UNIVERSITY OF NORDLAND

MASTER THESIS

A new micro diet with big potential to improve first feeding of lumpsucker *Cyclopterus lumpus*

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Abstract

Through the great and increasing interest in developing a micro diet for marine larvae during the last decade, earlier weaning of larvae onto artificial diets could be successfully done, but a total replacement of live feed has not been accomplished yet. Hence, the improvement and developing of a micro diet is not complete. The aim of this study was to evaluate a new developed micro diet by investigating the larval performance (growth and survival) and the larval quality (fatty acid composition) of lumpsucker (*Cyclopterus lumpus*) from first feeding till 67 days post hatch (DPH) compared to using standard feeds (commercial micro diet, *Artemia* and frozen zooplankton).

Introducing the new feed increased total length (TL) of larvae compared to the other feeding groups and increased dry weight (DW) compared to the commercial micro diet and frozen zooplankton ($p<0.001$). During *Artemia* feeding larvae showed higher TL compared to using the commercial micro diet ($p<0.001$). At the end, after 67 DPH, larvae fed with the new feed showed a higher TL (24.79±2.92 mm) and DW (71.3±20.87mg) than larvae fed with the commercial feed ($p<0.05$). No differences between TL (23.46±3.33mm, 24.08±2.61mm) and DW (54.88±23.27mg, 63.22±21.02mg) of larvae fed with commercial feed and *Artemia* was found at 67 DPH. A very poor growth during the trial showed larvae fed with frozen zooplankton. Larvae had smallest TL (10.82±1.13mm) and DW (9.19±12.17mg) at 67 DPH ($p<0.001$). Highest survival (95.52±0.7%) was observed in larvae fed with new feed ($p<0.05$), while survival between larvae of the commercial diet (89.35±3.5 %) and *Artemia* (89.85±2.96%) feeding was not different. Frozen zooplankton influenced survival of larvae negatively and less than 1% of the larvae survived ($p<0.001$). Fatty acid composition of the feed was reflected in the fatty acid composition of the lumpsucker larvae with highest linoleic acid levels in larvae fed with the commercial diet and highest EPA levels in larvae fed with frozen zooplankton ($p<0.05$). However, all larvae showed the recommended levels of EPA and DHA. Also total lipid content varied with the diet and larvae fed with commercial feed showed the highest lipid content ($p<0.05$).

The results of this study showed that there is room for improvement in first feeding of lumpsucker larvae. A 29.9% increase in growth of larvae compared to standard procedures and a 95% survival rate would be beneficial for the lumpsucker industry, an important and increasing industry in producing promising cleaner fish for salmon farms.

Sammendrag

Gjennom den store og økende interessen for å utvikle en mikrodiett for marine larver gjennom det siste tiåret, er nå tidligere tilvenning til formulert diett mulig, men en total erstatning av levende fôr har det ennå ikke lyktes å gjennomføre. Dermed er utvikling og forbedring av en mikrodiett fortsatt ikke komplett. Målet med denne studien var å evaluere en nylig utviklet mikrodiett ved å undersøke larve prestasjon (vekst og overlevelse) og larve kvalitet (fettsyre komposisjon) på rognkjeks (*Cyclopterus lumpus*) fra startfôring til 67 dager etter klekking (DEK) sammenlignet med standard fôr (kommersiell mikrodiett, *Artemia* og fryst dyreplankton).

Introduksjon av det nye fôret førte til økt total lengde (TL) av larver sammenlignet med larvegrupper gitt andre fôrtyper, samt økt tørrvekt (TV) sammenlignet med kommersiell mikrodiett og fryst dyreplankton ($p<0.001$). Larver gitt *Artemia* fikk økt TL sammenlignet med larver gitt kommersiell mikrodiett ($p<0.001$).Ved slutten av forsøksperioden 67 DEK, hadde larver gitt det nye fôret høyere TL (24.79±2.92 mm) og TV (71.3±20.87mg) sammenlignet med larver gitt det kommersielle fôret ($p<0.05$). Ingen forskjell mellom TL (23.46±3.33mm, 24.08±2.61mm) and TV (54.88±23.27mg, 63.22±21.02mg) ble observert på larver gitt kommersielt fôr og *Artemia* ved slutten av forsøksperioden 67 DEK. Larver gitt fryst dyreplankton hadde lav vekst gjennom førsøksperioden og hadde lavest TL (10.82±1.13mm) og TV (9.19±12.17mg) ved 67 DEK ($p<0.001$). Høyest overlevelse (95.52±0.7%) ble observert på larver gitt det nye fôret ($p<0.05$), men ingen forskjell på overlevelse ble observert mellom larver gitt kommersiell diett (89.35±3.5 %) og *Artemia* (89.85±2.96%)Fryst dyreplankton påvirket overlevelse av larver negativt og mindre enn 1% av larvene overlevde ($p<0.001$). Fettsyre sammensetning i rognkjekslarvene var reflektert av fôrets fettsyresammensetning med høyest linolsyrenivå i larver gitt kommersielt fôr og høyest EPA nivå i larver gitt fryst dyreplankton ($p<0.05$). Likevel, alle larvene hadde ønsket EPA og DHA nivå.Totalt fettinnhold varierte også mellom ulike fôrtyper og larver gitt kommersiell diett hadde høyest fettinnhold ($p<0.05$).

Dette forsøket viser at det er rom for forbedring I startfôring av rognkjekslarver. En 29.9% økning i vekst av larver sammenlignet med standard prosedyrer og 95% overlevelse kunne vært gunstig for rognkjeks industrien, en viktig og voksende industri for produksjon av en lovende rensefiskart for lakseoppdrett.

Table of contents

List of Figures

List of Tables

List of Appendix Figures

List of Appendix Tables

Introduction

Status of first feeding in aquaculture

To support a steady growing and sustainable aquaculture industry, supplying fish farms with healthy and good quality larvae and juveniles from intensive hatcheries rather than from wild stocks must be guaranteed. One big challenge in hatcheries is feeding through the larval stages and it is considered as one of the bottlenecks in aquaculture (Holt 2011, Piccinetti et al. 2014). Currently, most of the aquaculture species depend on live feed at start feeding, and only a few species can be fed with formulated micro diets post yolk sack stage (Holt 2011). Rotifers (*Brachionus* spp.) and brine shrimps (*Artemia* spp.) are commonly used as live feed in hatcheries as they are cheaper and easier to produce than copepods which are the natural prey for marine fish larvae. One main challenge with rotifers and *Artemia* is their lack of important nutrients. Due to the close relationship between larval nutrition at first feeding and larval development, larvae in hatcheries can show a lot of deformities and high mortality caused by nutritional deficiency (Cahu and Zambonino 2001). Enrichments can increase the nutritional value of the live feed and therefore increase survival of larvae, but still do not show the same results as larvae fed with copepods (Mæhre et al. 2013). Additionally, marine fish larvae have a much higher growth rate when fed with copepods, then using rotifers or *Artemia* (Hamre 2006, Mæhre et al. 2013). A specialized artificial micro diet for marine fish larvae that has an optimal nutritional value would be a big improvement for marine hatcheries. This diet could decrease mortality and deformities of larvae, but could also increase their growth rate. Additionally, it also reduces labor and economic cost compared to producing live feed. Such a micro diet, especially for small marine fish larvae, has not been developed yet. Although there is a great interest and effort in developing a micro diet for marine larvae during the last decade, a total replacement of live feed has not been successfully achieved yet. For now, the development stage of the larva at hatch or better at time where it feeds exogenous is the decisive factor of which feed, artificial feed or live feed can be used.

Development stage of marine fish larvae at fist feeding

There are large variations of the development stage at hatch between species and therefore also at start feeding. Larvae can have a direct development and show fully developed fins and a mature digestive system at hatch (precocial larvae), other larvae are poorly developed at hatch (altricial larvae) (Fuiman and Werner 2009). Many marine species and especially

species which are important for aquaculture have altricial larvae. This means at start feeding larvae have a small mouth gap which limits the size of feed, not fully pigmented eyes which hinders effective capturing of feed and have an immature digestive system which limits the digestion and absorption of nutrients. These factors make first feeding challenging, especially when developing and using artificial diets.

Advantages of using live prey are their size, their color, their swimming behavior and specific chemicals released from the prey, which are assumed to attract the larvae and increase the ingestions rate (Aragão et al 2004, Kolkovski 2008, Conceição et al. 2010). To be identified as feed from larvae and to stimulate the ingestion of the feed, those physical and chemical attractants have to be considered in artificial micro diets. Next to it, the availability of feed and whether the larvae are able to assimilate those nutrients are important topics at first feeding. Here enzyme activity in the digestive system of early marine larvae plays a major role.

In many species is the stomach at start feeding undifferentiated and not functional, therefore acid digestion and pepsin expression is missing (Holt 2011). On the other hand, the liver, pancreas and gallbladder are developed and becoming functional at start feeding (Holt 2011). Hence, the pancreas supplies the mid gut of the early larvae with digestive enzymes which are necessary for the digestion and absorption of proteins (trypsin), lipids (bile salt-activated lipase, phosphoslipase) and carbohydrates (amylase) (Infante and Cahu 2007, Holt 2011). However, enzyme activity of larvae is considered to be relatively low and for an optimal digestion enzymes need to be in adequate concentration (Kolkovski 2008). It is suggested that exogenous enzymes from live prey are beneficial for larval enzyme activity and can stimulate the digestive process (Izquierdo et al. 2000, Holt 2011). Contrariwise it is also assumed as larvae do not miss digestive enzyme at first feeding and that the importance of those enzymes from live feed are negligible (Cahu and Zambonino 2001, Holt 2011).

An advantage of using live prey is that the nutrients are in a highly digestible form and consist of relatively high amounts of free amino acids (Holt 2011). On the other hand, in formulated feeds usually fishmeal is used as protein source, structural proteins which show low digestibility in fish larvae (Hamre 2006). However, the composition of the diet can increase the activity of some enzymes and also influence the maturation of the digestive system. Thus the low trypsin secretion, when using feed that consists fishmeal, can be strongly increased by using a mix of protein and hydrolysate or free amino acids (Aragão et al. 2004, Holt 2011).

When it comes to lipids it is assumed that fish larvae can digest phospholipids much better than triglycerides and should therefore be included in the larvae's diet (Infante and Cahu 2007). Additionally, the sight, the smell and the presence of feed can trigger pancreatic secretion. Next to the availability of nutrients and the ability to assimilate those, it is important to know the nutritional requirements of fish larvae to get the right composition of the feed.

Nutritional requirements of marine fish larvae with special focus on fatty acids

The National Research Council (NRC 2011) provides details about the nutritional requirements for many adult fish and juveniles. On the other hand for marine fish larvae, especially cold water species, information about the exact requirements are lacking or do not exist. Yet, requirements for larvae are considered to be higher than for adults. For macronutrients like fatty acid or amino acids usually the composition of the ovaries, eggs, yolksac larvae and their naturel prey is analyzed to get a better understanding of the right composition of nutrients (Rønnestad et al. 1999, Sargent et al. 1999, Hamre 2006, Holt 2011). Special attention in larval nutrition is paid to the essential fatty acids which play a crucial role for larvae's growth and survival, but live prey which is used in hatcheries show high deficiencies.

Lipids

Lipids are the major source of energy, but also play an important role as structural components (Holt 2011). In copepods the lipid content normally varies between 6-16%, whereas in *Artemia*, the lipid content is higher in the range of 20-30 %, depending on the diet or enrichment (Hamre 2006). Important for marine fish are the highly unsaturated fatty acid (HUFA) docosahexaenoic acid (DHA, 22:6*n*-3), eicosapentaenoic acid (EPA, 20:5*n*-3) and arachidonic acid (ARA, 20:4n-6) (Sargent et al. 1999). Marine fish cannot synthesize DHA, EPA or ARA directly or from shorter fatty acid chains, due to the limited elongase and Δ-5 desaturase activity. Therefore they are essential for fish and have to be included in the diet. DHA is a crucial structure component for intermembrane reactions and processes and is important for the membrane fluidity, in stress control, in the immune system development and for growth (Holt 2011). It is considered as the most essential fatty acid. EPA and ARA are precursors for eicosanoids, which are involved in the hormone release, neural and immune function and osmoregulation (Holt 2011). Additionally, EPA shows also importance in the correct function of blood cells in marine fish (Holt 2011). Deficiencies or imbalance can lead

to poor growth, increasing mortality, abnormal pigmentation, immune deficiency and skeleton deformities (Holt 2011).

In copepods 20-40% of the fatty acids are DHA, 5-20 % are EPA and less 1% are ARA (Hamre 2006). Hence, the natural prey of marine fish larvae has a high amount of DHA and EPA. *Artemia* and rotifers on the other hand show a low amount of DHA or EPA and in an unfavorable ratio (Holt 2011). Therefore newly hatched nauplii have to be enriched with marine fish oils or with single oil derived from marine organism to increase the HUFA level (Sargent et al. 1997). Here, increasing EPA levels can be more easily achieved, while increasing DHA levels and the right balance between those seems to be more difficult (Conceição et al. 2010). Holt (2011) considers that 3% of the formulated feeds or live feeds dry weight should be HUFAs. 0.5% to 2.5% of this diet should be DHA, 0.7-1.6% EPA and 0.5 to 1.2% APA. The ratio of DHA, EPA and ARA in the diet must be considered as they compete for incorporation into the membrane. The optimal ratio between DHA and EPA is assumed to be 2:1and between EPA and ARA 5:1 (Bell et al. 2003, Holt 2011). This can vary between species. The balance between phospholipids and triacylglycerol in the feed has to be considered as well. In copepods the main essential fatty acid carrier are phospholipids, whereas *Artemia* and rotifers show a low amount of phospholipids (Conceição et al. 2010). Including 3 to 12 % phospholipids in the feed showed better larval survival, a higher enzyme activity and fewer deformities (Izquierdo et al. 2000, Hamre 2006, Infante and Cahu 2007).

Proteins

Proteins are important for growth (deposition in muscles) and as energy source during the larval stage and are mainly absorbed as free amino acid (Holt 2011). Marine pelagic eggs show an amino acid content of 50% and larval diets containing 50-70% protein show the best growth for marine larvae (Cahu and Zambonino 2001, Aragão et al 2004). On the other hand live prey in hatcheries show a lower amount and their free amino acid content is affected by their diet or enrichment (Aragão et al. 2004, Conceição et al. 2010). The total protein content in *Artemia* varies between 36-41% and in rotifers between 24-61% (Table 1 in Hamre 2006).

An essential amino acid profile for marine fish larvae and their required level is difficult to determine and does not fully exist (Cahu and Zambonino 2001). Nonetheless, 10 amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) are suggested to be essential for various fish larvae (Holt 2011). The optimal level of amino acids can change during the larval stage and with increasing age. It is

assumed that larvae can distinguish between essential amino acid, which are used for growth and non-essential amino acid, which than are used for energy. Hence, the right profile is the key to good growth and development. Most live prey do not fulfill the protein requirements and are lacking proteins or show protein imbalance (Holt 2011).

A big challenge for formulated feed is the leakage of water soluble proteins and other water soluble nutrients. 20 to 40% of the proteins and 50% of the free amino acids are lost two minutes after introducing the feed in water (Hamre 2006). After longer suspension up to 50% of the proteins and 80-90% of the free amino acid are lost. Additionally to a reduction of the nutrition value, a high protein lost can also lead to fouling of culture medium (Langdon 2003). High nutritional leakage is found in micro-bound diets, where components of the diet are held together with a binder and do not have any coating or wall (Langdon 2003, Holt 2011). Nevertheless, micro-bound diets are most commonly used as they are relatively easy and cheap to produce (Holt 2011). Additionally, they show a higher digestibility and their attraction trough the high leakage is assumed to be of advantage (Kolkovski 2008). Increasing the amount of water soluble nutrients in the diet could help to cover the nutritional lost (Holt 2011). There is an increased interest in developing embedded micro diets that can reduce the leakage of water-soluble nutrients. Coating and microencapsulation are two techniques. While coated micro diets are micro-bound diets that have a coating layer of lipid or lipoproteins, do encapsulated diets consist of a wall (cross-linked protein-wall or lipid-wall) that separates the dietary components from the surrounding (Kolkovski 2008). A challenge in developing and using encapsulated micro diets is the reduced or no digestibility of the feed as fish larvae are not able to break down the capsuling wall with its digestive enzymes (Langdon 2003). In cross-linked protein-walled capsules the digestibility is higher than for lipid-walled capsuled (Holt 2011). Then again, they still have a rather high leakage of water-soluble nutrient and are expensive to produce (Langdon 2003, Holt 2011). Lipid-walled microcapsules on the other hand show low nutrient lost, are inexpensive and easy to produce, but larvae struggle with the digestion of the lipid wall (Langdon 2003, Holt 2011).

Vitamins

Even less information of the nutritional requirements exists when it comes to vitamins. Vitamins are essential and complex organic compounds needed in many metabolic pathways. They cannot be synthesized by the larvae and therefore need to be introduced through the feed (Holt 2011). Lipid-soluble vitamins are absorbed with digestible fat in the intestine and can be

stored in the fish body (National Research Council 2011). Vitamin A is one of the essential lipid-soluble vitamins and plays an important role in the larval vision and their gene expression regulation (Holt 2011). Those processes can interact with the amount of HUFAs (Zambonino and Cahu, 2007). A deficiency of vitamin A causes multiple forms of skeleton deformities and different pigmentation pattern of the larvae (Hamre et al. 2010, Holt 2011). Copepods and *Artemia* have a very small amount of vitamin A, but show both a very high level of carotenoids to full fill the needs of vitamin A (Hamre 2006). Vitamin A is very affected by the mill machinery of the artificial feed production and therefore must be protected with a coating layer, a matrix and antioxidants (National Research Council 2011).

Vitamin C is one of the essential water-soluble vitamins. Water-soluble vitamins have to be provided in the diet constantly as those cannot be stored in the fish body and high doses will be excreted (Holt 2011). Vitamin C plays an important role as cofactor for hydroxylation reaction and influences the immune system, wound repair and stress response (Holt 2011). The lack of vitamin C during the early life stages causes spine deformities like scoliosis and lordosis (Cahu et al.2003). High concentrations of vitamin C in ovaries, eggs and copepods suggest a high request of vitamin C during the early stages (Hamre 2006, Holt 2011). Enriching live prey, who show a general low amount, with vitamin C results in higher survival and less deformities of the fish larvae (Kolkovski et al. 2000, Cahu et al. 2003, Busch et al. 2010).Vitamin C is very unstable and is easily destroyed through the process of producing artificial diets and must be added at the end of the process or in higher amounts. Additionally, water soluble vitamins show the same problems as water soluble proteins in formulated feed and can easily be lost.

Lumpsucker in aquaculture

Infections with the ectoparasitic sea lice, *Lepeophtheirus salmonis*, are one of the big challenges in salmon farming in the northern hemisphere. Not only is the impact of sea lice on the physiology of the infected farmed fish with further consequences for the growth and the mortality rate a main issue, just as well is the impact of sea lice on wild populations (Costello 2009a). It is assumed that through the release of large amounts of sea lice from sea pens into the environment wild salmon is threatened (Costello 2009a).

A common way of delousing salmon is using chemicals in form of immersion baths and in-feed treatment (Costello 2009b). Those delousing chemicals are very effective, but are assumed to be environmental harmful, show a risk that sea lice develop resistance against the

chemicals and are highly expensive (Denholm et al. 2002, Haya at al. 2005, Costello 2009b). In 2006 the estimated costs for sea lice control in Norwegian salmon farming were over 130 Million Euro (Costello 2009b). Today the production of Atlantic salmon, *Salmo salar*, in Norway almost doubled (FAO 2012). Therefore current costs for delousing should be assumed even higher than estimated for 2006.

Cleaner fish like the lumpsucker, *Cyclopterus lumpus*, have shown to be successful in keeping the sea lice level low (Imsland et al. 2014). Next to its effectiveness and efficiency, cleaner fish are economically beneficial and environmental friendly, compared to chemical treatments. Using cleaner fish instead of chemicals can reduce the costs of delousing to over 70% (Kvenseth and Andreassen 2008). Therefore, there is an increasing interest in culturing lumpsucker to have a controlled and steady supply for salmon farms.

