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Effect of maturation diets on the reproductive quality of pikeperch, *Sander lucioperca* (Linnaeus, 1758)

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DEDICATION

I dedicate this work to my beloved mother, Mrs Alodia Bibangamba, my senior brother, Mr Philbert Nyinondi, and my lovely daughter, Janelle. May Almighty God bless you all for your love, support and unending encouragements.

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My greatest gratitude goes to the Almighty God whose grace and mercy brought me this far.

LIST OF ABBREVIATIONS AND UNITS

€	Euro
ºC	Degree celcius
%	Percentage
Ml	Mililitre
µm	Micrometer
±	Approximately
/	Per
ANOVA	Analysis of variance
ARA	Arachidonic acid
C	Carbon
cm	Centimeter
Cpm	Counts per minute
CSI	Cumulative stress index
dph	Day post hatching
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
EFA	Essential fatty acid
FA	Fatty acid
FAO	Food & agriculture organization
FAME	Fatty acid methyl ester
g	Gram
GC	Gas chromatography
GSI	Gonadosomatic index
GV	Germinal vesicle
h	Hour
HIS	Hepatosomatic index
HUFA	Highly unsaturated fatty acid
IU/ml	International Unit per milliliter
IU/Kg b.w	International Unit per kilogram bodyweight
v/v	Volume per volume

kg	Kilogram
L	Liter
LCPUFA	Long chain polyunsaturated fatty acid
Mg	Miligram
mg/g DW	Miligram per gram dry weight
Min	Minute
Ml	Mililiter
Mm	Milimeter
mt/year	Metric ton/year
MUFA	Monounsaturated fatty acid
ng/ml	Nanogram/mililitre
pg/µl	Pikogram per microliter
SS	Standard solution
TAG	Triacylglycerol
TL	Total lipid

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ABSTRACT

Commercially dry diets allow greater control over the composition of biochemical components and reduce the risks of disease introduction, which are significant concerns when using the wet fish diets. However, satisfying the dietary lipid and FA requirements of pikeperch (Sander lucioperca) broodstock using artificial diets has proved difficult, particularly with respect to their highly unsaturated fatty acid HUFA composition. The aim of this study was to evaluate the effect of maturation diet on reproductive quality of pikeperch broodstock. Three diets were tested: sturgeon feed (aller aqua), seabass feed (nutreco) and goldfish (Wild 2014). The diets were given to fish for six months (aller aqua and nutreco) and 1 year (goldfish), group wild 2015 broodstock was used as control. Samples were taken from broodstock at pre-spawning (march) and at-spawning (April). The reproductive performance of pikeperch broodstock from the wild and hatchery broodstock was examined and larval quality on 1dph larvae (weight, length, total lipid, fatty acid and resistances to osmotic and starvation stress tests) were evaluated. Mean GSI, total lipid in gonad and oocyte diameter was significantly higher in wild 2015 and wild 2014 females. No significant difference was observed in HIS, total FAME and fecundity (absolute and relative) among experimental broodstock, while VSI was significantly lower in wild 2015 broodstock. Increase in GSI, fecundity, total lipid and total FAME within a group was observed at spawning. Oocyte maturation stages showed that wild broodstock were more mature than the other two groups fed dry diet. At spawning, the n-6/n-3 ratio of gonad from wild 2015 was about 2-fold higher than wild 2014 and 3-fold than aller aqua and nutreco fed broodstock. . The ratio of DHA/EPA/ARA in gonad of was recorded approximately 4/1/1 for wild 2015, 17/5/1 for wild 2014, 26/9/1 for nutreco fed and 28/10/1 for aller aqua fed broodstock. This indicates a noticeable deficiency of ARA in hatchery broodstock. Larval quality differed between larvae from wild 2015 and wild 2014 females. PUFA consisted mainly of n-6 series in eggs and larvae from wild 2015 females, while they consisted mainly of long chains of n-3 series in eggs and larvae from Wild 2014 females. CSI showed that larvae from gold fish fed (wild 2014) were more resistant to stress (osmotic and starvation) than larvae from wild 2015 females. Finally, this study has shown that a commercial pelleted diet for sturgeon and seabass are not suitable for pikeperch broodstock.

1. INTRODUCTION

1.1 Background information

Pikeperch, *Sander lucioperca* (L.) is one of the promising species in European inland aquaculture. It is a popular game fish with high-quality meat due to its low intramuscular bones (Nyina-Wamwiza *et al*., 2005). Recently, global captures are characterized by significant fluctuations with a decreasing trend whereas market demand of fish is growing (FAO 2013; Zakes, 2012). This has led the species to be considered as potential for intensive aquaculture in recirculation aquaculture systems (RAS) (Hermelink *et al*., 2013). Despite research efforts on pikeperch reproduction, production of stocking material (summer fry and fingerings) still relies mainly on wild breeders captured just before the spawning period (Ronyai, 2007; Zakes & Demska-Zakes, 2005). As a temperate fish species, pikeperch is an annual spawner with a synchronous oocyte development, reproduce only once a year at temperatures around 8–16 C, with the lowest range generally being 8–10 C. The process of intense yolk accumulation (vitellogenesis) is initiated normally late October and early November when both photoperiod and temperature decreases. During the subsequent 4–6 months the oocytes attain their full maturation ready to be deposited during spawning season between April and June (Lappalainen *et al*., 2003). Pikeperch as an annual spawner, an economical all year around production in RAS is hindered. Consequently, the induction of out-of-season spawning of adult pikeperch was subject of several aquaculture researches (Steffens et al., 1996; Demska-Zakes &Zakes, 2002; Zakes & Szczepkowski, 2004; Kaszubowski, 2005). Failure of spawning in captivity have been documented, hence demanding occasional hormone treatment using carp pituitary extract, human chorionic gonadotropin, and gonadotropin-releasing hormones or analogs to induce spawning (Demska-Zakes &Zakes, 2002; Kaszubowski, 2005; Ronyai, 2007).

Nutrition has been one of poorly understood aspect in pikeperch culture, and it has been demonstrated that this species can be fed commercial feed used in salmonids, seabass and turbot culture. However, the knowledge of nutritional requirement of this species refers primarily to proximate composition of the diet (Nyina-Wamwiza *et al*., 2005; Schulz *et al*., 2007; Wang et al., 2009). In fish, eggs and larval size, biochemical composition, and early growth ability performance are known to be related to reproductive performance and nutrition of breeders

(Izquierdo *et al*., 2001). Feed composition of broodstock has been reported to greatly affect egg quality mainly because it influences both the physiological processes during gametogenesis and the biochemical composition of eggs (Sargent, 1995). For example, arachidonic (ARA), eicosaenoic (EPA) and docosahexaenoic acids (DHA) participate to both regulation of prostaglandin synthesis in breeder and the development of brain and retina in larvae (Sargent, 1995). Therefore, further development of pikeperch production in captivity requires the supply of healthy and quality egg, larval and juveniles which can only be attained by better understanding of nutritional requirement of broodstock.

1.2 Problem statement

The reproductive quality of pikeperch breeders reared under hatchery conditions considerably differs from that of wild caught breeders. This could be due to the nutritional status of hatchery broodstock which consequently affects egg and larval quality. This reproduction gap between hatchery and wild breeders need to be better understood in order to develop a strategy that will ensure the production of high quality egg and larvae in culture systems

1.3 OBJECTIVES

1.3.1 Main objective

The aim of the present study was to investigate the effect of three (3) hatchery broodstock diet for pikeperch on reproductive output and larvae performance in comparison with wild breeders. The intent of the study is to attain better insight into the composition of an ideal pikeperch broodstock diet for hatchery breeders.

1.3.2 Specific objectives

a) To determine and compare the fecundity of broodstock, both absolute and relative fecundities
b) To compare and evaluate pikeperch broodstock gonadosomatic, heptosomatic and viscerosomatic indices as the reflection of fish maturity
c) To compare the nutritional status of pikeperch broodstock by evaluating total lipids and fatty acid composition of broodstock from liver, gonads and visceral fats.
d) To assess broodstock maturity by checking oocyte maturation stages and to compare different assessment methods.
e) To compare larval quality by measuring weight, length and analysing fat content.
f) To assess larval quality by submitting to stress test (salinity and starvation test)

2. LITERATURE REVIEW

2.1 Global aquaculture status

Aquaculture is the fastest growing sector in food production across the world, and it currently contributes about 42.2% in global fish food production (FAO, 2014). The growth in aquaculture production during the last four decades coincided with increasing effort of aquaculture research and development. Aquaculture research essential focuses on investigating the range of water parameters, nutrient requirements, feeds, use of therapeautants, production system and technology for aquatic species that are currently raised in captivity world-wide (Yossa & Verdegem, 2014). World aquaculture production attained an all-time high production of 90.4 million tonnes (live weight equivalent) in 2012 worth US$ 144.4 billion, including 66.6 million tonnes of food fish and 23.8 million tonnes of aquatic algae (FAO, 2014). The overall growth in aquaculture production remains relatively strong owing to the increasing of demand for food fish. World food fish aquaculture expanded at an average annual rate of 6.2% in the period of 2000-2012, which is more slowly than in the period of 1980-1990 (10.8%) and 1990-2000 (9.5%). However, the world food fish aquaculture production doubled from 32.4 million tonnes in 2000 to 66.6 million tonnes in 2012 (FAO, 2014)

Aquaculture development happens to be imbalanced and its production distribution is uneven, with Asia accounting about 88% of the world aquaculture production by volume and China remaining as the top producer with 61.7% share of the world total production. Worldwide, 15 top countries produced 92.7% of all farmed food fish in 2012 (Table 1).

