	18.4336	1.2412	18.1686	0.1326
C18:2n6	20.43	0.62	1.0220	0.0103	1.4792	0.2017	19.3315	1.1089	23.6670	0.6087
C18:3n6	0.30	0.04	0.5401	0.0397	0.9611	0.2547	0.2305	0.0000	0.1622	0.0000
C20:2n6	0.36	0.01	0.2448	0.0039	0.1761	0.0539	0.2871	0.0223	0.3305	0.0041
C20:3n6	0.00	0.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C20:4n6(ARA)	1.44	0.01	0.9503	0.0040	1.0347	0.1802	1.4394	0.0296	1.5583	0.0213
C22:5n6	2.13	0.39	0.2880	0.0001	3.7993	0.6418	2.9074	1.4433	1.5910	0.0983

Σ SFA	20.81	1.76	23.5218	2.0061	23.2487	1.7130	23.9077	1.9821	23.3648	2.0551
Σ MUFA	17.18	0.78	21.8613	0.9629	16.2317	0.7480	16.4395	0.7760	16.8862	0.7901
Σ PUFA (n-6)	24.66	3.28	3.0452	0.1670	7.4503	0.5597	24.1960	3.0919	27.3090	3.8337
Σ PUFA (n-3)	30.37	2.50	44.8222	3.5765	32.3147	2.7322	28.1223	2.2906	27.7544	2.2616
Σ FA	93.01	1.00	93.2505	1.0989	79.2454	0.8569	92.6655	0.9720	95.3145	1.0571
(n-3) / (n-6)	1.23	(-)	14.7190	(-)	4.3374	(-)	1.1623	(-)	1.0163	(-)
DHA/EPA	2.50	(-)	1.5958	(-)	2.1847	(-)	2.6138	(-)	2.6006	(-)
EPA/ARA	5.54	(-)	17.3684	(-)	9.4163	(-)	4.8994	(-)	4.4834	(-)

Appendix 17. Fatty acids of lumpsucker larvae fed with Artemia (Art)