Early life stages of lumpsucker

Lumpsucker eggs are small (2.2-2.6 mm), soft and sticky at spawning (Davenport 1985). During the first 48 hours in seawater the demersal eggs harden and build an egg-mass which is usually guarded by the lumpsucker male (Davenport 1985). Embryonic development last for 250-300 day degrees (Davenport 1985, Arctic Cleanerfish AS). Newly hatched larvae are relatively large (5.5-7mm) and show a continuous finfold, functional eyes, mouth and pectoral fins, and a relatively well-developed digestive system (Fig.1)(Davenport 1985, Benfey and Methven 1986, Brown 1986, Moring 2001, Ingólfsson and Kristjánsson 2002).

With the ventral adhesive disk, which is also functional at hatch, lumpsucker larvae are able to attach themselves immediately to seaweeds or stones (Davenport 1985, Brown 1986). Between or attached to seaweed are larvae and juveniles commonly found in the first months as larvae can there feed without exposing themselves to predators (Brown 1986, Ingólfsson and Kristjánsson 2002). Larvae begin to feed before the yolk sac is completely absorbed (total yolk sac exhaustion is after 10 to 15 days) and feed mainly on small organisms associated with seaweed but also on planktonic organisms (Ingólfsson and Kristjánsson 2002). In the beginning crustacean larvae and halacarid mites are consumed, while later larvae feed on harpacticoids, amphipods and isopods (Ingólfsson, A. and B. K. Kristjánsson (2002). When lumpsucker are about one year, they shift into pelagic habitats, where they are not attached to seaweed any longer (Ingólfsson and Kristjánsson 2002).

Lumpsucker are, next to its aquaculture importance, a good species for studying first feeding. Larvae are relatively well developed at hatch compared to other marine species and are able to feed on artificial feed straight away (Davenport 1985). Therefore most hatcheries can use commercially available artificial micro diets at start feeding.

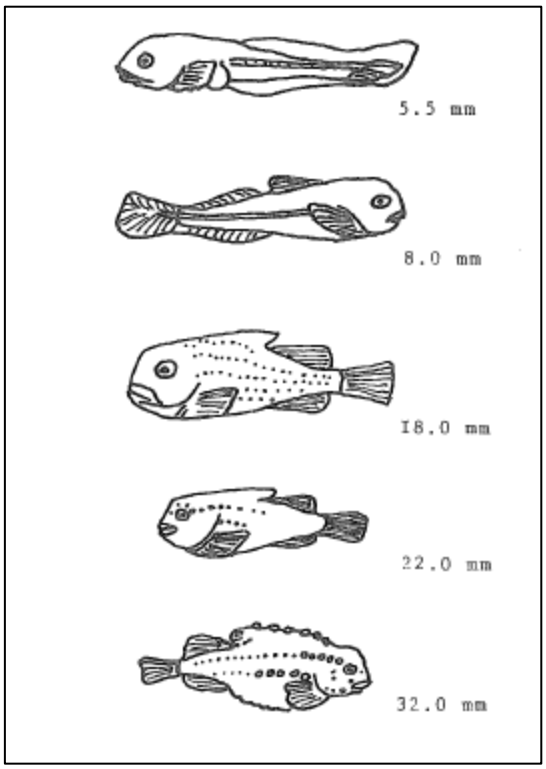


Figure 1. Lumpsucker larvae and juveniles (Davenport 1985)

Aim

The main aim of this study was to investigate a newly developed micro diet especially adapted to early life stages of marine fish in aquaculture. New and improved ingredients in the new micro diet should improve the performance and the quality of the lumpsucker larvae compared to the standard procedure, feeding with commercial diet. Growth and survival (larval performance), as well as fatty acid composition (larval quality) were compared between lumpsucker larvae reared under different feeding regimes. Next to the new formulated micro diet, a standard commercial micro diet, *Artemia* as live prey and a relatively new feeding method: frozen zooplankton are used. Results could give valuable information on how to improve micro diets in the future and therefore improve lumpsucker hatcheries, but also other marine hatcheries.

Materials and Methods

Experimental Set up

Approximately 3000 newly hatched lumpsucker larvae from Arctic Cleanerfish AS were stocked in each of the 12 black and flat bottomed 100L tanks, filled with 80L filtered seawater, at Mørkvedbukta Research Station (University of Nordland) (Fig.2). The water flow was 2L per minute for the first weeks and was increased to 6L per minute after week 4. Larvae were reared under continuous low light condition. The average temperature was 10.3 ± 0.6 °C and the average oxygen level was 96.0 ± 3.2 % (Appendix Table I). After start feeding, the tanks were daily cleaned via siphoning. Duration of the experiment was 67 days, starting with the arrival of the newly hatched larvae on the 30th of May and ending at 4th of August 2015.

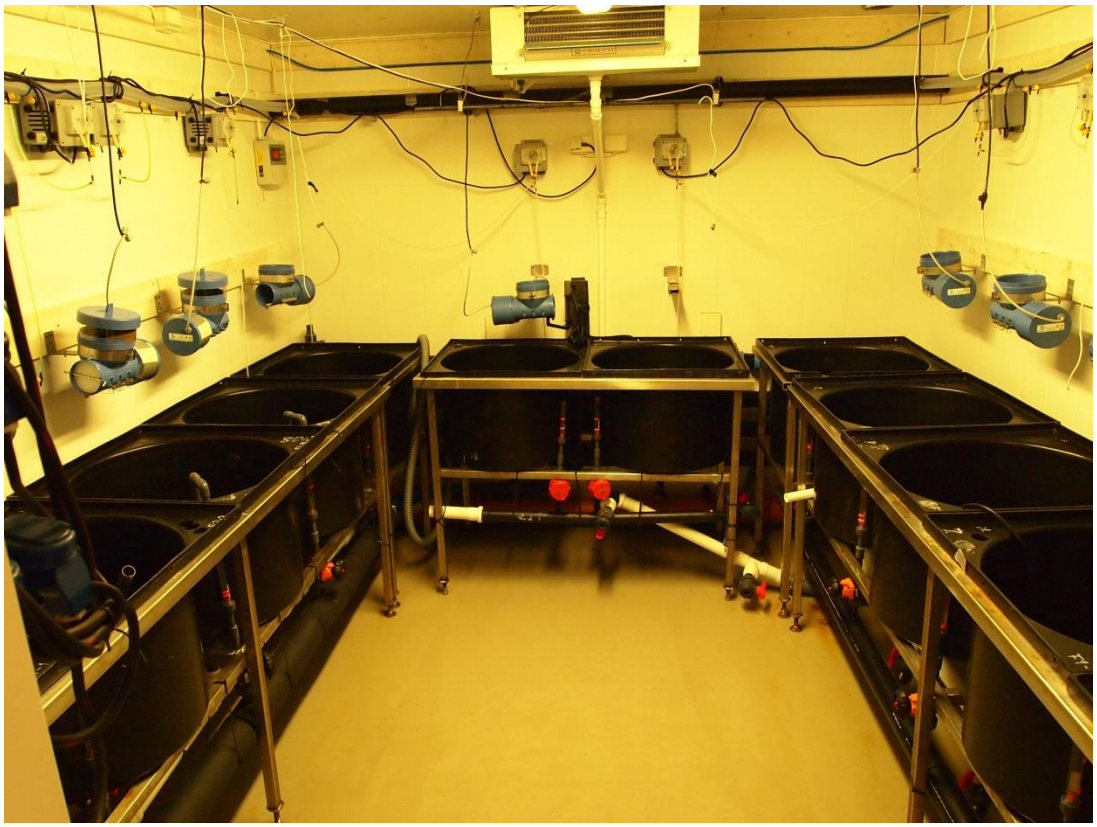


Figure 2. Rearing tanks of the lumpsucker larvae with automatic feeder and feeding pumps.

Feeding regime

In the present study four different feeds were used: a commercial formulated dry feed (dry feed), *Artemia* as live feed, a new formulated dry feed (new feed) and frozen zooplankton

(zooplankton). In four different feeding regimes those feed were introduced into the tanks starting at 5 days post hatch (DPH). Details are given in Table 1. Group 1 was the control group, where larvae were fed with dry feed from 5 DPH and through the whole experiment. Larvae of the other three groups got for one week *Artemia* and in the second week a co-feeding of *Artemia* and an additional feed (Table 1). After *Artemia* feeding stopped larvae were fed with just the additional feed which was introduced in the second week. Due to a limited amount of new feed, larvae in group 3 were fed with a mix of new feed and dry feed for 3 weeks and then just dry feed till the end of the experiment. In this mix, the dry feed was the main feed and only a small amount of new feed was used (20:1).

Table 1. Feeding regime of the four different groups (G) during the trial. Grey highlighted are sampling points at DPH and weeks post feeding (WPF).

DPH	WPF	G1	G2	G3	G4
		Control	**Dry feed**	**New Feed**	**Zooplankton**
5	0	Dry feed	*Artemia*	*Artemia*	*Artemia*
12	1	Dry feed	*Artemia*/Dryfeed	*Artemia*/New feed	*Artemia*/Zooplankton
19	2	Dry feed	Dry feed	Dry feed/New feed	Zooplankton
26	3	Dry feed	Dry feed	Dry feed/New feed	Zooplankton
33	4	Dry feed	Dry feed	Dry feed/New feed	Zooplankton
40	5	Dry feed	Dry feed	Dry feed	Zooplankton
47	6	Dry feed	Dry feed	Dry feed	Zooplankton
54	7	Dry feed	Dry feed	Dry feed	Zooplankton
61	8	Dry feed	Dry feed	Dry feed	Zooplankton
67	9	Dry feed	Dry feed	Dry feed	Zooplankton

Three replicates for each of the four groups were randomly distributed in the experiment room (Fig.2). In all the 12 tanks the larvae were fed till saturation. During daytime from 8 till 15, feed was introduced via hand feeding, while the rest of the day larvae were feed with automatic feeders (dry feed) or feeding pumps (*Artemia* and zooplankton) . The size of the feed was increased with increasing size of the larvae, starting from 100 µm at the beginning up to 500 µm at the later stages (Appendix Table II).

Feed

Commercial formulated dry feed

The feed company advertises that the micro diet is made of fish meal, lecithin, wheat gluten, fish oil, vitamins and mineral premixes. It promises a high amount of hydrolyzed protein and phospholipid content. According to the manufacture the dry feed contained 55% proteins, 15% lipids (14.3% of the lipid are HUFAs), 13.5% ash, 5 % fibre and 2 % phosphorus (skretting.no).

Live feed Artemia

Artemia were cultured and enriched daily using 100L hatching and enrichment tanks, which were heated and provided with strong aeration and light. *Artemia* cysts (from Inve Aquaculture) were incubated in 60L filtered seawater. After 24h hatched nauplii were washed with seawater, separated from unhatched cyst and empty shells and then transferred into the enrichment tank. *Artemia* were then enriched with Ori-Green (from Skretting) for 24h. Before introducing into the lumpsucker larvae tanks, enriched *Artemia* were washed with seawater.

New formulated dryfeed

The new feed was made by the University of Nordand and Bioforsk in Bodø. Main content of this micro diet was remains of raw materials from the fish industry, macro algae products and naturel zooplankton. To prevent any major leakage of nutrients, feed was embedded. Formula and methodology to prepare the new feed are not public available.

Frozen Zooplankton

Zooplankton was harvested from populations of wild zooplankton at the coast of Nordland, Norway. First zooplankton was frozen using liquid nitrogen and stored at minus 35 °C. Afterwards feed was embedded and then frozen again. Details about methodology of embedding are not public available (Planktonic AS, Herøy). Before introducing the frozen plankton feed into the larvae tanks, it was thawed and mixed with seawater.

Sampling

All sample points were conducted similarly and 30 larvae were taken from each of the 12 tanks for the later analyses. Reference sampling was on 3 DPH prior to the larvae being start fed, to get the initial measurements and data of the larvae. The later samplings were first

weekly; 1, 2, 3 and 4 weeks after first feeding (5 DPH), while the last samples were taken at 6 and 9 weeks after first feeding (Table 1). Therefore data for 12, 19, 26, 32, 47 and 67 DPH exists after the initial sampling.

The 30 larvae were collected with a plastic pipette till 47 DPH and later with a small net into a glass beaker filled with sea water. Larvae were than anesthetized with MS 222 and rinsed afterwards in distilled water. All 30 larvae were photographed under the microscope (Olympus SZ-12) with the software program Cell A for later length measurements and deformities check. 10 of those 30 larvae were then transferred into pre weighted tin capsules and put into a minus 20 °C freezer for dry weight measurements. The other 20 larvae were transferred into a plastic tube and kept in a minus 80 °C freezer for the fatty acid analyze.

Data analysis

Length

To determine the length of the larvae, the total length TL (from the mouth to the end of the fin fold/tail fin) was measured. At the last sampling day (67 DPH) larvae were too big for using the microscope and a ruler was used for length measurements. Additionally, all larvae were checked for any deformities.

Dry weight

At 3 DPH, the initial measurement, 5 larvae were pooled and put in one tin capsule for dry weight (DW) measurements. At the following samplings, larvae were individually transferred into each one tin capsule. The larvae in the tin capsules were dried at 60°C for 24 h in the Drying oven (Termaks Drying oven) and then weight on a microbalance (Mettler Toledo Microbalance UMX2). Tin capsules were pre-weighted before larvae were added and therefore individual dry weight could be calculated. To investigate the weight specific growth rate (SGR), all individual dry weights from one tank at one sampling point where summed up. To calculate the weight specific growth rate (SGR), the following equation was used:

$$SGR= (\ln W_t - \ln W_i)/t,$$

where W_i and W_t are the initial DW (5DPH) and the DW after t days (Houde 1989).

Fatty acid composition

For the fatty acid analyze all the 20 larvae from one tank were pooled in one plastic tube. The preparation to determinate the fatty acid composition with gas chromatography is labor intensive. Therefore only two sampling points, larvae from 4 weeks of feeding (33 DPH) and from the end of trial (67 DPH), were used. Additionally, the four different feeds were analyzed. At first, samples were freeze dried for 24 h in the freezer drier (VirTis BenchTop K), homogenized and then dived into two replicates. For extraction of lipids the method based on Bligh and Dyer (1959) was used and total lipid content of each sample could be determined. For the hydrolysis of lipids the method based on Metcalfe et al. (1966) was used. Afterwards fatty acids could be identified with the gas chromatograph SCION 436-GC and by reference of a known standard. Fatty acids were measured by peak integration and expressed as relative area percentage on the total fatty acid area. To calculate the amount of fatty acids per 100g sample the following equation was used:

Fatty acid (g/100g sample) = (Total lipid content (g/100g sample)/100) * Fatty acid (% of total fatty acid content)

Survival

Mortality of larvae was daily recorded. At the end of the trial fish in each tank was counted and the exact total number of larvae at start could be assigned. To calculate the survival rate, the following equation was used:

$$\text{Survival rate (\%)} = (100/ N_{Start})*N_{End},$$

where N_{Start} and N_{End} are the number of individuals at start and end of the trial.

Statistical analysis

Length, dry weight, specific growth rate and fatty acid composition data are presented as mean ± standard deviation. Survival data were expressed as percentage, but for the statistical analyze data were transformed using data=arcsin $\sqrt{P}$ to get a binomial distribution. All data (except the SGR, which was not statistically analyzed) were analyzed with one-way parametric ANOVA followed by Tukey's test using the software package Statistica 10.0. A p-value of < 0.05 was regarded as statistically significant. Exact p-values of Tukey's test are given in Appendix Table III-VII.

Results

Growth

TL and DW

Larvae from tanks of the same feeding group showed no significant difference in TL and DW during the whole trial. Only at the last sampling (67 DPH) larvae from one tank of the control group showed a significant difference ($F_{(12,165)}$=7.5, p<0.001) in DW compared to the other two control tanks (Appendix Fig.I). All data of the TL and DW is summarized in the Appendix Table VIII. Before start feeding (3 DPH) the initial TL and DW of larvae were not significantly different between the four feeding groups (Fig. 3). Larvae were between 6.71±0.25 to 6.75±0.19mm and 1.39±0.16 to 1.43±0.21mg.

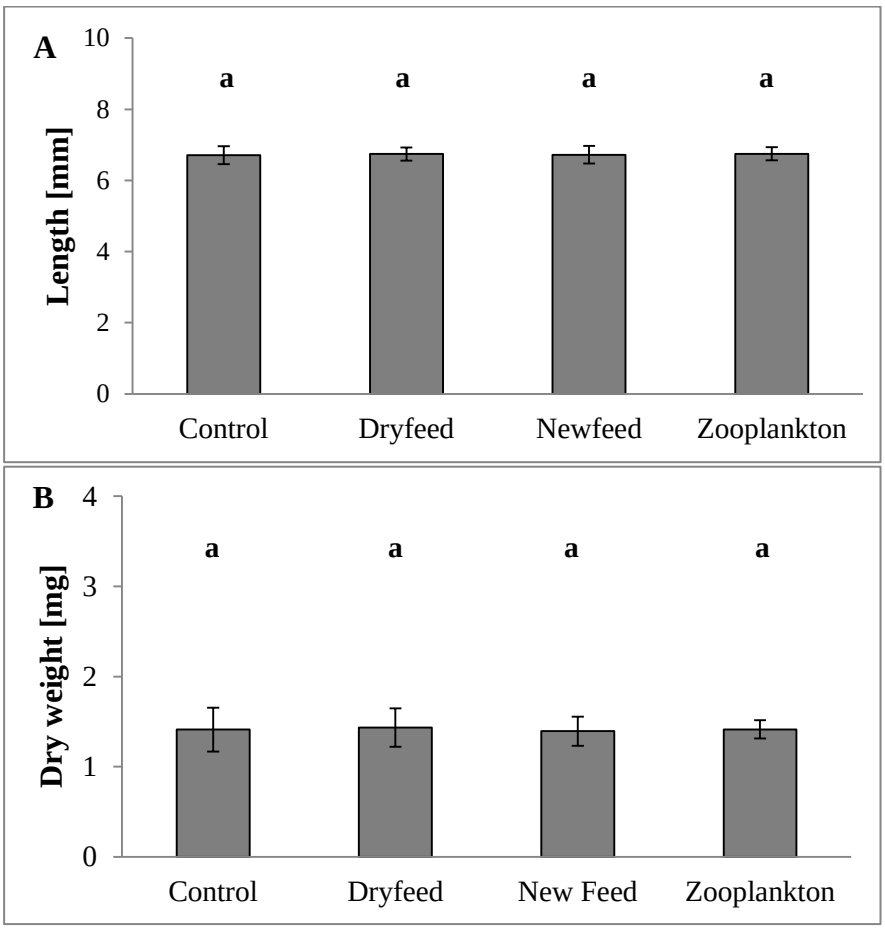


Figure 3. Total length (n=360) in mm (A) and dry weight (n=120) in mg (B) of lumpsucker larvae of the four different feeding groups at 3 DPH. No significant difference in total length and dry weight (p>0.05).

At 12 DPH, after the first week of feeding, there were significant ($F_{(3,356)=}$63.0, p<0.001) differences between the TL of larvae from the different feeding groups. The TL of larvae from the control group was 6.89±0.31mm, while larvae of the other groups were significant larger (p<0.001, Fig. 4). No significant differences in TL and DW between those three groups were seen. The DW of larvae in the control group was 1.61±0.76 mg, while the DW of larvae from the other groups were approximately 0.25 mg larger, but not significant.

