GENT UNIVERSITY

FACULTY OF BIOSCIENCE ENGINEARING

Academic year (2011-2012)

Weaning of pikeperch (*Sander lucioperca* L.) larvae and the importance of highly unsaturated fatty acids

Martin Sserwadda

Author

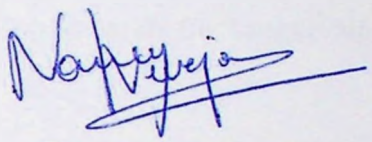
Promoter: Dr.ir. Nancy Nevejan

Tutor: Stijn Van Hoestenberghe

Thesis submitted in partial fulfillment of the requirements for the academic degree of Master of Science in Aquaculture

Copyright

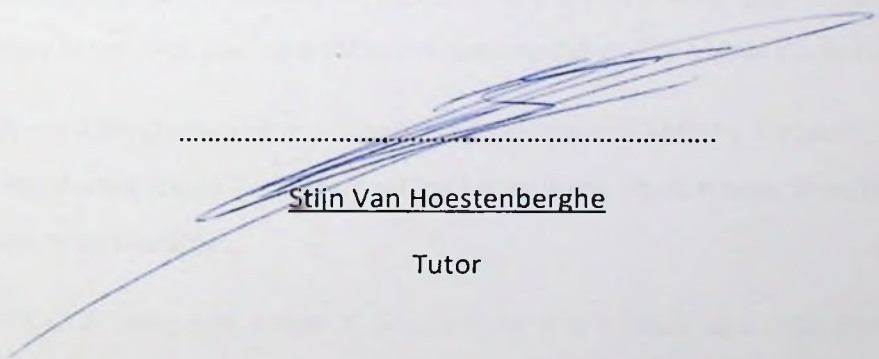
Gent University, 24, August 2012.



.....

Dr. ir. Nancy Nevejan

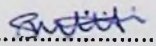
Promoter



.....

Stijn Van Hoestenberghe

Tutor



.....

Martin Sserwadda

Author

Acknowledgements

I express my gratitude to my promoter, Dr. Ir. Nancy Nevejan for her tireless guidance and corrections that made this thesis successful. I acknowledge the assistance from the Artemia reference centre laboratory staff during my two year study programme. I also acknowledge my tutor Stijn Van Hoestenberghe from Catholic University of Leuven for his technical advice and hands on experience that helped me a lot to improve my culture system. To Tom and Geert, am truly grateful for your technical assistance during the course of my experiment. I also acknowledge Johan Aerts and his professor Sarah De Saeger for their help with the cortisol samples.

I would like to thank VLIRUOS for giving me a chance to fulfill my childhood dream ,by providing all the necessary financial assistance that has allowed me to complete my study program. To the Director General of National Agricultural Research Organisation and the Director of National Fisheries Resources Research Institute, I will always hold you in high regard for granting me a study leave with pay to enable me accomplish my two year study program.

Special thanks to my wife Stella and my daughters Martina and Martha for their love, patience and support to me during these two years when I was away from home. Indeed without you this wouldn't have been possible.

To the ALMIGHTY GOD who has made it possible for my friends and I to accomplish what brought us here at University of Gent.

Abstract

Weaning of pikeperch (*Sander lucioperca* L.) larvae and the importance of highly unsaturated fatty acids was examined in two consecutive experiments. In the first experiment, 16day old larvae (wetweight:5mg;dryweight:0.82mg;length:9.52mm) were reared in 10-l cylindrical-conical tanks in a recirculating system (20±1° C) at a stocking density of 60 larvae litre^{-1} and fed either enriched *Artemia* or non enriched *Artemia* nauplii for 10 consecutive days. Enrichment was achieved with either DHA selco® or ICES 30/0.6 emulsions. Larval growth was similar for all treatments, with the best growth (mean wet weight: 55.1±20.8mg) obtained by fish larvae fed DHA enriched *Artemia* nauplii and the lowest growth (wet weight: 16.7±10.3mg) in fish fed non enriched *Artemia* nauplii. The specific growth rate ranged between 19.2±2.0 - 24.0±2.0%day^{-1} while survival rate ranged between 6.8±0.7 – 8.0±0.7 with larvae fed on DHA enriched *Artemia* and EPA enriched *Artemia* respectively. FAME composition in larvae at 26 days post hatch (dph) was similar although larvae fed DHA enriched *Artemia* had the highest FAME content (94.3mg.gDW^{-1}) and the lowest FAME content was (84.8mg.gDW^{-1}) for those that received non enriched *Artemia* nauplii. In the second experiment, 26 day old larvae obtained from experiment1 were reared under similar culture conditions but at a lower stocking density of 9 larvae litre^{-1} and were fed exclusively with artificial feed (AgloNorse®, O-Range® and Vegetable test diet), under five treatments (DHA- AgloNorse® (DHA-A),DHA- O-Range® (DHA-O),DHA-Vegetable (DHA-V),EPA- AgloNorse® (EPA-A),NON- Vegetable (NON-V)) for a period of 13 days. Significant differences were observed in larvae that received DHA-A and NON-V and DHA-O treatments while those under EPA-A and DHA-V seemed non significant. The best growth (wet weight: 103.2±7.3mg) and lowest growth (wet weight: 52.1±15.7mg) was obtained by fish larvae that received DHA-A and NON-V treatments respectively. Larvae that received dietary HUFAs (DHA-A, EPA-A and DHA-O) throughout the experimental period had the highest DHA and EPA levels, between 17.9 – 22.3mg.gDW^{-1} and 5.0 – 5.3mg.gDW^{-1} respectively. When exposed to stress by confinement in 2l-beakers with either culture water or saline water (15ppt), larvae at 26dph expressed more cortisol than larvae at 39dph.

Keywords: Pikeperch; *Sander lucioperca*; Weaning; Fatty acids; Cortisol; Stress; *Artemia*

Table of contents

List of figures

List of tables

List of abbreviations

FAO	Food and Agricultural Organisation
RAS	Recirculation Aquaculture System
ACTH	Adrenocorticotropic hormone
HUFAs	Highly unsaturated fatty acids
MUFAs	Mono unsaturated fatty acids
SFAs	Saturated fatty acids
PUFAs	Polly unsaturated fatty acids
EFAs	Essential fatty acids
EPA	Eicosapentaenoic acid
DHA	Decosahexaenoic acid
ARA	Arachidonic acid
LIN	Linoleic acid
ALA	α-Linolenic acid
FA	Fatty acid
FAME	Fatty acid methyl ester
GSL	Great salt lake
Sgr	Specific growth rate
WW	Wet weight
DW	Dry weight

ANOVA	Analysis of variance
SPE	Solid phase extraction
Rpm	Revolution per minute
PBS	Phosphate buffer solution
PL	Phospholipid
FO	Fish oil
POO	Pure olive oil
HPI	Hypothalamus-interrenal axis
ARC	Laboratory of aquaculture and *Artemia* reference center
Pers.comm	Personal communication
Hr	Hour
Fig	Figure

Introduction

Perciformes like pikeperch (*Sander lucioperca*) are increasingly focused on, in an effort to diversify European inland aquaculture (Wang et al., 2009) (figure 1). Pikeperch is highly rated by customers due to its meat's nutritional and organoleptic qualities and fast growth rates (Schulz et al., 2007). Pikeperch has also been used as a biomanipulation tool in order to reduce the number of unwanted fish, usually cyprinids (Lappalainen et al.,2003). In addition pikeperch is also highly regarded as a sport fish and a candidate for re-stocking of natural habitats (Schulz et al., 2008). However this demand cannot be met by capture fisheries as wild stocks have been greatly over harvested and suggested production of the species in aquaculture to meet this demand. Pikeperch fishery is a major fishery in Finland, Russia and Estonia but the landings are generally decreasing year by year (figure 2).

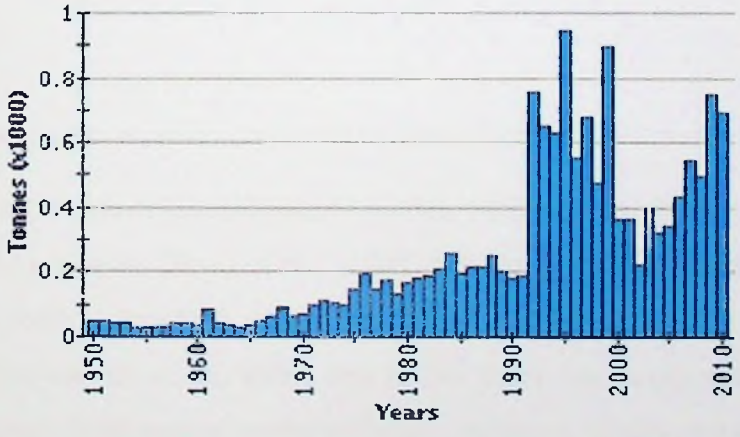


Figure 1.Global aquaculture production for *Sander lucioperca* (FAO FISHERY STATISTIC)

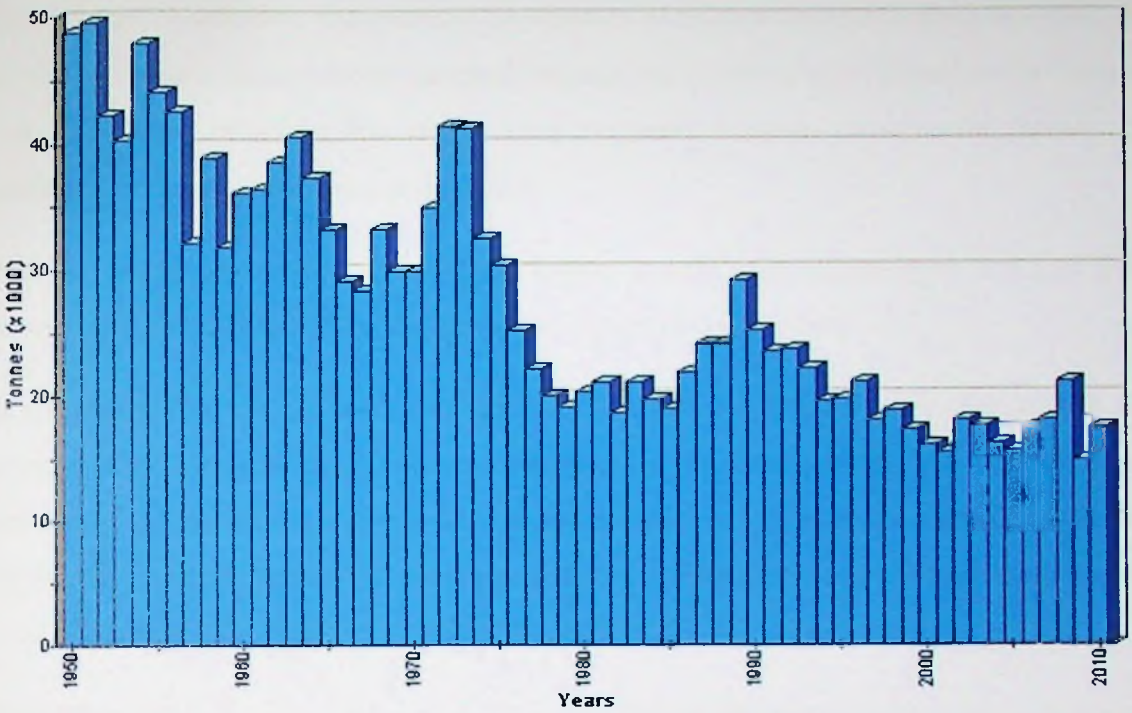


Figure 2.Global capture production for *Sander lucioperca* (FAO Fishery Statistic)

The pikeperch (*S.lucioperca*) is widely distributed in Eastern and Central European inland waters and estuaries (Koed et al., 2002). Pikeperch occurs mainly in both fresh and brackish waters, typically inhabiting lakes and rivers belonging to the Black sea, the Caspian drainages (Lappalainen et al., 2003) and in the Baltic sea (Lappalainen et al.,2003). Pikeperch has also been introduced to Western Europe ,Western Turkey and Morocco (Lappalainen et al., 2003).