Table 1: Farmed food fish production by top 15 producers and main groups of farmed species in the year 2012 (Source: FAO 2014)

Producer	Finfish		Crustaceans	Molluscs	Other species	National total	Share in world total
	Inland aquaculture	Mariculture					
	(Tonnes)			(Tonnes)			(Percentage)
China	23 341 134	1 028 399	3 592 588	12 343 169	803 016	41 108 306	61.7
India	3 812 420	84 164	299 926	12 905	...	4 209 415	6.3
Viet Nam	2 091 200	51 000	513 100	400 000	30 200	3 085 500	4.6
Indonesia	2 097 407	582 077	387 698	...	477	3 067 660	4.6
Bangladesh	1 525 672	63 220	137 174	...	...	1 726 066	2.6
Norway	85	1 319 033	...	2 001	...	1 321 119	2.0
Thailand	380 986	19 994	623 660	205 192	4 045	1 233 877	1.9
Chile	59 527	758 587	...	253 307	...	1 071 421	1.6
Egypt	1 016 629	...	1 109	...	...	1 017 738	1.5
Myanmar	822 589	1 868	58 981	...	1 731	885 169	1.3
Philippines	310 042	361 722	72 822	46 308	...	790 894	1.2
Brazil	611 343	...	74 415	20 699	1 005	707 461	1.1
Japan	33 957	250 472	1 596	345 914	1 108	633 047	1.0
Republic of Korea	14 099	76 307	2 838	373 488	17 672	484 404	0.7
United States of America	185 598	21 169	44 928	168 329	...	420 024	0.6
Top 15 subtotal	**36 302 688**	**4 618 012**	**5 810 835**	**14 171 312**	**859 254**	**61 762 101**	**92.7**
Rest of world	2 296 562	933 893	635 983	999 426	5 288	4 871 152	7.3
World	**38 599 250**	**5 551 905**	**6 446 818**	**15 170 738**	**864 542**	**66 633 253**	**100**

Note: the symbol "..." means the production volume is regarded as negligibly low

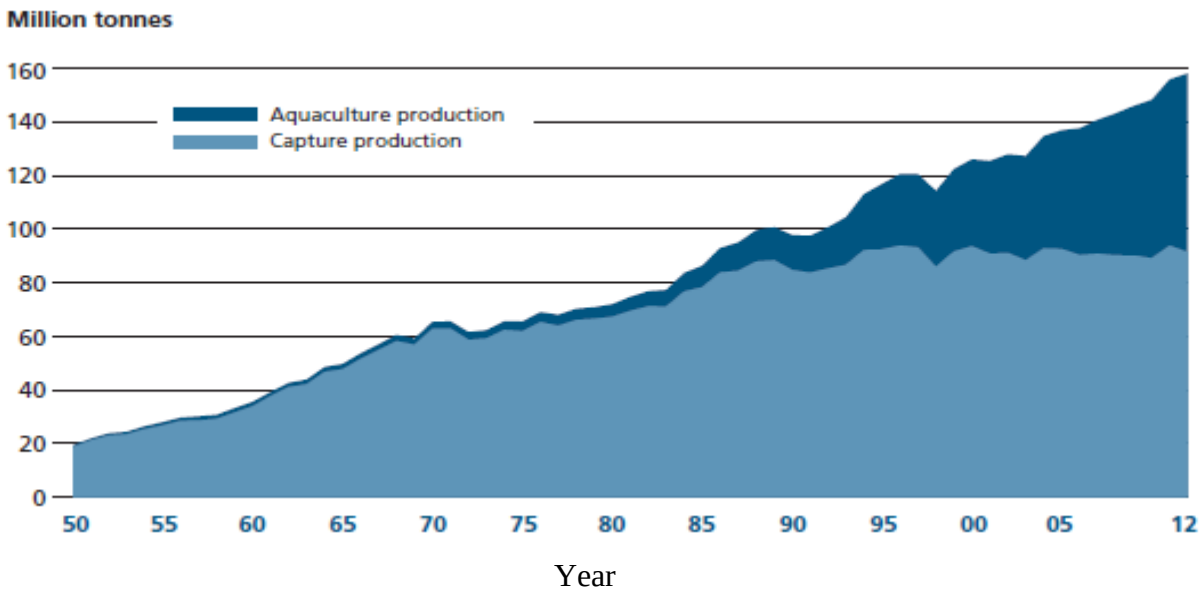

Figure 1: World capture fisheries and Aquaculture production (Source: FAO 2014)

2.2 Pikeperch general overview

Pikeperch, *Sander lucioperca* (Linnaeus, 1758) is a long slender fish with elongated body and pointed snout that grows to attain a body weights of 2-5kg and legths of 50-70cm, the maximum length of 130cm and body weights of 12-18kg has been reported (FAO, 2013; Zakes, 2012). Pikeperch has two dorsal fins, the first spiny and separated by a narrow interspace from the second. Anal fin has 2-3 spines rays and 11-13 soft rays, lateral line with 84-95 scales. Pelvic fins widely spaced, the distance between them almost as the base of one fin. It has one caudal fin and no spines in the gill cover (Akbarzadeh *et al.,* 2009). Wild Pikeperch has greenish-grey or brown colour on the back and sides becoming lighter on the lower sides and white on the belly (FAO, 2014)

Pikeperch is taxonomically classified in kingdom animalia, phylum chordata, class actinopterygii, order perciformes, family percidae, genus sander and species *S. Lucioperca* (Kottelat & Freyhof, 2007).

Figure 2: Adult pikeperch, *Sander lucioperca* Linnaeus, 1758 (source; Poland fish, 2011)

Synonyms of pikeperch, *Sander lucioperca*

Lucioperca linnei, (Malm, 1877)

Lucioperca sandra (Cuvier, 1828)

Stizostedion luciperca (Linnaeus, 1758)

2.2.1 Origin and geographical distribution

Pikeperch is semi-anadromous cold-water fish that commonly inhibits both freshwater and brackish waters in estuaries and costal zones of catchment areas of the Caspian, Aral, Baltic, Black and North seas (Lappalaine *et al*., 2003: Akbarzadeh *et al*., 2009). It is also found in large, turbid rivers and eutrophic lakes. It feeds mainly on gregarious and pelagic fishes (Lappalaine *et al*., 2003). Pikeperch is native to Eastern Europe (from Netherlands to Caspian Sea), but has been introduced to the Rhine catchment and to England. It is now widespread in France and Western Europe, and is rapidly extending its range in Eastern and Central England as well as waters of Northern Africa (Algeria, Morocco and Tunisia), North America and Asia (China) (Kottelat & Freyhof, 2007; FAO, 2014).

2.2.2 Life cycle and spawning

Pikeperch attain first sexual maturity at 3-10 years of age, usually at 4 years. Individuals migrate to brackish water for foraging but move to freshwater for habitats, the migrations up to 250 km have been recorded (Lappalaine *et al.*, 2003; FAO, 2012). Spawning season of pikeperch occurs in April to May with some exceptional which spawn from late February until July, depending on the temperatures of spawning grounds. In the spawning ground, male construct a nest by cleaning it from mud. The nest has the diameter of about 50 cm and depth of 5-10 cm. Sometimes the nest contains plant roots and other plant materials on which eggs are deposited. During spawning the female remains over the nest while the male circles rapidly around, at about 1 meter from the nest. Then the male takes a vertical orientation and both swim around swiftly while eggs and sperm are released. The female leaves the nest after all eggs are released while the male defends the nest and aerates the eggs with his pectorals. Females spawn once a year, laying all the eggs at one time. Feeding larvae are positively phototactic and feed on pelagic organisms after they leave the nest for open water (Lappalaine *et al*., 2003; FAO, 2012).

Before spawning pikeperch male become darker than female (Akbarzadeh *et al*., 2009) and female have a more enlarged abdomen that produce 170-230 eggs/ g body weight and the incubation time ranges from 3 days at 20C to 11 days at 10C. Reabsorption of yolk sac supplies and lipid droplet is finished at total body length of 5.8 to 6.5mm while the scales begin to lie

down at a total body length of 23 to 28mm primarily on the caudal peduncles (Pre- Casanova & Cano, 2014)

2.3 Aquaculture of pikeperch in Europe

Pikeperch is considered to have a high potential for inland aquaculture diversification in Europe (Wang *et al.*, 2009; Zarski *et al.*, 2012). The market value of pikeperch is high in comparison with various freshwater fishes, its farm gate price range from 8 to 11€ kg^{-1}. Decline of global wild pikeperch production from 48,800 tonnes in 1950 to 18,098 tonnes in 2013 (Figure 3) has strengthened the demand for pikeperch stocking materials (summer fry or fingerling) (FAO, 2014). This increasing demand over the last decade, has led to the creation of several new farms in Europe that produce pikeperch in Recirculating Aquaculture System (RAS) (Mylonas & Robles, 2014). Year-round production of pikeperch requires constant high temperatures (24-26°C) to ensure relatively high growth rates (i.e. production of 1-2 kg fish in 15 -18 months from non-selected strains) and this is only feasible in recirculating aquaculture system (RAS). These RAS also allow high densities of 80-100 kg /m^3 (Luczynski *et al.*, 2007; Dalsgaard *et al.*, 2013)

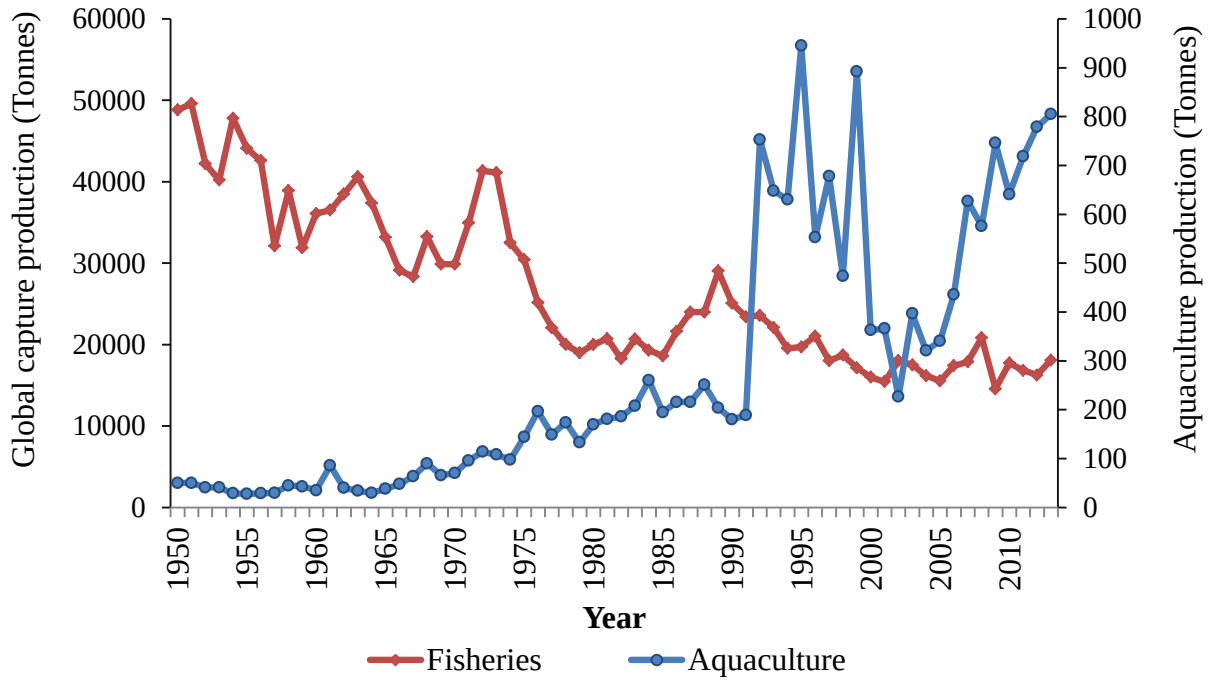

Figure 3: Global fisheries capture and aquaculture production of pikeperch, *Sander lucioperca* (source: FAO FishStat, 2014)

Although pikeperch aquaculture is growing, larvae production still relies on wild breeders whose spawning is induced by hormone treatment or manipulation of photoperiod and rearing temperature (Luczynski *et al.,* 2007; Zakes &Demska-Zakes, 2009; Wuertz *et al*., 2013). Recently, a substantial breakthrough was achieved by out-of-season reproduction (Wuertz *et al*., 2013). However, in comparison with other freshwater fishes, production of pikeperch under captivity is still modest. The major bottlenecks for further expansion of pikeperch culture today include their high sensitivity to stressors such as handling and husbandry practices that result in high and sudden mortalities, low larval survival (typical 5-10%), high incidence of deformities and lack of knowledge on the genetic variability of the used broodstocks (Mylonas & Robles, 2014)**.**

2.4 Reproduction of Pikeperch in captivity

2.4.1 Methods of Reproduction

The increasing demand for pikeperch fry and fingerling for culture and the highly variable effectiveness of methods used to date for reproduction of pikeperch, have prompted several groups of scientists to study and develop more effective methods for reproducing this species. Classification of methods can be done based on stocking density and culture system or based on the method used to handle and manage the broodstock. According to Luczynski *et al, (*2007) three methods of pikeperch reproduction are propagated.