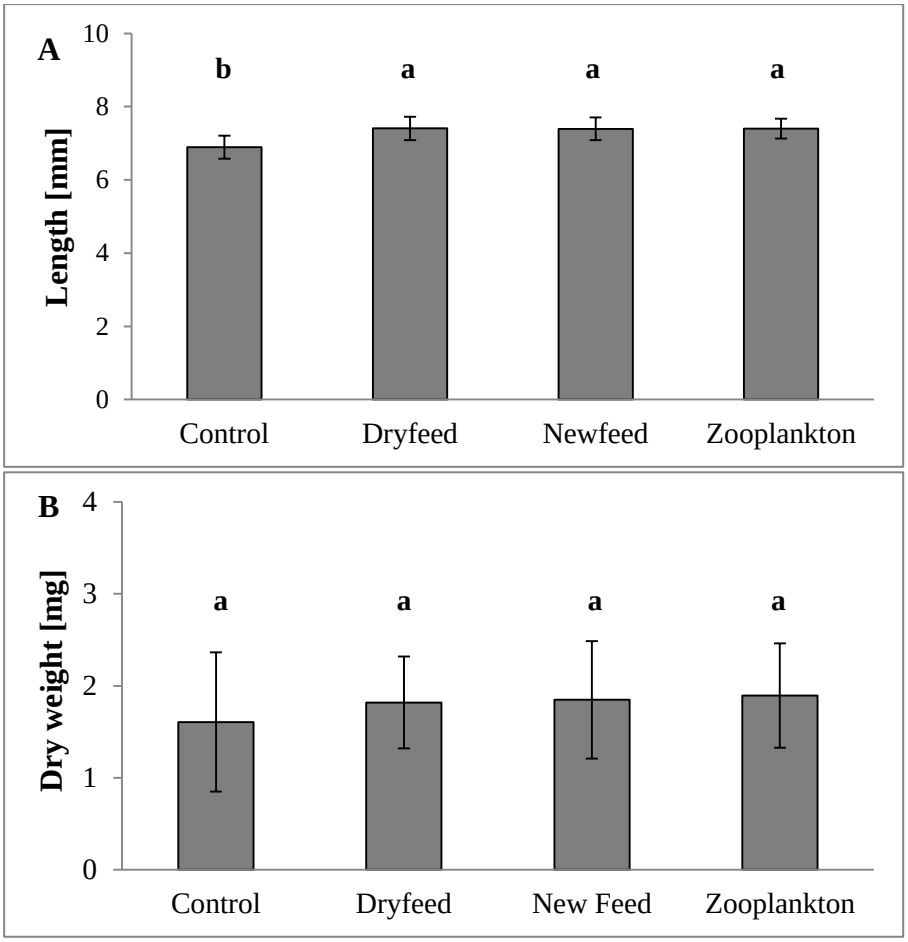


Figure 4. Total length (n=360) in mm (A) and dry weight (n=120) in mg (B) of lumpsucker larvae of the four different feeding groups at 12 DPH. Bars sharing the same letter showed no significant differences. No significant difference in dry weight (p>0.05).

Differences in larval size between the groups became clearer at 19 DPH. Those differences were significant both for TL and DW ($F_{(3,356)=}$58.1, p<0.001 and $F_{(3,116)=}$5.2, p=0.002, respectively). The new feed and zooplankton group larvae showed the significant highest TL with 8.30±0.47 mm and 8.52±0.47mm, respectively (p<0.001). Larvae of the control group showed the significant smallest TL (7.62 ± 0.54 mm) and lowest DW (2.07±0.82 mg) compared to larvae of the other three feeding groups (Fig.5). The significant highest DW

(2.85±0.99 mm) compared to the control was observed in larvae of the new feed group ($p<0.05$).

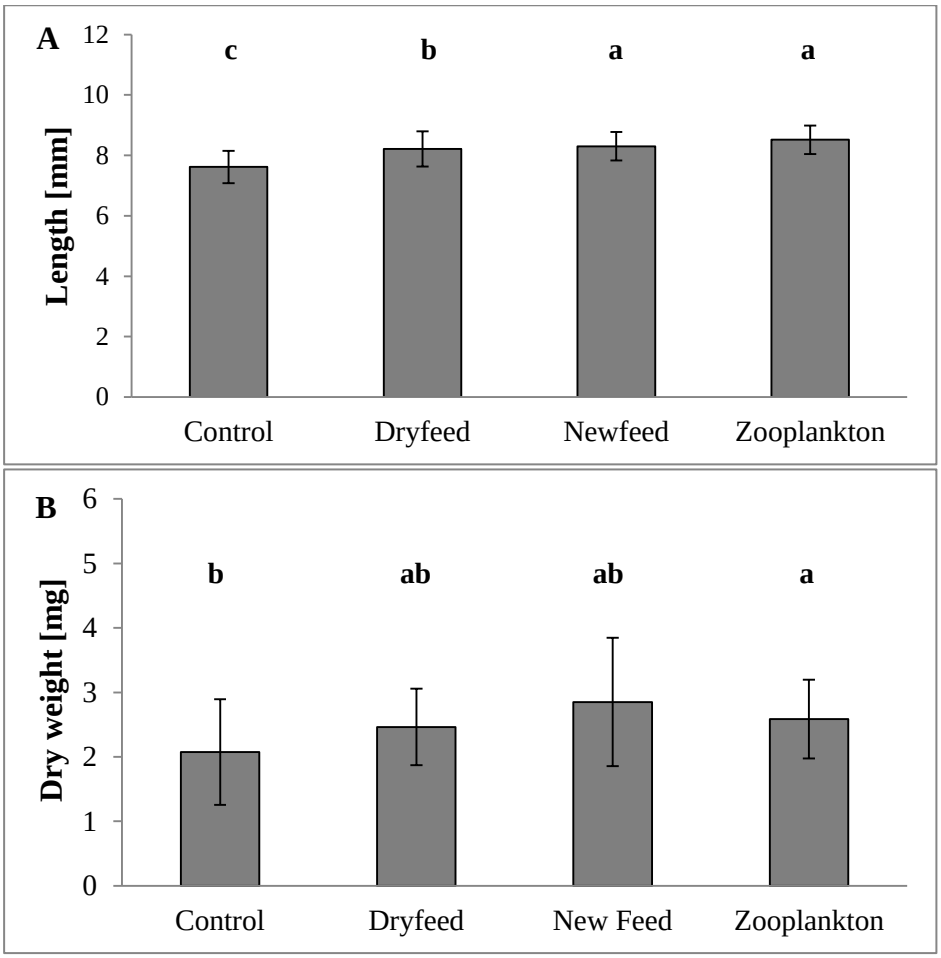


Figure 5. Total length (n=360) in mm (A) and dry weight (n=120) in mg (B) of lumpsucker larvae of the four different feeding groups at 19 DPH. Bars sharing the same letter showed no significant differences.

Significant (TL:$F_{(3,356)}$=10.8, $p<0.001$ and DW:$F_{(3,116)}$=9.0, $p<0.001$) differences between TL and DW of larvae of the four groups were also seen at 26 DPH. The largest larvae after 3 weeks of feeding were larvae from the new feed group and had a significant higher TL (9.18±0.61 mm) and DW (3.17±0.6 mg) than the control and the zooplankton group (Fig. 6, Appendix Table III,IV). Larvae from the control group remained the smallest larvae compared with the other three feeding groups, having the smallest TL (8.63± 0.79 mm) and DW (2.25±0.55 mg, Fig. 6). However, at 26 DPH the TL and DW from larvae from zooplankton group did not differ significantly from the control group.

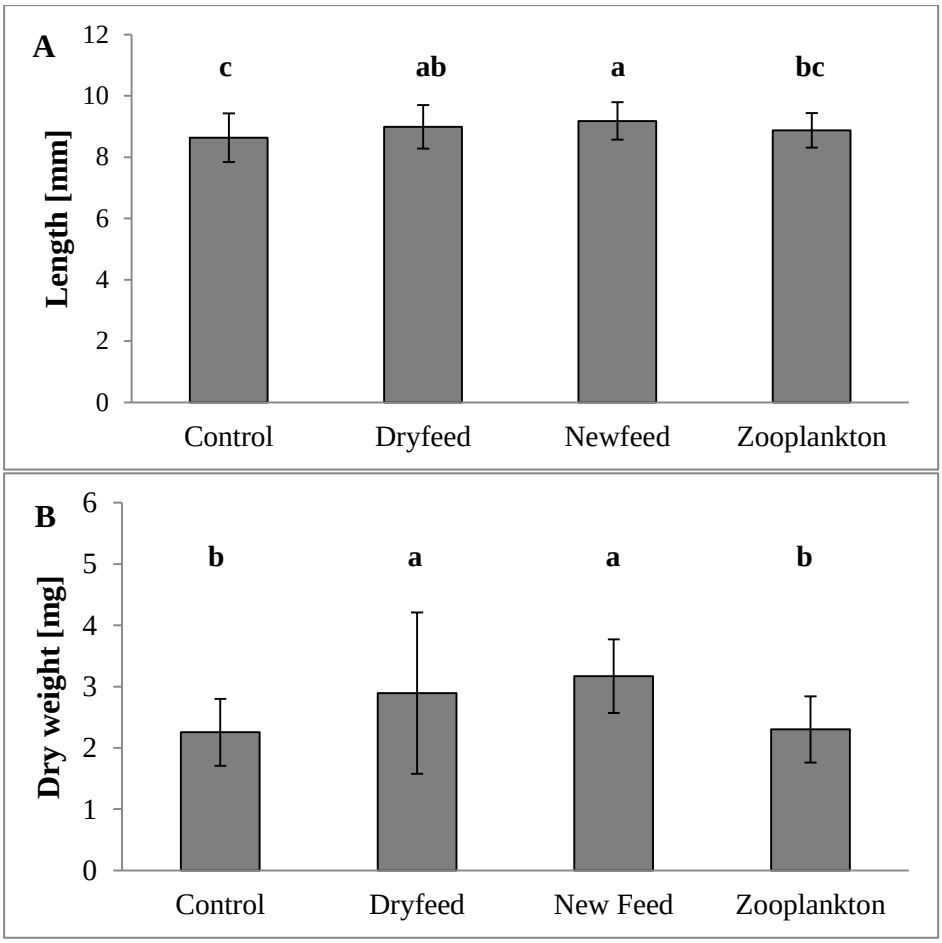


Figure 6. Total length (n=360) in mm (A) and dry weight (n=120) in mg (B) of lumpsucker larvae of the four different feeding groups at 26 DPH. Bars sharing the same letter showed no significant differences.

At 33 DPH, the differences between TL and DW of the different groups were still significant ($F_{(3,356)}$=101.1, p<0.001 and $F_{(3,116)}$=26.3, p<0.001, respectively). In contrast to 26 DPH, larvae fed with zooplankton showed the significant smallest larvae (TL=9.03±0.76mm; DW= 2.57±1.12mg) compared to the other three groups (p<0.001, Fig. 7). Differences in TL and DW between those three groups were not significant (Fig. 7).

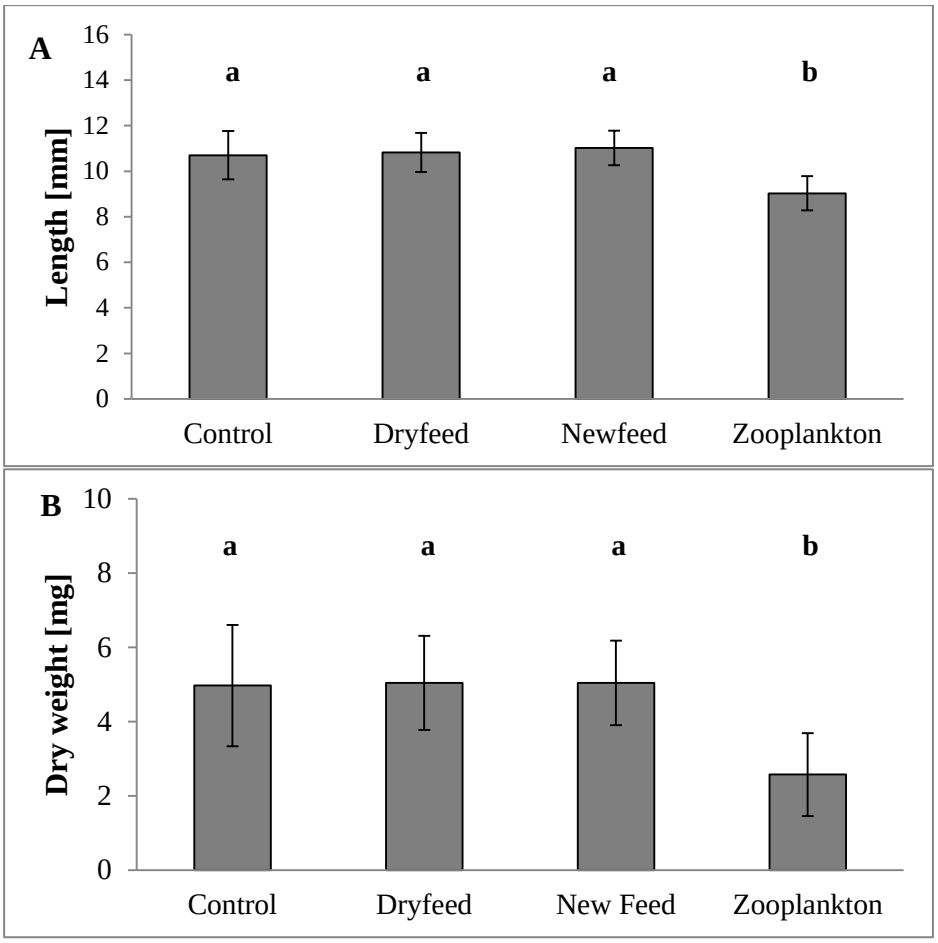


Figure 7. Total length (n=360) in mm (A) and dry weight (n=120) in mg (B) of lumpsucker larvae of the four different feeding groups at 33 DPH. Bars sharing the same letter showed no significant differences.

Differences in TL and DW between the groups were also significant at 47 DPH (TL:$F_{(3,356)}$=272.9, p<0.001 and DW:$F_{(3,116)}$=65.0, p<0.001). Larvae of the new feed group showed the largest larvae (Fig. 8). The TL (15.86±1.38mm) was significant higher than from larvae of the other three groups (p<0.001). Second largest larvae were larvae from the dry feed group, which had significant higher TL (15.05 ± 0.86mm) than larvae from the control and zooplankton group (p<0.001). On the other hand larvae fed with frozen zooplankton showed the smallest larvae (TL= 10.11±0.88mm, DW=3.49±1.56 mg, p<0.001). The larvae of the zooplankton group had a five times smaller DW than larvae from the new feed group, 3.49±1.56 vs. 16.82±3.78 mg. However, DW from the other three groups did not differ significant.

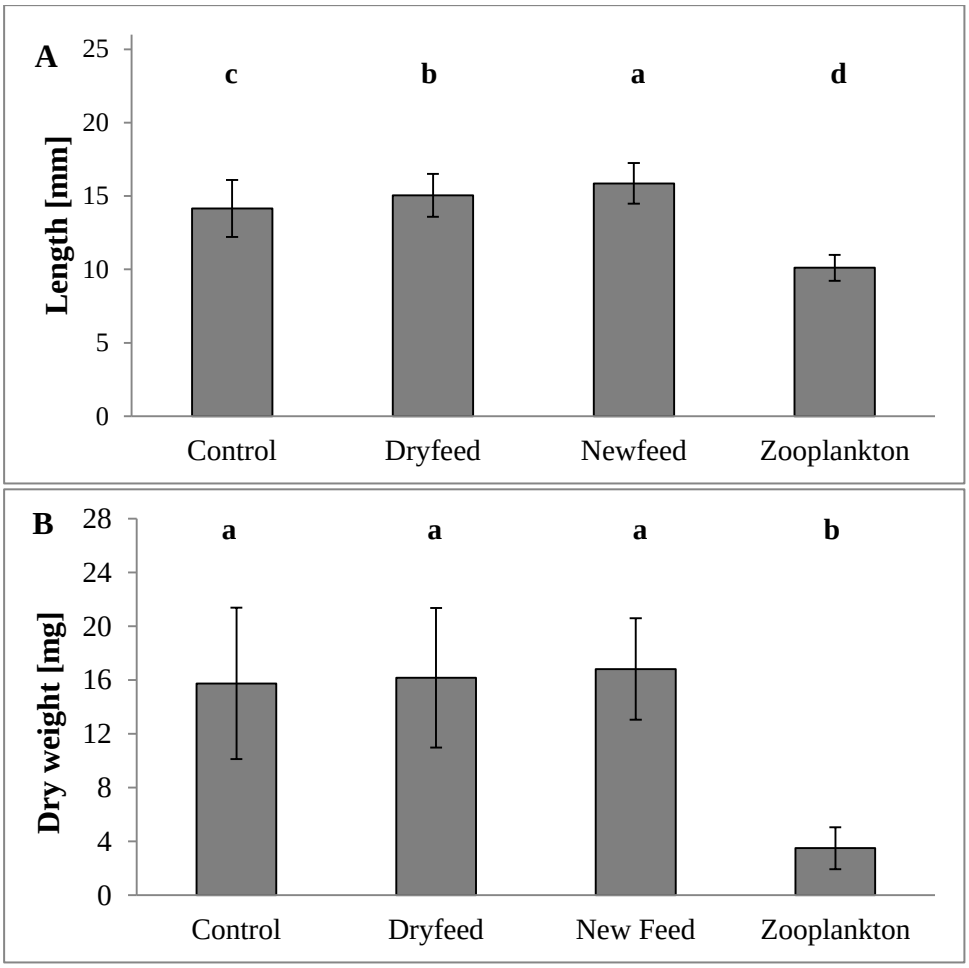


Figure 8. Total length (n=360) in mm (A) and dry weight (n=120) in mg (B) of lumpsucker larvae of the four different feeding groups at 47 DPH. Bars sharing the same letter showed no significant differences.

At the end of the trial, at 67 DPH the TL and DW differed between the feeding groups significant ($F_{(3,356)}$=578.3, p<0.001 and $F_{(3,116)}$=52.9, p<0.001, respectively). Larvae of the new feed group were the largest larvae with a TL of 24.79±2.92 mm and a DW of 71.3±20.87 mg (Fig. 9). This was significant larger than larvae of the control (p<0.05) and the zooplankton group (p<0.001), but not compared to larvae of the dry feed group. The significant smallest (TL= 10.82±1.13 mm; DW= 9.19±12.17mg) larvae after 9 weeks of feeding were larvae fed with frozen zooplankton (p<0.001, Fig. 9). The TL of larvae of the zooplankton group was 2.3 times and its DW was 7.7 times smaller than larvae of the new feed group.

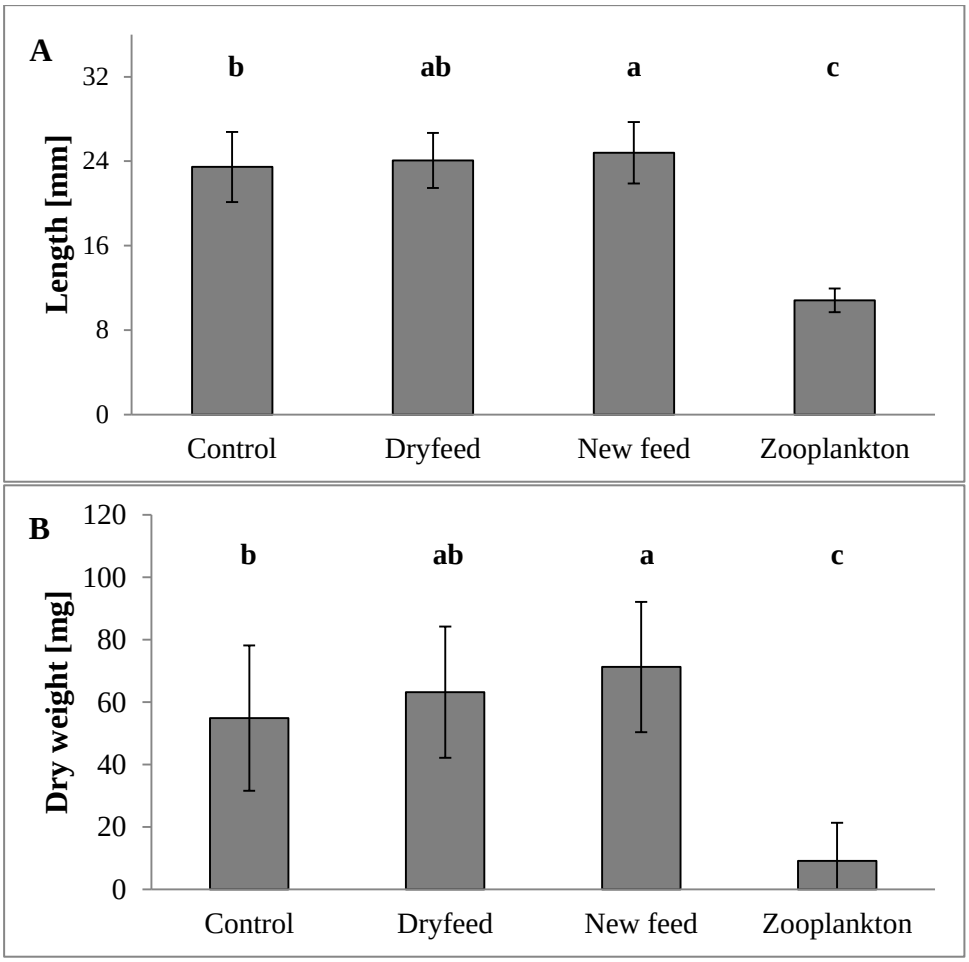


Figure 9. Total length (n=360) in mm (A) and dry weight (n=120) in mg (B) of lumpsucker larvae of the four different feeding groups at 67 DPH. Bars sharing the same letter showed no significant differences.