Pikeperch is a fresh water species but can tolerate some salinity levels (Lappalainen et al.,2003 ; Koed et al., 2002). Eggs can hatch successfully in water with salinity lower than 3 %. Juveniles and adult pikeperch can tolerate salinity up to 10% and 12% respectively (Koed et al., 2002).

Spawning of pikeperch is temperature dependent and usually happens at 10-14 $^{\circ}$ C during April and May. The eggs are sticky in nature and are usually deposited on substrates like roots, rocks and other hard structures at the bottom (Koed et al., 2002). Hatched larvae usually don't require rotifers as starter feed but from onset of exogenous feeding prefer nauplii of copepods,

which are soon replaced by the copepodite stages and mature copepods (Peterka et al., 2003). When the larvae are about 15mm standard length, diet is mainly contributed to by cladocerans and at 20-40mm, selection for larger prey (*Leptodora kidtii*, chironomid larvae) occurs (Lappalainen et al.,2003; Peterka et al., 2003).

The fish is highly carnivorous feeding on fishes, insects and crustaceans and can attain a maximum length of 130 cm and a weight of 12- 18 kg (FAO Statistics).

Since pikeperch is rich in polyunsaturated fatty acids (PUFA), it is a very popular fish among consumers that promote healthy foods (Jankowska et al., 2003). In Europe there has been a remarkable effort to increase pikeperch production both in aquaculture and wild fisheries due to the demand by both the consumer (large size fish, 2-4 kg each) and the restocking (0+ and 1-year old fish) market (Kestemont, 2003).

To meet this ever increasing demand, a lot of studies on pikeperch culture have been carried out (Zakes, 1997; Ljinggren et al., 2003; Peterka et al., 2003; Schulz et al., 2005; Schulz et al., 2008; Kestemont, 2003). Larviculture for pikeperch was mainly done on an extensive and semi intensive scale but is recently done in intensive systems as the fish has gained attention as a potential and suitable new species for intensive fish farming (Nyina-Wamwiza et al., 2005). Larvae can now be reared in intensive systems like recirculation systems (RAS) on live feeds and also on substitution of live feeds with compound diets (Ostazawska et al., 2005; Kestemont, 2007; Szkudlarek et al., 2007; Lund and Steenfeldt, 2009). Pikeperch nutritional requirement studies such as fatty acid requirements have mainly focused on juveniles (Molnar et al., 2006; Lund and Steenfeldt, 2009). Fatty acids are known to have effects on the metabolism of fish as they supply energy and are components of membranes, pheromones and hormones (Schulz et al., 2005). Bell & Tocher (1989) documented that most fish larvae use lipids and fatty acids as their major energy sources. During organogenesis, essential polyunsaturated fatty acids (PUFA) are used as structural components of cell membranes (Lund et al., 2009). These modulate a number of cell processes like membrane transport, receptor function, and enzymatic activities and are precursors for highly active molecules (Bell & Tocher, 1989; Navarro and Sergent, 1992;

Ganga et al., 2006; Lund et al., 2009). Hamza et al. (2008) showed that there is a positive correlation between increasing dietary phospholipids and larval final weight in pikeperch.

Most freshwater fish including pikeperch have the ability to elongate and desaturate fatty acids with 18 carbons to PUFA (Buzzi et al., 1995; Lund et al., 2009). The essential fatty acids for fish are broadly categorized into two distinct groups: the n-3 and n-6 groups; the poly-unsaturated fatty acids (PUFA) with carbon chain length of 18 and highly unsaturated fatty acids (HUFA) with carbon chain lengths of 20 and 22, of both n-3 and n-6 series (Smith et al., 2003).

A major difference seems to exist in the essential fatty acid requirement for both fresh water fish and marine fish (Hamza et al., 2008). Fresh water fish need either linoleic acid (LA, 18:2n-6) or linolenic acid (ALA, 18:3n-3) or both these fatty acids, while marine fish need the highly unsaturated fatty acids especially EPA and DHA (Buzzi et al., 1997; Hamza et al., 2008). Sargent (2002) showed that fish like all vertebrates cannot synthesize de novo polyunsaturated fatty acids (PUFA) and therefore the need to provide these essential fatty acids in the diets. Poor growth and low feed conversion are two common signs of essential fatty acid deficiency that have been reported in several freshwater and marine species. Knowledge on essential fatty acid requirements for pikeperch larvae are still limited (Lund et al., 2009). Abi-Ayad et al. (2004) showed that starved pikeperch larvae utilize DHA and EPA, but also monounsaturated and saturated fatty acids as metabolic substrates. This suggested a lower physiological essential fatty acid requirement in pikeperch larvae, a fact that is supported by comparisons with Eurasian perch larvae (*Perca fluviatilis*), where DHA is spared to the detriment of other fatty acids for formation of new cells.

Research into suitable alternative sources of fat other than fish oil is on the increase and oils such as vegetable oils are of increasing interest to aquaculture (Lund and Steennfeldt, 2009). However Montero (2003) showed that vegetable oils and their deficiency in HUFAs may change the metabolism of the fish and cause increased sensitivity to stress. Lund and Steennfeldt (2009) demonstrated that pikeperch larvae reared on pure olive oil- based enrichment (POO) experienced a high mortality of 88% whereas no significant differences in survival of larvae

reared of EFA (HUFA) supplemented diets or fish oil (FO) diets was observed. It has been shown that in fish, highly unsaturated fatty acids increased the stress resistance such as handling, temperature variations and salinity changes (Furuita et al., 1999). Kestemont et al. (2007) showed that higher essential fatty acid content may increase cell fluidity and result in better adaption and resistance to suddenly exposed environmental temperature or osmotic stress conditions in pikeperch larvae.

The feeding requirements of fish larvae in nature are reflected by the composition of the zooplankton consumed (Ostaszewska et al., 2006). *Artemia* is normally given as the first natural food in fish larval culture, yet it does not meet all nutritional requirements of fish larvae due to insufficient content of polyunsaturated fatty acids (PUFA) (Lavens et al., 1995; Ostaszewska et al., 2006). Watanabe et al. (1983) showed that enrichment of *Artemia* nauplii before feeding them to fish larvae improved the concentrations of essential nutrients. Freshwater fish are less sensitive to variable fatty acid levels in *Artemia* nauplii but their fatty acid composition may affect growth in these species. Osteszewska et al. (2006) showed that fatty acid profile in pikeperch larvae reflected the dietary fatty acid profiles in the feed just as is the case with other fish species. Pikeperch is able to elongate and desaturate n-3 fatty acids (Ostaszewska et al., 2006; Jankowska et al., 2003). Therefore linoleic acid and linolenic acid may satisfy the essential fatty acid requirements in this species. Ostaszewska (2006) was able to show that DHA biosynthesis in pikeperch requires the involvement of the enzymatic complex allowing DHA synthesis from EPA by 24:6n-3 and peroxisomal β-oxidation, a process similar in Eurasian perch, *Perca fluviatilis* (Kestemont et al., 2001). Lund and Steennfeldt (2009) observed no significant differences in survival of larvae between pikeperch larvae that were feed essential fatty acid supplemented diets or fish oil diets. This could be attributed to the presence of DHA, EPA and ARA which are the main fatty acids in fish oil but also the products of linoleic acid or linolenic acid which are essential fatty acids for freshwater fish. Hamza et al. (2008) found out that increasing dietary phospholipid levels has a positive effect on growth of pikeperch larvae. This is in agreement with other freshwater species like common carp (Fontagne et al., 2000) and

marine species like European sea bass and gilthead sea bream (Izquierdo et al., 2001; Gisbert et al., 2005).

The transition of the pro-larvae (yolk dependent larvae) to exogenous feeding is a critical event in the early stage of development of percid fish as failure to accomplish first feeding results in mortality (Kestemont et al., 2006). Weaning age often occurs at the moment at which the stomach becomes functional with a switch from an exclusively intestinal digestion to mainly a stomachal digestive activity (Kestemont et al., 2003).

Larviculture of pikeperch normally is characterized by three important critical stages were a lot of mortality is observed. The first peak of mortality is normally observed at the end of exogenous feeding when yolk reserves are depleted. It usually occurs at 4-6 days post hatching (dph). The second peak of mortality happens between days 8-16 largely attributed to swim bladder inflation and the third peak to cannibalism at 22-34 dph (Szkudlarek et al., 2007). The second peak of mortality (8-16 dph) could also be attributed to starvation of the larvae that reach the point of no return and choose to starve to death (Hamza et al., 2007, 2008). This point of no return is a phenomenon where the fish larvae refuse to eat despite food being available leading to massive mortality.

Kestemont et al. (2003) showed that perch larvae have already developed efficient pancreatic and intestinal enzymes at very early larval stages, suggesting that a compound diet could be provided very early. Osteszewska et al. (2006) demonstrated that pikeperch larvae can be weaned directly onto a suitable commercial diet. Hamza et al. (2007) on the other hand showed that digestive capacities of pikeperch larvae was largely affected by diet and weaning time.

Hamza et al. (2008) showed that phospholipid supplementation improves growth and activities of digestive enzymes in pike perch larvae significantly, when they observed that an increase in dietary PL from 14g.kg^{-1} to 95g.kg^{-1} led to 50% enhancement in weight in 34 day old larvae and the earlier maturation of digestive structures.