2.4.1.1 Natural spawning

This is the oldest method carried out in ponds where one set of breeders (spawner set – 1 ♀ + 2 ♂) per 1-4 ha is released into earthen carp ponds. After spawning, the fish are left in the pond for 6-8 weeks until enough stocking material (summer fry or fingerling depending on initial stocking densities) has been obtained (Steffens *et al.,* 1996). Sometimes breeders use pond ground as the spawning substrate but more often artificial nests are applied. The size of the nest should correspond to that of the female since pikeperch are very fecund fish spawning more than 100,000 eggs per kg of female. After spawning the nests with eggs are transported to a hatchery or another pond (Lappalaine *et al*., 2003; Luczynski *et al.,* 2007; Zakes & Demska-Zakes, 2009).

2.4.1.2 Semi-artificial reproduction

This method requires the preparation of spawning nests, made of either natural or synthetic materials (Demska-Zakes & Zakes., 2002). As in natural reproduction, the size of spawning nest should correspond to that of the female breeder. The broodstock spawn in cages placed in ponds or lakes, or the fish are placed in plastic tanks equipped with spawning nests and with water temperature between 10-14°C. Nests are checked twice (morning and evening) and after spawning, breeders are removed from the cage or tank. The nests with eggs may be transported to the hatchery and placed in the incubation tank (recommended temperature 16-20°C; Steffens *et al.*, 1996), or the famer may leave them in the cage to hatch (Luczynski *et al.*, 2007). It is possible to administer spawning hormone to broodstock before transferring them to the tanks or cages.

2.4.1.3 Artificial reproduction

This is the most reliable method for obtaining high number of larvae (Luczynski *et al.*, 2007). After being transported to the hatchery, the broodstocks animals are sorted by sex and females are sorted by their degree of maturity (Ronyai, 2007). A catheter is used to sample eggs by insertion in the female genital opening and sucking eggs, which are then fixed in Serra's fixative to assess the position of the nucleus (Luczynski *et al.*, 2007; Zakes &Demska-Zakes, 2009; Zarski *et al*., 2012).

Off-season- reproduction is the newest method of artificial reproduction of pikeperch and is used at hatcheries equipped with RAS and cooling systems for reducing water temperatures. Fish are stimulated environmentally (temperature and photoperiod). Thermal stimulation lasts for 18 weeks: 8 weeks cooling phase (20-8 °C), 6 weeks chilling phase (8-4-8 °C) and 4 weeks warming phase (8-12 °C) (Zakes & Szczepkowski, 2004)

The knowledge on the physiological processes in fish has facilitated the use of substances that induce maturation and reproduction even out of season. Studies on percids reproduction (perch, pikeperch) showed that using carp pituitary extract (CPE), human chorionic gonadotropin (HCG) and luteinizing hormone-releasing hormone (LH-RH) or super-active analogs (LH-RHa, with or without dopamine antagonist) stimulate maturity (Kurcharczyk *et al.*, 1996; Demska-Zakes & Zakes., 2002; Kaszubowski, 2005; Ronyai, 2007; Sosinski, 2007; Zarskis *et al.*, 2012).

Female showed the spawning rate above 80% when they were intramuscular injected with HCG (400-600 IU Kg $^{-1}$ b.w) (Zakes &Demska-Zakes, 2009. Several authors recommended administering the total dose of HCG (400-700 IU Kg $^{-1}$ b.w) in three to five injections (Steffens *et al.*, 1996; Kaszubowski, 2005; Wang *et al*., 2009). However, Zakes &Demska-Zakes (2005, 2009), did not find effect on the number of injection or the size of HCG doses (400 0r 700 IU Kg $^{-1}$ b.w), administered in two portion) on the spawning rate of pikeperch, when female of different maturation stage where injected same dose, mature female needed few number of injection of the same dose to induce spawning than less mature female. These author including Ronyai (2007) recommended that the size of the dose and the number of injection should be determined based on the maturity of the female.

2.4.2 Gonadal development and Oocyte maturation stages

To understand mechanisms that govern reproduction in pikeperch, a basic understanding of the gonadal and hormonal changes during the productive cycle is required. Photoperiod and temperature are generally considered as the most important cues in gametogenesis and spawning in pikeperch (Migaud *et al*., 2002).

An egg is the final product of oocyte growth and differentiation. In teleost, numerous circulating endocrine and locally-acting paracrine and autocrine factors regulate the oocyte development and maturation stages. However, it is the pituitary gonadotropins (luteinizing hormone, LH, and follicle-stimulating hormone, FSH) and sex steroids, that control the development of the oocyte and its surrounding follicle layers and ensure coordination of the various processes that are involved in the production of fertilizable egg (Lubzens *et al.,* 2010).

Major stages during oocyte development include formation of primordial germ-cells (PGCs), transformation of PGCs into oogonia and then their transformation into primary oocyte with the onset of meiosis. This is followed by the massive growth of the oocyte during vitellogenesis, whereby the oocyte accumulates nutritional reserves needed for the development of the embryo**.** At this period the oocyte also accumulates RNA and completes the differentiation of its cellular and non-cellular envelopes. During this time the oocyte remains in meiotic arrest, at the end of prophase and in the diplotene stage (Lubzens *et al.,* 2010).

Maturation processes are characterised by reduced or stopping of endocytosis, resumption of meiosis, germinal vesicles breakdown (GVBD), the formation of a monolayer of cortical alveoli under oolemma, and yolk platelet dissolution and pelegophil oocyte that undergo hydration. The first mitotic division give rise to two cells differing in size, the small cell with first polar body degenerate and the large secondary oocyte is formed, and finally ovulation takes place at the end of the maturation process. The secondary oocyte is then extruded from its surrounding follicular cell layer and moves into the ovarian lumen. At this stage the female gamete is known as ovum (Patino & Sullivan, 2002; Lubzens *et al.*, 2010).

The usual division of oocyte into different stages is rather artificial as oocyte development is a dynamic process and it is difficult to identify the beginning or the end of the process. However, categorization of maturation stages in pre-ovulatory oocytes is crucial in determination of the effectiveness of pikeperch production under controlled conditions. There are several opinions about the classification of maturation stages. Zakes &Demska-Zakes (2009) classified oocyte maturation stages by considering only the position of germinal. However, this classification was reported insufficient to describe the changes that occur during the final oocyte maturation (FOM). Thus, Kucharczyk *et al*. (2007) in a description of the maturational stages in pikeperch included information on the importance of oil droplet morphology in pre-ovulatory oocytes as an important indicator. The new classification of oocyte maturation stages developed by Zarski *et al*. (2012), is based on two morphological indicators: germinal vesicle migration or its breakdown (GVBD) and different oil droplet coalescence rates. This categorization covers seven stages (from I to VII) – from the final stage of vitellogenesis to ovulation;

Stage I – the germinal vesicle (GV) is situated in the oocyte centre, and oil droplets are poorly visible

Stage II – the beginning of GV migration (GV in majority is located very close to the centre of oocyte) and the beginning of coalescence of oil droplets, which are very well visible

Stage III – migrating GV (reached half the oocyte diameter) and oil droplets are clearly visible

Stage IV – the GV is located above half the oocyte and a large oil droplet is clearly visible (the droplet diameter is greater than the GV diameter and it reaches the size of about 1/3 of the oocyte diameter) with visible smaller droplets

Stage V – the GV is located above half the oocyte, and one large (size of about half the oocyte diameter) oil droplet is clearly visible

Stage VI – oocytes sample taken for analysis shortly after correction are macroscopically transparent; no visible GV after they are placed in Serra's solution (following GVBD) and oocytes at the pre-ovulation stage

Stage VII – ovulation

Ovulation in species such as pikeperch or Eurasian perch, *Perca fluviatilis* (L.), has been obtained without hormonal stimulation. However, its application significantly affects ovulation rate and synchronization of ovulation in wild spawners, which have similar maturation stages. Asynchronous reproduction has a negative impact on production as it may lead to differentiation in the size of reared larvae and intensification of cannibalism. Hence, a precise system determining the oocyte maturity and the latency time between hormonal stimulation and ovulation is very important in aquaculture (Zarski *et al.,* 2012).

Histology offers a powerful tool in the study of reproductive health of fishes. Gonadal histology is used to analyse changes in gonad such as the thickness of the vitelline envelope at various stages, yolk appearances and sertoli cell proliferation, a clear indication of different maturation stages of fish oocyte could be attained by histology. Pikeperch show synchronous oocyte growth (Lappalaine *et al*., 2003; Wang *et al*., 2009). Determination of oocyte maturation stages in pikeperch has been done by several authors (Migaud *et al*., 2003; Zakes &Demska-Zakes., 2009; Zarski *et al.*, 2012), although these authors did not perform ovarian histology of pikeperch.

Staging of female gonads based on three morphological indicators, migration of germinal vesicle or its breakdown (GVBD), oil droplet coalescence rates and yolk globules (Selman *et al*., 1993: Lubzens *et al*., 2010). This categorization covers seven stages, from the final stage of vitellogenesis to ovulation.

Stage 1- Vitellogenic oocyte characterised by nucleus surrounded by opaque area, nucleus enlarges and nucleoli pushed from the nucleus. The nucleus is in the central position.

Stage 2 - Yolk globules form a homogenous mass with some lipid droplets inside the cytoplasm. Germinal Vesicle (GV) starts to migrate from its central position to the periphery (sometimes still in the center)

Stage 3 - migrating GV (reached half the oocyte diameter) and lipid droplets increase in size (coalescence)

Stage 4 - the GV is located above half the oocyte with one large oil droplet and several small (size of lipid droplet is bigger than GV of about half the oocyte diameter).

Stage 5 - GV completes its migration towards the periphery (animal pole) and a large lipid droplet (or sometimes several) GV is located at the vegetable pole

Stage 6 - GV breaks down (oocyte characterized by a large lipid droplet)

Stage 7 - Ovulation

Stage 8 - Oviposition, the emptied ovary contains primary oocytes

2.4.3 Importance of broodstock nutrition on fish reproduction

The quality of fish gametes can be highly variable due to a significant number of external factors or broodstock management practices in both wild and aquaculture conditions. Nutrition plays an important role in reproduction of most organisms including aquatic organisms such as fish. The availability of high quality eggs, fry and weaned juveniles has been identified as main bottlenecks of pikeperch culture (Kucharczyk *et al*., 2007; Kestemont *et al*., 2008). Hence, research focusing on the improvement of broodstock management and its effects on egg and larval quality is considered a priority from both the scientific and technical perspectives (Mylonas & Robles, 2014).

The nutritional status of broodstock has been reported to have effect on the quality of reproduction of farmed fish including the chemical composition of eggs, fertilization, hatching rates and larval survival rates (Bell *et al*., 1997; Izquierdo *et al*., 2001; Henrotte *et al*., 2008; Wang *et al*., 2009; Henrotte *et al*., 2010). Morphological and biochemical parameters such as lipids (Sargent *et al*., 2002), amino acids (Ronnestad & Fynh, 1993) or vitamins (Ronnestad *et al*., 1997) have been identified as major indicator of egg quality.