Growth rate

Larvae from all feeding groups increased their TL and DW significant during the 67 days ($F_{(6,2492)}$= 5393.0, p<0.001, Fig.10), but the feed had a significant effect on the growth of larvae (TL:$F_{(3,2492)}$=819.2, p<0.001 and DW:$F_{(3,716)}$=51.5, p<0.001). The growth in TL and DW of larvae of the same feeding group showed similar curves, but weight seemed to be a better indicator for growth than length at the later stages (Fig. 10). The largest differences in DW were seen at the end of the trial at 67DPH, but started to be present at 33 to 47 DPH (Fig. 10).

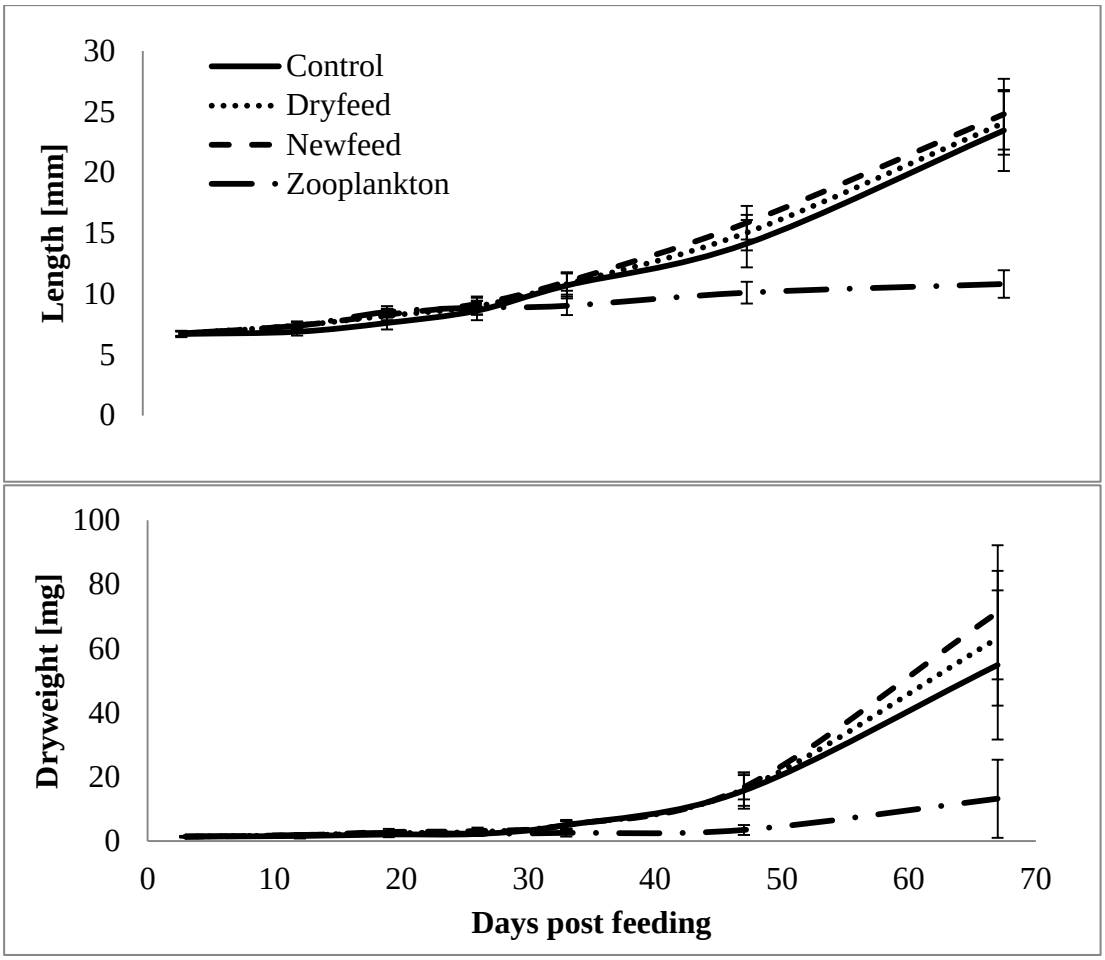


Figure 10. Growth in length and dry weight of lumpsucker larvae reared under four different feeding regimes during the first 67 days. Continuous line = control, dotted line = dry feed, dashed line = new feed, alternating line = zooplankton.

A closer look at the first 33 DPH showed that starting with the first week of feeding (5 till 12 DPH) differences in the growth between the feeding groups could be detected (Fig.11). In this first week, the SGR of larvae from the control (0.013±0.03 mg/day) was just half of the SGR of larvae from the other groups and continued to be the lowest till 26 DPH (Table 2). At 26 DPH all the groups showed a decrease in SGR, with the strongest decrease in the zooplankton group (Table 2, Fig.11).

After 26 DPH the growth increased strongly in larvae of the control, dry feed and new feed group (Fig. 10, 11). Larvae of the control group showed the highest growth in that week and therefore had a similar SGR (0.042±0.01 mg/day) like the other 2 groups at 33DPH (Table 2). Larvae from the zooplankton group on the other hand showed a stagnation in growth (0.020±0.00 mg/day), which continued till 47DPH (Table 2, Fig. 10). At the end of the trial the SGR of larvae from that group increased the SGR to 0.027±0.02 mg/day, but showed the

lowest growth compared to all the other groups (Table 2). The growth of larvae from the control, dry feed and new feed group increased till 47DPH and continued to increase sharp till 67DPH, especially in DW (Fig.10). In this time window, higher SGR were observed in all the three groups compared to earlier sample points, while the highest SGR in the whole the trial was at 67 DPH (Table 2). The highest growth (0.061±0.00 mg/day) was detected in larvae from the new feed group (Table 2, Fig. 10).

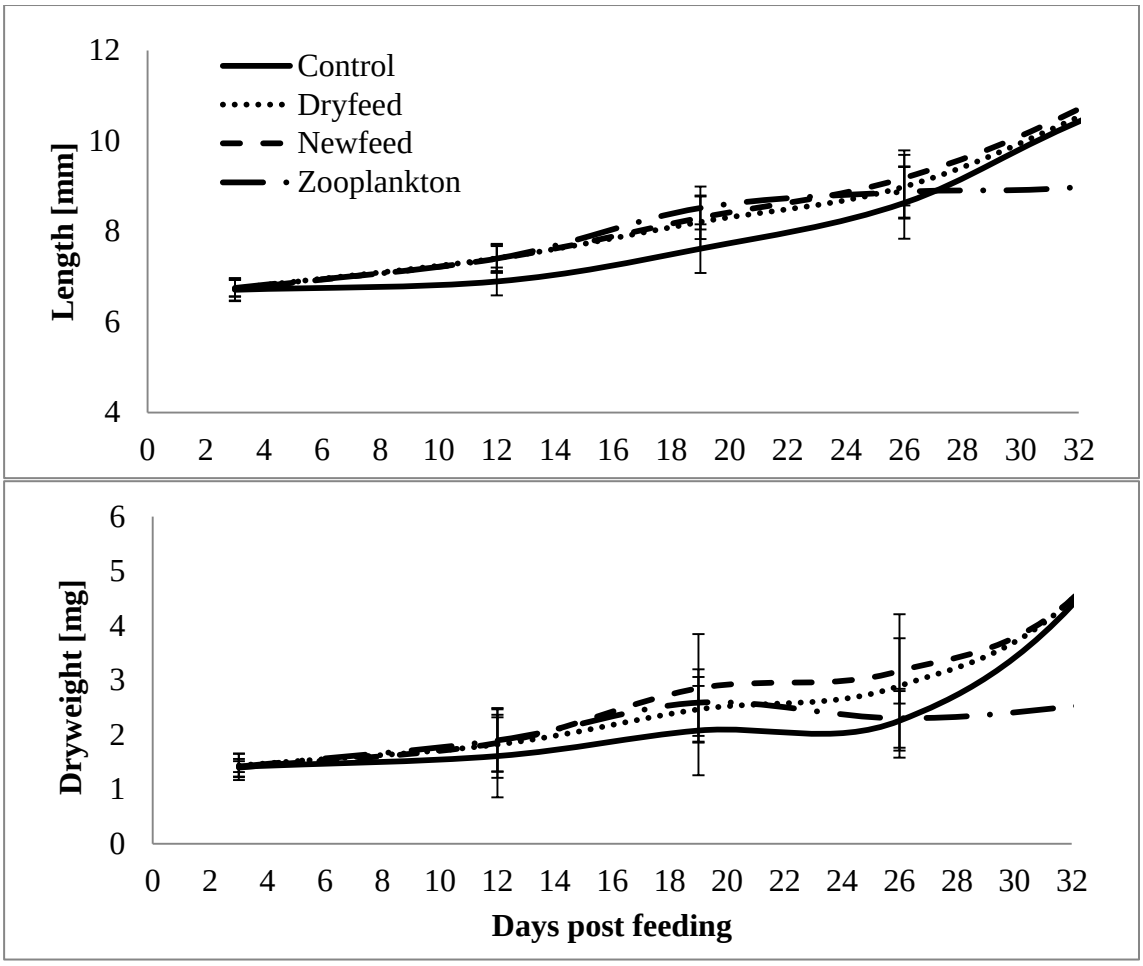


Figure 11. Growth in length and dry weight of lumpsucker larvae reared under four different feeding regimes during the first 32 days. Continuous line = control, dotted line = dry feed, dashed line = new feed, alternating line = zooplankton

Table 2. Specific growth rate (mg/d) of lumpsucker larvae reared under four different feeding regimes during the first 67 days.

DPH	12	19	26	33	47	67
Control	0.013±0.03	0.022±0.02	0.020±0.01	0.042±0.01	0.055±0.00	0.057±0.00
Dryfeed	0.026±0.03	0.034±0.01	0.031±0.01	0.042±0.00	0.055±0.00	0.059±0.00
New Feed	0.029±0.02	0.043±0.01	0.036±0.00	0.043±0.01	0.057±0.00	0.061±0.00
Zooplankton	0.030±0.04	0.038±0.02	0.021±0.00	0.020±0.00	0.020±0.01	0.027±0.02

Survival

Variances of the survival data were homogenous and survival of larvae from tanks of the same feeding groups were not significant different from each other. On the other hand, the feed had a significant effect on the survival of lumpsucker larvae ($F_{(3,8)}$=852.0, $p<0.001$). Survival at the end of the trial (67DPH) of larvae from the control, dry feed and new feed group was in general very high with more than 89% survival (Fig.12). The significant highest survival was seen in larvae fed with new feed ($p<0.05$). In this group over 95 % of the larvae survived. Larvae fed with zooplankton had the significant lowest survival rate with 0.09 % ($p<0.001$).

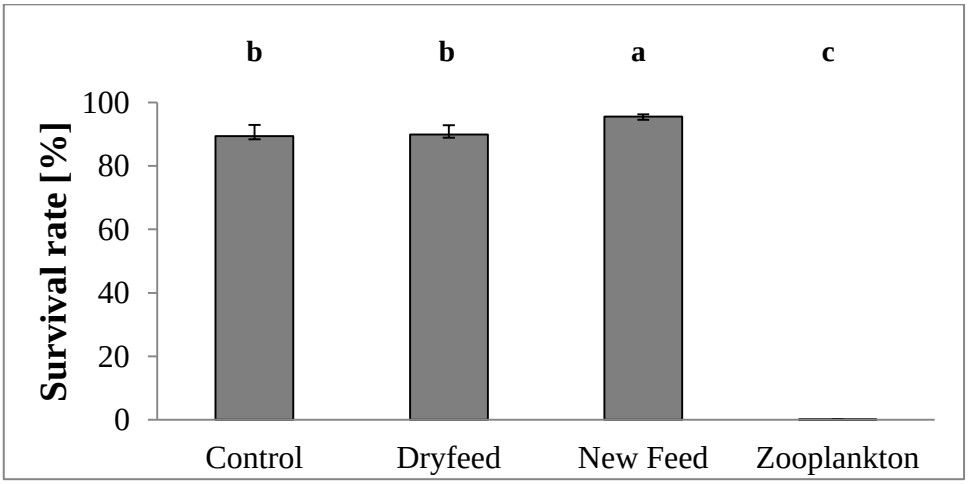


Figure 12. Survival rate of lumpsucker larvae after 67 DPH reared under different feeding regimes. Bars sharing the same letter showed no significant differences.

Highest mortality of the larvae from the control, dry feed and new feed group occurred during the first 4 weeks of feeding (Fig. 13). A closer look at those first weeks showed that the survival of larvae from the control and the new feed group started to decrease around 17 DPH (Fig.14). Larvae of the control showed a steep increase in mortality till 26 DPH, while larvae fed with new feed showed less mortality, but prolonged till 32 DPH. A similar flatter survival curve had larvae of the dry feed group (Fig. 14). In contrary to the larvae from the new feed group, this period of higher mortality was over 4 weeks, from 9 DPH till 35 DPH. After those first weeks almost no mortality could be observed in the control, dry feed and new feed tanks (Fig. 13). The survival of larvae from the zooplankton group started to decrease at the same time and in the same degree as larvae of the new feed group (Fig. 14). However, at 24 DPH, survival decreased steeply and even more drastic around 30 DPH. After 40 DPH almost no larvae were left in the tanks.

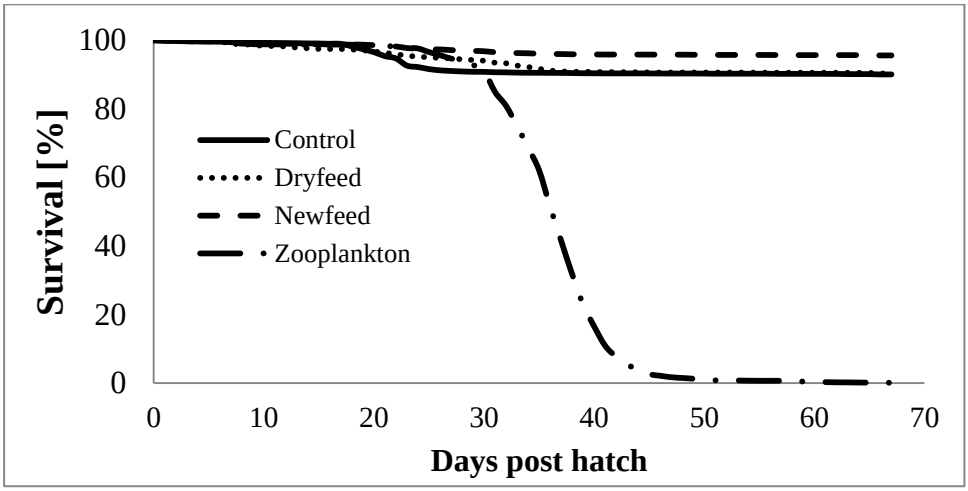


Figure 13. Survival rate of lumpsucker larvae reared under four different feeding regimes during the first 67 days. Continuous line = control, dotted line = dry feed, dashed line = new feed, alternating line = zooplankton.

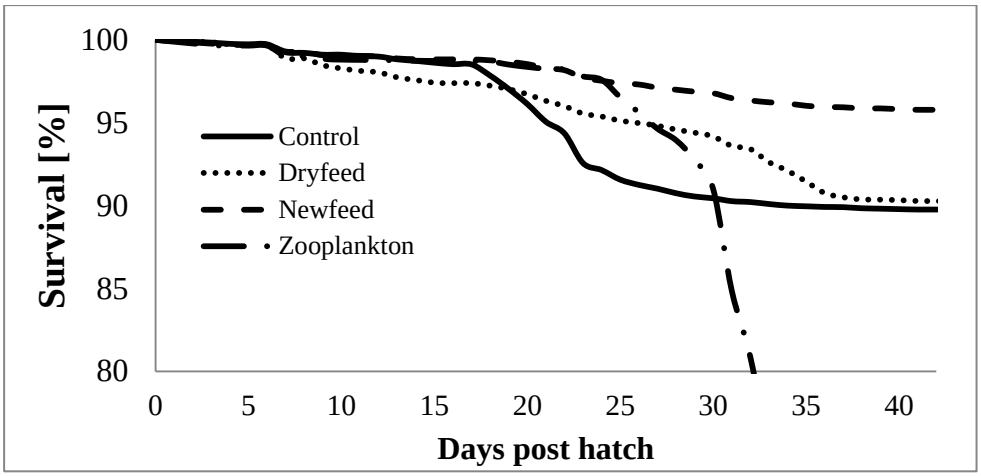


Figure 14. Survival rate of lumpsucker larvae reared under four different feeding regimes during the first 40 days. Continuous line = control, dotted line = dry feed, dashed line = new feed, alternating line = zooplankton.

Fatty Acid Composition

Feed

Unfortunately there are no results available from the fatty acid analyze of *Artemia*, but for the other three feeds it was possible to get results from the analysis. In the dry feed the most abounded fatty acid was C18:2n-6 with 5.42±0.31 g/100g sample, followed by C16:0 with 3.38±0.18 g/100g sample and C18:1n-9 with 2.34±0.13 g/100g sample (Fig. 15). The most abounded fatty acids in the new feed and in frozen zooplankton were C22:6n-3 (DHA) with 2.22±0.05 and 1.94±0.24g/100g sample, C20:5n-3 (EPA) with 1.49±0.05 and 2.13±0.29 g/100g sample, and C16:0 with 1.22±0.06 and 1.26±0.17g/100g sample, respectively (Fig. 15).

There were significant differences in the amounts of C16:0 ($F_{(2,9)}$= 278.2, $p<0.001$), C18:0 ($F_{(2,9)}$= 181.4, $p<0.001$), C19:0 ($F_{(2,9)}$= 462.4, $p<0.001$), C18:1n-9 ($F_{(2,9)}$= 814.3, $p<0.001$), C18:2n-6 ($F_{(2,9)}$= 1097, $p<0.001$), C20:5n-3 ($F_{(2,9)}$= 28.4, $p<0.001$) and C22:6n-3 ($F_{(2,9)}$= 5.4, $p<0.05$) between the different feeds. Comparing the three feeds with each other showed that dry feed had significant higher amounts of C16:0, C18:0 (0.67±0.03 g/100g sample), C19:0 (0.60±0.02 g/100g sample), C18:1n-9 and C18:2n-6 ($p<0.001$). Especially big differences in C18:2n-6, C16:0, C18:1n-9 were seen (Fig. 15). The new feed had the lowest amounts of C18:0 (0.11±0.06g/100g sample, $p<0.001$) and C18:1n-9 (0.16±0.02 g/100g sample,

p<0.001), but was the only feed of the three which showed C20:4n-6 (ARA, 0.01±0.01). Zooplankton had the lowest amounts of C19:0 (0.05±0.03 g/100g sample, p<0.001) and compared to the dry feed a lower amount of C22:6n-3 (p<0.05). On the other hand, the highest amount of 20:5n-3 was observed in the frozen zooplankton compared to the dry feed (p<0.001) and the new feed (p<0.05).