Pikeperch don't require rotifers as starter feed but prefer nauplii of copepods, which are soon replaced by copepodite stages and mature copepods (Peterka et al., 2002). However *Artemia* is the most widely used natural feed source in aquaculture with over 2000 metric tonnes of dry cysts are marketed worldwide for onsite hatching (Van Stappen, 2011). Enrichment of *Artemia* nauplii with vitamin C and HUFA is usually recommended and is essential for fast growing larvae and to prevent scoliosis, lordosis and maxillary deformities in pikeperch (Kestemont et al., 2003).

It has been reported that some freshwater fish species, like *Clarias gariepinus* and *Heterobranchus longifilis*, can be directly weaned to artificial diets right from the onset of exogenous feeding (Hung et al., 2001). Larvae of Asian catfish were able to feed 48 hours after hatching at a temperature of 28-30 $^{\circ}$ C even though the yolk sac was not completely absorbed. Hung et al.(2001) attributed this to the fact that the larvae have a mouth size large enough to ingest different types of natural diets. Ostaszewska et al. (2005) was able to show an improved survival and growth of pikeperch larvae with only dry feed, although all previous studies demonstrated that pikeperch larvae can be successfully reared on both live feed and formulated feed but poor survival and growth rates are obtained when using solely formulated feed (Kestemont et al., 2007).

A study carried out to assess growth rate, survival rate, cannibalism and deformities in pikeperch larvae showed that the highest mean survival rate (24.8%) was obtained in the control groups fed with *Artemia* nauplii only , while the mean survival in weaned groups was 17.7, 15.3 and 13.3% at day 12,day 19, and day 26 respectively (Kestemont et al., 2007). This shows that early weaning is possible in pikeperch larval rearing. Kestemont et al. (2007) was also able to show that pikeperch larvae receiving a formulated feed for freshwater fish were able to survive better when compared with larvae fed with a formulated feed for marine fish although mean final weight and specific growth rate were significantly higher in fish fed with live food than in those fed formulated diets. Ostaszewska et al. (2005) suggested that the use of commercial and experimental feeds as a starter diet in pikeperch larvae rearing could be meaningful since pikeperch larvae are able to synthesize and transport lipids by 12 dph. High

quality dry feed is readily accepted, digested and absorbed by the fish very early in its early development. Ostaszewska et al. (2005) presented pikeperch larvae with commercial microdiets and live Artemia nauplii and for both diets observed similar survival and growth rates. He further proved that pikeperch larvae readily ingested, digested and assimilated exogenous food without addition of natural food.

Profitability of fish farming activities is mainly dependant on optimization of growth. Growth of a fish species can be influenced by a number of factors among which temperature and feeding being the most important (Gardeur et al., 2007). Temperature regulates metabolic activity within which growth is maximal and most percid fishes display fastest growth at relatively warm temperatures (Wang et al., 2009). For pikeperch the optimal temperature has been documented to be within the range of 24-30 ^{o} C (Wang et al., 2009).

Arachidonic acid (ARA) is thought to be the precursor to eicosanoids such as prostaglandins, which are released in response to signals associated with tissue damage or stress (Van Anholt et al., 2004). These prostaglandins are involved in the control of osmoregulatory processes and the regulation of the stress-induced hypothalamus-pituitary-interrenal (HPI) axis (Van Anholt et al., 2004). It is this axis that facilitates the release of cortisol, the main corticosteroid in teleost fish and is important when a fish is trying to adapt to a stressor (Van Anholt et al., 2004). Several authors (Specker & Schreck 1980; Schreck 1982; Maule et al., 1989; Campbell et al., 1994; Mc-Cormick et al., 1998) have stated that prolonged stress and elevated corticosteroid levels can have adverse effects on survival, growth, reproduction, immune function and general fitness (Grant& Schreck, 2002).

The objective of this present study was to investigate the weaning of pikeperch (*Sander lucioperca*) larvae and the effects of three different diets. The importance of HUFAs in relation to growth, survival and acute stress response of pikeperch larvae was also investigated.

Materials and methods

Two months before the arrival of fish larvae, a biofilter of 200l of water capacity was set up. The plastic tank was filled half way with micro beads that provided surface for bacterial attachment. Strong aeration was provided in order to provide oxygen and to maintain the micro beads in constant motion. The micro beads were washed with a disinfectant and dried overnight before putting them in the tank. The system was heated with two heaters to maintain a temperature of 19 ± 1°C . The system was inoculated with both *Nitrosomonas* and *Nitrobacter*, collected from the ARC stock culture. For this period, 10 mL of NH_4Cl_2 were added once a day after checking the concentration of both Ammonium-nitrogen and Nitrite-nitrogen. The tank was covered to avoid light.

Hatching of *Artemia* cysts was performed according to standard methods developed at the ARC (Lavens and Sorgeloos, 1996). Great Salt Lake *Artemia* (GSL) cysts were hatched at a concentration of 2g of cysts/l at 28° C under continuous aeration and light in 40l of water. After 24 hours, Instar 1 nauplii were harvested and washed with seawater. For enrichment, Instar 1 nauplii were re-introduced into 10l tanks. Before enrichment, the lipid emulsion was thoroughly mixed to prevent formation of lipid globules in the tanks. Enrichment was done at 28 ° C under continuous aeration and light for 24 hours. After 24 hours the enriched nauplii were harvested and rinsed with sea water to remove the excess fats in order to prevent foam and film formation in the fish rearing tanks. To enrich the *Artemia*, two lipid emulsions, Easy DHA Selco® (INVE Aquaculture, Dendermonde, Belgium) rich in DHA (156.2mg.g^{-1}, DHA/EPA = 3.3) and the ICES 30/0.6 (INVE Aquaculture, Dendermonde, Belgium) rich in EPA (128.3mg.g^{-1}, DHA/EPA = 0.7) were added to the tanks at a concentration of 0.6 g.$10l^{-1}$ at t=0.

During this study, two sets of fish larvae were received. The first batch of fish larvae were 4 days post hatching (dph), and were purchased from Viskweek centrum in Leende (The Netherlands) and transported from Netherlands to Belgium. Upon arrival they were put in a large tank to acclimatize to the laboratory conditions. The next day these larvae were counted one by one and stocked in the 15 rearing tanks. One group of 9 tanks was fed with Easy DHA-

enriched *Artemia*, a second group of 3 tanks was fed with EPA- enriched *Artemia* and the last group of 3 tanks was fed with non-enriched *Artemia*. It was noticed that the larvae exhibited a high wall-clinging behaviour and that the majority of them was not eating. During the next two weeks there was a lot of mortality and hardly any *Artemia* could be detected in the intestines of the larvae. It was then decided to terminate the experiment and prepare for a new batch of fish larvae.

This time, older pikeperch larvae (16dph) were purchased from Viskweek centrum in Leende (The Netherlands) and transported from Netherlands to Belgium. Fish larvae were acclimated to the laboratory conditions of the Laboratory of Aquaculture & Artemia Reference Center (ARC), counted one by one and stocked into 15 fish rearing tanks of 10 l capacity. Tanks were stocked with 600 larvae. They were fed some non-enriched Great Salt Lake (GSL) *Artemia* in the evening as advised by the supplier. These larvae had been fed only Instar 1 GSL *Artemia* at the hatchery before arrival to the ARC laboratory. These larvae were divided into three treatments and fed with DHA enriched *Artemia*, EPA enriched *Artemia* and non-enriched *Artemia* for 10 days. At the end of the live feed phase, the remaining larvae of each treatment were pooled and counted manually one by one. The larvae were then restocked at a concentration of 9 larvae.litre^{-1} and starved for one day before weaning the larvae with 100% dry feed that lasted for 13 days. The co-feeding strategy was not applied, in accordance to the protocol of the commercial hatchery Aquapri in Denmark (pers. comm.).

The rearing facility which consisted of 15 conical tanks of 10 l was connected to a recirculation water system. Tanks had separate inlet taps and 500µm outlet filters. Aeration was provided through the biofilter tank and there was no aeration provided in the fish culture tanks. All effluent tank-water passed a sedimentation tank before biofilter treatment and reuse (Fig. 3). The water flow was 6 l.h^{-1}. Photoperiod was set at 20 hours light and 4 hours darkness for the first two weeks, afterwards the light (100 lux) was switched on for 24 hr. Temperature was maintained at 19 ±1°C. Ammonium and nitrite concentration were monitored daily using the Merck test kits. Oxygen level was kept above 7mg.l^{-1} by adding fresh water to the recirculation system and was measured using the SCHOTT FIELDLAB OXI oxygen probe.

The study was designed to assess the effect of 5 different dietary regimes on the survival and growth of pikeperch larvae (Table 1). All tests were done in triplicate. Two commercial diets for weaning marine fish larvae, AgloNorse®(K/S Tromso Fiskeindustri A/S &Co, Norway) mainly used in commercial hatcheries in Norway and O-Range®(INVE Aquaculture, Dendermonde, Belgium), a weaning feed for sea bass and sea bream were tested. They have a high PUFA content of 50and 30mg.g^{-1} DW respectively. They were compared with a homemade vegetable test feed from KULeuven (Table 2).

Table 1: Experimental treatment groups

Treatment	*Artemia* Enrichment medium	Lipid composition	Dry feed	Remark
DHA-V	Easy DHA selco	DHA/EPA=2	Vegetable	High in PUFAs but no HUFA in dry feed
DHA-A	Easy DHA selco	DHA/EPA=2	Aglonorse	
DHA-O	Easy DHA selco	DHA/EPA=2	Orange	
EPA-A	ICES 30/0.6(EPA)	DHA/EPA=0.7	Aglonorse	Very low DHA amount in emulsion but high levels of PUFAs in dry feed
Non -V	No enrichment	DHA/EPA=0	Vegetable	No HUFAs supplied to the fish larvae

The different treatments were chosen to answer the following study questions:

- Which commercial diet gives the best results for pike perch larvae? (DHA-A versus DHA-O)
- Is there a need for HUFAs after the live feed phase or is the fish able to produce enough HUFAs to meet its requirements? (DHA-V versus DHA-A and DHA-0)

- Which HUFA, DHA or EPA, is preferably supplemented to pike perch larvae in order to promote growth and survival? (DHA-A versus EPA-A)
- Are 16dph pikeperch larvae able to elongate and desaturate throughout the whole larval phase or do they need HUFA supplementation in early days? (DHA-V versus Non-V)
- Does the addition of HUFAs affect the stress resistance of pike perch larvae?