2.4.3.1 Importance of lipids in reproduction

Lipids are organic constituents of the fish body that play an important role as source of energy and essential fatty acids (EFA). The requirement of essential fatty acids (EFA) as membrane components is to maintain both structure and function of the cell membrane (Sargent *et al.,* 1995; Bell *et al*., 1997; Sargent *et al*., 2002). The major lipid storage sites of fish are liver, mesenteric fat and muscles. Diets high in lipid content lead to fat deposit in the body cavity and perivisceral organs (e.g. liver) in lean fishes while fatty fishes deposit in muscles. Therefore, the liver of lean fish is commonly fattier that fatty fishes. In spring- spawning fishes such as pikeperch, gonads mature during winter when food supplies are limited and feeding rate is reduced. As a result of that, the nutrients for gonad maturation have to be drawn from other organs such as muscles, liver and visceral fats. The lipid reserve in fish eggs has been identified to play major role in larval development, both as substrate for metabolism and as structural components in membrane biogenesis (Sargent, 1995; Henrotte *et al.*, 2010).

The n-3 highly unsaturated fatty acids (HUFAs), especially eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA) are essential in broodstock diets, having critical function as the main component of phospholipids of the cell membranes (Izquierdo *et al*., 2001)

The studies by Lavens *et al*. (1999) and Furuita *et al*. (2002) showed that deficiency of n-3 HUFAs negatively affect egg quality, possibly due to the fact that developing eggs and larval stages of the fish probably have greater requirements for n-3 HUFA, because of the preponderance of n-3 HUFA in their neural and visual tissue which predominates in the early stages of development. For that reason, any deficiency in these particular fatty acids can cause developmental abnormalities in the neural system and may affect their success as visual predators at the onset of first feeding (Bell *et al.,* 1995a, b). Furthermore, Bruce *et al*. (1999) and Sargent (1995) reported that n-6 HUFAs also play an important role in fish reproduction. They demonstrated that both EPA and ARA (arachidonic acid; 20:4 n-6) are involved in cell mediated functions and are precursors of eicosanoids which has an impact on the reproduction. Sargent *et al*, (2002) and Henrotte *et al*. (2010), also described that the eicosanoid production is influenced by the cellular ratio of EPA/ARA. ARA is the chief precursor of the eicosanoids, including the generation of series II prostaglandins (PGs). However, EPA competitively interferes with

eicosanoid production from ARA, because both series II and series III PGs are catalysed by the same cyclooxygenase and lipoxygenase.

The study on Eurasian perch, *Perca fluviatilis* by Abi-Ayad *et al.* (1997) pointed out the importance of DHA and EPA levels in the broodstock diet without taking into consideration the importance of HUFAs from the n-6 family. However, Henrotte *et al*. (2008) have shown that an n-3/n-6 ratio of 5/1 is associated with low reproductive performance in Eurasian perch, specifically regarding spawning rate, but also the fertilization rate, while enriched diet containing a ratio n-3/n-6 between 0.8 and 2.5 improved fertilization and spawning rate. To obtain better reproductive performance and egg quality, Kestemont *et al*. (2008) suggested manipulation of FA composition of broodstock diet especially ARA while an excess of dietary EPA should be avoided in perch. They demonstrated that artificial diet with adequate ratio of DHA:EPA:ARA (2:1:1) can provide similar spawning performance and larva quality as that diet composed of forage fish, while inappropriate ratios significantly impaired reproductive performances. They especially emphasize the importance of ARA in a broodstock diet for pikeperch and warn for an excess of dietary EPA.

2.4.3.2 Importance of protein in reproduction

Protein is another dietary nutrient which has been found to affect the reproductive performance of fish. It constitutes the most abundant nutrient in fish eggs and is a main energy source during embryonic development of fishes (Ronnestad *et al*., 1992). Furthermore, growth of the embryo mainly involves deposition of protein. A diet containing sufficient amount of protein with a balanced composition of amino acids is vital for the supplementation of protein and amino acids for the embryo. For example, Izquierdo *et al*. (2001) reported that low protein–high calorie diet caused a reduction in red seabream reproductive performance while a broodstock diet well balanced in essential amino acids (amount of a a), improved vitellogenesis. Moreover, reduction of dietary protein levels from 51% to 34% together with an increase in dietary carbohydrate levels from 10% to 32% was reported to reduce egg viability in seabass (Cerdá *et al*., 1994). The diets low in protein levels showed to cause alterations in GnRH release in seabass broodstock during spawning and plasma hormonal levels of the gonadotropin GtH II, which is known to play an important role in regulation of oocyte maturation and ovulation (Navas *et al.*, 1997).

2.4.4 Egg size and Fecundity

Pikeperch has relative small eggs compare to other percids of the same size such as walleye and perch. The diameter of pikeperch eggs range from 0.5 to 1.4mm with average of 0.9 mm, while that of walleye, (*Sander vitreus*) range between 1.4 to 2.1mm and that of Eurasian perch (*Perca fluviatilis*) range between 1.0 to 1.9mm (Becer & Ikiz, 1999). The egg size also seems to affect the potential survival of both egg and larvae. The largest and the best quality egg were found in 5-7 years old repeat spawning female (943-2525 g), while larvae from small eggs showed low viability after hatching (Schlumberger & Proteau, 1996).

Fecundity is influenced by the broodstock diet. Under good food conditions, the gonadosomatic index (GSI: weight of gonad x 100/ weight of eviscerated fish) of large females can reach 22% directly before spawning (Becer & Ikiz, 1999). Two fecundity estimates are generally calculated, absolute fecundity and relative fecundity. The absolute fecundity is the number of eggs in a female fish and relative fecundity is the number of eggs per g female. Both estimates are function of the size of pikeperch. Kisior &Wandzel (2001) found lowest number of eggs in the youngest, lightest and shortest females. The absolute fecundity was found to be closely related to the length of the fish, while no clear relationship was found between relative fecundity and length. Relative fecundity of pikeperch broodstock range between 48 and 467 eggs /g female with the average between 150 and 400 egg/g female (Kisior &Wandzel, 2001; Lappalainen *et al*., 2003).

2.5 Larval quality and stress test

Larval survival, growth and tolerance to unfavourable environmental conditions in the culture system depend very much on their quality (Koedijk *et al*., 2012). The persistent variation in the quality of the larvae and their unpredictable future has led the aquaculturists to assess the quality of the larvae before its introduction into the culture system by exposing larvae to high salinities or starvation test. High quality seeds have been reported to guarantee high growth and resistance to stress when exposed to unfriendly environment (Ashraf *et al*., 2010). Stress tests are mostly applied in early larval stages and they generally involve the exposure of larvae to adverse environmental conditions. A starvation stress test reflects energy reserve of the larvae egg yolk, and a salinity test is the commonly used method to determine the quality of larvae. The salinity of water used to test larvae quality depends on the larval stage and species to be tested.

Salinity stress tests have previously been developed to detect indirect differences in physiological condition of larval fish between treatment groups in nutritional studies when no differences exist in survival and growth. For example, in the nutritional study of striped Bass, (*Morone saxatilis)* and inland silver sides, (*Menidia beryllina*) larvae, salinity test used to assess the quality when no difference found in survival and growth (Ashraf *et al*., 2010). However, the development of similar methods for other species cultured have been useful not only for research purposes such as evaluation of diet effectiveness, but also as a simple tool for the farmer to assess the quality of the fry before stocking (Dhert *et al*, 1990).

3. MATERIALS AND METHODS

A: PIKEPERCH BROODSTOCK

3.1 Location of the study

This study took place at the facilities of the aquaculture center of the provincial research institute Inagro in Rumbeke-Beitem, Belgium. Analysis of the samples was done at the Laboratory of Aquaculture & Artemia Reference Center (ARC) of Ghent University and ovarian histology was carried out at the Faculty of Veterinary Medicine, Ghent University, Belgium.

3.2 Origin of broodstock animal

The hatchery broodstock was reared under controlled conditions in the recirculating aquaculture system (RAS) of Inagro since August 2013.They grew up feeding on pellets specifically formulated for turbot fish and were kept at a temperature of 24°C until early November 2014. Early November 2014, fish were separated into two groups and each group switched to a broodstock diet. The first group was fed commercial sturgeon broodstock feed (Aller Aqua) and the second group on commercial seabass broodstock feed (Nutreco). During winter fish did not eat a lot due to the low temperature of 6-7°C, hence the quantity of feed given, determined by their consumption amounted to approximately 500g for 30 fish per week. The ration was adjusted daily according to the quantity of uneaten feed removed from the tanks. The pellet feeds were distributed by automatic feeders. Inagro has no exact data on feeding quantities and frequencies. The hatchery broodstock had gone through specific temperature regime to induce maturation (Figure 4).

This wild pikeperch group counts two groups, a first group consist of wild broodstock that was caught last year in spring (March 2014) and used for reproduction at Inagro in that same year. Since then, they were kept in holding tanks .The fish were fed goldfish which in its turn was fed koi feed. This group received the same temperature regime as the farmed fish to induce maturation. The second group consists of newly caught wild pikeperch in April, 2015. This group did not receive any feed until spawning. Maturation depended on their food reserves. Manipulation of temperature was done on early May to induce spawning. The temperature increased from 11°C to 15°C by daily increase of 1-3°C.

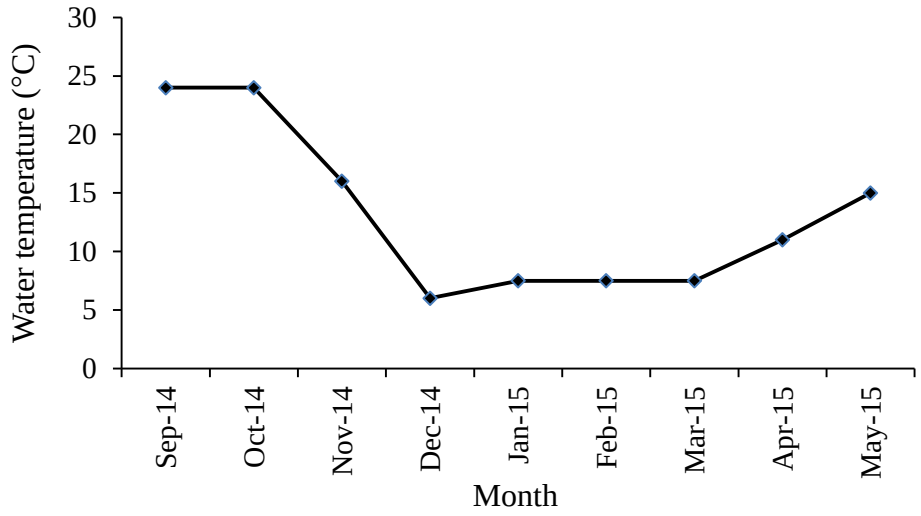

Figure 4: Water temperature regime to induce maturation in hatchery and wild broodstock

3.4 Hormonal treatment

Farmed broodstock feeding on commercial feeds did not spawn spontaneously by temperature manipulation hence a spawning agent was applied. Female broodstock were inter-muscularly injected with 400 IU/ kg of human chorionic gonadotropin (HCG) hormone (Figure 5, right). Female were first placed in a holding container with anaesthesia (MS-222) until they were full anaesthezed to avoid stressing them (Figure 5, left), then after injection female were put in fresh water for recovery.