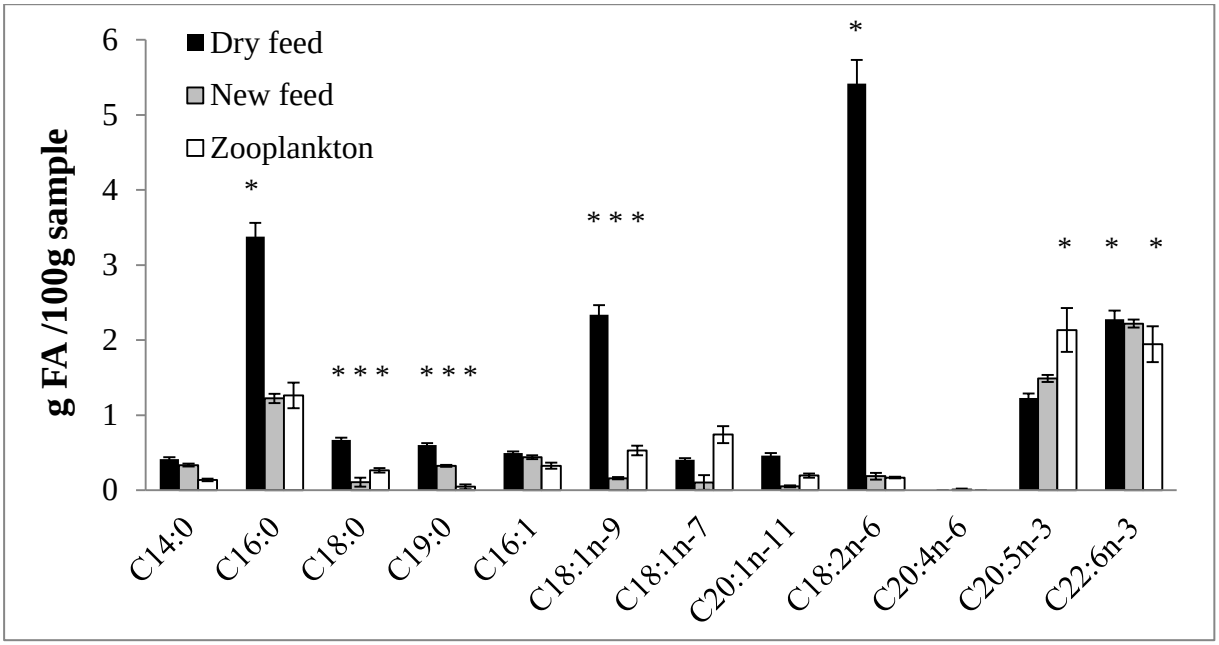


Figure 15. Fatty acid composition (n=12) in g of fatty acid (FA) per 100g sample of the three feed types. Dry feed=black, new feed=light grey and frozen zooplankton=white. Stars are showing significant differences. FA<0.2 g/100g (except ARA) are excluded from the graph, but are given in the Appendix Table IX.

Significant differences were also seen between the total lipid content ($F_{(2,9)}$= 299.8, p<0.001), the total amount of n-6 HUFAs ($F_{(2,9)}$= 823.8, p<0.001), the ratio of n-6/n-3 ($F_{(2,9)}$= 1400, p<0.001) and the ratio of DHA/EPA ($F_{(2,9)}$= 9.628, p<0.001) of the three different feed types. The total lipid content of the dry feed (20.25±0.84 g/100g) was significant higher than of the new and zooplankton (Table 3, p<0.001). Also the highest total amount of n-6 HUFAs (5.53±0.36 g/100g sample) and the highest n-6/n-3 ratio (1.47±0.08) was found in the dry feed (p<0.001). A significant lower DHA/EPA ratio of 0.91±0.02 showed the frozen zooplankton compared to the dry feed and the new feed which had a DHA/EPA ratio of 1.85±0.02 (p<0.001) and 1.49±0.02 (p<0.05), respectively.

Table 3. Total lipid content, total n-6 HUFAs, total n-3 HUFAs (in g per 100g sample) and ratio of n-6/n-3 HUFAs and ratio of DHA/EPA of the dry feed, new feed and frozen zooplankton. Values sharing the same or no letter showed no significant differences.

FA (g/100g)	Dry feed	New feed	Zooplankton
Total lipid	20.25±0.84[a]	8.78±0.32[b]	8.89±0.99[b]
n-6 HUFAs	5.53±0.36[a]	0.24±0.06[b]	0.26±0.01[b]
n-3 HUFAs	3.75±0.18	3.83±0.06	4.12±0.53
n-6 / n-3 ratio	1.47±0.08[a]	0.06±0.02[b]	0.06±0.01[b]
DHA/EPA ratio	1.85±0.02[a]	1.49±0.02[b]	0.91±0.02[c]

Larvae of 33 DPH

At 33 DPH the most abundant fatty acid in the fatty acid profile of larvae of the control, dry feed and new feed group was C18:2n-6 (3.21±0.28, 2.55±0.05 and 2.56±0.10 g/100g sample), followed by C22:6n-3 (2.63±0.15, 2.30±0.04 and 2.34±0.08 g/100g sample), C16:0 (2.60±0.16, 2.21±0.02 and 2.28±0.05 g/100g sample) and C18:1n-9 (2.26±0.18, 2.06±0.03 and 2.10±0.15 g/100g sample, Fig 16), respectively. In larvae of the zooplankton group on the other hand, the most abundant fatty acids were C22:6n-3(2.97±0.53 g/100g sample), C16:0 (1.78±0.01 g/100g sample), C18:1n-9 (1.58±0.29 g/100g sample) and 20:5n-3(1.17±0.11 g/100g sample).

Having a closer look at the amounts, there were significant differences between the levels of C16:0 ($F_{(3,8)}$=42.6, $p<0.001$), C18:0 ($F_{(3,8)}$=40.0, $p<0.001$), C18:1n-9 ($F_{(3,8)}$=7.4, $p<0.05$), C18:2n-6 ($F_{(3,8)}$=162.1, $p<0.001$) and C20:5n-3($F_{(3,8)}$=8.4, $p<0.05$) in lumpsucker larvae of the different feeding groups. Larvae of the dry feed and new feed group had no significant difference between fatty acid compositions and showed similar profiles (Fig. 16). Larvae of the control group had quite a similar fatty acid profile as larvae of the dry feed and new feed group, nevertheless showed a significant higher amount of C16:0 (2.60±0.16g/100g, $p<0.05$), C18:0 (0.83±0.05g/100g, $p<0.05$) and C18:2n-6 (3.21±0.29g/100g, $p<0.05$). On the other hand in larvae fed with zooplankton a different fatty acid profile was observed (Fig. 16). Larvae had significant lower amounts of C16:0 (1.78±0.06g/100g, $p<0.001$), C18:1n-9 (1.58±0.29g/100g, $p<0.05$) and C18:2n-6 (0.4±0.13g/100g, $p<0.001$) than larvae of the other

groups, but highest C18:0 (1.06 ±0.05g/100g, $p<0.001$) and C20:5n-3 levels (1.17 ± 0.11g/100g, $p<0.05$). No C20:4n-6 was detected in larvae of the zooplankton group, which could be found in small amounts in larvae of the other groups (0.19±0.01g/100g).

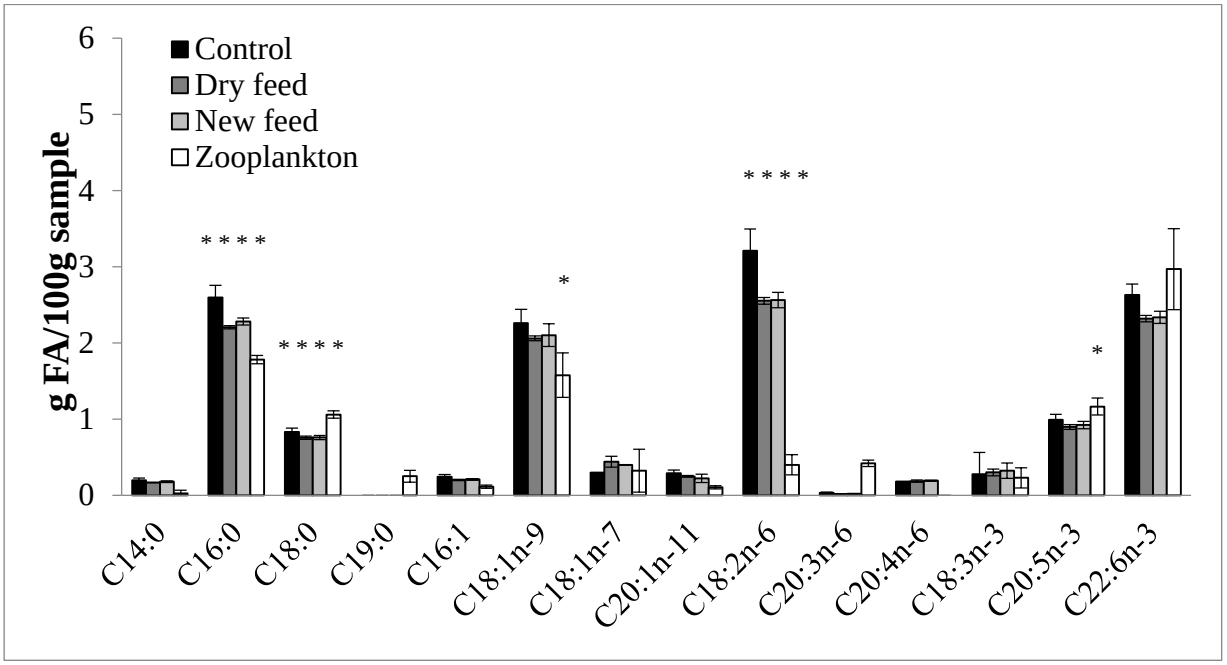


Figure 16. Fatty acid composition (n=12) in g of fatty acid (FA) per 100g sample of lumpsucker larvae reared under four different feeding regimes at 33 DPH. Black= control, dark grey= dry feed, light grey = new feed, white = zooplan. ton. Stars are showing significant differences, but no significant difference between dry feed and new feed group. FA<0.2 g/100g (except ARA) are excluded from the graph, but are given in the Appendix Table X.

There were also significant differences between the amounts of total lipid content ($F_{(3,8)}=14.0$, $p<0.001$), total n-6 HUFAs ($F_{(3,8)}= 130.6$, $p<0.001$) and the ratio of n6/n3 ($F_{(3,8)}=523.8$, $p<0.001$) of lumpsucker larvae of the different feeding groups (Table 4). Larvae of the control group had the significant highest total lipid content (15.03±0.78 g/100g, p-values in Appendix Table VII). They also showed the significant highest amount of n-6 HUFAs (3.48±0.29g/100g) and the significant highest n-6/n-3 ratio (0.86±0.02) compared to larvae of the other groups (p-values in the Appendix Table VII). In larvae of the zooplankton group the lowest amount of n-6 HUFAs (0.82±0.16g/100g, $p<0.001$) and the significant highest n-6/n-3 ratio could be detected (0.18±0.03, $p<0.001$).

Table 4. Table. Total lipid content, total n-6 HUFAs, total n-3 HUFAs (in g per 100g sample) and ratio of n-6/n-3 HUFAs and ratio of DHA/EPA of lumpsucker larvae reared under four different feeding regimes at 33 DPH. Values sharing the same or no letter showed no significant differences.

FA (g/100g)	Control	Dry Feed	New Feed	Zooplankton
Total lipid	15.03±0.78 [a]	13.22±0.18 [b]	13.36±0.44 [b]	12.65±0.26 [b]
n-6 HUFAs	3.48±0.29 [a]	2.79±0.06 [b]	2.82±0.11 [b]	0.82±0.16 [c]
n-3 HUFAs	4.04±0.25	3.62±0.08	3.71±0.15	4.50±0.6
n-6 / n-3 ratio	0.86±0.02 [a]	0.77±0.02 [b]	0.76±0.01 [b]	0.18±0.03 [c]
DHA/EPA ratio	2.65±0.05	2.59±0.09	2.53±0.06	2.54±0.37

Larvae of 67 DPH

As survival of larvae from the zooplankton group was extremely low, analyzing samples of the last week from this group would not have given scientific accurate results. Therefore, at 67 DPH only results of larvae from the other groups were analyzed and presented. The most abundant fatty acids in larvae of the control, dry feed and new feed group were C18:1n-9 (4.10±0.05, 4.16±0.42 and 4.30±0.57 g/100 g sample), C22:6n-3 (3.68±0.04, 3.60±0.26, 3.31±0.22 g/100 g sample), C18:2n-6 (3.23±0.08, 3.19±0.24 and 3.23±0.13 g/100 g sample) and C16:0 (3.15±0.08, 3.01±0.15 and 3.07±0.11), respectively (Fig. 17). Fatty acid composition was not significant different between larvae of those three groups. There were also no differences between the total lipid content, total n-3 HUFAs and total n-6 HUFAs as well as between the ratios of n-6/n-3 HUFAs and DHA/EPA (Table 5).

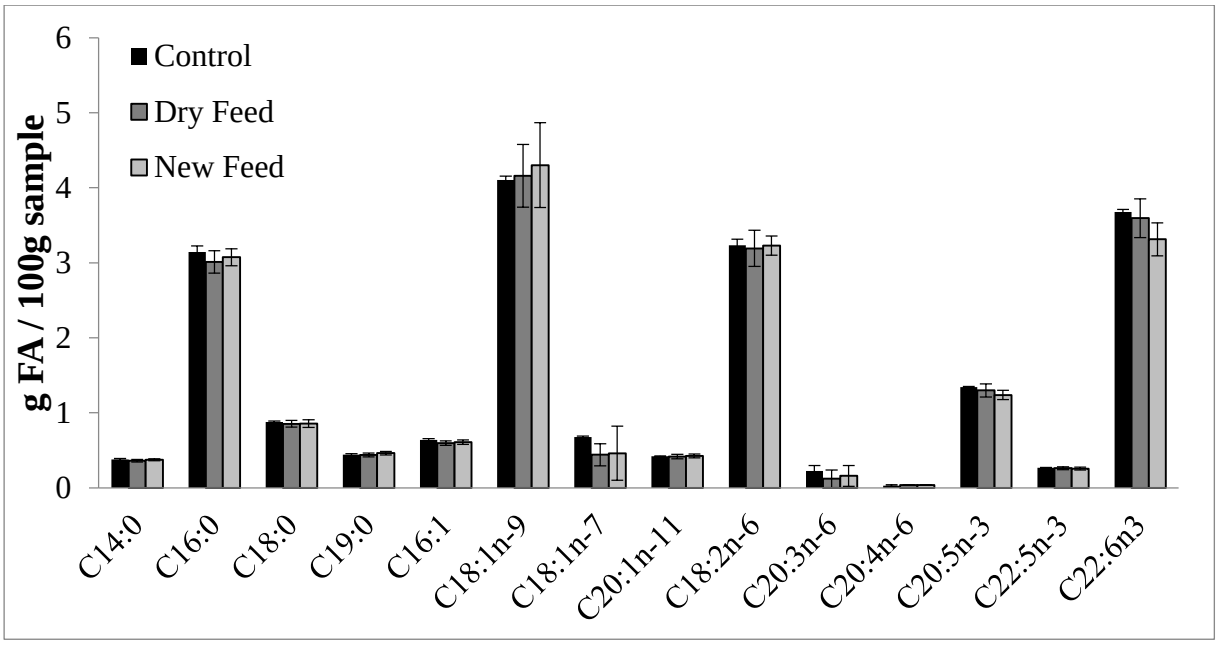


Figure 17. Fatty acid composition (n=12) in g of fatty acid (FA) per 100g sample of lumpsucker larvae reared under three different feeding regimes at 67 DPH. Black= control, dark grey= dry feed, light grey = new feed. No significant difference in fatty acid composition. FA<0.2 g/100g (except ARA) are excluded from the graph, but are given in the Appendix Table XI.

Table 5. Table. Total lipid content, total n-6 HUFAs, total n-3 HUFAs (in g per 100g sample) and ratio of n-6/n-3 HUFAs and ratio of DHA/EPA of lumpsucker larvae reared under three different feeding regimes at 67 DPH. Values sharing the same or no letter showed no significant differences.

FA (g/100g)	Control	Dry Feed	New Feed
Total	22.03±0.41	21.36±1.2	21.36±1.17
n-6 HUFAs	3.67±0.06	3.53±0.19	3.60±0.18
n-3 HUFAs	5.32±0.31	5.18±0.36	4.84±0.3
n-6 / n-3	0.69±0.01	0.68±0.03	0.75±0.05
DHA/EPA	2.74±0.01	2.77±0.02	2.67±0.06

Deformities and other observations

In all the feeding groups were no deformities of lumpsucker larvae observed during the 67 days.

On the other hand, during analyzing the pictures of larvae for the length measurements, feed could be detected in the gut as larvae are relatively transparent on the ventral side. This was a general observation and will not be included in the results as there is no other data supporting this and the gut content could not be quantified. Anyhow, those observations might be important for the discussion of the current study, but also for future studies.

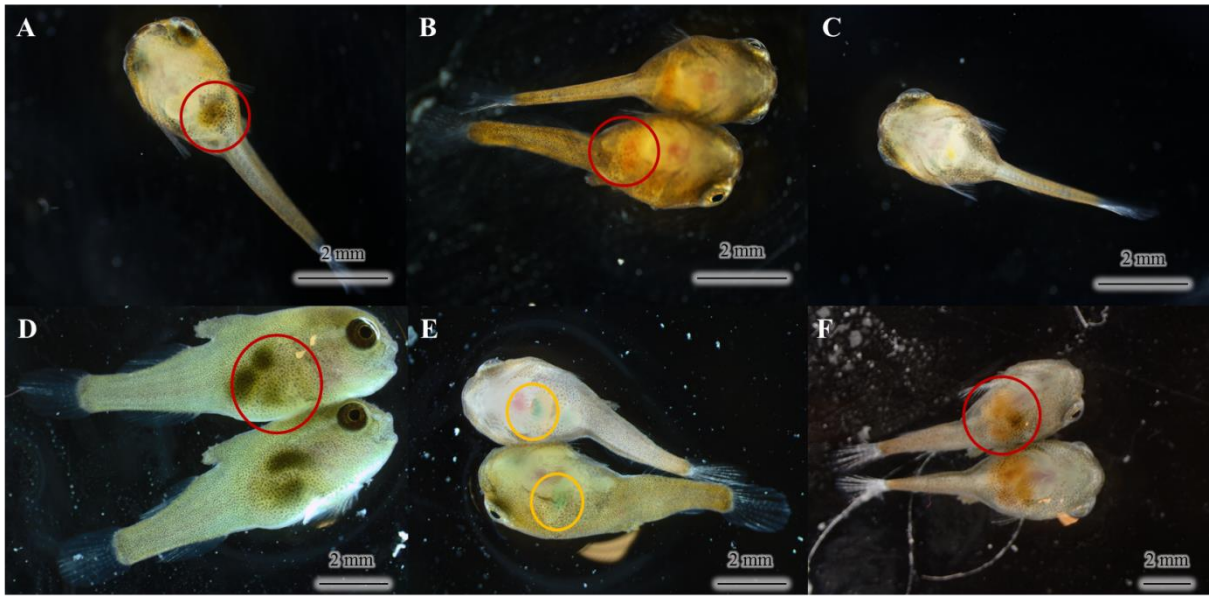


Figure 18. General observations of gut content of lumpsucker larvae. A,B: Ventral appearance of larvae fed with dryfeed (A) and Artemia (B) at 12 DPH. C: Ventral appearance of larvae with empty gut (with orange colored bill in gall bladder) at 12 DPH. D: Lateral appearance of larvae fed with dryfeed at 33 DPH. E: Ventral appearance of larvae fed with zooplankton at 33 DPH. Larvae show small or no amount of feed and green colored bill in gall bladder (yellow circle).F: Ventral appearance of larvae fed with zooplankton at 67 DPH. Red circles mark the feed in the gut of the larvae.