Table 2.Proximate composition of the different dry diets

Parameter	AgloNorse®	O-Range®	Vegetable
Protein (%)	59	55	44
Fat (%)	16-18	13	19
Ash (%)	10	13	8
Fibre (%)	1	1	3.9
Moisture (%)	8-9		4

Feeding was done automatically by a peristaltic pump (Fig. 4) during the *Artemia* phase while the dry feeds were hand-fed. All tanks were cleaned once every morning before feeding by siphoning and any mortalities seen were recorded daily. Water loss due to evaporation was replaced with some fresh water. Water flow through the system was aided with two water pumps. The enriched and non-enriched *Artemia* soup was cold stored (4° C) in order to lower the metabolic activities and maintain enrichment (Fig. 4).

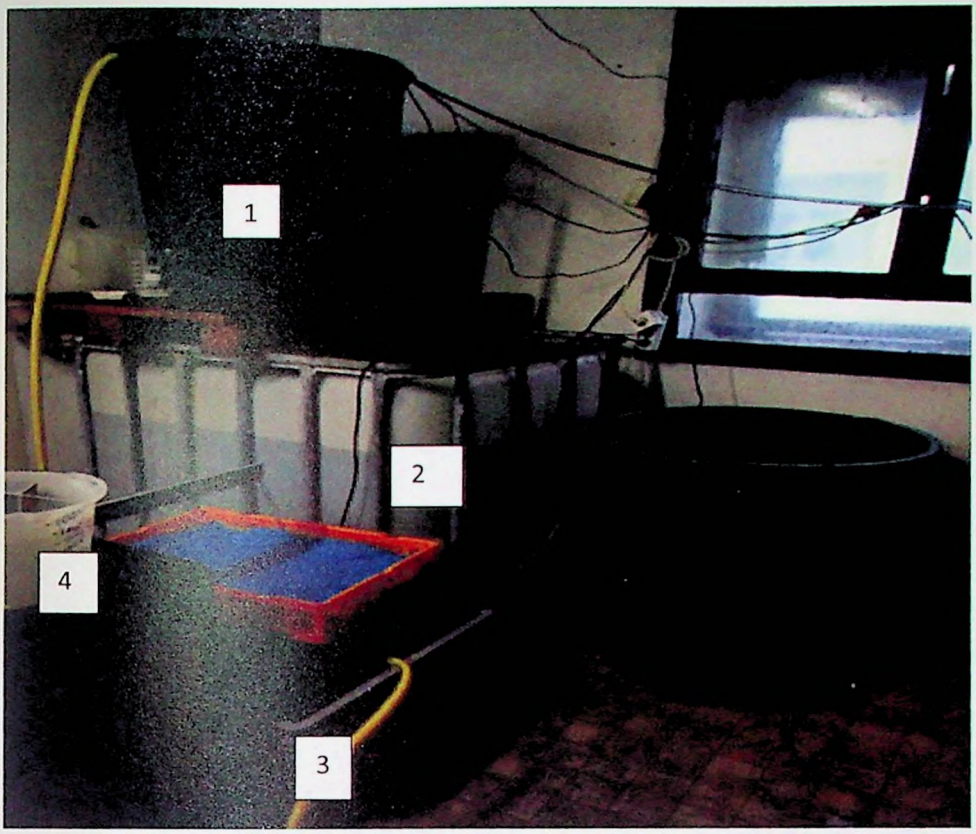


Figure 3. The experimental set up, showing the biofilter (1), reservoir tank (2),sedimentation tank (3) and a fish rearing tank (4)

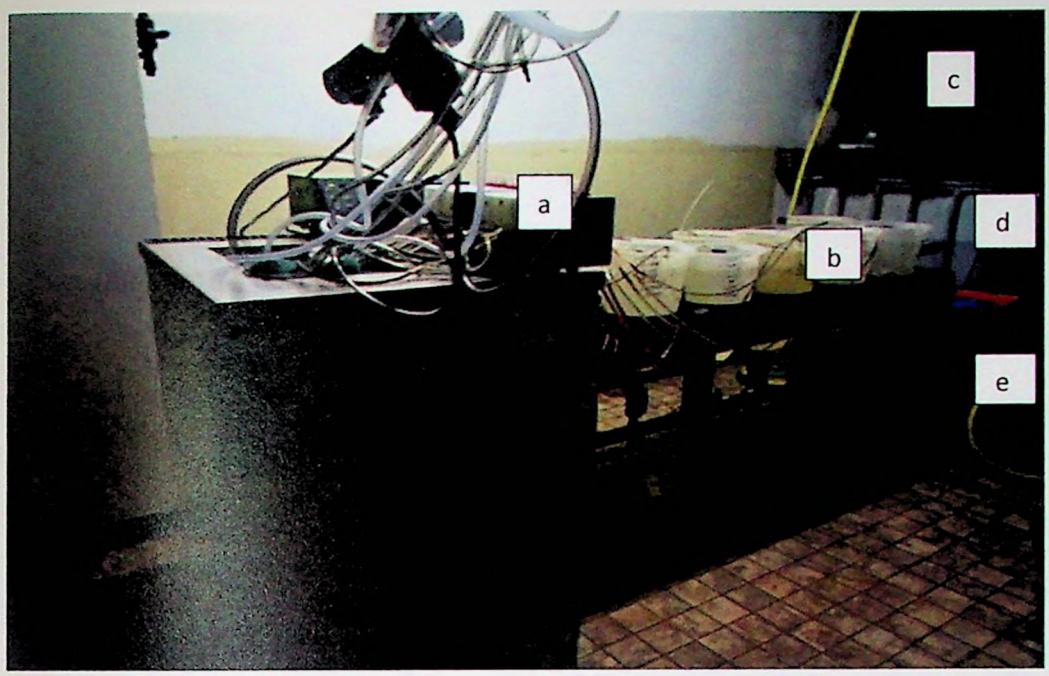


Figure 4. The experimental set-up, showing the peristaltic pump(a), fish rearing tanks(b), biofilter tank(c),reservoir tank(d) and the sedimentation tank(e)

Samples of fish larvae were taken to measure total length, wet weight (WW) and dry weight(DW). These were taken at t=16dph, t=26dph and t=39dph. Total length was measured using an electronic digital vernier caliper(S/N 91035453) except for the initial fish samples, where total length (distance from upper lip till end of tail) was measured at the Department of Morphology (Faculty of Veterinary Science, Ghent University) with a specialized software CellD. The WW was measured using a microbalance, after removal of excess water with paper tissue. The DW was measured using a microbalance after drying at 103 $^{\circ}$ C for 4 hours. A total of 200 fish larvae were sampled for wet and dry weight and 10 fish larvae for total length measurement at day 16. At 26 dph, 15 fish larvae were sampled from each treatment group (5 fish larvae.tank^{-1}) except for the group fed DHA enriched Artemia were 3 tanks were picked arbitrally as the source of the samples. At 39 dph, 20 fish larvae were sampled from DHA-A, 10 fish larvae for DHA-O, 10 fish larvae for DHA-V, 20 fish larvae for EPA-A and 10 fish larvae for NON-V treatment for wet weight, dry weight and length measurement. It should be noted that at 39dph, samples were taken after pooling of the replicate tanks of same treatment.

Fatty acid analysis was carried out on larvae at the end of the live feed phase at 26dph and on larvae at 39dph on termination of the experiment. At dph 26, a total of 15 larvae were sampled. Collection of these samples was done after pooling all the replicate tanks of the same treatment. From each treatment, 5 larvae were collected. On day 39 post hatching, all replicates were pooled per treatment before sampling and a total of 37 larvae were sampled from the 5 treatment groups (DHA-A=5,DHA-V=11,EPA-A=10,NON-V=6 and DHA-O=5). The larval tissue was then homogenized using a Polytron®tissue homogenizer. The fatty acid composition was determined using the direct esterification method (Lepage and Roy, 1984)

On dph 26 and 39 , larvae were subjected to an acute salinity stress. A total of 10 larvae randomly selected from each treatment, after pooling all the remaining fish larvae, were rapidly transferred in triplicate 2l beakers with either culture water or 15g$NaCl.l^{-1}$ water. The larvae were observed for 30 minutes and mortalities recorded after which the remaining live larvae of the replicate trials were pooled and preserved for body cortisol content analysis. They were dried using tissue paper, transferred to falcon tubes and stored at -80 $^{\circ}$ C.

The cortisol was extracted using diethyl ether followed by tetrachlormethane as described by Hiroi et al.(1997). The larval fish sample was weighed and kept on ice. This was followed with the addition of 1.5ml of ice cold phosphate buffer saline (PBS). The sample was then homogenized using a POLYTRON® homogenizer. The homogenizer was cleaned with PBS every after homogenizing each sample. To the homogenized sample 3ml of ether was added and the contents were mixed by shaking the test tube vigorously for 2 minutes. The above step of adding ether was done twice. The top (ether) layer was removed and put into a clean test tube and stored at -80° C for 12 hours. After the cold storage, the ether layer was collected in a new clean test tube and dried at room temperature. This was done using a BUCHI R-114 Rotavapor. To the dried residue, 300µl of tetrachloromethane was added and mixed for 4 minutes to reconstitute it. PBS containing 0.1% gelatin(300 µl) was then added, and mixed for 2 minutes. After centrifugation (1500 rpm, 10 min, 4 $^{\circ}$ C), the aqueous layer was collected and stored in aliquots for cortisol determination. A centrifugation of 1500 rpm was used instead of the 3000 rpm to avoid breakage of the test tubes. After extraction, the samples were kept in the freezer

at -18°C till analysis. After thawing, the samples were first purified by Solid Phase Extraction (SPE). After drying and resuspension in 100 µl of solvent, 40 µl was injected in the UPLC-MS/MS for chromatographical analysis. This new method, developed by Mr. J. Aerts (PhD, johan.aerts@ilvo.vlaanderen.be), Faculty of Pharmaceutical Sciences, Department of Bio-analysis has been validated on hair samples and will soon be published. No validation for pike perch samples has been done for this study.

Table 3.Feeding strategy and time scheme

Age of pikeperch larvae	feed	light	Feeding regime	Stocking density
Day 16-26	GSL Artemia	20-4	Constant feeding with peristaltic pump for 18 hours	60 larvae/litre
Day 26-27	No co-feeding; starvation	18 hours		
Day 27-39	Dry feed (15% of total biomass)	24/0	Every 2-3 hours only during day	9 larvae/litre

The dry feed was estimated at 15% of total biomass at an estimated SGR of 10%day^{-1}

Statistical analysis

Statistical analysis was based on one way analysis of variance (ANOVA). Significant differences between treatments were determined using a post-hoc Tukey HSD multiple comparisons test with a significance level at $p<0.05$. For data that was not homogeneously distributed a non parametric test- Kruskal-Wallis was used instead of ANOVA. Analyses were performed using S+ statistical package software.