Figure 5: Broodstock in anaesthesia bath (left) Female broodstock hormone injection (right)

3.5 Sample collection

This study had two sampling campaigns; the first sampling took place expected spawning and the second one week before spawning was expected. One week before taking samples, all broodstock were sorted by sex then tagged for easy identification, every tag had an unique code to identify the fish and its sex. Samples were collected from four different groups of pikeperch broodstock.

Group 1: Reared pikeperch fed with Sturgeon feed (Aller aqua pellets)

Group 2: Reared pikeperch fed with sea bass feed (Nutreco pellets)

Group 3: Wild pikeperch caught in 2014 fed goldfish

Group 4: Wild pikeperch caught in 2015 (unfed)

On 25th March group 1, 2 and 4 were sampled, where group 3 was only sampled once (one week before spawning) due to shortage of broodstock. The second sampling on 30th April, all four groups were sampled. A total of five female from each group were used at each sampling campaign.

The tagged females were isolated in a holding tank. Then each fish was captured using a towel to stop movement. Eggs were collected by inserting a catheter on the female genital opening and sucking out the eggs. The eggs were then placed in test tube containing clearing fluid (Serra's fixative solution: ethanol 96%, formalin, glacial acetic acid, 6:3:1v/v)

In the next step the female was killed by a blow on the head (Figure 6, right), then weighted and measured for standard and total length. Body organs were collected (gonads, liver and visceral fats) (Figure 7, left), weighed and stored into ice till they arrived at the ARC (Figure 7 right). At the ARC samples were stored at the temperature of -30°C until analysis.

Figure 6: Selection of female broodstock (left), killing fish by a blow on the head (right)

Figure 7: Collecting organs for analysis (left) collected samples into icebox (right)

3.6 Fecundity analysis

The fecundity analysis was done by counting egg on gravimetric basis. A small portion of egg was taken and preserved in 4% formalin. Three subsample of 0.1g each were taken and eggs were counted under microscope and the average number of eggs was determined. Calculations of both absolute and relative fecundity for each fish were done by using the formula of Bagenal & Barum, (1978) below;

Absolute fecundity = Average number of eggs x Gonad weight

Relative fecundity = (Average number of eggs x Gonad weight) / weight of fish

3.7 Gonadosomatic, viscerosomatic and hepatosomatic Indices

The percentage (%) gonadosomatic index (GSI), viscerosomatic index (VSI) and hepatosomatic Index (HIS) were calculated using the formula as described by Kristan *et al*, (2012) below;

GSI = (Gonad weight / Body weight) x 100

HIS = (Liver weight / Body weight) x 100

VSI = (Visceral weight / Body weight) x 100

3.8 Determination of total lipid

The method of total lipid extraction and purification by Folch *et al*., (1957) was followed. The collected sample was dried for 4h at the temperature of 103°C. A small portion of 200mg (dry weight) was taken in a 50ml telflon tube then 4ml methanol (MeOH) was added. The mixed sample was homogenised for 1 minute at a speed of 7rpm. Then 8ml of chloroform ($CHCl_3$) was added and homogenised for 1 minute at a speed of 8rpm. The mixer was rinsed with 2ml solvent mix ($CHCl_3$: MeOH-2:1) followed by centrifugation for 15 min at a speed 5rpm. Upper layer was removed into other glass tube, and then 6ml of solvent mix was added to the residue and shaken for 1 min followed by centrifugation for 15 min at speed 5rpm. Thereafter, the upper layer was poured in the glass tube with the previous upper layer obtained and 5ml potassium chloride (KCl 0.88%) was added to the total extract. After shaking well and centrifuging for 5 min at speed 3rpm, the upper layer was pipetted and thrown away and the bottom layer was kept and dried to which $NaSO_4$ was added. The solution was collected into a glass flask of 50ml (previously rinsed with acetone and dried for 30 min in vacuum desiccator with pump). Then it was rinsed with chloroform and filtered. The solvent was evaporated with the rotavapor and finally flushe to dryness with nitrogen. The glass tube was put into a vacuum desiccator for 30 min. The tube was weighed along with fat and fat weight was calculated by subtraction the weight of the tube and percentage fat was determined on dry weight basis. To enable the comparison of data independent from the weight of the fish total, percentage lipid content was standardized per kilo of female (TL/kg female).

3.9 Determination of Fatty acid Methyl Ester (FAME)

FAME analysis was done following the method of Lepage and Roy, (1984) for esterification of fatty acids. A small sample was weighed and placed into the bottom of a 35 ml glass tube with a Teflon lined screw cup, 100 µl of the on the internal standard solution containing 4.78255 mg/ml 20:2(n-6) acid dissolved in isooctane was added. 5ml of methanol/toluene mixture (3:2) was then added in the mixture followed by adding 5ml of freshly prepared acetylchloride/methanol mixture (1:20). The tube was flushed with nitrogen gas and closed tightly. After shaking carefully the glass tube was put in the boiling water bath (100°C) for 1 h with regularly shaking for every 10 min. After 1h the tubes were cooled down and then 5ml of distilled water and 5ml of hexane was added. Centrifugation for 5 min was done and the upper (hexane) layer was transferred into another glass tube. Hexane extraction was repeated two times more, each time using 5ml of hexane pre tube. The combined hexane phases were dried by vacuum filtering in a 50ml pearshaped flask over a 4 cm diameter filter filled for one third with anhydrous sodium sulphate powder. The tube was rinsed and filtered for several times with small quantities of hexane (± 5) until the flask is filled up. The solvent were evaporated on a rotary evaporator at 35°C followed by flushing to dryness with Nitrogen gas and the flask was weighed again. The dried Fatty acid Methyl Esters were finally dissolved in 0.5ml isooctane and transferred in a 2ml glass vial with Teflon lined screw cap. The vial was flushed with nitrogen and the sample was stored in freezer at -30°C until injection. For the actual Gas Chromatography (GC) analysis, 0.25µm of dilution was injected in iso-octane containing ± 2mg FAME's/ml. the individual FAME amount were calculated using the known amount of the internal standard as reference.

3.10 Determination of protein content of feed

The protein content in the broodstock feed was determined using the Kjeldahl methods. The sample to be analysed was weighed and 0.2g (dry weight) was brought into the digestion flask. 5 g of selenium mixture (catalyst) and 25 ml of sulphuric acid (H_2SO_4) was added into the digestion flask containing the sample. After warming up the flask containing the sample mixture for 10 min the mixture was set up to full heating allowing the sample digestion. The heat was lowed when the condensation started at the top of the tube. The sample was heated in low heat until the liquid was clear green or yellowish, then the heating was stopped and allow the sample to cool down. After the digestion has been completed the digestion flask was connected to a

receiving flask by a tube. 125 ml of water was added to the solution in the digestion flask, and then solution was made alkaline by addition of 32% sodium hydroxide (NaOH) to a total volume of 250 ml. The distillate solution was collected from the digestion flask through the tube into the receiving flask (Erlenmeyer flask) containing 150 ml boric acid (2%), indicator methyl red and bromo cresol green. The nitrogen content was then estimated by titration of the ammonium borate formed with standard hydrochloric acid (HCl), using methyl red as an indicator to determine the end-point of the reaction. The following equation can be used to determine the percentage protein of the sample.

%Protein = 6.25 x %Nitrogen.

3.11 Oocyte maturation stage

3.11.1 Microscopic analysis of oocyte

Two small samples of eggs were taken from each female and observed under Graphtec-digitizer, KD 3320 microscope to determine the stage of maturation. The first sample was taken using the catheter and the second sample was taken direct from the gonad. The new classification method of pre-ovulatory oocyte maturation stage suitable of pikeperch eggs (Zarski *et al.,* (2012). This classification is based on the migration of Germinal Vesicle (GV) from the centre of the oocytes to the periphery accompanied with the oil droplet. Egg maturation stages were divided into seven stages (six pre-ovulatory stages and Ovulation regarded as a seventh stage). The female was assigned a maturation stage depending on the most abundant observed stage.

3.11.2 Histological analysis of oocyte

A fragment from the ovary of each female was removed and fixed in Bouin solution for 48hrs and then was replaced in 70% ethanol until analysis. Small ovarian sample was put into the cassette, marked and then put into the processing machine. The ovaries were dehydrated through a series of graded ethanol (70%, 80%, 96% & 100%), and then ethanol was replaced by cleaning agent xylene (xylene I and II) and finally paraffin. This process was automated in a tissue processor STP 420D Microm. The process was followed by embedding samples in paraffin wax using an embedding centre EC 350-1 and EC 350-2 Microm. The paraffin blocks were made by filling the mold with small amount of paraffin then the ovaries were transferred from the cassette into the mold containing paraffin which then was placed on a cold plate where the paraffin

solidifies to form blocks after 30min (Figure 11). The ovaries in paraffin blocks were sectioned using HM 360 microtome with 5µm thickness and the gap of 100µm between sections. Sectioned samples were placed in 37°C water bath then was placed on glass slide and stained with Hematoxylin and Eosin (H&E) stain. A total of 9 sections were prepared for each ovary. The stained sections were examined by light microscopy, using a microscope (Olympus BX61) equipped with digital camera (Olympus, DP50) and image analysis software (Cell F, Olympus Staging of female gonads was performed according to Selman *et al*. (1993) and Lubzens *et al*. (2010). Determination of different oocyte stages was based on counting 50 oocytes cut through the nucleus. Measurement of oocyte diameter for eggs cut through nucleus was recorded.

Figure 8: Sample into mold filled with paraffin (left), samples on cold plates(middle) and samples in paraffin blocks (Right)

The two methods for used to determine oocyte maturation stages (microscopic and histological) were compared to see to what extend they are compatible or differ.

B: PIKEPERCH LARVAE

3.12 Larvae analysis

Assessing larval quality, 2dph (day post hatch) larvae were packed in oxygenated polyethylene bags at Inagro and then were transported to the ARC laboratory.

3.12.1 Larval length analysis

A total of 50 larvae from each group were taken for length analysis. Larvae were fixed into 10% formalin 12hrs (from the day they arrived at ARC laboratory until analysis). Length measurement was carried out by using Graphtec-digitizer, KD 3320 microscope together with Euromex cold light illuminator EKI. The total length of larvae was recorded by reading the line drown on the larvae picture taken.

3.12.2 Larvae weight analysis

Three subsample containing 200 larvae were taken from each group for weight analysis. Pre-weighted aluminium cups were used to obtain wet weight using Sortorious, ISO 9001 weighing balance. Then the samples were placed in the oven at 103°C for 4 h for dehydration. The larvae dry weight was obtained by subtracting the weight of empty cup from total dry weight.

3.12.3 Larvae stress test

Stress test with starvation and salinity was done for 10 days with 2 dph larvae to check the larvae quality from broodstock animals that were fed different feed. Larvae of only two broodstocks groups, Wild 2014 and Wild 2015 were tested, because the other groups did not spawn.