Discussion

Lumpsucker larvae

Only little information exists about the early life stages of lumpsucker. The size of newly hatched larvae, which has been reported, is similar to the present study (Davenport 1985, Benfey and Methven 1986, Moring 2001). On the other hand growth of the larvae in this study seemed to be better compared with previous published literature. Benfey and Methven (1986) reared larvae, which just grew 1.3 mm and 7.1 mg in the first 33 days, while the lumpsucker larvae of Brown et al. (1997) were just 8 mm after 50 days. Temperature and the feeding regime (both *Artemia* and a commercial feed) were similar between the literature and the present study. Pulse feeding, like it was done in the present study can improve growth of lumpsucker larvae compared to continuous feeding (Brown et al. 1997). Light conditions, prey density, fish density or in general good rearing conditions during the present study could be reasons for the better growth performance.

Most other cultured marine species like Atlantic cod (*Gadus morhua*) (Puvanendran and Brown 2002, Hall et al. 2004), European sea bass (*Dicentrarchus labrax*) (Houde 1989, Villamizar et al. 2009) and gilthead sea bream (*Sparus aurata*) (Houde 1989, Fernandez-Diaz and Yúfera 1997) and Atlantic halibut (*Hippoglossus hippoglossus*) (Pittman et al. 1990, Olsen et al. 1999) show smaller, lighter and less developed larvae at hatch than lumpsucker. First feeding of the above mentioned species is even more challenging and therefore most of them rely on live prey. Nevertheless, the growth rate seems to be much higher (Houde 1989, Olsen et al 1999, Hamre et al. 2002, Puvanendran and Brown 2002). On the other hand when larvae of European sea bass (Cahu et al. 1999) and gilthead sea bream (Fernandez-Diaz and Yúfera 1997, Yúfera et al 2000) are fed with artificial diets, they show almost similar SGR compared to the lumpsucker larvae of the present study. The spotted wolffish (*Anarhichas minor*) is one of the few marine species that have larger larvae at hatch than the lumpsucker (Falk-Petersen et al. 1999, Hansen and Falk-Petersen 2001). They are well developed and can therefore feed on artificial feed straight away and the growth rate is similar to the lumpsucker larvae in present study (Falk-Petersen et al. 1999, Hansen and Falk-Petersen 2001).

The TL of lumpsucker larvae in this study increased linearly, while the DW increased exponentially. Therefore, larvae grow mostly in length the first weeks, while later on DW is a

better indicator for growth in lumpsucker larvae. The first 4 weeks (till 28 DPH) the DW increased slowly and showed even a decrease in SGR in the 4th week after hatch. An exponential growth in larval weight is also observed for other cultured marine species (Olsen et al 1999, Hansen and Falk-Petersen 2002, Puvanendran and Brown 2002). Larvae of Atlantic cod (Hamre et al. 2002, Puvanendran and Brown 2002), European sea bass (Cahu et al 1999), Atlantic halibut and spotted wolffish (Hansen and Falk-Petersen 2002) show a steep increase in weight after 20 to 25 DPH. Compared to lumpsucker larvae of the current study, this is one week earlier.

Larvae of Atlantic cod (Puvanendran and Brown 2002), European sea bass (Cahu et al 1999), gilthead sea bream (Fernandez-Diaz and Yúfera 1997, Yúfera et al 2000) and Atlantic halibut (Olsen et al 1999) show in general a much lower survival rate (20 to 60 %) than lumpsucker from the present study. Thus, the trend with the highest mortality during the first weeks is sheared with the lumpsucker larvae of the present study. In addition, Williams and Brown (1991) observed a peak in mortality of lumpsucker larvae at the 4th week after hatch. Therefore, the first weeks and especially the 4th week seemed to be a critical week in lumpsucker larvae. After this critical week, the DW increased steep and survival was stable with almost no mortality in the present study.

The nutritional value of the different feed

Commercial dry feed

A closer look at the commercial dry feed showed that the most abundant fatty acids, C18:2n-6 (linoleic acid), C16:0 (palmitic acid) and C18:1n-9 (oleic acid), are mainly found in vegetable oils which are used in fish feed to replace fish oil (Bell et al 2002, Izquierdo et al. 2003, Hamre 2006). Especially phospholipids from soybean lecithin are commonly used and contain large amounts of linoleic acid, the highest abundant fatty acid in dry feed (Sargent et al. 1997, Cahu and Zambonino 2001, Hamre 2006). The dry feed company uses soya beans as ingredients for their feeds and it could be suggested that this was also used in the dry feed of this study (skretting.com). Phospholipids are beneficial for the digestion of fatty acids as mention in the introduction and diets containing up to 6.6% phospholipids from soybean lecithin showed good growth rates for other marine fish larvae (Cahu and Zambonino 2001). Increased phospholipid levels in the diet improved also lipid and EPA retention in fish larvae

(Izquierdo et al 2000). On the other hand with increasing non-marine phospholipids or other non-marine fatty acids in the diet, the fatty acid composition can get unfavorable and can influence the fatty acid profile of the fish, especially marine species which are limited in converting shorter C18-chains into HUFAs (Izquierdo et al. 2003, Hamre 2006, Holt 2011).

Nevertheless, the amount of the two important essential fatty acids for marine fish larvae, C20:5n-3 (EPA) and C22:6n-3 (DHA), showed the upper recommended levels for marine feed (Holt 2011). As well was the ratio of DHA and EPA the closest to the recommended ratio of 2:1 compared to the other two feed of this study (Holt 2011). One important aspect which had to be considered was that in the present study results of fatty acids were given as g per 100 g sample. Looking at the EPA and DHA percentage to the total fatty acid amount showed less than half of the amount recommended and found in copepods (Sargent et al 1999, Bell et al 2003, Evjemo et al 2003, Holt 2011). Nevertheless, the total lipid content of the dry feed was much higher than the total lipid content of copepods and therefore showed the required EPA and DHA levels (Hamre 2006, Holt 2011). Lipids are the major energy source in larvae and a diet with a higher lipid level can therefore deliver more energy (Cahu and Zambonino 2001). Additionally high lipid contents of 15% or even up to 25% showed to be beneficial in reducing cannibalism in cod hatcheries and nurseries (Hamre 2006). Especially saturated and monounsaturated fatty acids are considered to be important for metabolic energy (Sargent et al. 1997, Sargent et al. 1999). Dry feed showed a higher amount of saturated and monounsaturated fatty acid than copepods, but also than the new feed and the frozen zooplankton (Evjemo et al 2003, Maehre et al. 2013). Too high amounts of saturated and mono-unsaturated fatty acids on the other hand could lead to an imbalance (Sargent et al. 1997, Sargent et al. 1999).

New feed

There were differences between the fatty acid composition between the new feed and the other two feed.The new feed seemed to contain no or less vegetable oils than the dry feed as fatty acids such as linoleic acid, oleic acid and palmitic acid were much less abundant. Therefore, the new feed showed a much lower n6/n3 ratio than dry feed and closer to the ratio found in copepods (Sargent et al.1999, Evjemo et al 2003). EPA and DHA were the most abundant fatty acids in the new feed like it is seen in copepods, but showed lower levels (Sargent et al. 1999, Bell et al. 2003, Holt 2011).Nevertheless, the amounts of EPA and DHA

were similar to the amounts in dry feed and showed also the recommended levels for marine fish larvae (Holt 2011). The ratio between DHA and EPA was less than the optimal ratio of 2:1 with 1.5:1. The new feed was the only feed in the current study where C20:4n-6 (ARA) was detected, but just small amounts. All together the new feed seemed to show a nutritional value close to marine larvae's natural prey in terms of fatty acids (Sargent et al. 1999, Evjemo et al 2003, Holt 2011, Maehre et al. 2013).

Frozen zooplankton

Looking at the fatty acid composition of zooplankton compared to other feeds showed that most differences were compared to the dry feed. New feed showed much less amounts of linoleic acid, oleic acid and palmitic acid as frozen zooplankton was harvested from the marine environment and was not enriched with any vegetable oils. DHA and EPA were the two most abundant fatty acids and their levels were as recommended (Holt 2011). Nevertheless, the amount of EPA was higher and the amount of DHA was lower than usually found in live copepods (Sargent et al 1999, Evjemo et al 2003, Holt 2011, Maehre et al. 2013). This led to the lowest DHA/EPA ratio in this study and compared to copepods (Sargent et al 1999, Evjemo et al 2003, Maehre et al. 2013). Compared to frozen copepods of other studies, frozen zooplankton of the current study showed much higher EPA and DHA value (Yu et al. 2012).

The Artemia effect- Larvae fed with Artemia

To see how live feed effects the lumpsucker larvae, larvae of the control and larvae of the dry feed group were compared. Being fed with *Artemia* did not affect the survival of larvae, but increased the growth of larvae during the first two weeks significantly. It can be suggested that both feeds were ingested immediately by the larvae as feed was detected in the gut of larvae from both control and *Artemia* tanks (tanks of the dry feed, new feed and zooplankton group) after one day of introducing the feed. Nevertheless, a lower ingestion rate or lower digestibility of the commercial feed compared to *Artemia* could have led to a poorer growth. No investigations on the ingestion rate or on digestibility were done in the present study to confirm this.

However, the nutritional values of the feeds in terms of fatty acids were studied. Comparison between dry feed and *Artemia* itself could not be made. Yet it is assumed that *Artemia* have a

rather low nutritional value, especially in terms of fatty acids (Holt 2011). Dry feed on the other hand seemed to have a relatively good fatty acid composition and showed good EPA and DHA levels. The fatty acid composition between larvae of the control and the dry feed group could be compared. Nevertheless, it has to be considered that *Artemia* feeding was finished at 19 DPH. Hence, larvae were feeding on dry feed for already two weeks at 33 DPH and for almost 7 weeks at 67 DPH. Fatty acid compositions of larvae do shift with the diets (Rosenlund et al 1997). Therefore, the influences of *Artemia* composition might not be visible anymore (Rosenlund et al 1997). At 33 DPH larvae of both groups showed a similar frequency of fatty acids, but the amount of fatty acids from vegetable oils, used in the dry feed, was less in larvae of the dry feed group compared to the control group. This could suggest that the *Artemia* composition consisted of lower levels of those fatty acids. Also a lower total lipid content of *Artemia* could be suggested as larvae of the dry feed group showed a lower total lipid content compared to larvae of the control group. At the end of the trial (67 DPH) larvae of the dry feed group did not show any differences in the fatty acid profile compared to larvae of the control group, which showed that the larval compositions shifted with the diet.

Larvae of both groups seemed to have a good EPA and DHA retention as larvae show similar amounts as in the dry feed at 33 DPH. At 67 larvae of the control and dry feed group seemed to show even higher levels of DHA than the dry feed. This and a higher DHA/EPA ratio in larvae at 33 and 67 DPH than in the feed could suggest a better DHA retention than EPA retention. On the other hand deposition of fatty acid of vegetable oils such as linoleic acid seemed to be reduced and larvae seemed to show less amounts than the feed at both sampling points. Maximizing the retention of EPA and especially DHA in larvae and on the other hand minimizing the retention of linoleic acid should support good growth, survival and no deformities in larvae even with a higher amount of n6 fatty acids in feed. Analyzing larvae samples from 12 or 19 DPH, at *Artemia* feeding, could give a better inside of the composition of *Artemia* and larvae fed with *Artemia*.

A general observation during the execution of the experiment was that a lot of larvae from the control tanks were active swimming during the first days of feeding. This was not the case in the groups that were fed with *Artemia* and most larvae were adhere to the tank walls. From this attached position (clinging) larvae are able to feed and do not need to swim around to capture prey (Brown 1986, Killenet al. 2007). In Brown (1986) lumpsucker larvae did not swim in the water column for the first three weeks after hatch. But this behavior seems to be

depending on the availability of prey or feed. Killen et al. (2007) reported better growth in tanks with clinging lumpsucker compared to tanks with active swimming larvae.
In the present study it seems that larvae in the control tanks did not capture enough feed while clinging and needed to swim to find and get more feed. Both groups had sufficient amount of feed, but dry feed was partly floating, most of it was sinking and then was accumulated at the bottom. *Artemia* on the other hand is swimming and therefore constantly available at the clinging position. Hence, the characteristic of dry feed in the water seems to be less favorable in the clinging position and larvae have to actively search for it. Searching and swimming for feed costs energy, energy which cannot be used for growth. Higher growth in tanks with clinging lumpsucker compared to tanks with swimming lumpsucker was also observed in Killen et al. (2007).

The new feed effect - Larvae fed with new feed

Larvae of the dry feed group were compared with the new feed group, to exclude the *Artemia* effect and to see the impact of the new feed on lumpsucker. The new feed had a significant effect on larval survival and growth. Larvae showed higher survival and since introducing the new feed highest TL and DW, with significant larger larvae at 19 DPH and 47DPH compared to larvae from the dry feed group. It has to be mentioned and considered that larvae just got small amounts of the new feed and just for 4 weeks, but it still showed a significant effect. Given that larvae just got small amounts of new feed the question is: was it actually the nutritional value which increased growth or maybe just stimuli of a substance in the feed which increased the intake?

There were nutritional differences between the new feed and commercial dry feed and new feed seemed to be closer to the nutritional value of copepods than the dry feed. However, it did not change the fatty acid composition of the larvae fed with new feed. Larvae showed no differences in the fatty acid profile compared to larvae of the dry feed group. Perhaps the amount of ingested feed was too little to have an impact on the fatty acid profile. Other nutritional differences, for instance in proteins or vitamins, between the new feed and the commercial dry feed could have led to better growth and survival of larvae fed with new feed. No investigations of other nutrients than the fatty acids were made in this study to confirm this.

However, the fatty acid profile of larvae did not vary and an actually ingestion of the new feed could not be detected in the gut of the sampled larvae, but needs to be investigated more clearly in further studies. Therefore, maybe not the nutritional value of the new feed increased growth and survival, but an attractant or stimuli in the new feed increased feed intake in general (of *Artemia* and later dry feed). Physical attractant such as the color and movement of the feed and chemical attractant such as the smell and taste of the feed can stimulate the feeding behavior of fish larvae (Aragao et al. 2004, Kolkovski et al.2008). The color of the new feed varied from the dry feed. The new feed was dark red, while the dry feed was green. Movement or sinking behavior of the two feeds were not studied, but no variation was noted during feeding. Anyhow, to stimulate the feed intake, especially if assuming the intake of other feed such as the commercial dry feed or *Artemia*, physical attractiveness of the new feed might have been negligible. Chemical stimuli on the other hand might have been of greater importance.

Free amino acids are considered to be chemical attractants for fish larvae (Aragao et al. 2004). A commercial dry feed that was coated with krill hydrolysate increased ingestion, growth and survival rate of fish larvae compared to larvae fed the same but non-coated dry feed or *Artemia* (Kolkovski et al. 2000). Interesting was that when adding krill hydrolysate directly into the water, when larvae were fed with non-coated diet, the ingestion rate increased by 200% compared to larvae being fed with non-coated diet alone. The ingestion rate between larvae where krill hydrolysate was added in the water and larvae fed with the krill-coated diet was not different (Kolkovski et al. 2000).The new feed in the present study, even though it was embedded, was developed in that way to show some leakage to be more attractive for the larvae. Therefore the smell of the new feed, compounds leaking from the new feed into the water, might have increased the feed search of the lumpsucker larvae, which than increased the feed intake (probably mostly of *Artemia* and dry feed). A higher ingestion rate might have increased growth and survival of the larvae in the present study. Future studies with focus on the ingestion rate of lumpsucker should be carried out to confirm this.

The frozen zooplankton effect - Larvae fed with frozen zooplankton

Frozen zooplankton had a highly significant negative effect on larval survival and growth. The survival rate was less than 1% and larvae showed a very poor growth compared to the other groups. Hence, it can be concluded that frozen zooplankton alone was not suitable or sufficient as diet for lumpsucker larvae. Poor growth and survival of larvae has been observed in other species which were fed with frozen or preserved zooplankton (Yu et al. 2012,

Piccinetti et al. 2014). It is suggested that the nutritional value of the frozen zooplankton is not sufficient enough, and that the thawing process of plankton might lead to a reduction of the nutritional value (Sharma and Chakrabarti 2000, Yu et al. 2012). Mostly water soluble nutrients, which have not been investigated in the present study, are lost, rather than fatty acids (Busch and Nordgreen 2011, not published). A leakage trial with the new feed in a seawater suspension with focus on water soluble nutrients should be carried out in the future to confirm this.

Nevertheless, feed was embedded in the present study and should not show high leakage and nutritional lost. It was even suggested that co-feeding with preserved copepods which led to a better larval performance was due to the better nutritional value compared to *Artemia* (Piccinetti et al. 2014). In the present study when co-fed with *Artemia* lumpsucker larvae showed a very good growth and survival. Larvae had the highest TL compared to the other groups during *Artemia* feeding. In Yu et al. 2012 the larvae fed with frozen copepods showed a better EPA and DHA retention than larvae fed with micro diets. Larvae in this study seemed to have a high retention of fatty acids in general as almost all fatty acid levels, including DHA levels, were higher than in the frozen zooplankton feed. On the other hand, EPA deposition was not as good and levels in larvae were almost half the amount than found in the feed. Nevertheless, the ratio of DHA/EPA in larvae was much better than in the frozen zooplankton and the amount of EPA and DHA was as recommended, but not as high in marine fish larvae fed with copepods (Sargent et al. 1999, Busch et al. 2010). Compared to other studies where larvae were fed with preserved copepods, larvae of this study showed better levels of EPA and DHA (Yu et al. 2012, Piccinetti et al. 2014). No ARA (C20:4n-6) could be detected nether in feed, nor in larvae.

This showed that not the nutritional value should lead to a poor survival and growth of lumpsucker larvae when fed with zooplankton alone. Little or no amounts of feed were observed in the gut of larvae from the zooplankton group after *Artemia* feeding stopped. Additionally, almost all of those larvae showed a gall bladder with green colored bile. This was seen only in a very few larvae from the other groups or in the previous weeks with *Artemia* feeding. Enlarged gallbladders with green colored bile were observed in adult whiting after 24 to 40 hours starvation and in Atlantic salmon juveniles after 96 hours starvation (Talbot and Higgins 1982, Robb 1992). Therefore it seems larvae of the zooplankton group could not detect or capture enough feed and starved after *Artemia* feeding stopped.

Sea bream larvae being fed with only preserved copepods did not survive past 20 DPH (Piccinetti et al. 2014). It was suggested that the fast sinking behavior of the preserved feed led to the poor performance of larvae when fed solely. A combined diet of preserved zooplankton and live prey covers the nutritional requirements via copepods, but feed is available longer through the live feed. Larvae in the present study were able to first feed on the frozen natural plankton and later when those sank to the bottom, feed on *Artemia*. After *Artemia* feeding stopped, growth and survival of lumpsucker larvae decreased drastic as the availability of the feed decreased.

Nevertheless, larvae were able to be weaned on the dry feed which is also sinking. Chemical or visually attractant should have been higher in frozen zooplankton than in dry feed as during the *Artemia* feeding larvae showed better TL than larvae from the dry feed group. This supports the idea that the availability and the sinking behavior might be the reason for the poor performance of larvae fed with frozen zooplankton. In the present study zooplankton stayed up to an hour/1.5 hours in the water column of the tank after introducing it. However, larger sized plankton sank quicker and were less available for the larvae. As the sinking time of dry feed was not investigated in the present study it can be just assumed that dry feed might have had a different sinking behavior and stayed longer in the water column. What was observed was, that some of the dry feed also accumulated on the surface and was sinking later and was therefore more available for larvae. In addition, larvae were also swimming to the surface and feeding directly from the floating feed, which was also observed in Benfey and Methven (1986).