Formulae for growth parameters

The specific growth rate (sgr, %) was calculated using the formula:

$$Sgr\ (\%) = (LNWt-LNW\alpha/t)*100$$

Wt: weight at time t

W_α: weight at time t=0

t: duration in days

3.0 Results

3.1 Growth results

The initial values for WW, DW and length of the 16dph larvae were 5mg, 0.82mg and 9.52mm respectively. There were no significant differences in WW, DW and length between the treatments at the end of the live feed phase (dph 26) (Table 4). This lack of significant differences could be attributed to the heterogeneous growth of fish larvae in the pooled tank samples.

Table 4.Summary of pikeperch larvae performance fed with Artemia first (10 days) followed by different weaning diets (13 days)

	Live feed phase (10 days, n=3)			Dry feed phase (13 days)				
	NON	EPA	DHA	NON-V	EPA-A	DHA-V	DHA-O	DHA-A
				n=2	n=4	n=2	n=2	n=4
WW(mg)/fish	39.2±10.3	34.2±9.9	55.1±20.8	52.1±15.7^{a}	78.8±16.1^{ab}	66.1±17.1^{ab}	58.0±9.5^{a}	103.2±7.3^{b}
Length (mm)	16.7±2.0	18.2±1.1	18.9±2.6	21.9±2.6	23.1±1.9	21.3±1.1	22.7±3.3	26.4±3.3
DW(mg)/fish	4.5±1.9	5.2±1.2	12.1±12.1	6.6±2.8	23.2±13.9	24.6±8.2	14.7±4.2	19.8±6.1
Ash (mg)/fish				7.9	11.9	8.0	8.3	11.3
Sgr (%day^{-1})	20.5±2.0	19.2±2.0	24.0±2.0	2.2±3.0	6.5±3.0	1.4±3.0	0.4±3.0	4.8±3.0
% survival	6.9±0.7	6.8±0.7	8.0±0.7	38.5±6.0	44.4±6.0	34.4±6.0	27.8±6.0	38.9±6.0

Values in a row with different letters indicate significant differences ($p<0.05$)

The larvae that were fed DHA- enriched *Artemia* nauplii had a tendency to grow better compared to the other two treatments, especially considering their DW (12.1mg±12.1).This is almost three times as high as the DW of the larvae that received the non-enriched (4.5mg ±1.9) *Artemia*. It should also be noticed that their SGR (24.0±2.0% day-1) was highest. Survival was low at the end of the live food phase and ranged between 6.8±0.7% and 8.0±0.7%.

During the dry feed phase, WW was found to be statistically different between larvae feed DHA-A,DHA-O and NON-V, with DHA-A performing better than the other two. There was no significant difference between larvae fed EPA-A or DHA-V and the other treatments.

The percentage survival ranged between 27.8±6.0%(DHA-O) and 44.4±6.0%(EPA-A) during the dry feed phase. In general the fish larvae that were weaned onto AgloNorse® had the highest survival rate followed by those fed with the vegetable diet and O-Range® diet respectively. The sgr is lowest for the fish receiving the O-Range diet (0.4±3.0%) and highest for the AgloNorse diet (4.8±3.0% – 6.5±3.0%). The vegetable diet results in intermediate growth results.

3.2 Fatty acid analysis

A selection of fatty acids present in the emulsions used for enrichment(mg.gDW^{-1}), clearly demonstrated that Easy DHA selco® is rich in DHA while the ICES 30/0.6 is particularly rich in EPA. Easy DHA selco® had 3.3 times more DHA than EPA while ICES 30/0.6 had a ratio of DHA:EPA equal to 0.7 (Table 5).

Table 5. Fatty acid composition of the emulsoins used for *Artemia* enrichment (mg.gDW^{-1}) (n=1)

Fatty acid	Easy DHA selco®	ICES 30/0.6
16:0	127.6	59.5
18:0	34.9	30.0
TOTAL SFA	162.5	89.5
16:1(n-7)	33.9	9.5
18:1(n-9)	137.4	85.8
TOTAL MUFA	171.3	95.3
18:2(n-6)	46.5	38.9
18:3(n-6)	1.2	1.1
20:3(n-6)	0.6	1.0
20:4(n-6)ARA	10.7	6.5

TOTAL (n-6)PUFA	70.5	51.6
18:3(n-3)	10.1	6.7
20.3(n-3)	1.4	0.6
20:5(n-3)EPA	47.7	128.3
22:6(n-3)DHA	156.2	94.1
TOTAL(n-3)PUFA	221.8	253.6
TOTAL mg FAME/gDW	790.9	637.9
DHA/EPA	3.3	0.7
ARA/DHA	0.07	0.07
ARA/EPA	0.22	0.05
(n-3)/(n-6)	3.15	4.91

The fatty acid profile of the *Artemia* receiving the respective emulsions reflected to a certain degree the profile of the emulsions : *Artemia* enriched with Easy DHA Selco had a DHA/EPA raio of 1.1 while the *Artemia* enriched with ICES 30/0.6 had a ratio of 0.2 (Table 6). The DHA value in the Easy DHA Selco-enriched *Artemia* is however rather low, although non-enriched *Artemia* are devoid of any DHA as expected. The total FAME content in non-enriched *Artemia* was also 37-42% lower than in the enriched *Artemia*.

Table 6.Fatty acid composition of *Artemia* fed to pikeperch larvae

(mg.gDW^{-1}) (n=1)

Fatty acid	*Artemia* DHA	*Artemia* EPA	NON ENRICHED *Artemia*
16:0	16.4	16.9	10.2
18:0	10.2	10.9	8.2
TOTAL SFA	26.6	27.8	18.4
16:1(n-7)	3.0	3.2	1.7
18:1(n-9)	27.2	29.1	18.4
TOTAL MUFA	30.2	31.3	20.1
18:2(n-6)	8.6	9.3	5.3
18:3(n-6)	1.1	1.0	0.6
20:3(n-6)	0.2	0.3	0.2
20:4(n-6)ARA	1.0	1.2	0.7
TOTAL(n-6)PUFA	11.3	12.2	6.9
18:3(n-3)	43.6	42.2	27.3
20:3(n-3)	2.2	2.1	1.5
20:5(n-3)EPA	4.8	13.1	2.1
22:6(n-3)DHA	5.3	3.0	
TOTAL(n-3)PUFA	11.9	21.0	5.0
Total mg FAME/gDW	160.2	174.1	102.1
DHA/EPA	1.1	0.2	
ARA/DHA	0.2	0.4	
ARA/EPA	0.2	0.1	0.3
(n-3)/(n-6)	1.1	1.7	0.7

Table 7.Fatty acid content of fish larvae at the end of the live feed phase(day 26) (mg.g DW^{-1}) (n=1)

Fatty acid	DHA ENRICHED	EPA ENRICHED	NON ENRICHED
16:0	15.3	13.9	14.0
18:0	7.2	7.2	6.6
TOTAL SFA	22.5	21.1	20.6
16:1(n-7)	1.3	1.0	1.2
18:1(n-9)	13.9	12.0	13.1
TOTAL MUFA	15.2	13.0	14.3
18:2(n-6)	4.3	3.2	3.9
18:3(n-6)	0.5	0.3	0.4
20:3(n-6)	0.7	0.5	0.8
20:4(n-6)ARA	2.2	2.4	2.1
TOTAL(n-6)PUFA	8.9	7.6	8.2
18:3(n-3)	7.8	5.2	6.3
20:3(n-3)	1.0	0.8	0.9
20:5(n-3)EPA	4.4	5.8	3.7
22:6(n-3)DHA	10.6	12.0	8.9
TOTAL(n-3)PUFA	20.9	22.8	18.3
TOTALmgFAME/gDW	94.3	86.7	84.8
DHA/EPA	2.4	2.1	2.4
DHA/ARA	4.8	5.0	4.2
EPA/ARA	2.0	2.4	1.8
(n-3)/(n-6)	2.3	3.0	2.2

The total FAME content in the fish larvae was very similar for the 3 treatments and varied between 84.8 mg.gDW^{-1} and 94.3 mg.gDW^{-1}. These results show that the fish larvae in all the treatments had more DHA than EPA and ARA. The results also show that the larvae maintained similar ratios of DHA/EPA, DHA/ARA and EPA/ARA Similarly, the level of 18:2(n-6) and 18:3 (n-3), possible precursor fatty acids to ARA and EPA/DHA in fish larvae receiving non-enriched *Artemia* was comparable to the ones that received enriched *Artemia*.

The analysis of the three weaning diets reveals that the vegetable diet possesses no ARA and only very small quantities of EPA and DHA (Table 8). On the other hand, it is exceptionally rich in oleic acid (40% of total FAME) and linoleic acid (25% of total FAME). AgloNorse® has almost double the amount of oleic acid (23.2 mg.gDW^{-1}) and 2.5 times more linoleic acid (23.5 mg.gDW^{-1}) than O-Range®. The EPA and DHA content is comparable between the two diets.

Table 8. Fatty acid composition of the dry diets (mg.gDW^{-1}) (n=1)

Fatty acid	AgloNorse®	O-Range®	Vegetable
16:0	27.8	24.7	15.9
18:0	5.0	7.1	4.9
TOTAL SFA	32.8	31.8	20.8
16:1(n-7)	5.8	6.0	2.1
18:1(n-9)	23.2	12.9	51.6
TOTAL MUFA	29.0	18.9	53.7
18:2(n-6)	23.5	9.5	32.1
18:3(n-6)	0.1	0.2	0.1
20:3(n-6)	0.0	0.2	0.0
20:4(n-6)ARA	0.8	1.5	0.0
TOTAL(n-6)PUFA	25.5	13.2	32.2
18:3(n-3)	3.0	2.4	4.8
20:3(n-3)	1.0	0.3	
20:5(n-3)EPA	9.6	8.4	0.2
22:6(n-3)DHA	16.1	17.1	0.5
TOTAL(n-3)PUFA	29.5	28.5	5.5
TOTALmgFAME/gDW	168.5	135.7	127.6
DHA/EPA	1.7	2.0	2.5
DHA/ARA	20.1	11.4	
EPA/ARA	12.0	5.6	
(n-3)/(n-6)	1.2	2.2	0.2

Table 9.Fatty acid composition of fish larvae at the end of the experiment (day 39) (mg.gDW^{-1})(n=1)