3.12.3.1 Starvation stress test

Starvation test was done with two types of water. The first type was rearing water (transport water of the larvae) and second type was synthetic freshwater (EPA 2002). Each type of water had three replicates each containing 20 larvae and 20ml of water. Dead larvae were removed from the experimental cups, counted and recorded in daily basis for 10 days. Percentage survival was calculated.

3.12.3.2 Osmotic stress test

In the osmotic stress test two control treatment (0g.l^{-1}) were compared (control 1- rearing water and control 2- synthetic freshwater) with five different treatment of salinity; 5g.l^{-1}, 10g l^{-1}, 15g.l^{-1}, 20g.l^{-1} and 25g.l^{-1}. The desired salinity was obtained by adjusting salinity of seawater (30.89g.l^{-1}) and synthetic freshwater (0g.l^{-1}), and then 2 ml of rearing water was added to all control and treatments. The total volume of water in each experimental cup was 22ml, a total of 10 larvae were placed in cups with different salinity and each treatment had three replicates. The water temperature range was 18±1°C.

Dead larvae were removed from the experimental cups, counted and recorded. Larvae were considered dead when they did not move the appendages completely. The average value of the replicated treatment obtained after individual addition of the cumulative mortalities in the consequent time interval (CSI) was calculated as it reflects the stress resistance of the larvae (Dhert et al, 1992). The higher the numeric value of the index, the more the larvae are stressed and less larvae quality, and vice versa.

3.13 Statistical Analysis

Statistical software IBM SPSS Statistics (Statistical Package for Social Sciences, acquired by International Business Machines Corporation), version 22 was used for data analysis. One way ANOVA was done for comparing means assuming equal variances. Homogeneity of variances was checked by Levene statistics. In case of violating homogeneity robust test of equality of means was checked by Welch and Brown-Forsythe test. The Duncan' POST-HOC TEST was used for multiple comparisons between means. In some cases, independent sample t- test at 95% confidence interval was undertaken to compare the means.

4. RESULTS

A: PIKEPERCH BROODSTOCK

4.1 Body weight, total and standard lengths

The mean total length, standard length and body weight of the wild 2015 broodstock was significantly higher than that of broodstock fed commercial feed (aller aqua and nutreco) before spawning period. At spawning, mean body weight, total length and standard length showed no significant difference between all four groups of experimental broodstock (Table 2).

When comparing the parameters before and at spawning within one group, it was found that the mean body weight of wild 2015 broodstock was significantly lower (1.75 ± 0.11) at spawning than before spawning (2.61 ± 0.22). No significant differences were observed in the mean body weight of other groups (aller aqua and nutreco fed broodstock). Mean total and standard lengths of all broodstock groups did not show significant difference at spawning compared to one month before.

Table 2: Mean body weight (kg,) total length (cm) and standard length (cm) between different group of pikeperch broodstock at spawning and before spawning period (n=5)

Broodstock group	Body weight (Kg)	Total length (cm)	Standard length (cm)
	Before spawning		
Aller aqua fed broodstock	1.75 ± 0.11^{Aa}	56.36 ± 0.93^{Aa}	50.54 ± 1.19^{Aa}
Nutreco Fed broodstock	2.00 ± 0.33^{Aa}	58.00 ± 3.03^{Aa}	52.10 ± 1.95^{Aa}
Wild 2015 broodstock	2.61 ± 0.22^{Ba}	61.60 ± 2.04^{Ba}	56.90 ± 2.04^{Aa}
	At spawning		
Aller aqua fed broodstock	1.91 ± 0.30^{Aa}	57.70 ± 2.54^{Aa}	52.90 ± 2.61^{Aa}
Nutreco Fed broodstock	2.00 ± 0.29^{Aa}	58.70 ± 3.46^{Aa}	53.90 ± 3.71^{Aa}
Wild 2015 broodstock	1.76 ± 0.78^{Ab}	55.60 ± 5.86^{Aa}	50.80 ± 6.22^{Aa}
Wild 2014 broodstock	1.61 ± 0.57^{A}	54.60 ± 5.86^{A}	50.20 ± 5.76^{A}

Note: Data are shown as mean ± SD. Value within column with different superscripts are significantly different ($P<0.05$). Capital letter superscripts compare between groups at same spawning period, small letter superscripts compare within a group at spawning and before spawning period.

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4.2 Gonad, liver and visceral fat weights analysis

Table 3 shows the mean weight of gonad, liver and visceral fat from four groups of experimental broodstock at spawning period and one month before. Before spawning period, mean weight values of gonad and liver of wild 2015 broodstock were significantly higher, while that of visceral fat was significantly lower than that of aller aqua and nutreco fed broodstock. No significant difference was found between aller aqua and nutreco fed broodstock.

At spawning, mean gonad weight of the wild 2015 broodstock was still significantly higher than that of other three broodstock groups. No significance difference was observed in mean liver weight for all four groups of broodstock. Nutreco diets leads to the highest visceral fat content, being significant different from the wild broodstock 2014 and 2015, while no significant difference was found with that of aller aqua fed broodstock. Wild 2015 broodstock had significantly lower mean visceral fat weight than all other treatments.

The increase in mean gonad weight for aller aqua and nutreco fed broodstock was observed at spawning period compared to one month before, while mean liver and visceral fat weight did not change significantly. For wild broodstock, both mean of liver and visceral fat weight were significantly lower at spawning compared to before spawning period, while no significant decrease in mean gonad weight was observed at spawning

Table 3: Mean gonad, liver and visceral fat weight (g) of four different female broodstock groups one month before spawning and at spawning period (n=5)

Broodstock group	Gonad weight (g)	Liver weight (g)	Visceral fat weight (g)
	Before spawning		
Aller Aqua fed broodstock	90.94 ± 21.98^{Aa}	25.20 ± 7.10^{Aa}	97.98 ± 21.64^{Aa}
Nutreco Fed broodstock	105.72 ± 51.13^{Aa}	29.18 ± 7.33^{Aa}	126.84 ± 40.84^{Aa}
Wild 2015 broodstock	267.60 ± 100.65^{Ba}	46.50 ± 11.31^{Ba}	37.88 ± 19.39^{Ba}
	At spawning		
Aller Aqua fed broodstock	154.02 ± 76.75^{Aa}	24.88 ± 15.86^{Aa}	102.28 ± 16.85^{ACa}
Nutreco Fed broodstock	154.00 ± 55.91^{Aa}	25.16 ± 3.83^{Aa}	135.46 ± 31.64^{Aa}
Wild 2015 broodstock	237.68 ± 164.52^{Ba}	23.38 ± 13.02^{Ab}	19.98 ± 20.44^{Bb}
Wild 2014 broodstock	172.44 ± 91.31^{A}	26.52 ± 9.09^{A}	89.34 ± 27.50^{C}

Note: Data are shown as mean ± SD. Value within column with different superscripts are significantly different ($P<0.05$). Capital letter superscripts compare between groups at same spawning period, small letter superscripts compare within a group at spawning and before period.

4.3 Broodstock gonadosomatic, hepatosomatic and viscerosomatic indices

Before spawning period, mean gonadosomatic index (GSI) of wild 2015 broodstock was significantly higher (10.37 ± 4.24) than aller aqua fed broodstock (5.23 ± 1.23) and nutreco fed broodstock (5.07 ± 1.85). No significant differences were observed in mean GSI between aller aqua and nutreco fed broodstock. In terms of mean hepatosomatic index (HIS), no significant difference was observed between all three experimental groups. Wild 2015 Broodstock was significantly lower in mean viscerosomatic index (VSI) (1.42 ± 0.60) compared to aller aqua fed broodstock (5.64 ± 1.35) and nutreco fed broodstock (6.29 ± 1.49) (Table 4).

At spawning, mean GSI was significantly higher for wild 2015 broodstock than that of aller aqua and nutreco fed broodstock, while no significant difference was observed with that of wild 2014 broodstock. No significant difference was found between wild 2014 broodstock mean GSI in comparison with aller aqua and nutreco fed broodstock. Mean HIS showed no significant difference between all four experimental groups. Mean VSI was found significantly lower in wild 2015 broodstock compare other groups of broodstock. No significant difference was observed between wild 2014 broodstock with that of aller aqua and nutreco fed broodstock.

Mean GSI values showed an increase at spawning compared to one month before, although the differences were not significant. Mean HIS and VSI of broodstock caught from the wild in 2015 was significantly lower at spawning compare to one month before spawning season. No significant difference were found in mean HIS and VIS of aller aqua and nutreco fed broodstock at spawning compared to one month before spawning.

Table 4: Mean GSI, HIS and VSI (%) of pikeperch broodstock one month before spawning and at spawning period (n=5)

Broodstock group	GSI (%)	HIS (%)	VSI (%)
	Before spawning		
Aller Aqua fed broodstock	5.23 ± 1.34^{Aa}	1.43 ± 0.36^{Aa}	5.64 ± 1.35^{Aa}
Nutreco Fed broodstock	5.07 ± 1.85^{Aa}	1.45 ± 0.20^{Aa}	6.29 ± 1.49^{Aa}
Wild 2015 broodstock	10.37 ± 4.24^{Ba}	1.77 ± 0.31^{Aa}	1.42 ± 0.60^{Ba}
	At spawning		
Aller Aqua fed broodstock	7.68 ± 3.23^{Aa}	1.25 ± 0.62^{Aa}	5.46 ± 1.14^{Aa}
Nutreco Fed broodstock	7.57 ± 2.02^{Aa}	1.26 ± 0.10^{Aa}	6.75 ± 1.27^{Aa}
Wild 2015 broodstock	12.50 ± 2.91^{Ba}	1.28 ± 0.18^{Ab}	0.94 ± 0.56^{Bb}
Wild 2014 broodstock	10.03 ± 4.06^{AB}	1.67 ± 0.24^{A}	5.76 ± 1.25^{A}

Note: Data are shown as mean ± SD. Value within column with different superscripts are significantly different ($P<0.05$). Capital letter superscripts compare between groups at same spawning period, small letter superscripts compare within a group at spawning and before period.

4.4 Relative and absolute fecundity

The differences in mean values of absolute and relative fecundity between different broodstock groups were not significant at both spawning and one month before spawning period. An increase in both fecundities (absolute and relative) was observed at spawning compared to one month before spawning, although the differences were not significant except for nutreco fed broodstock (Figure 12).