Interesting was that at 47 DPH and 67 DPH larvae showed a full gut and no green colored gall bladder anymore. This suggests that larvae were able to feed on zooplankton more successfully later on. It might be that lumpsucker larvae changed their foraging behavior to grazing on the bottom of the tank. No investigations on feeding behavior were made in the present study and future studies have to be done to confirm this. In the future the intervals between feeding should be shorter, but with smaller amounts to not contaminate the tank. Feed would be longer available for the larvae and might lead to a higher feed intake. On the other hand, maybe the density of the feed would not be enough. For now, the best way of feeding larvae with frozen zooplankton is via co-feeding with live prey or with artificial feed.

Long term differences in larval growth

After 26 DPH, larvae of the control group showed a better growth than larvae of the dry feed and new feed group, and all had similar TL and DW at 33 DPH. Larvae of the control group which captured and digested the dry feed for already 3 weeks seemed to have advantages after the critical 4th week over larvae from the dry feed and new feed group where dry feed was later introduced.

On the other hand, larvae of the dry feed group seemed to be better weaned onto dry feed than larvae from the new feed group. Co-feeding is assumed to prepare larvae to accept the artificial diet better when live feed is withdrawn (Rosenlund et al. 1997, Engrola et al. 2009, Holt 2011). Therefore, after *Artemia* feeding stopped larvae of the dry feed group might have had better preconditions to capture, ingest and digest the dry feed than larvae of the new feed group. On the other hand better weaning onto artificial feed usually implies that when co-fed the feed was actually ingested by the larvae (Rosenlund et al. 1997). A general observation was that when the larvae were co-fed with *Artemia* for one week no dry feed could be observed in the gut of the sampled larvae. This suggests that larvae were just ingesting little or no amounts of dry feed and mostly feeding on *Artemia*. Nevertheless, it seems to be advantageous introducing the feed before weaning even if larvae just ingested little or no amounts during co-feeding. Larvae which were more accustomed to dry feed showed better growth during that period.

Interesting was that two weeks later, at 47 DPH, and at the end of the trial the growth rate between larvae of all three groups was different again, even though all three groups had the same feed from then. Hence, even though feeding differently was for a short time, differences in growth can be manifested then and can even increase further on, after feeding stopped. Long term differences in growth of fish larvae have been observed in other studies too (Mæhre et al. 2013). It would be of high interest to continue the research of long term differences in growth even after 2 months and see if those differences are also seen after half a year or a year.

Conclusion

Despite the fact that the development of an artificial micro diet to completely replace live feed is not finished, the commercial dry feed which was used in the present study showed very good results, with high survival, good growth and no deformities of the lumpsucker larvae. Therefore this feed, which is commonly used in lumpsucker hatcheries in Norway, seems to be a sufficient feed for lumpsucker larvae. Larvae are able to recognize, to ingest and digest the feed. High survival, good growth and no deformities suggest that feed covers the major nutritional requirements. The results of the fatty acid composition confirm this.

Nevertheless, there was a 15.2% increase in growth in DW of larvae when using additionally live prey like *Artemia* at start feeding. Using additionally *Artemia* and the new feed increased growth by 29.9% and showed a 95% survival rate. Therefore, even though the commercial feed is good, there is room for improvement in the diet and for the lumpsucker industry. Especially using the new feed additionally to standard procedures is easy and not labor intensive for lumpsucker hatcheries, compared to adding *Artemia* production. Higher survival and an earlier transfer to salmon farms are economically beneficial for the lumpsucker farms. Additionally, lumpsucker juveniles fed with new feed might be more robust compared to standard reared juveniles. A more robust lumpsucker in sea cages is of advantage for salmon farmers. Stress tests, for example exposing lumpsucker in a hypersaline environment, could confirm in future studies the robustness of lumpsucker reared under different feeds.

Future perspectives

In this present study not all of the aspects of lumpsucker nutrition could be covered. In future ontogenetic studies should be carried out to understand the development of the organs in lumpsucker in general, but also to investigate the differences in development while using different feed. Histologic analyses of the digestive organs, but also digestive enzyme analyses should be carried out. Additionally ingestions rate studies with stomach content analyses should be carried out in future to confirm the observation made in this study about the ingested feed. This would also help to get a better understanding of how much of the feed will be actually ingested and how attractive the feed is.

All larvae samples for the fatty acid analyze were analyzed without any starvation period and larvae might have had a full gut when analyzing. Hence, this could have influenced the results of all the larval fatty acid compositions and should be considered in future fatty acid analyses. Next to fatty acid composition, future studies could also focus on other nutrients to understand better the compositions of the feed and larvae and reasons for better larval growth. Then more replicates should be used to improve the statistic results. Future growth studies should also be more focused on the DW, than on TL. Therefore more replicates should be used for DW to improve the statistics in DW.

References

Aragão, C., Conceição, L. E., Dinis, M. T. and Fyhn, H. J. (2004). Amino acid pools of rotifers and Artemia under different conditions: nutritional implications for fish larvae. Aquaculture, 234(1), 429-445.

Bell, J. G., McEvoy, L. A., Estevez, A., Shields, R. J. and Sargent, J. R. (2003). Optimising lipid nutrition in first-feeding flatfish larvae. Aquaculture, 227(1), 211-220.

Benfey, T. J. and Methven, D.A. (1986). Pilot-scale rearing of larval and juvenile lumpfish (*Cyclopterus lumpus* L.), with some notes on early development. Aquaculture 56(3–4): 301-306.

Bligh, E. G. and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology, 37(8), 911-917.

Brown, J. (1986). The development of feeding behaviour in the lumpfish, *Cyclopterus lumpus*. Journal of Fish Biology 29(sA): 171-178.

Brown, J. A., Wiseman, D. and Kean, P. (1997). The use of behavioural observations in the larviculture of cold-water marine fish."Aquaculture 155(1–4): 297-306.

Busch, K. E. T., Falk-Petersen, I. B., Peruzzi, S., Rist, N. A. and Hamre, K. (2010). Natural zooplankton as larval feed in intensive rearing systems for juvenile production of Atlantic cod (*Gadus morhua* L.). Aquaculture Research, 41(12), 1727-1740.

Busch, K. and Nordgreen, A. (2010) Frossent plankton som fôr for torskelarver i oppdrett. not published.

Cahu, C. L., Infante, J. Z., Quazuguel, P. and Le Gall, M. M. (1999). Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass *Dicentrarchus labrax* larvae. Aquaculture, 171(1), 109-119.

Cahu, C. and Infante, J. Z. (2001). Substitution of live food by formulated diets in marine fish

larvae. Aquaculture, 200(1), 161-180.

Cahu, C., Infante, J.Z. and Takeuchi, T. (2003). Nutritional components affecting skeletal development in fish larvae. Aquaculture, 227(1), 245-258.

Conceição, L. E., Yúfera, M., Makridis, P., Morais, S. and Dinis, M. T. (2010). Live feeds for early stages of fish rearing. Aquaculture Research, 41(5), 613-640.

Costello, M. J. (2009a). How sea lice from salmon farms may cause wild salmonid declines in Europe and North America and be a threat to fishes elsewhere. Proceedings of the Royal Society B: Biological Sciences, rspb20090771.

Costello, M. J. (2009b). The global economic cost of sea lice to the salmonid farming industry. Journal of fish diseases, 32(1), 115-118.

Davenport, J. (1985). Synopsis of biological data on the lumpsucker, *Cyclopterus lumpus* (Linnaeus, 1758) (No. 147). Food & Agriculture Org..

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

Engrola, S., Figueira, L., Conceição, L. E., Gavaia, P. J., Ribeiro, L. and Dinis, M. T. (2009). Co-feeding in Senegalese sole larvae with inert diet from mouth opening promotes growth at weaning. Aquaculture, 288(3), 264-272.

Evjemo, J. O., Reitan, K. I. and 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.

Falk-Petersen, I. B., Hansen, T. K., Fieler, R. And Sunde, L. M. (1999). Cultivation of the spotted wolffish *Anarhichas minor* (Olafsen)–a new candidate for cold-water fish farming. Aquaculture Research, 30(9), 711-718.

FAO (2012). Fishery Information Data and Statistics Unit. Fishstat Plus v. 2.30. Rome: FAO.

Fernandez-Diaz, C. and Yúfera, M. (1997). Detecting growth in gilthead seabream, *Sparus aurata* L., larvae fed microcapsules. Aquaculture, 153(1), 93-102.

Fuiman, L. A. and Werner, R. G. (Eds.). (2009). Fishery science: the unique contributions of early life stages. John Wiley & Sons.

Hall, T. E., Smith, P. and Johnston, I. A. (2004). Stages of embryonic development in the Atlantic cod *Gadus morhua*. Journal of morphology, 259(3), 255-270.

Hamre, K., Opstad, I., Espe, M., Solbakken, J., Hemre, G. I. and Pittman, K. (2002). Nutrient composition and metamorphosis success of Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae fed natural zooplankton or Artemia. Aquaculture Nutrition, 8(2), 139-148.

Hamre, K. (2006). Nutrition in cod (*Gadus morhua*) larvae and juveniles. ICES Journal of Marine Science: Journal du Conseil, 63(2), 267-274.

Hansen, T. K. and Falk-Petersen, I. B. (2001). The influence of rearing temperature on early development and growth of spotted wolffish *Anarhichas minor* (Olafsen). Aquaculture Research, 32(5), 369-378.

Hansen, T. K. and Falk-Petersen, I. B. (2002). Growth and survival of first-feeding spotted wolffish (*Anarhichas minor* Olafsen) at various temperature regimes.Aquaculture Research, 33(14), 1119-1127.

Haya, K., Burridge, L. E., Davies, I. M. and Ervik, A. (2005). A review and assessment of environmental risk of chemicals used for the treatment of sea lice infestations of cultured salmon. In Environmental effects of marine finfish aquaculture (pp. 305-340). Springer Berlin Heidelberg.

Holt, G. J. (2011). Larval fish nutrition. John Wiley & Sons.

Houde, E. D. (1989). Comparative growth, mortality, and energetics of marine fish larvae:

temperature and implied latitudinal effects. *Fishery Bulletin*, *87*(3), 471-495.

Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Foss, A., Vikingstad, E. and Elvegård, T. A. (2014). The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon *(Salmo salar* L.). Aquaculture, 424, 18-23.

Infante, J. Z. and Cahu, C. L. (2007). Dietary modulation of some digestive enzymes and metabolic processes in developing marine fish: applications to diet formulation. Aquaculture, 268(1), 98-105.Izquierdo et al. 2000

Ingólfsson, A. and B. K. Kristjánsson (2002). "Diet of juvenile lumpsucker *Cyclopterus lumpus* (Cyclopteridae) in floating seaweed: effects of ontogeny and prey availability." Journal Information 2002(2).

Izquierdo, M. S., Socorro, J., Arantzamendi, L. and Hernández-Cruz, C. M. (2000). Recent advances in lipid nutrition in fish larvae. Fish Physiology and Biochemistry, 22(2), 97-107.

Izquierdo, M. S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L. and Rosenlund, G. (2003). Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. Aquaculture Nutrition, 9(6), 397-407.

Kvenseth, P. G. and Andreassen, J. (2008). Wrasse, sea lice and economy. Norsk Fiskeoppdrett, 27-33.

Killen, S. S., Brown, J. A. and 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.

Kolkovski, S., Czesny, S. and Dabrowski, K. (2000). Use of krill hydrolysate as a feed attractant for fish larvae and juveniles. *Journal of the World Aquaculture Society*, *31*(1), 81-88.

Kolkovski, S. (2008). Advances in marine fish larvae diets. Cruz-Suárez E, Ricque D, Tapia M, Nieto MG, Villarreal LDA, Lazo JP et al, 20-45.

Langdon, C. (2003). Microparticle types for delivering nutrients to marine fish larvae. Aquaculture, 227(1), 259-275.

Metcalfe, L. D., Schmitz, A. A. and Pelka, J. R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Analytical chemistry, 38(3), 514-515.

Moring, J. R. (2001). Intertidal growth of larval and juvenile lumpfish in Maine: A 20-year assessment. Northeastern Naturalist 8(3): 347-354.

Mæhre, H. K., Hamre, K. and Elvevoll, E. O. (2013). Nutrient evaluation of rotifers and zooplankton: feed for marine fish larvae. *Aquaculture Nutrition*, *19*(3), 301-311.

NRC (2011). Nutrient Requirements of Fish and Shrimp. National Research Council.

Olsen, A. I., Attramadal, Y., Jensen, A. and Olsen, Y. (1999). Influence of size and nutritional value of *Artemia franciscana* on growth and quality of halibut larvae (*Hippoglossus hippoglossus*) during the live feed period. Aquaculture, 179(1), 475-487.

Piccinetti, C. C., Tulli, F., Tokle, N. E., Cardinaletti, G. and Olivotto, I. (2014). The use of preserved copepods in sea bream small-scale culture: biometric, biochemical and molecular implications. *Aquaculture Nutrition*, *20*(1), 90-100.

Pittman, K., Skiftesvik, A. B. And Berg, L. (1990). Morphological and behavioural development of halibut, *Hippoglossus hippoglossus* (L.) larvae. Journal of Fish Biology, 37(3), 455-472.

Puvanendran, V. and Brown, J. A. (2002). Foraging, growth and survival of Atlantic cod larvae reared in different light intensities and photoperiods. Aquaculture, 214(1), 131-151.

Robb, A. P. (1992). Changes in the gall bladder of whiting (*Merlangius merlangus*) in relation

to recent feeding history. ICES Journal of Marine Science: Journal du Conseil, 49(4), 431-436.

Rosenlund, G., Stoss, J. and Talbot, C. (1997). Co-feeding marine fish larvae with inert and live diets. Aquaculture, 155(1), 183-191.

Rønnestad, I., Thorsen, A. and Finn, R. N. (1999). Fish larval nutrition: a review of recent advances in the roles of amino acids. Aquaculture, 177(1), 201-216.

Sargent, J. R., McEvoy, L. A. and Bell, J. G. (1997). Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture, 155(1), 117-127.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J. and Tocher, D. (1999). Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture, 179(1), 217-229.

Sharma, J. G. and Chakrabarti, R. (2000). Replacement of live-food with refrigerated-plankton food for *Cyprinus carpio* (L.) larvae cultured. Current Science, 79(2).

Talbot, C. and Higgins, P. J. (1982). Observations on the gall bladder of juvenile Atlantic salmon *Salmo salar* L., in relation to feeding. Journal of Fish Biology, 21(6), 663-669.

Villamizar, N., García-Alcazar, A. and Sánchez-Vázquez, F. J. (2009). Effect of light spectrum and photoperiod on the growth, development and survival of European sea bass (*Dicentrarchus labrax*) larvae. Aquaculture, 292(1), 80-86.

Williams, P. J. and J. A. Brown (1991). Developmental changes in foraging-predator avoidance trade-offs in larval lumpfish *Cyclopterus lumpus*. Marine Ecology Progress Series 76: 53-60.

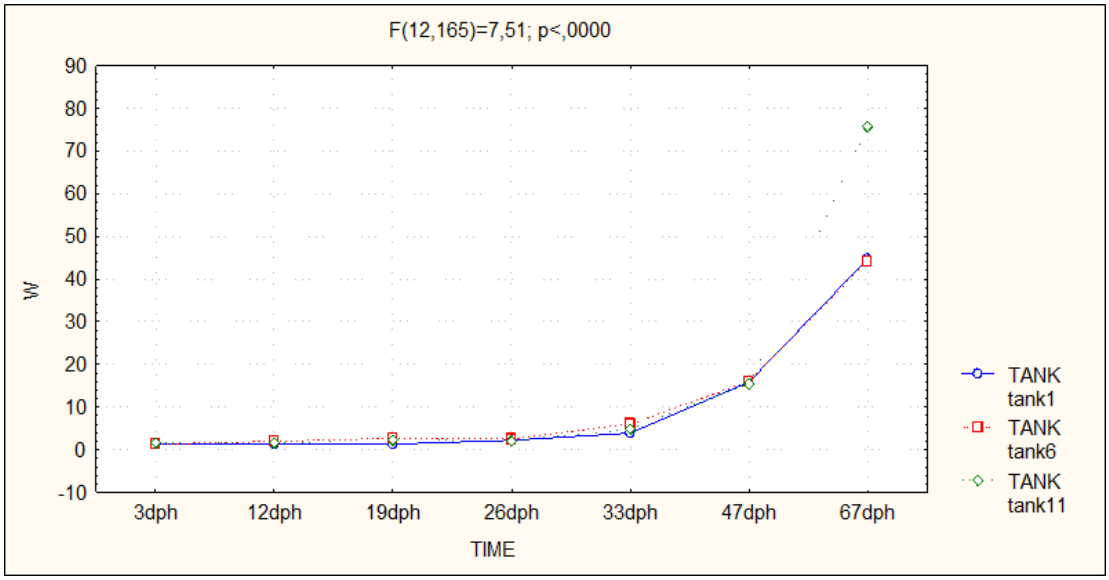
Yu, H. R., Mai, K. S., Ma, H. M., Ai, Q. H., Duan, Q. Y., Zambonino-Infante, J. L. and Cahu,

C. L. (2012). Evaluation of microdiets versus frozen copepods on growth, survival and selected chemical composition of large yellow croaker Pseudosciaena crocea larvae. 水生生物学报, 36(1).

Yúfera Fernandez-Diaz, C., ,Pascual, E., Sarasquete, M. C.and Moyano, F. J. (2000). Towards an inert diet for first-feeding gilthead seabream *Sparus aurata* L. larvae. Aquaculture Nutrition, 6, 143-152.

Appendices

Appendix Figures



Appendix Figure 1. DW (W) in mg of Lumpsucker larvae from the tanks of the control group.

Appendix Tables

Appendix Table I. Temperature (T), Degree Days (DD) and Oxygen (O) during the feeding trial of lumpsucker larvae. Grey highlighted are sampling points.

DPH	T [°C]	DD	O [%]	DPH	T [°C]	DD	O [%]
1	8.5	8.5	98	36	10.5	362.5	97
2	8.5	17.0	98	37	10.5	373.0	98
3	8.5	25.5	98	38	10.8	383.8	102
4	8.5	34.0	98	39	10.5	394.3	96
5	8.5	42.5	98	40	10.7	405.0	101
6	10.2	52.7	98	41	10.5	415.5	95
7	10.3	63.0	99	42	10.5	426.0	93
8	10.3	73.3	99	43	10.5	436.5	93
9	10.3	83.6	98	44	10.3	446.8	94
10	10.2	93.8	98	45	10.4	457.2	93
11	10.1	103.9	98	46	10.7	467.9	93
12	10.3	114.2	83	47	10.6	478.5	91
13	10.4	124.6	96	48	10.3	488.8	91
14	10.1	134.7	98	49	10.5	499.3	91
15	10.3	145.0	98	50	10.3	509.6	92
16	9.6	154.6	96	51	10.4	520.0	91
17	10.0	164.6	96	52	10.6	530.6	91
18	10.2	174.8	94	53	10.5	541.1	96
19	12.0	186.8	96	54	10.6	551.7	95
20	10.8	197.6	98	55	10.5	562.2	95
21	10.2	207.8	97	56	10.6	572.8	95
22	10.2	218.0	98	57	10.5	583.3	94
23	10.2	228.2	98	58	10.5	593.8	95
24	10.2	238.4	98	59	10.5	604.3	95
25	10.3	248.7	102	60	10.5	614.8	94
26	10.2	258.9	99	61	10.5	625.3	95
27	10.2	269.1	98	62	10.4	635.7	94
28	10.2	279.3	98	63	10.4	646.1	95
29	10.5	289.8	100	64	10.5	656.6	94
30	10.4	300.2	99	65	10.4	667.0	92
31	10.3	310.5	98	66	10.4	677.4	93
32	10.3	320.8	99	67	10.4	687.8	93
33	10.4	331.2	97				
34	10.3	341.5	98				
35	10.5	352.0	100				

Appendix Table II. Size of the different feeds during the feeding trial of lumpsucker larvae. Grey highlighted are sampling points.