Fatty acid	NON-V	DHA-A	EPA-A	DHA-V	DHA-O
16:0	8.5	17.4	15.5	9.8	15.6
18:0	5.0	5.3	5.0	6.0	5.2
TOTAL SFA	13.5	22.7	20.5	15.8	20.8
16:1(n-7)	0.7	1.9	1.7	0.9	2.7
18:1(n-9)	7.7	11.5	10.6	8.0	9.4
TOTAL MUFA	8.4	13.4	12.3	8.9	12.1
18:2(n-6)	2.1	8.7	7.8	2.1	5.0
18:3(n-6)	0.2	0.2	0.2	0.2	0.2
20:3(n-6)	0.4	0.2	0.2	0.4	0.1
20:4(n-6)ARA	2.6	1.6	1.6	2.7	1.9
TOTAL(n-6)PUFA	6.4	11.6	10.8	7.2	9.0
18:3(n-3)	1.0	1.3	1.4	1.4	1.1
20:3(n-3)	0.3	0.2	0.2	0.3	0.3
20:5(n-3)EPA	2.0	5.3	5.0	2.5	5.0
22:6(n-3)DHA	10.9	20.3	17.9	14.2	22.3
TOTAL(n-3)PUFA	15.1	27.7	24.9	19.0	29.3
TOTALmgFAME/gDW	54.6	96.6	89.3	65.4	88.4
DHA/EPA	5.5	3.8	3.6	5.7	4.5
DHA/ARA	4.2	12.7	11.2	5.3	11.7
EPA/ARA	0.8	3.3	3.1	0.9	2.6
(n-3)/(n-6)	2.4	2.4	2.3	2.6	3.3

The results show that pikeperch are interested in maintaining a certain constant percentage of DHA . The results suggest that linoleic acid(18:2(n-6)) was converted to ARA as indicated by the low levels of linoleic acid in both NON-V and DHA-V treatments, despite the high level of this fatty acid in the Vegetable diet (Table 8).

Table 10. A comparison of ARA, EPA and DHA content ($mg.gDW^{-1}$) in fish at day 26 and day 39 and the EFA index

Fattyacid mg.gDW (%)	FISH AT DAY 26			FISH AT END OF EXPERIMENT (35dph)				
	DHA	EPA	NON-V	DHA-A	DHA-O	DHA-V	EPA-A	NON-V
20:4(n-6)ARA	2.2	2.4	2.1	1.6	1.9	2.7	1.6	2.6
20:5(n-3)EPA	4.4	5.8	3.7	5.3	5.0	2.5	5.0	2.0
22:6(n-3)DHA	10.6	12.0	8.9	20.3	22.3	14.2	17.9	10.9
18:1(n-9)/22:6(n-3)	1.3	1	1.5	0.6	0.4	0.6	0.6	0.7

3.3 Cortisol results

All the fish that were subjected to the acute saline stress survived the exposure and so no mortality was recorded.

At both samplings, the larvae expressed detectable levels of cortisol. In general the trend was that when fish are put into the stressfull condition (saline water at 15ppt) they expressed more cortisol than when they were just confined in a glass beaker of culture water. This was not the case for the fish larvae under the DHA-A treatment which seemed to release more cortisol

under culture water than saline water (figure 7). In Figure 6, fish larvae that got DHA enriched *Artemia* seemed to express more cortisol under the culture water than in the saline water.

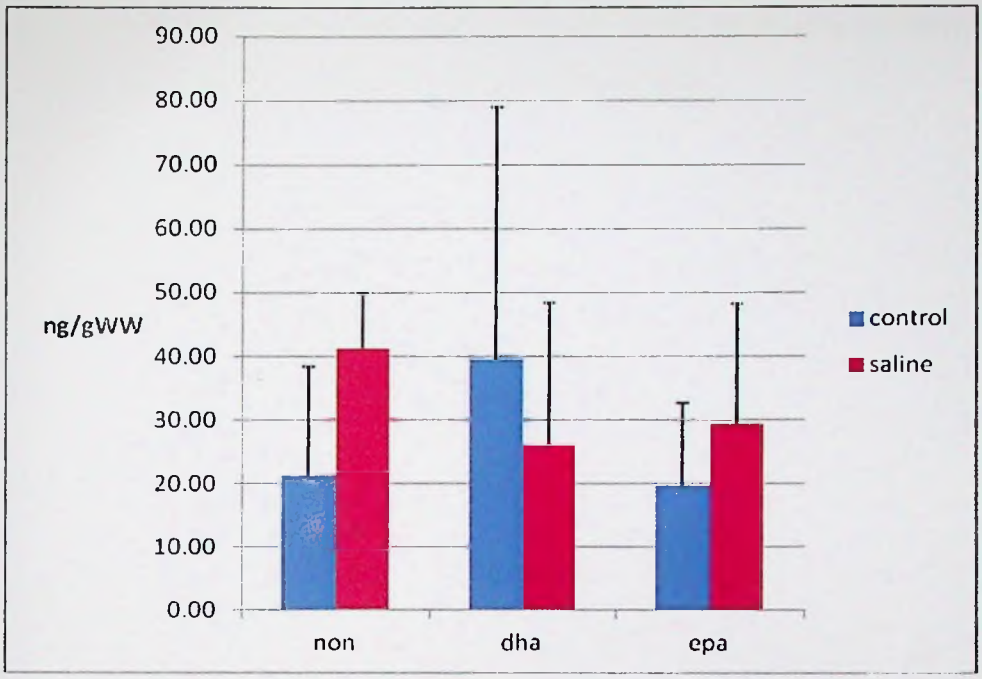


Figure 5. Cortisol levels (ng/gWW) at 26dph salinity stress test

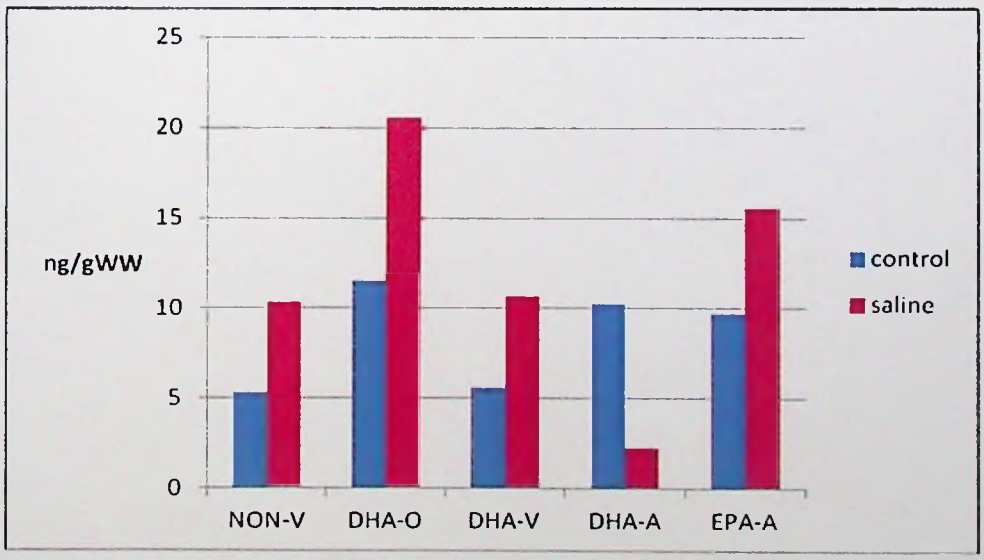


Figure 6.Cortisol levels (ng/gWW) at 39dph salinity test

The results show that fish larvae under treatment DHA-O, had the highest level of cortisol released (21ng/gWW) followed by treatment EPA-A (16ng/gWW) while DHA-V and NON-V treatments had intermeditate results (11 and10.5ng/gWW respectively) and DHA-A treatment had the least amount of cortisol (3ng/gWW) released.

DISCUSSION OF RESULTS

In the first batch of fish (starting with pikeperch larvae of 4dph), the larvae were seen to cling on the tank sides and they hardly ate and died masively over a period of 2 weeks. This was in aggreement to what other authors have observed as the second peak of mortality (Hamza et al., 2007, 2008).

During the live feed phase (duration 10 days) of the second batch (starting with pikeperch larvae of 16dph), the specific growth rate ranged between 19.2±2.0%.day^{-1} and 24.0±2.0%.day^{-1}. These results compare well with Lund and Steenfeldt (2011) who found SGR for pike perch larvae of 20.8%.day^{-1} to 22.3%.day^{-1} from dph 3 till dph 21. They fed the larvae with varying dietary fatty acid enriched *Artemia*. Zakes and Szkudlarek (2007) on the other hand obtained only a SGR of 1.9%day^{-1} - 2.7%day^{-1} with 4 dph pikeperch fed unenriched *Artemia* for 14 days. In their experiment, the 4dph fish larvae were hand-fed with non-enriched *Artemia* nauplii and on 16dph the quantity of *Artemia* offered at each feeding was gradually decreased by 50% daily untill the *Artemia* had been fully replaced by the artificial diet on 18 dph. Kestemont et al. (2007) found an SGR of 7.54%.day^{-1} for 26 dph old pikeperch larvae that were fed Vitamin C and HUFA enriched *Artemia* before weaning them.

The larvae that received DHA enriched *Artemia* had a tendency to grow faster, although the high standard deviation of the samples distorts somewhat the results. However, looking at the results after the weaning period with AgloNorse®, the significant difference between EPA-A and DHA-A, confirms this tendency. There was no significant difference in FAME composition in the 26 dph larvae after 10 days of feeding with either enriched or non enriched *Artemia*, although larvae that received DHA enriched *Artemia* had the highest total FAME content of 94.3 mg.gDW^{-1}, while those that received either EPA-enriched or NON-enriched *Artemia* had 86.7 mg.gDW^{-1} and 84.8 mg.gDW^{-1} respectively. The fish larvae maintained a constant percentage of DHA of 11.2%,13.8% and 10.5% when fed DHA-enriched, EPA-enriched and NON-enriched *Artemia* respectively. The fact that the larvae receiving DHA enriched Artemia had a tendency to perform better, can be possibly attributed to the fact that pikeperch, being a fresh water

fish, is able to elongate and desaturate fatty acids to highly unsaturated fatty acids (HUFAs) which promote growth (Ostaszewska et al., 2006) but at a cost. Desaturation and elongation of EPA to DHA costs energy so the larvae fed with either EPA-enriched or NON- enriched *Artemia* had to divert energy from growth which is reflected in reduced weight and length.