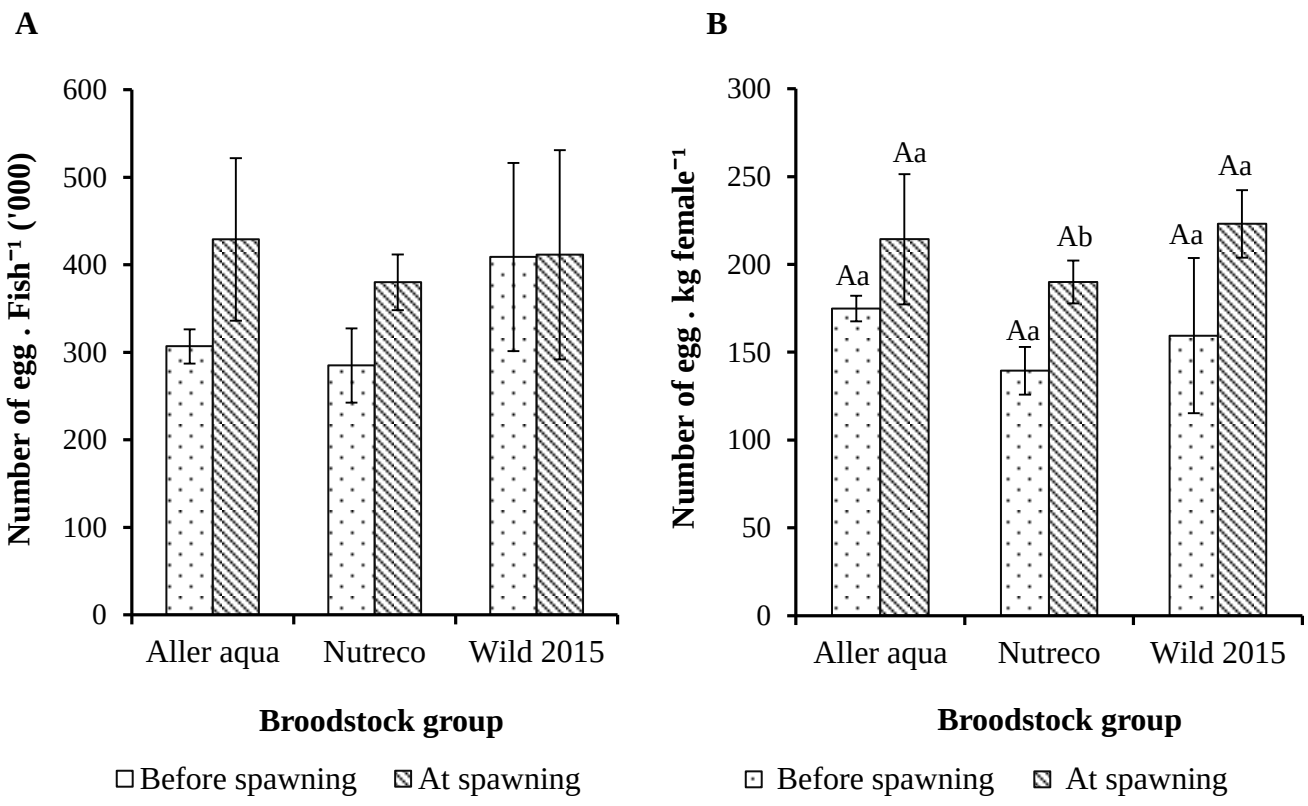


Figure 9: Absolute fecundity (**A**) and relative fecundity (**B**) of pikeperch broodstock at spawning and one month before spawning period. Different letters within a group denotes significant difference (P<0.05). Capital letter (A) compare between groups, while small letter (a) compare within group

4.5 Oocyte maturation stage

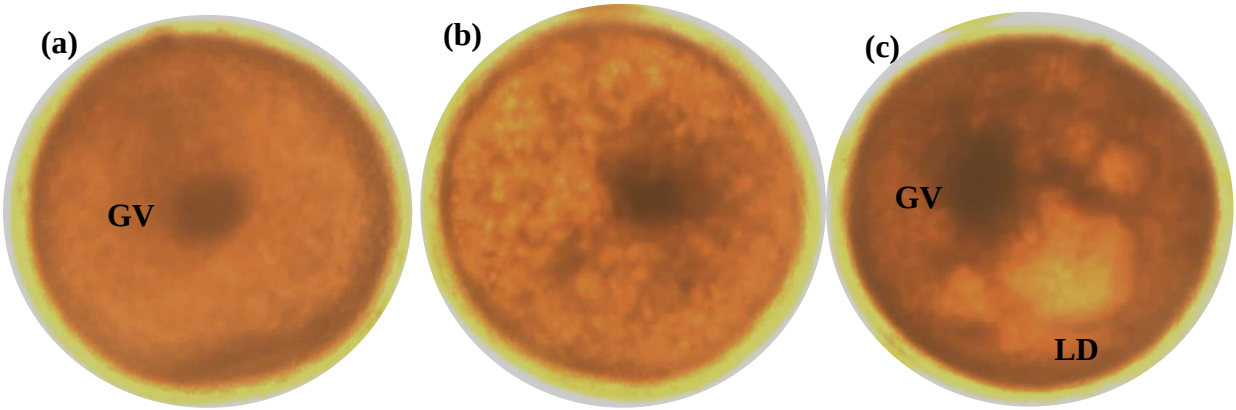
4.5.1 Microscopic analysis of oocyte maturation

Female broodstock feeding on same feed were found to have different oocyte maturation stages. The highest number of females feeding on commercial pellets (aller aqua and nutreco) was found to be in stage II before spawning period while wild 2015 females all were in stage III. At spawning, most females feeding on commercial feed were found to be in stage III of maturation while female caught from the wild in 2014 (fed goldfish) and 2015 mostly were stage III and IV. One female from wild 2014 broodstock group had the most matured oocyte stage V (Table 5). There was no difference on the oocyte maturation stage between the eggs taken direct from the gonads and the eggs taken by using catheter..

Table 5: Preovulatory oocyte maturation stages in pikeperch broodstock from four different groups checked at spawning period and one month before spawning period (n=10)

	Oocyte maturation stage				
Broodstock group	Fish 1	Fish 2	Fish 3	Fish 4	fish 5
Aller aqua fed broodstock before spawning	II	I	II	II	II
Aller aqua fed broodstock at spawning	III	III	III	II	III
Nutreco fed broodstock before spawning	II	II	II	II	II
Nutreco fed broodstock at spawning	III	II	III	III	III
Wild 2015 broodstock before spawning	III	III	III	III	III
Wild 2015 broodstock at spawning	IV	IV	III	II	IV
Wild 2014 broodstock at spawning	III	II	V	IV	III

Note: When different stages were found in a female, she was assigned the most abundant stage



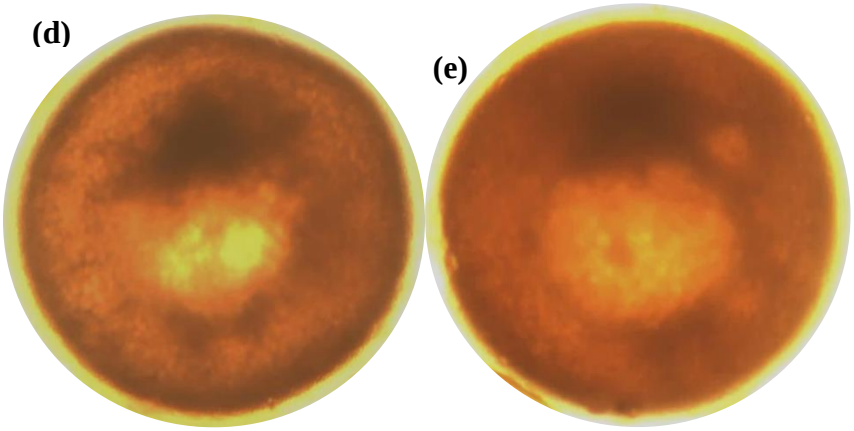


Figure 10: Preovulatory oocyte maturation stages in pikeperch after cytoplasm clarification with serra's solution:(a) stage I, (b) stage II, (c) stage III, (d) stage IV and (e) stage V. Oocyte were cathetered shortly after the fish were caught and other were taken direct from the gonads (GV: Geminal vesicle, LD: Lipid droplet).

4.5.2 Histological analysis of oocyte maturation

Maturation stages were classified based on morphological features; germinal vesicle migration and breakdown, coalescence of lipid droplet and yolk globules. Before spawning period, ovaries containing oocytes with the nucleus in the central position (stage 2) were mostly found in nutreco and aller aqua fed broodstock. Stage 1 and a lot of protoplasmic oocyte (primary oocyte) were observed in one of the aller aqua fed broodstock ovary. Most oocytes of the wild 2015 broodstock had a migrated germinal vesicle to the peripheral position with several large oil droplets (stage 3).

At spawning stage 4 was the most abundant stage in wild broodstock (wild 2014 and 2015), while stage 3 was abundant in hatchery broodstock. Stage 5 was observed in one of the wild 2014 broodstock. No atretic oocytes (vitelline envelope breakdown with yolk reabsorption after spawning) were observed among experimental broodstock groups. Different oocyte maturation stages are as shown in figure 14 below.

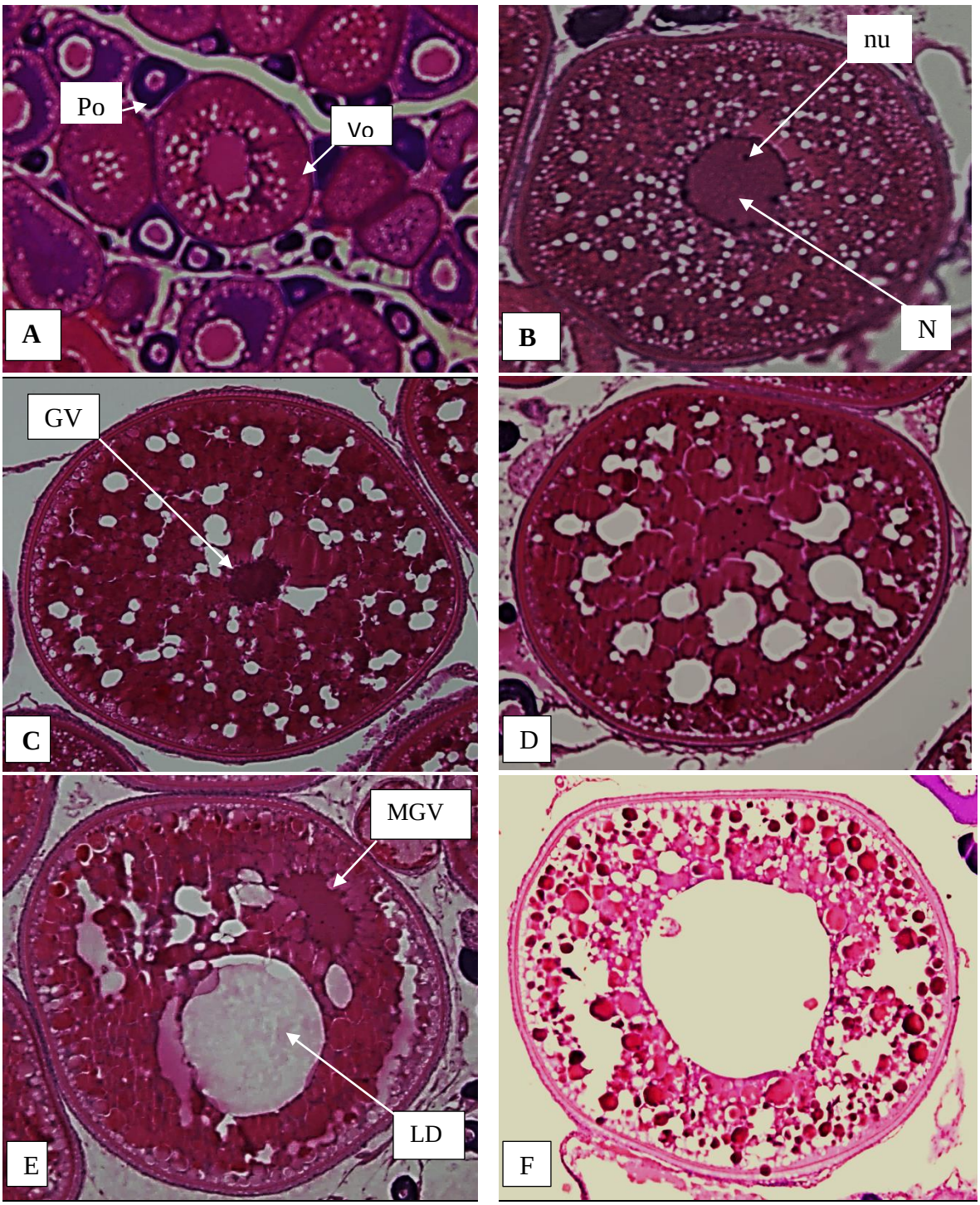


Figure 11: Ovarian histology of pikeperch preovuratory oocyte maturation stages.A: Perinucleolar stage of primary oocyte. B: Cortical alvelous stage (Stage 1). C: GV at the center and mass lipid droplet (stage 2). D: Migrated GV and few large lipid droplet (stage 3). E: GV at the periphery with lipid droplet in the centre (stage 4). F: Geminal vesicle breakdown (stage 5). **Po**- primary oocyte, **Vo**- vitellogenic oocyte**, N**- nucleus, nu- nucleoli, **GV,** geminal vesicle, **MGV**- migrated geminal vesicle and **LP**- lipid droplet. 10x