Fatty acids of lumpsucker larvae fed with Artemia(Art) from 2dph to 34 dph. Fatty acids are in % of total fatty acids and SD is +/- standad deviation										
Age (dph)	**9**	**± SE**	**15**	**± SE**	**21**	**± SE**	**29**	**± SE**	**34**	**± SE**
C14:0	1.0087	0.0197	0.9317	0.0672	0.8034	0.0845	0.7968	0.0270	0.8377	0.0359
C15:0	0.3517	0.0262	0.3622	0.0180	0.3667	0.0167	0.3311	0.0134	0.2758	0.0081
C16:0	14.5466	0.4363	14.1271	0.4176	13.2232	0.1307	14.3801	1.3342	16.3782	0.1076
C17:0	0.3270	0.0810	0.4610	0.0350	0.7152	0.0323	0.4903	0.1178	0.4259	0.0214
C18:0	5.5632	0.3192	5.6274	0.2698	5.8335	0.1513	5.1960	0.1241	4.7429	0.1608
C20:0	0.0964	0.0098	0.0717	0.0128	0.0982	0.0067	0.0956	0.0037	0.0907	0.0097
C21:0	0.2971	0.0000	0.2953	0.0344	0.1815	0.0039	0.1954	0.0169	0.1039	0.0122
C22:0	0.3229	0.0695	0.3007	0.0213	0.2880	0.0189	0.3069	0.0236	0.2205	0.0239
C14:1n5	0.0424	0.0224	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0106	0.0013
C16:1n9	0.4125	0.0100	0.3684	0.0185	0.4761	0.0443	0.0000	0.0000	0.3598	0.0387
C16:1n7	1.5643	0.0574	2.2174	0.3028	3.6352	0.1214	2.4857	0.4966	2.3001	0.0729
C16:1n5	0.0000	0.0000	0.1721	0.0000	0.0000	0.0000	0.0000	0.0000	0.1649	0.0000
C17:1n7	0.3491	0.0299	0.4884	0.0455	0.8353	0.0334	0.6269	0.1960	0.5199	0.0390
C18:1n9	13.4122	0.4720	13.8364	0.1157	13.4477	0.0871	10.7892	0.2654	12.0629	0.1081
C18:1n7	3.6636	0.1541	4.9948	0.4118	7.5236	0.0104	5.5060	1.3014	4.4784	0.2339
C18:3n4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C20:1n9	2.0839	0.2605	1.7515	0.0483	0.9242	0.0057	0.6819	0.1839	0.8666	0.0328
C20:1n7	0.3367	0.0000	0.2641	0.0104	0.0000	0.0000	0.0000	0.0000	0.0879	0.0000
C20:3n4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:1n11	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:1n9	0.3986	0.1279	0.2604	0.0151	0.1994	0.0116	0.0873	0.0113	0.1134	0.0092
C24:1	0.2443	0.0482	0.3111	0.0156	0.2881	0.0193	0.2679	0.0849	0.3572	0.0445
C16:4n3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C18:3n3	0.9028	0.0216	1.3896	0.1960	2.6083	0.0034	1.7925	0.0071	2.0315	0.0449
C18:4n3	0.6982	0.1444	0.6880	0.1137	0.5052	0.0126	0.4182	0.0285	0.4202	0.0197
C20:3n3	0.0979	0.0233	0.1313	0.0376	0.1129	0.0019	0.0753	0.0033	0.0860	0.0034
C20:4n3	0.6201	0.0684	0.5853	0.0072	0.4363	0.0008	0.1779	0.0082	0.2397	0.0060
C20:5n3(EPA)	13.3624	0.7823	12.9829	0.7004	12.0458	0.0052	8.9503	0.7575	8.4761	0.1365
C22:5n3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:6n3(DHA)	22.8177	1.2554	22.1937	1.8357	17.2158	0.0282	14.4775	1.6563	17.6134	0.4457
C18:2n6	1.6010	0.0890	2.0555	0.2212	3.5936	0.0111	7.1374	6.6129	15.9281	0.8965
C18:3n6	0.9098	0.3365	0.1397	0.0162	0.2792	0.0119	1.8270	0.8912	0.0986	0.0179
C20:2n6	0.1999	0.0116	0.1668	0.0174	0.1507	0.0105	0.1231	0.0880	0.2333	0.0024
C20:3n6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C20:4n6(ARA)	1.7896	0.1557	2.7505	0.1996	4.6733	0.0027	4.2360	1.4584	3.5204	0.2386
C22:5n6	1.5702	0.3315	2.5657	0.1711	3.8666	0.1569	2.6968	0.7201	2.1149	0.0867

Σ SFA	22.5136	1.7961	22.1771	1.7483	21.5097	1.6500	21.7921	1.7705	23.0756	2.0054
Σ MUFA	22.5077	0.0886	24.6648	0.0457	27.3296	0.0419	20.4449	0.1925	21.3217	0.0232
Σ PUFA (n-6)	6.0705	0.3140	7.6782	0.5353	12.5633	0.8850	16.0204	1.1066	21.8954	2.5215
Σ PUFA (n-3)	38.4989	3.0344	37.9708	2.9388	32.9243	2.3651	25.8917	1.9334	28.8669	2.2452
Σ FA	95.9327	0.9581	92.4909	0.9429	96.9476	0.8389	84.1491	0.7459	95.1595	0.9256
(n-3) / (n-6)	6.3420	(-)	4.9453	(-)	2.6207	(-)	1.6162	(-)	1.3184	(-)
DHA/EPA	1.7076	(-)	1.7095	(-)	1.4292	(-)	1.6175	(-)	2.0780	(-)
EPA/ARA	7.4668	(-)	4.7202	(-)	2.5776	(-)	2.1129	(-)	2.4077	(-)