The survival percentage after the live feed phase between 6.8±0.7 and 8±0.7%. This is a low survival as compared to other studies by Kestemont et al.(2007) and Zakes and Szkudlarek (2007) who obtained survival rates of 24.8±2.1% when 11dph pikeperch larvae were fed *Artemia* nauplii and 72.3±1.0 to 79.2±0.8% when 4dph pikeperch larvae were fed a mixture of *Artemia* nauplii and Lansy®artificial feed respectively. This can be attributed to a high bacterial load in the culturing water during the first 5 days. It was made obvious by the milky colour of the water. This led us to add at least one litre of freshwater to the culture tanks every morning after cleaning and to keep the water level in the reserve tank higher to increase the circulation of water through the system. Other possible causes of high mortality mentioned in literature are failure of swim bladder inflation and complete yolk sac absorption(14-16 dph) and (Diaz et al., 2002; Kestemont et al., 2007), which was not observed in our case. At this stage when the yolk sac and oil globule are completely resolved, the nutrient reserves of the fish larvae are exhausted and so an external food source must be in place to supply the nutritional requirements of the fish larvae (Kestemont et al., 2007). However Osteszewska et al. (2005) was able to directly wean 5-day old pikeperch larvae onto artificial diets and obtained survival rates that ranged from 21.6% to 54.4%.

During the weaning experiment, pikeperch had a specific growth rate ranging from 0.41±3%.day^{-1} to 6.47±3%.day^{-1}. These values are very low as compared to findings of other authors. Ostaszewska and Boruta (2006) obtained SGR of 10.87%day^{-1} - 11.29%day^{-1} when pikeperch larvae of 5mg were fed with dry diets of varying fatty acid composition while Hamza et al. (2008) obtained SGR of 14.1%day^{-1} - 15.8%day^{-1} for larvae that are 34dph. Zakes and Demska-Zakes (2006) obtained SGR of 15.8%.day^{-1} to 16.9%.day^{-1} when pikeperch larvae of 0.7mg were fed with a mixed diet of formulated feed and *Artemia*. The specific growth rate in our experiment can be explained by the fact that our fish were already 26dph, weighing 39-

55mg when weaning started. It is known that the older the fish, the lower the specific growth rate (Nevjan, 2010).

During the weaning experiment the pikeperch larvae were divided into five treatment groups and fed commercial and formulated dry diets for a period of 13 days.The survival rate achieved in the weaning experiment ranged from 27.78±6.0% to 44.44±6.0% with the best result for treatment EPA-A and the worst for DHA-O, although not significantly different. Other studies on pikeperch have similar survival rates. Hamza et al. (2008) obtained survival rates of 33±3.1% to 36±2.5% when pikeperch larvae were feed different dietary phospholipid levels for 24 days from 10 to 34 dph. Ostaszewska and Boruta (2006) had survival rates ranging from 50.8% to 54.4% when they reared pikeperch larvae of 0.5mg from day 5 till day 47 post-hatching. Kestemont et al. (2007) obtained survival rates ranging from 33.7% to 77.4% when 19 dph larvae were kept for 18 days on dry diets.

At the end of the weaning period the final body weight was found to be highly significantly different among the larvae that received DHA-A on the one hand and NON-V or DHA-O on the other hand ($p<0.007$). Larvae that received AgloNorse® perfomed best, followed by those receiving the vegetable diet. The O-Range® diet performed worst, not only in terms of WW, but also in terms of survival and sgr (although not significantly). This poor performance could be attributed to the poor acceptance of the feed by the fish larvae and also the lowest fat percentage (table 2). During the weaning experiment, fish that were given EPA enriched *Artemia* prior to weaning with AgloNorse®, grew at the same rate as those that were given DHA enriched *Artemia*. They could not however make up for the lagging behind during the life feed phase. During the dry feed phase, the larvae still showed a tendency to maintain a constant percentage of DHA that ranged from 20%-25%. (Table 9).

Pikeperch like most of the other fresh water fish species are able to synthesize both these n-3 and n-6 HUFA using Δ5 and Δ6 desaturases and elongases (Sergent et al.,1995). Both these enzymes are responsible for elongation and desaturation of α-linolenic (ALA,18:3n-3) and linoleic acid (LIN,18:2n-6) to EPA and DHA and ARA respectively (Sergent et al.,1995). This is

clearly observed in our experiment as the larvae that did not received HUFAs (NON-V and DHA-V treatments) were able to convert LIN (18:2n-6) to ARA, as indicated by the relatively high presence of ARA in the fish (Table 8) and the low levels of linoleic acid (2.1 mg.gDW^{-1}), in comparison to the AgloNorse® treatment (7.8 – 8.7 mg.gDW^{-1}), despite the high level of this fatty acid in the Vegetable diet (32 mg.gDW^{-1}). The DHA/ARA ratio is two to three times lower than in fish receiving O-Range® or AgloNorse®, while none was present in the Vegetable diet.

It is also important to note that larvae are also able to convert EPA to DHA using enzymes Δ6 desaturase enzyme (Sergent et al.,1995). The larvae that received dietatary HUFAs (DHA-A, EPA-A and DHA-O) throughout had the highest and very similar EPA and DHA levels, between 5.0 - 5.3 mg.gDW^{-1} and 17.9 - 22.3 mg.gDW^{-1} respectively. Intermediate values are recorded in fish that belonged to the treatment DHA-V and as expected, the larvae that did not receive any HUFA during the experiment (NON-V) had the lowest concentration of DHA (10.9 mg.gDW^{-1}) and EPA (2 mg.gDW^{-1}). There is no evidence that the pikeperch larvae were capable of elongating and desaturating linolenic acid, although an elongation and desaturation of EPA to DHA can be suspected. Since the fish of the Non-V treatment grew about 30% in weight during the dry feed phase, and the DHA content increased with 22% to 10.9 mg.gDW^{-1}, instead of dropping with 30% from 8.9 to 6.2 mg.gDW^{-1}, an internal resource had to be available. EPA could easily have been that resource since the level dropped with 46% from 3.7 to 2 mg.gDW^{-1}.

Since the two desaturation enymes are involved in the conversion of EPA to DHA and LIN to ARA respectively, this results in some sort of competition for the enymes between these two families of fatty acids hence the importance of both the absolute concentrations and dietary proportions between n-3 and n-6 PUFA (Sergent et al.,1997). In other words the fish try to maintain absolute concentrations of both the n-3 and n-6 fatty acids in order to limit the competion for the desaturase and elongase enzymes. If one fatty acid concentration greatly exceeds the other, then suppresion of the desaturation process of the less abundant FA will occur (Ahlgren et al.,2009).

The ratio DHA/EPA in fish larvae increases from 2.1-2.4 mg.gDW^{-1} at day 26 to 3.6-5.7 mg.gDW^{-1} at day 39 (Table7 & 9 respectively). This could imply that DHA was prefentially retained in the larval lipids at the expense of other fatty acids. This is in aggreement with a previous study on Eurasian perch (Kestemont et al., 2002). This shows that in pikeperch the competition between fatty acid families of 18:3(n-3) and 18:2(n-6) for Δ6 and Δ5 desaturase enzymes tends to favour 18:3(n-3) family (Sergent et al.,1995; Kestemont et.,2002).

Cortisol is often used in determing a fish's response to stress and this is mainly through its effects on metabolism and osmoregulation processes (Ganga et al., 2006). Therefore the increase in cortisol levels is regarded as a reliable method in differentiating stressed and non-stressed fish (Ganga et al., 2006). The fatty acids DHA, EPA and ARA all promote stress resistance and DHA does this even to a higher extent than EPA and ARA (Furuita et al.,1999). The results obtained in this study seem to confirm this since no mortality was seen in fish subjected to a salinity test reflecting the high DHA and EPA content in the fish larvae of all treatments although NON-V had only half the values of those found in fish larvae under DHA-A treatment. The cortisol results of both day 26 and day 39, seem to indicate an increased level of cortisol in their tissue when the fish are put in the salt water except for treatments DHA enriched *Artemia* and DHA-A respectively where the reverse was observed. For both larval sets (26 and 39 dph), these treatments led to the best growth performances and in our case this seems to be indicative of the physiological conditions that influence responses to stress in contrast to the findings of Lund and Steenfeldt (2009). Furthermore, DHA-O larvae at 39dph had the highest level of cortisol expressed when compared with the other treatments, while they performed worst. Another explanation could be that O-Range® diet had the highest amount of ARA (1.5mg.gDW^{-1}) and the second highest amount of EPA (8.4mg.gDW^{-1}). Both these HUFAs, ARA and EPA, are seen to promote adrenocorticotropic hormone (ACTH)-induced cortisol production in sea bream interrenal cells and guppies (Gang et al., 2006).

For larvae 39 dph, the NON-V and DHA-V fed larvae expressed less cortisol in culture water when compared to the larvae that received HUFA rich diets (AgloNorse® and O-Range®) and also after saltwater exposure with exception of the DHA-A treatment. This was also described

by Lund and Steenfeldt (2009), when they fed pikeperch larvae with pure olive oil enriched *Artemia*. There was less production of cortisol in absence of HUFA. This may indicate that HUFA may play a role in the ability of the fish larvae to synthesise cortisol (Lund and Steenfeldt, 2009).

In conclusion, our results show that aglonorse was the best performing feed for the pikeperch larvae. There is no need to provide HUFAs after the live feed phase as the fish is able to elongate and desaturate PUFAs to make own HUFAs. Growth and survival of pikeperch can be promoted by both DHA and or EPA supplementation. Pikeperch larvae are able to elongate and desaturate PUFAs throught the whole larval period.