DPH	Dry feed	New feed	Zooplankton
5	100 µm	no new feed	no zooplankton
12	100/300 µm	100-200 µm	150 µm
19	100/300 µm	100-200 µm	150 µm
26	300 µm	200-500 µm	150 µm
33	300/500 µm	200-500 µm	150 µm
40	500 µm	no new feed	1-400 µm
47	500 µm	no new feed	1-400 µm
54	500 µm	no new feed	2-500 µm
61	500 µm	no new feed	2-500 µm
67	500 µm	no new feed	2-500 µm

Appendix Table III. P-values of the Tukey's test from the TL data of the lumpsucker larvae. Red highlighted shows significant differences.

Days post hatch	Feeding regime	p-values of the Tukey's test			
12 DPH		{1}	{2}	{3}	{4}
		6.893483	7.406301	7.401400	7.400536
	Control {1}		7.689E-06	7.689E-06	7.689E-06
	Dryfeed {2}	7.689E-06		0.999549389	0.99926782
	Newfeed {3}	7.689E-06	0.99954939		0.9999975
	Zooplankton {4}	7.689E-06	0.99926782	0.999997497	
19 DPH		{1}	{2}	{3}	{4}
		7.617889	9.555767	8.306111	8.517655
	Control {1}		7.689E-06	2.91467E-05	7.689E-06
	Dryfeed {2}	7.689E-06		7.689E-06	7.689E-06
	Newfeed {3}	2.9147E-05	7.689E-06		0.486157
	Zooplankton {4}	7.689E-06	7.689E-06	0.486157	
26 DPH		{1}	{2}	{3}	{4}
		8.629066	8.987878	9.188845	8.872600
	Control {1}		0.00206918	7.86781E-06	0.07331502
	Dryfeed {2}	0.00206918		0.188998997	0.66121256
	Newfeed {3}	7.8678E-06	0.188999		0.00908643
	Zooplankton {4}	0.07331502	0.66121256	0.00908643	
33 DPH		{1}	{2}	{3}	{4}
		10.70138	10.82084	11.02534	9.029777
	Control {1}		0.79224372	0.059209347	7.689E-06
	Dryfeed {2}	0.79224372		0.38952899	7.689E-06
	Newfeed {3}	0.05920935	0.38952899		7.689E-06
	Zooplankton {4}	7.689E-06	7.689E-06	7.689E-06	
47 DPH		{1}	{2}	{3}	{4}
		14.14685	15.05133	15.87588	10.11010
	Control {1}		0.00021511	7.689E-06	7.689E-06
	Dryfeed {2}	0.00021511		0.000967205	7.689E-06
	Newfeed {3}	7.689E-06	0.0009672		7.689E-06
	Zooplankton {4}	7.689E-06	7.689E-06	7.689E-06	
67 DPH		{1}	{2}	{3}	{4}
		23.45555	24.07778	24.78889	10.82222
	Control {1}		0.38570666	0.003746808	7.689E-06
	Dryfeed {2}	0.38570666		0.266459465	7.689E-06
	Newfeed {3}	0.00374681	0.26645947		7.689E-06
	Zooplankton {4}	7.689E-06	7.689E-06	7.689E-06	

Appendix Table IV. P-values of the Tukey's test from the DW data of the lumpsucker larvae. Red highlighted shows significant differences

Days post hatch	Feeding regime	p-values of the Tukey's test			
19 DPH		{1}	{2}	{3}	{4}
		2.074709	2.463583	2.850273	2.586661
	Control {1}		0.213331103	0.001058638	0.055328488
	Dryfeed {2}	0.2133311		0.217718005	0.926506341
	New feed {3}	0.00105864	0.217718005		0.551082134
	Zooplankton {4}	0.05532849	0.926506341	0.551082134	
26 DPH		{1}	{2}	{3}	{4}
		2.254430	2.893853	3.168554	2.301287
	Control {1}		0.01623863	0.000312984	0.996222794
	Dryfeed {2}	0.01623863		0.566140175	0.030193806
	New feed {3}	0.00031298	0.566140175		0.000557721
	Zooplankton {4}	0.99622279	0.030193806	0.000557721	
33 DPH		{1}	{2}	{3}	{4}
		4.970327	5.042923	5.043994	2.574037
	Control {1}		0.996519923	0.996365488	0.000136375
	Dryfeed {2}	0.99651992		1	0.000136375
	New feed {3}	0.99636549	1		0.000136375
	Zooplankton {4}	0.00013638	0.000136375	0.000136375	
47 DPH		{1}	{2}	{3}	{4}
		15.75197	16.17717	16.82378	3.491093
	Control {1}		0.981454194	0.775088131	0.000136375
	Dryfeed {2}	0.98145419		0.939090967	0.000136375
	New feed {3}	0.77508813	0.939090967		0.000136375
	Zooplankton {4}	0.00013638	0.000136375	0.000136375	
67 DPH		{1}	{2}	{3}	{4}
		54.88334	63.22333	71.30000	9.193334
	Control {1}		0.364845753	0.009190023	0.000136375
	Dryfeed {2}	0.36484575		0.393742681	0.000136375
	New feed {3}	0.00919002	0.393742681		0.000136375
	Zooplankton {4}	0.00013638	0.000136375	0.000136375	

Appendix Table V. P-values of the Tukey's test from the survival data of the lumpsucker larvae. Red highlighted shows significant differences.

Feeding regime	p-values of the Tukey's test			
	{1} M=124.91	{2} M=124.80	{3} M=135.71	{4} M=0.0000
Control {1}		0.99998681	0.03488506	0.00023067
Dry feed {2}	0.9999868		0.03332282	0.00023067
New feed {3}	0.0348851	0.03332282		0.00023067
Zooplankton {4}	0.0002307	0.00023067	0.00023067	

Appendix Table VI. P-values of the Tukey's test from the fatty acid composition data of three different feed types. Red highlighted shows significant differences.

Fatty acid	Feeding regime	p-values of the Tukey test		
C16:0		{1}	{2}	{3}
	Dryfeed {1}		0.0000	0.0000
	New feed {2}	0.0000		0.9136617
	Zooplankton {3}	0.0000	0.9136617	
C18:0		{1}	{2}	{3}
	Dryfeed {1}		0.0000	0.0000009
	New feed {2}	0.0000		0.0013583
	Zooplankton {3}	0.0000009	0.0013583	
C19:0		{1}	{2}	{3}
	Dryfeed {1}		3.00E-07	0.00E+00
	New feed {2}	3.00E-07		2.00E-07
	Zooplankton {3}	0.00E+00	2.00E-07	
C18:1n-9		{1}	{2}	{3}
	Dryfeed {1}		0.0000	0.0000
	New feed {2}	0.0000		0.0003385
	Zooplankton {3}	0.0000	0.0003385	
C18:1n-7		{1}	{2}	{3}
	Dryfeed {1}		0.0016566	0.0005954
	New feed {2}	0.0016566		0.0000043
	Zooplankton {3}	0.0005954	0.0000043	
C18:2n-6		{1}	{2}	{3}
	Dryfeed {1}		0.0000	0.0000
	New feed {2}	0.0000		0.9899472
	Zooplankton {3}	0.0000	0.9899472	
C20:5n-3		{1}	{2}	{3}
	Dryfeed {1}		0.1440104	0.0001181
	New feed {2}	0.1440104		0.0014293
	Zooplankton {3}	0.0001181	0.0014293	
C22:6n-3		{1}	{2}	{3}
	Dryfeed {1}		0.8627489	0.0327471
	New feed {2}	0.8627489		0.0738275
	Zooplankton {3}	0.0327471	0.0738275	
N6 HUFA		{1}	{2}	{3}
	Dryfeed {1}		0.0000	0.0000

	New feed {2}	0.0000		0.9848546
	Zooplankton {3}	0.0000	0.9848546	
N6/N3		{1}	{2}	{3}
	Dryfeed {1}		0.000	0.0000
	New feed {2}	0.0000		0.9963756
	Zooplankton {3}	0.0000	0.9963756	
DHA/EPA		{1}	{2}	{3}
	Dryfeed {1}		0.0846811	0.0005395
	New feed {2}	0.0846811		0.0072594
	Zooplankton {3}	0.0005395	0.0072594	
TOTAL		{1}	{2}	{3}
	Dryfeed {1}		0.0000	0.0000
	New feed {2}	0.0000		0.9696819
	Zooplankton {3}	0.0000	0.9696819	

Appendix Table VII. P-values of the Tukey's test from the fatty acid composition data of the lumpsucker larvae at 33 DPH. Red highlighted shows significant differences.

Fatty acid	Feeding regime	p-values of the Tukey test			
C16:0		{1}	{2}	{3}	{4}
		M=2.5967	M=2.2059	M=2.2833	M=1.7819
	Control_ {1}		0.0006682	0.0025902	3.62E-06
	DF_L {2}	0.0006682		0.3181963	0.0003922
	NF_L {3}	0.0025902	0.3181963		0.0001254
	ZP_L {4}	3.62E-06	0.0003922	0.0001254	
C18:0		{1}	{2}	{3}	{4}
		M=.83333	M=.75787	M=.76000	M=1.0611
	Control_ {1}		0.0461357	0.0511939	0.0001003
	DF_L {2}	0.0461357		0.9485006	1.269E-05
	NF_L {3}	0.0511939	0.9485006		1.337E-05
	ZP_L {4}	0.0001003	1.269E-05	1.337E-05	
C18:1N-9		{1}	{2}	{3}	{4}
		M=2.2633	M=2.0620	M=2.1033	M=1.5772
	Control_ {1}		0.225714	0.3273761	0.0020743
	DF_L {2}	0.225714		0.7943815	0.0133822
	NF_L {3}	0.3273761	0.7943815		0.0089534
	ZP_L {4}	0.0020743	0.0133822	0.0089534	
C18:2N-6		{1}	{2}	{3}	{4}
		M=3.2100	M=2.5548	M=2.5633	M=.40175
	Control_ {1}		0.0013386	0.0014501	3.209E-08
	DF_L {2}	0.0013386		0.9517739	2.566E-07
	NF_L {3}	0.0014501	0.9517739		2.488E-07
	ZP_L {4}	3.209E-08	2.566E-07	2.488E-07	
C20:5N-3		{1}	{2}	{3}	{4}
		M=.99333	M=.89820	M=.92333	M=1.1668
	Control_ {1}		0.1456505	0.2696325	0.0187171
	DF_L {2}	0.1456505		0.6814448	0.0018704
	NF_L {3}	0.2696325	0.6814448		0.0033176
	ZP_L {4}	0.0187171	0.0018704	0.0033176	
N6 HUFA		{1}	{2}	{3}	{4}
		M=3.4767	M=2.7947	M=2.8167	M=.82379
	Control_ {1}		0.00135	0.0016479	6.949E-08
	DF_L {2}	0.00135		0.880878	7.016E-07
	NF_L {3}	0.0016479	0.880878		6.441E-07
	ZP_L {4}	6.949E-08	7.016E-07	6.441E-07	
N6/N3		{1}	{2}	{3}	{4}
		M=.85992	M=.76570	M=.75993	M=.18430
	Control_ {1}		0.0011395	0.0007839	4.437E-10
	DF_L {2}	0.0011395		0.7700108	1.464E-09

	NF_L	{3}	0.0007839	0.7700108		1.584E-09
	ZP_L	{4}	4.437E-10	1.464E-09	1.584E-09	
TOTAL			{1}	{2}	{3}	{4}
			M=15.033	M=13.217	M=13.363	M=12.650
	Control_	{1}		0.0015606	0.0025744	0.0002732
	DF_L	{2}	0.0015606		0.7147776	0.1817936
	NF_L	{3}	0.0025744	0.7147776		0.1028857
	ZP_L	{4}	0.0002732	0.1817936	0.1028857	

Appendix Table VIII. TL in mm and DW in mg of lumpsucker larvae reared under four different feeding regimes during the first 67 days.

TL [mm]	Control	Dryfeed	Newfeed	Zooplankton
3 DPH	6.71 ± 0.25	6.74 ± 0.18	6.72 ± 0.25	6.75 ± 0.19
12 DPH	6.89 ± 0.31	7.41 ± 0.32	7.39±0.31	7.40±0.27
19 DPH	7.62 ± 0.54	8.21 ± 0.58	8.30±0.47	8.52±0.47
26 DPH	8.63± 0.79	8.99 ± 0.71	9.18±0.61	8.87±0.57
33 DPH	10.70± 1.06	10.82 ± 0.86	11.02±0.76	9.03±0.76
47 DPH	14.15± 1.95	15.05 ± 0.86	15.86±1.38	10.11±0.88
67 DPH	23.46± 3.33	24.08 ± 2.61	24.79±2.92	10.82±1.13
DW [mg]	Control	Dryfeed	Newfeed	Zooplankton
3 DPH	1.41 ± 0.24	1.43 ± 0.21	1.39 ± 0.16	1.41 ± 0.10
12 DPH	1.61±0.76	1.81±0.50	1.85±0.64	1.89±0.57
19 DPH	2.07±0.82	2.46±0.59	2.85±0.99	2.59±0.61
26 DPH	2.25±0.55	2.89±1.32	3.17±0.6	2.30±0.54
33 DPH	4.97±1.63	5.04±1.27	5.04±1.14	2.57±1.12
47 DPH	15.75±5.64	16.18±5.20	16.82±3.78	3.49±1.56
67 DPH	54.88±23.27	63.22±21.02	71.3±20.87	9.19±12.17

Appendix Table IX. Full fatty acid profile (g/100g sample) of the dry feed, new feed and frozen zooplankton.

FA	Dry feed	New feed	Zooplankton
C14:0	0.42±0.02	0.33±0.02	0.14±0.02
C14:1	0.02±0.01	0.04±0.02	0.02±0.01
C15:0	0.05±0.00	0.03±0.02	0.02±0.00
C16:0	3.38±0.18	1.22±0.06	1.26±0.17
C16:1	0.50±0.02	0.44±0.03	0.33±0.04
C17:0	0.11±0.02	0.04±0.01	0.03±0.03
C18:0	0.67±0.03	0.11±0.06	0.26±0.03
C18:1n-11	0.01±0.01	0.03±0.03	0.00±0.01
C18:1n-9	2.34±0.13	0.16±0.02	0.53±0.06
C18:1n-7	0.41±0.02	0.10±0.10	0.74±0.11
C18:2n-6	5.42±0.31	0.19±0.04	0.17±0.01
C18:3n-6	0.00	0.01±0.01	0.00
C18:3n-3	0.08±0.02	0.03±0.03	0.04±0.05
C19:0	0.60±0.02	0.33±0.01	0.05±0.03
C20:0	0.05±0.00	0.03±0.04	0.00
C20:1n-11	0.46±0.03	0.05±0.01	0.19±0.03
C20:2n-11	0.04±0.00	0.06±0.04	0.03±0.03
C20:3n-11	0.07±0.02	0.01±0.03	0.03±0.04
C20:3n-6	0.03±0.06	0.02±0.03	0.09±0.01
C20:4n-6	0.00	0.01±0.01	0.00
C20:5n-3	1.23±0.06	1.49±0.05	2.13±0.29
C22:1n-9	0.05±0.01	0.00	0.02±0.02
C22:4n-6	0.08±0.00	0.01±0.02	0.00
C22:5n-3	0.17±0.01	0.10±0.00	0.00
C22:6n-3	2.28±0.12	2.22±0.05	1.94±0.24
C24:1n-9	0.04±0.05	0.03±0.02	0.00
UNKNOWN	1.72±0.12	1.70±0.03	0.91±0.17

Appendix Table X. Full fatty acid profile (g/100g sample) of lumpsucker larvae reared under four different feeding regimes at 33 DPH.

Fatty acid	Control	Dryfeed	Newfeed	Zooplankton
C14:0	0.20±0.03	0.17±0.01	0.18±0.01	0.02±0.04
C14:1	0.00	0.00	0.00	0.01±0.01
C15:0	0.00	0.00	0.00	0.19±0.01
C16:0	2.60±0.16	2.21±0.02	2.28±0.05	1.78±0.01
C16:1	0.24±0.03	0.20±0.01	0.21±0.01	0.11±0.01
C17:0	0.00	0.00	0.00	0.10±0.01
C18:0	0.83±0.05	0.75±0.02	0.76±0.03	1.06±0.01
C18:1n-11	0.00	0.00	0.00	0.00
C18:1n-9	2.26±0.18	2.06±0.03	2.10±0.15	1.58±0.29
C18:1n-7	0.30±0.00	0.44±0.07	0.40±0.00	0.33±0.28
C18:2n-6	3.21±0.28	2.55±0.05	2.56±0.10	0.40±0.13
C18:3n-6	0.03±0.00	0.02±0.01	0.02±0.00	0.00
C18:3n-3	0.28±0.03	0.30±0.02	0.32±0.02	0.23±0.07
C19:0	0.00	0.00	0.00	0.25±0.08
C20:0	0.13±0.10	0.01±0.01	0.02±0.01	0.00
C20:1n-11	0.29±0.04	0.25±0.01	0.22±0.06	0.11±0.02
C20:2n-11	0.02±0.00	0.04±0.01	0.03±0.01	0.01±0.02
C20:3n-11	0.00	0.00	0.00	0.22±0.07
C20:3n-6	0.04±0.01	0.02±0.01	0.02±0.00	0.42±0.04
C20:4n-6	0.18±0.01	0.19±0.02	0.19±0.01	0.00
C20:5n-3	0.99±0.07	0.89±0.03	0.92±0.05	1.17±0.11
C22:1n-9	0.17±0.01	0.10±0.08	0.14±0.00	0.00
C22:4n-6	0.02±0.00	0.02±0.01	0.02±0.00	0.00
C22:5n-3	0.14±0.00	0.13±0.01	0.12±0.01	0.13±0.11
C22:6n3	2.63±0.15	2.30±0.04	2.34±0.08	2.97±0.53
C24:1n-9	0.00	0.00	0.00	0.00
UNKNOWN	0.50±0.01	0.53±0.01	0.50±0.10	1.55±0.43

Appendix Table XI. Full fatty acid profile (g/100g) of lumpsucker larvae reared under three different feeding regimes at 67 DPH.

Fatty acid	Control	Dryfeed	Newfeed
C14:0	0.38±0.01	0.36±0.02	0.37±0.01
C14:1	0.02±0.01	0.03±0.01	0.02±0.01
C15:0	0.09±0.01	0.08±0.01	0.08±0.01
C16:0	3.15±0.08	3.01±0.15	3.07±0.11
C16:1	0.64±0.02	0.60±0.03	0.61±0.03
C17:0	0.15±0.01	0.15±0.01	0.15±0.01
C18:0	0.88±0.01	0.85±0.05	0.86±0.05
C18:1n-11	0.03±0.01	0.02±0.01	0.03±0.01
C18:1n-9	4.10±0.05	4.16±0.42	4.30±0.57
C18:1n-7	0.68±0.01	0.44±0.15	0.46±0.36
C18:2n-6	3.23±0.08	3.19±0.24	3.23±0.13
C18:3n-6	0.03±0.01	0.04±0.01	0.04±0.01
C18:3n-3	0.03±0.02	0.02±0.01	0.03±0.01
C19:0	0.44±0.01	0.44±0.03	0.46±0.02
C20:0	0.02±0.01	0.03±0.01	0.03±0.01
C20:1n-11	0.42±0.01	0.42±0.03	0.43±0.03
C20:2n-11	0.06±0.02	0.06±0.03	0.07±0.02
C20:3n-11	0.03±0.01	0.03±0.01	0.04±0.01
C20:3n-6	0.22±0.08	0.12±0.12	0.16±0.14
C20:4n-6	0.03±0.01	0.04±0.01	0.04±0.01
C20:5n-3	1.34±0.01	1.30±0.09	1.24±0.06
C22:1n-9	0.04±0.01	0.04±0.01	0.04±0.01
C22:4n-6	0.15±0.01	0.14±0.01	0.14±0.01
C22:5n-3	0.27±0.01	0.26±0.02	0.26±0.02
C22:6n3	3.68±0.04	3.60±0.26	3.31±0.22
C24:1n-9	0.05±0.02	0.06±0.01	0.07±0.01
UNKNOWN	1.86±0.18	1.87±0.20	1.82±0.15