Appendix 18. Fatty acids of lumpsucker larvae fed with Copepods and Cirripedia(Cop/Cir)

Fatty aci+A1:K36ds of lumpsucker larvae fed with Cop/Cir from 2dph to 34 dph. Fatty acids are in % of total fatty acids and SD is +/- standad deviation										
Age (dph)	**9**	**± SE**	**15**	**± SE**	**21**	**± SE**	**29**	**± SE**	**34**	**± SE**
C14:0	1.1006	0.1120	1.0750	0.0096	0.7976	0.0286	0.8931	0.0965	0.8703	0.041028
C15:0	0.3283	0.0008	0.4214	0.0225	0.2849	0.0286	0.3161	0.0580	0.3449	0.023145
C16:0	15.9905	0.7809	18.1261	0.9402	16.4872	0.5191	16.9079	0.6994	17.0418	1.620508
C17:0	0.4148	0.0705	0.3407	0.0355	0.3882	0.0039	0.0000	0.0000	0.1887	0
C18:0	5.4442	0.5852	7.5218	0.1175	6.0791	0.0016	5.5650	0.3846	6.1568	0.180287
C20:0	0.0597	0.0000	0.0000	0.0000	0.0615	0.0068	0.0787	0.0091	0.1193	0
C21:0	0.0000	0.0000	0.0000	0.0000	0.3945	0.0000	0.6944	0.0000	0.2108	0
C22:0	0.5276	0.0431	1.0798	0.2313	0.7172	0.3684	0.5066	0.1923	1.0271	0.308017
C14:1n5	0.0117	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
C16:1n9	0.4078	0.0000	0.0000	0.0000	0.3445	0.0684	0.2647	0.0638	0.2754	0
C16:1n7	1.2019	0.0923	0.8585	0.0812	1.5515	0.0808	1.3501	0.0918	1.0233	0.044395
C16:1n5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
C17:1n7	0.2326	0.0634	0.1387	0.0622	0.1792	0.0130	0.2370	0.0255	0.1784	0.003904
C18:1n9	14.2861	0.4127	13.5220	0.5231	10.3188	0.9610	10.7443	0.5651	11.2902	0.003785
C18:1n7	3.0142	0.0162	3.3111	0.2808	4.9543	0.2726	3.3017	0.3175	2.6248	0.091951
C18:3n4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
C20:1n9	2.4342	0.5230	1.5987	0.3094	1.0984	0.2145	0.8430	0.0895	1.0777	0.327558
C20:1n7	0.3661	0.0000	0.0000	0.0000	0.5000	0.0000	0.1879	0.0095	0.0000	0
C20:3n4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
C22:1n11	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
C22:1n9	0.3133	0.0691	0.2326	0.0000	0.1758	0.0323	0.1469	0.0292	0.2669	0.138434
C24:1	0.3838	0.0217	0.3892	0.0626	0.5435	0.1343	0.3395	0.0452	0.6027	0.259514
C16:4n3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
C18:3n3	0.6363	0.0228	0.3399	0.0451	0.3337	0.0206	1.2737	0.0965	1.2275	0.08354
C18:4n3	0.6448	0.1525	0.3080	0.0365	0.2736	0.0767	0.4321	0.0924	0.3176	0.048316
C20:3n3	0.0777	0.0314	0.0000	0.0000	0.0895	0.0000	0.0792	0.0130	0.0000	0
C20:4n3	0.6509	0.1018	0.3805	0.0836	0.2119	0.0467	0.1678	0.0254	0.1749	0
C20:5n3(EPA)	15.0143	0.9336	12.4579	0.0301	14.1509	0.9884	8.8924	0.6678	8.5454	0.510337
C22:5n3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
C22:6n3(DHA)	26.5401	0.3298	23.9519	0.4279	25.1666	1.3084	19.7517	2.0084	19.2890	0.018858
C18:2n6	1.7410	0.4911	1.4179	0.2147	0.8092	0.0330	13.7427	0.8743	13.8695	2.931052
C18:3n6	0.1237	0.0220	0.3071	0.0532	0.1032	0.0000	0.0000	0.0000	0.5237	0.236606
C20:2n6	0.2462	0.0200	0.1906	0.0195	0.1890	0.0056	0.2369	0.0138	0.2674	0.001292
C20:3n6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
C20:4n6(ARA)	0.9590	0.0736	1.0162	0.0336	1.2138	0.1936	1.3633	0.0797	1.2222	0.063319
C22:5n6	0.9927	0.7617	3.3290	0.3495	2.6759	1.0577	2.1424	0.4892	3.1549	0.433945
Σ SFA	23.8658	1.9636	28.5649	2.2589	25.2103	2.0305	24.9620	2.0728	25.9598	2.0964
Σ MUFA	22.6518	0.2036	20.0508	0.0934	19.6660	0.2827	17.4151	0.1307	17.3395	0.1160
Σ PUFA (n-6)	4.0626	0.2745	6.2608	0.5074	4.9911	0.4156	17.4854	2.1940	19.0377	2.1890