References

Abi-Ayad, Boutiba Z. S.-M.E.-A, Melard. C. and Kestemont P. (2004). Dynamics of total body fatty acids during early ontogeny of pikeperch (*Sander lucioperca*) larvae. Fish Physiology and Biochemistry 30, 129-136

Ahlgren G., Vrede T. and Goedkoop W. Chapter 7; Fatty acid ratios in freshwater fish, zooplankton and zoobenthos- are there specific optima? Lipids in Aquatic Ecosystems. (2009)

BellV.M and TocherD.R. (1989). Molecular species composition of the major phospholipids in brain and retina from rainbow trout (*Salmo gairdneri*). Biochemistry. Journal. 264, 909-915

Buzzi M, Henderson. R.J and Sergent .J.R. (1995). The desaturation and elongation of linolenic acid and eicosapentaenoic acid by hepatocytes and liver microsomes from rainbow trout (*Oncorhynchus mykiss*) fed diets containing fish oil or olive oil. Biochimica et Biophysica Acta 1299, 235-244

Demska-Zakes. K and Zakes. Z. (1997). Effect of 17 α-methyl testosterone on gonadal differentiation in pikeperch, *Stizostedion lucioperca* L. Aquaculture Research 28, 59-63

Diaz J.P., Mani-Ponset L., Blasco C. and Robert Connes. (2002). Cytological detection of the main phases of lipid metabolism during early post-embryonic development in three teleost species: *Dicentrarchus labrax, Sparus aurata* and *Stizostedion lucioperca*. Aquatic Living Resources 15, 169-178

Feist Grant and Schreck Carl B. (2002). Ontogeny of the stress response in Chinook Salmon, *Oncorhynchus tshawytascha*. Fish Physiology and Biochemistry 25, 31-40

Frankiewicz P., Zalewski M., Schiemer F. and Darbrowski K. (1997).Vertical distribution of planktivorous 0+pikeperch,*Stizostedion lucioperca* (L),in relation to particulate or filter feeding. Fisheries Management and Ecology 4, 93-101

FOA FISHERY STATISTICS (2012): www.fao.org/fishery/culturedspecies/*Sander lucioperca*/en

Fontagne S., Burtaire L., Corraze G. and Bergot P. (2000). Effects of dietary medium-chain triacylglycerols (tricaprylin and tricaproin) and phospholipid supply on survival, growth and lipid metabolism in common carp (*Cyprinus carpoi L.*) larvae. Aquaculture 190, 289-303

Furuita H., Kooichi K. and Takeuchi T. (1999). Effect of different levels of eicosapentaenoic acid and docosahexaenoic acid in *Artemia* nauplii on growth, survival and salinity tolerance of larvae of the Japanese flounder, Paralichthys olivaceus. Aquaculture 170, 59-69

Ganga R., Tort L., Acerete L., Montero D. and Izquierdo M.S. (2006). Modulation of ACTH-induced cortisol release by polyunsaturated fatty acids in interrenal cells from gilthead seabream, *Sparus aurata*. Journal of Endocrinology 190, 39-45

Gardeur J.N., Mathis N., Kobilinsky A. and Brun-Bellut J. (2007). Simultaneous effects of nutritional and environmental factors on growth and flesh quality of *Perca fluviatilis* using a fractional factorial design study. Aquaculture 273, 50-63

Gilbert Van Stappen .(2011). MSc.Aquaculture notes: Live feed and larvi culture

Gisbert E., Villeneuve L., Zambonino Infante J.L., Quazuguel P. and Cahu C.L.(2005). Dietary phospholipids are more efficient than neutral lipids for long chain polyunsaturated fatty acid supply in European sea bass *Dicentrarchus labrax* development. Lipids 40,1-10

Hamza N., Mhetli M., Khemis I.B, Cahu C. and Kestemont P. (2008). Effect of dietary phospholipid levels on performance, enzyme activities and fatty acid composition of pikeperch (*Sander lucioperca*) larvae. Aquaculture 275, 274-282

Hamza N. , Xueliang X., Maboudou J., Imorou Toko I. and Kestemont P . (2007). Effect of weaning age and diet on pikeperch larviculture. Aquaculture 264, 197-204

Hiroi J., Sakakura Y., Tagawa M., Seikai T. and Tanaka M.(1997). Developmental changes in low – salinity tolerance and responses of prolactin, cortisol and thyroid hormones to low- salinity environment in larvae and juveniles of Japanese founder, *Paralichthys olicaceus*. Zoological Sciences 14, 987-992

Hung L.T. , Tuan N.A. , Cacot P.P. and Lazard J.(2001).Larval rearing of Asian catfish

Izquierdo M.S., Fernandez-Palacios H. and Tacon A.G.J. (2001). Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197, 25-42

Jankowska B., Zakes Z., Zmijewski T. and Szczepkowski M. (2003). A comparison of selected quality features of the tissue and slaughter yield of wild and cultivated pikeperch *Sander lucioperca* L.). European Food Research Technology217,401-405

Kestemont P. , Xueliang X., Gersande B., Melard C., Gielen M., Brun-Bellut J. and Fontaine P.(2003). Feeding and nutrition in European percid fishes- a review

Kestemont P., Imorou Toko I., Maboudou J. and Xueliang X. (2003). Larval study on pikeperch *Stizostedion lucioperca*: effects of weaning age and diets (live and formulated) on survival, growth, and cannibalism, deformity and stress resistance

Kestemont P., Jourdan S., Houbart M., Melard C., Paspatis M., Fontaine P., Cuvier A., Kentouri M. and Baras E.(2003). Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: biotic and abiotic influences. Aquaculture 227, 333-356.

Koed A., Balleby K. and Mejlhede P. (2002). Migratory behaviour of adult pikeperch (*Sander lucioperca*) in a lowland river. Hydrobiologia 483, 175-184

Lappalainen J., Dorner H. and Wysujack K.(2003). Reproduction biology of pike perch (*Sander lucioperca*(L.)) – a review. Ecology of Freshwater Fish 12, 95-106

Lavens P. and Sorgeloos P.(1996). Manual on the Production and Use of Live Food or Aquaculture. Food and Agriculture Organization,Rome,Italy,ISBN-10:9251039348,295

Lepage G. and Roy C.C. (1984). Improved recovery of fatty acid through direct transesterification without prior extraction or purification. Journal of Lipid Research 25, 1391-1396

Ljinggren L., Staffan F., Falk S., Linden B. and Mendes J. (2003). Weaning of juvenile pikeperch, *Stizostedion lucioperca L.*, and perch, *Perca fluviatilis L.*, to formulated feed. Aquaculture Research 34, 281-287

Lund I. and Steenfeldt S.J. (2011). The effects of dietary long-chain essential fatty acids on growth and stress tolerance in pikeperch larvae (*Sander lucioperca L.)* Aquaculture Nutrition 17, 191-199

Molnar T., Szabo A., Szabo G., Szabo C. and Hancz C.(2006). Effect of different dietary fat content and fat type on the growth and body composition of intensively reared pikeperch *Sander lucioperca* (L.) Aquaculture Nutrition 12, 173-182

MolnarT., Hancz C., Bodis M., Muller T., Bercsenyi M. and Horn P. (2004). The effect of initial stocking density on growth and survival of pike-perch fingerlings reared under intensive conditions. Aquaculture International 12, 181-189

Montero D., Kalinowski T.,Obach A., Robaina L., Tort L., Caballero M.J. and Izquierdo M.S. (2003). Vegetable lipid sources for gilthead seabream (*Sparus aurata*): effects on fish health. Aquaculture 225, 353-370

Nevejan N. (2010). MSc.Aquaculture notes: Fish culture techniques

Navarro C.J., Bell V. M., Amat F. and Sargent .R.J. (1992). The fatty acid composition of phospholipids from brine shrimp, *Artemia* spp., eyes. Comparative Biochemistry and Physiology Vol.103B, No.1, pp.89-91

Nyina-Wamwiza L., Xueliang L. X., Gersande B. and Kestemont P.(2005). Effect of dietary protein, lipid and carbohydrate ratio on growth, feed efficiency and body composition of pikeperch *Sander lucioperca* fingerlings. Aquaculture Research 36, 486-492

Ostaszewska T. and Boruta A. (2006). The effect of diet on the fatty acid composition and liver histology of pikeperch (*Sander lucioperca* (L.)) larvae. Archives of Polish Fisheries Vol.14, Fasc.1, 53-66

Ostaszewska T., Dabrowski K., Czuminska K., Wanda Olech and Olejniczak M. (2005). Rearing of pike perch larvae using formulated diets- first success with starter feeds. Aquaculture Research 36, 1167-1176

Peterka J., Matena J. and Lipka J. (2003). The diet and growth of larval and juvenile pikeperch (*Stizostedion lucioperca* (L.)): A comparative study of fishponds and a reservoir. Aquaculture International 11, 337-348

Rodriguez C., Cejas J.R., Martin M.V., Badia P., Samper M. and Lorenzo A. (1997). Influence of n-3 highly unsaturated fatty acid deficiency on the lipid composition of broodstock gilthead seabream (*Sparus aurata* L.) and on egg quality. Fish Physiology and Biochemistry 18, 177-187

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

Sargent J., Bell G., Lesley M.E., Tocher D., and Estevez A. (1999). Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191-199

Schulz C., Huber M., Ogunji J. and Rennert B. (2008). Effects of varying dietary protein to lipid ratios on growth performance and body composition of juvenile pike perch (*sander lucioperca*). Aquaculture Nutrition,14,166-173

Schulz C., Gunther S., Wirth M. and Rennert B. (2006). Growth performance and body composition of pike perch (*Sander lucioperca*) fed varying formulated and natural diets. Aquaculture International 14,577-586

Schulz C., Knaus U., Wirth M. and Rennert B. (2005). Effects of varying dietary fatty acid profile on growth performance, fatty acid ,body and tissue composition of juvenile pike perch (*Sander lucioperca*). Aquaculture Nutrition 11, 403-413

Schulz C., Bohm M., Wirth M. and Rennert B. (2007). Effect of dietary protein on growth, feed conversion, body composition and survival of pike perch fingerlings (*Sander lucioperca*). Aquaculture Nutrition 13, 373-380

Smith G.G., Thompson P.A., Ritar A.J. and Dunstan G.A. (2003). Effects of starvation and feeding on the fatty acid profiles of stage 1 phyllosoma of the spiny lobster, *Jesus edwardsii*. Aquaculture Research 34, 419-426

Van Anholt R.D., Koven W.M., Lutzky S. and Wendelaar Bonga S.E. (2004). Dietary supplementation with Arachidonic acid alters stress response of gilthead seabream (*Sparus aurata*) larvae. Aquaculture 238, 369-383

Wang N., Xueliang X. and Kestemont P. (2009). Effect of temperature and feeding frequency on growth performances, feed efficiency and body composition of pikeperch juveniles (*Sander lucioperca*)

Watanabe T., Tamiya T., Oka A., Hirata M., Kitajima C. and Fujita S.(1983). Improvements of dietary value of live foods for fish larvae by feeding them on (ω3) highly unsaturated fatty acids and fat- soluble vitamins. Bulletin Japan Social Science Fish 49, 471-479

Xueliang X. and Kestemont P. (2002). Lipid Metabolism and FA Composition in Tissues of Eurasian Perch *Perca fluviatilis* as Influenced by Dietary Fats. Lipids 37, 297-304

Zakes Z., Kowalska A., Jankowska B. and Siwicki A. (2011). Substituting vegetable oil for fish oil in pikeperch diets: the impact on growth, internal organ histology, blood biochemical parameters, and proximate composition. Aquaculture Nutrition 17, e148-e163

Zakes Z., Kowalska A., Jankowska B. and Demska-Zakes. (2011).Effect of different dietary lipid levels on growth performance, slaughter yield, chemical composition and histology of liver and intestine of pikeperch *Sander lucioperca*. Czech Journal of Animal Science 56, (3),136-149

Zakes. Z. and Szkudlarek M. (2007). Effect of stocking density on survival and growth performance of pikeperch, Sander lucioperca (L.), larvae under controlled conditions. Aquaculture International 15,67-81