Σ PUFA (n-3)	43.5641	3.5199	37.4382	3.1454	40.2262	3.3583	30.5970	2.5143	29.5543	2.4551
Σ FA	94.1443	1.0865	92.3147	1.0372	90.0936	1.0121	90.4594	0.9347	91.8913	0.9331
(n-3) / (n-6)	10.7233	(-)	5.9797	(-)	8.0596	(-)	1.7499	(-)	1.5524	(-)
DHA/EPA	1.7677	(-)	1.9226	(-)	1.7784	(-)	2.2212	(-)	2.2572	(-)
EPA/ARA	15.6562	(-)	12.2590	(-)	11.6582	(-)	6.5225	(-)	6.9916	(-)

Appendix 19. Fatty acids of lumpsucker larvae fed with Copepods(Cop/FD)

Fatty acids of lumpsucker larvae fed with Copepods(Cop/FD) from 2dph to 34 dph. Fatty acids are in % of total fatty acids and SD is +/- standad deviation										
Age (dph)	2	± SE	9	± SE	15	± SE	21	± SE	34	± SE
C14:0	0.8877	0.1280	1.0128	0.0134	0.7054	0.0051	0.7533	0.0319	0.9004	0.0198
C15:0	0.3095	0.0352	0.3343	0.0105	0.2797	0.0107	0.3161	0.0565	0.2758	0.0413
C16:0	13.6170	0.3327	17.5212	0.1189	17.3507	0.2348	15.2472	0.6031	17.3530	0.2813
C17:0	0.3473	0.0458	0.4250	0.0460	0.6201	0.0174	0.9230	0.6942	0.2076	0.0265
C18:0	5.3931	0.5740	5.9799	0.0247	5.6125	0.1212	4.9410	0.3197	4.7915	0.4260
C20:0	0.0760	0.0178	0.0565	0.0023	0.0480	0.0057	0.1130	0.0168	0.1117	0.0003
C21:0	0.4058	0.0569	0.0000	0.0000	0.1577	0.0000	0.1359	0.0000	0.1636	0.0378
C22:0	0.6327	0.1350	0.4324	0.0364	0.2142	0.0181	0.3845	0.21575793	0.5172	0.2413
C14:1n5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0353	0.0000	0.0000	0.0000
C16:1n9	0.3169	0.0552	0.4365	0.0234	0.4548	0.0063	0.2612	0.0144	0.3040	0.0496
C16:1n7	1.0714	0.0081	1.1227	0.0123	1.2659	0.0160	1.1335	0.0682	1.2974	0.1395
C16:1n5	0.0841	0.0048	0.0000	0.0000	0.0804	0.0173	0.2093	0.0000	0.1052	0.0000
C17:1n7	0.2304	0.0055	0.2543	0.0052	0.2589	0.0361	0.1940	0.0363	0.1973	0.0274
C18:1n9	13.5641	0.0040	13.0628	0.0721	11.7391	0.0970	10.0781	0.5541	10.9623	0.8750
C18:1n7	2.9049	0.1635	2.8203	0.0016	2.7663	0.0197	1.9615	0.1986	2.0656	0.1400
C18:3n4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C20:1n9	2.0701	0.1010	1.7169	0.0469	1.2106	0.0319	1.0243	0.0487	0.9404	0.1667
C20:1n7	0.0000	0.0000	0.2150	0.0118	0.0000	0.0000	0.0000	0.0000	0.0692	0.0000
C20:3n4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:1n11	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:1n9	0.4697	0.0510	0.2626	0.0472	0.1811	0.0180	0.1609	0.0234	0.1392	0.0489
C24:1	0.3677	0.0084	0.3839	0.0269	0.3501	0.0651	0.3368	0.0047	0.3765	0.0929
C16:4n3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C18:3n3	0.5279	0.0954	0.5685	0.0048	0.6451	0.0099	1.5121	0.0873	1.7325	0.0843
C18:4n3	0.6288	0.0326	0.5109	0.0194	0.4557	0.0142	0.3004	0.0000	0.3888	0.0446
C20:3n3	0.1206	0.0270	0.0814	0.0089	0.1020	0.0065	0.0858	0.0000	0.0689	0.0253
C20:4n3	0.6659	0.0511	0.4800	0.0004	0.5060	0.0201	0.2232	0.0233	0.1902	0.0095
C20:5n3(EPA)	14.0112	0.8379	13.7881	0.0058	12.9522	0.3214	6.2704	0.4445	6.8559	0.5419
C22:5n3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C22:6n3(DHA)	24.1712	1.7405	26.3672	0.0585	29.9057	1.4447	16.6448	0.5035	18.2085	0.3947
C18:2n6	1.3264	0.2628	1.6854	0.0029	1.4097	0.0405	17.1200	1.7156	22.2662	1.6548
C18:3n6	0.2279	0.1268	0.1250	0.0517	0.2571	0.0000	0.0000	0.0000	0.1130	0.0495
C20:2n6	0.2118	0.0368	0.2127	0.0081	0.2355	0.0086	0.2265	0.0166	0.3122	0.0531
C20:3n6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C20:4n6(ARA)	0.8151	0.1806	1.0895	0.0037	1.4244	0.0404	1.3093	0.0870	1.4702	0.0510
C22:5n6	2.3152	0.6052	1.3327	0.0896	1.2749	0.1618	1.1012	0.4047	2.2585	1.0324
Σ SFA	21.6690	1.6765	25.7622	2.1601	24.9883	2.1361	22.8141	1.8590	24.3208	2.1187

Σ MUFA	21.0793	0.0962	20.2750	0.0782	18.3071	0.0729	15.3948	0.7416	16.4570	0.9597
Σ PUFA (n-6)	4.8962	0.3599	4.4453	0.2928	4.6016	0.2729	19.7569	2.7750	26.4203	3.5908
Σ PUFA (n-3)	40.1257	3.2195	41.7960	3.4533	44.5667	3.8143	25.0367	2.0721	27.4448	2.2662
Σ FA	87.7702	0.9876	92.2785	1.6108	92.4637	1.1377	83.0026	0.8823	95.6816	1.0357
(n-3) / (n-6)	8.1952	(-)	9.4024	(-)	9.6851	(-)	1.2672	(-)	1.0388	(-)
DHA/EPA	1.7251	(-)	1.9123	(-)	2.3089	(-)	2.6545	(-)	2.6559	(-)
EPA/ARA	17.1903	(-)	12.6550	(-)	9.0931	(-)	4.7892	(-)	4.6631	(-)