4.6 Oocyte diameter

Before spawning period, mean oocyte diameter of the wild 2015 females was significantly higher than broodstock fed on commercial feed (aller aqua and nutreco). At spawning, no significant difference was observed in the mean oocyte diameter between wild 2015 and wild 2014 Both broodstock caught from the wild had significantly higher mean oocyte diameter compared to that of broodstock fed commercial feed (aller aqua and nutreco) (Table 6).

The mean oocyte diameter values of aller aqua and nutreco fed broodstock were significantly higher at spawning compared to one month before spawning period. Broodstock caught from the wild in 2015showed no significant different in the mean oocyte diameter at spawning compared to month before (Figure 14).

The relationship between oocyte diameter and maturation stage showed that as the oocyte matures also its diameter increases. Based on a total of 2250 eggs measured a strong correlation (R =0.9564) between the oocyte diameter and the Oocyte maturation stage was found (Figure 15).

Table 6: Mean oocyte diameter (µm) of individual pikeperch broodstock, at spawning and one month before spawning period (n=50)

	Oocyte diameter (µm) before spawning					
Broodstock Group	**Fish 1**	**Fish 2**	**Fish 3**	**Fish 4**	**Fish 5**	**Mean**
Aller aqua fed broodstock	643 ± 50	453 ± 51	611 ± 34	639 ± 47	414 ± 40	592 ± 79[a]
Nutreco fed broodstock	598 ± 32	599 ± 32	605 ± 37	661 ± 24	681 ± 23	629 ± 39[a]
Wild broodstock 2015	843 ± 36	898± 30	878 ± 28	761 ± 30	741 ± 30	824 ± 70[b]
	Oocyte diameter (µm) at spawning					
Aller aqua fed broodstock	748 ± 30	750 ± 32	722 ± 30	718 ± 33	682 ± 36	724 ± 28[a]
Nutreco fed broodstock	731 ± 33	766 ± 28	687 ± 30	655 ± 32	745 ± 28	720 ± 45[a]
Wild broodstock 2015	867 ± 26	827 ± 20	838 ± 25	790 ± 31	901 ± 28	844 ± 42[b]
wild broodstock 2014	884 ± 43	656 ± 38	949 ± 31	930 ± 21	911 ± 35	866 ± 120[b]

Note: Data are shown as mean ± SD. Value within column with different superscripts are significantly different ($P<0.05$, n=50)

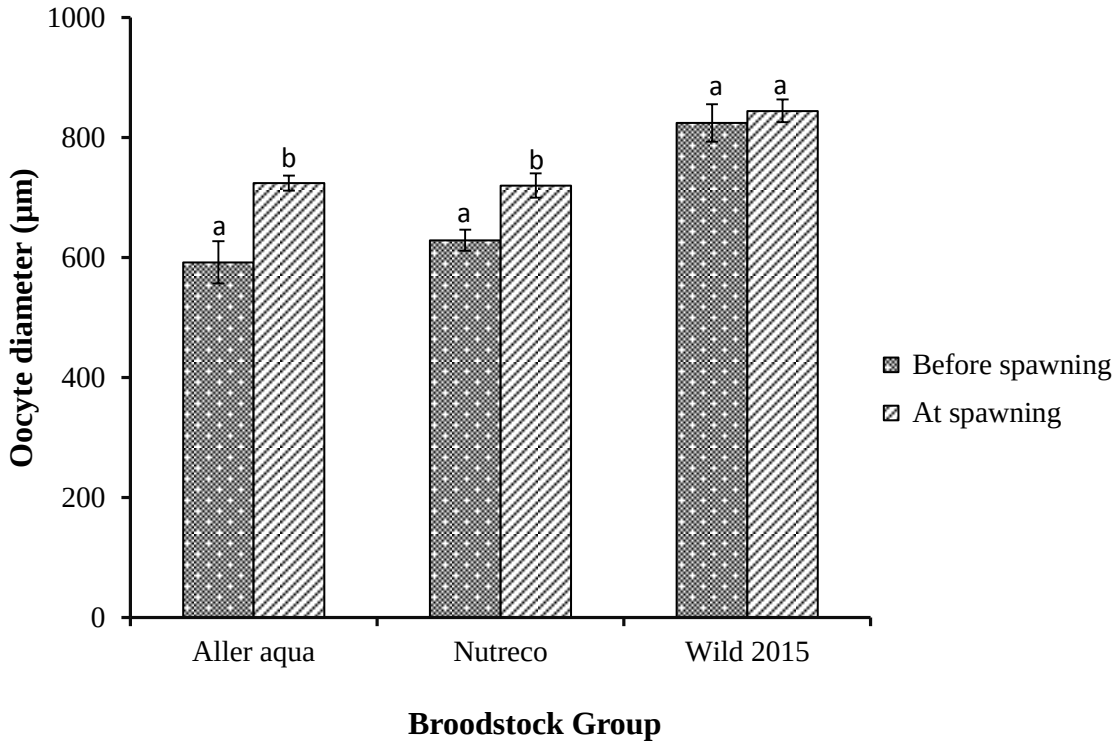


Figure 12: Oocyte diameter of pikeperch broodstock at spawning compared to a month before spawning period. Different letters within a group denote significant difference (P<0.05)

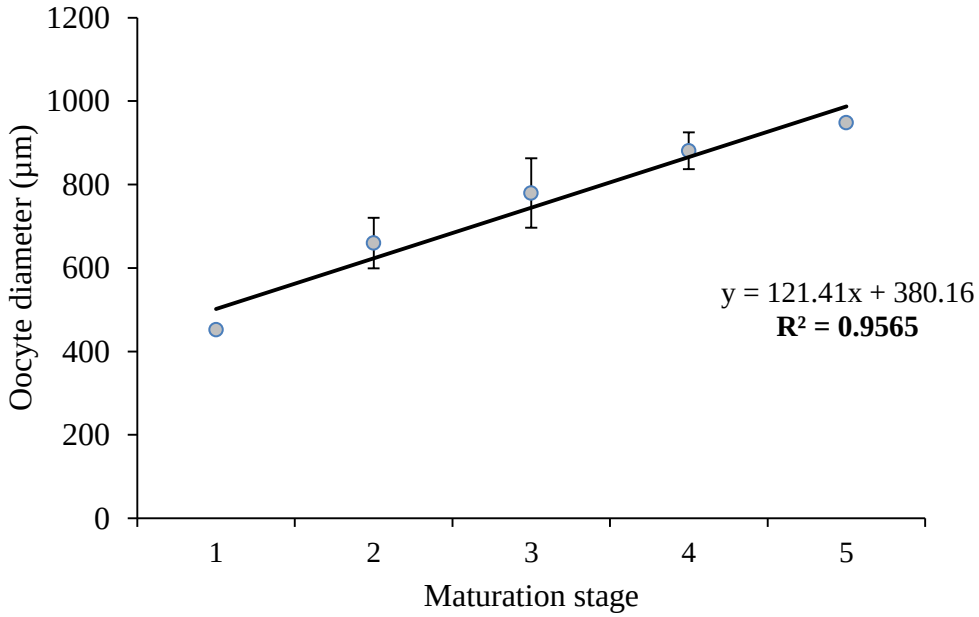


Figure 13: The relationship between oocyte maturation stage and oocyte diameter (µm)

4.7 Total lipid analysis

A month before spawning time, mean total lipid (g/kg fish) content in gonad was significantly higher in wild 2015 broodstock, while that of visceral fat was significantly lower compared to mean total lipid content of broodstock fed commercial diets (aller aqua and nutreco). No significant differences were observed in mean total lipid content of liver between all three experimental groups. At spawning, no significant difference was found in mean total lipid content of gonad between all groups of broodstock. Mean total lipid content of the liver was significantly higher in the broodstock caught from the wild in 2014 than that of aller aqua and nutreco fed broodstock, while no significant difference was found with that of broodstock caught from the wild in 2015 (Table 7).

Table 7: Total lipid (g/kg fish) of different pikeperch broodstock groups at spawning and one month before spawning period (n=5)

	Gonad	**Liver**	**Visceral Fat**
Broodstock group	Before spawning		
Aller Aqua fed broodstock	16.56 ± 5.04^{a}	2.98 ± 0.71^{a}	55.83 ± 12.89^{a}
Nutreco Fed broodstock	18.18 ± 6.27^{a}	3.74 ± 1.75^{a}	61.81 ± 13.90^{a}
Wild 2015 broodstock	33.64 ± 14.22^{b}	2.29 ± 1.02^{a}	13.23 ± 5.47^{b}
	At spawning		
Aller Aqua fed broodstock	24.19 ± 10.49^{a}	3.29 ± 0.83^{ab}	53.25 ± 10.79^{a}
Nutreco Fed broodstock	23.75 ± 6.78^{a}	2.11 ± 0.68^{a}	66.52 ± 14.45^{a}
Wild 2015 broodstock	39.64 ± 11.18^{a}	2.10 ± 0.51^{a}	9.16 ± 5.14^{b}
Wild 2014 broodstock	35.18 ± 15.41^{a}	4.37 ± 1.35^{b}	55.63 ± 10.68^{a}

Note: Data are shown as mean ± SD expressed as TL/kg female. Value within column with different superscripts are significantly different ($P<0.05$, n=5)

Total lipid content of gonad was found to be higher in all three broodstock groups at spawning period compared to a month before, although the differences were not significant (Figure 17A). In liver, total lipid was found significantly lower at spawning in nutreco fed broodstock compared to a moth before. No significant differences were observed in liver total lipid content of the wild 2015 and aller aqua fed broodstock at spawning compared to a one mot before (Figure 17B). In visceral fat, total lipid content showed no significant difference in all broodstock groups at spawning compared to one month before spawning period (Figure 17C).

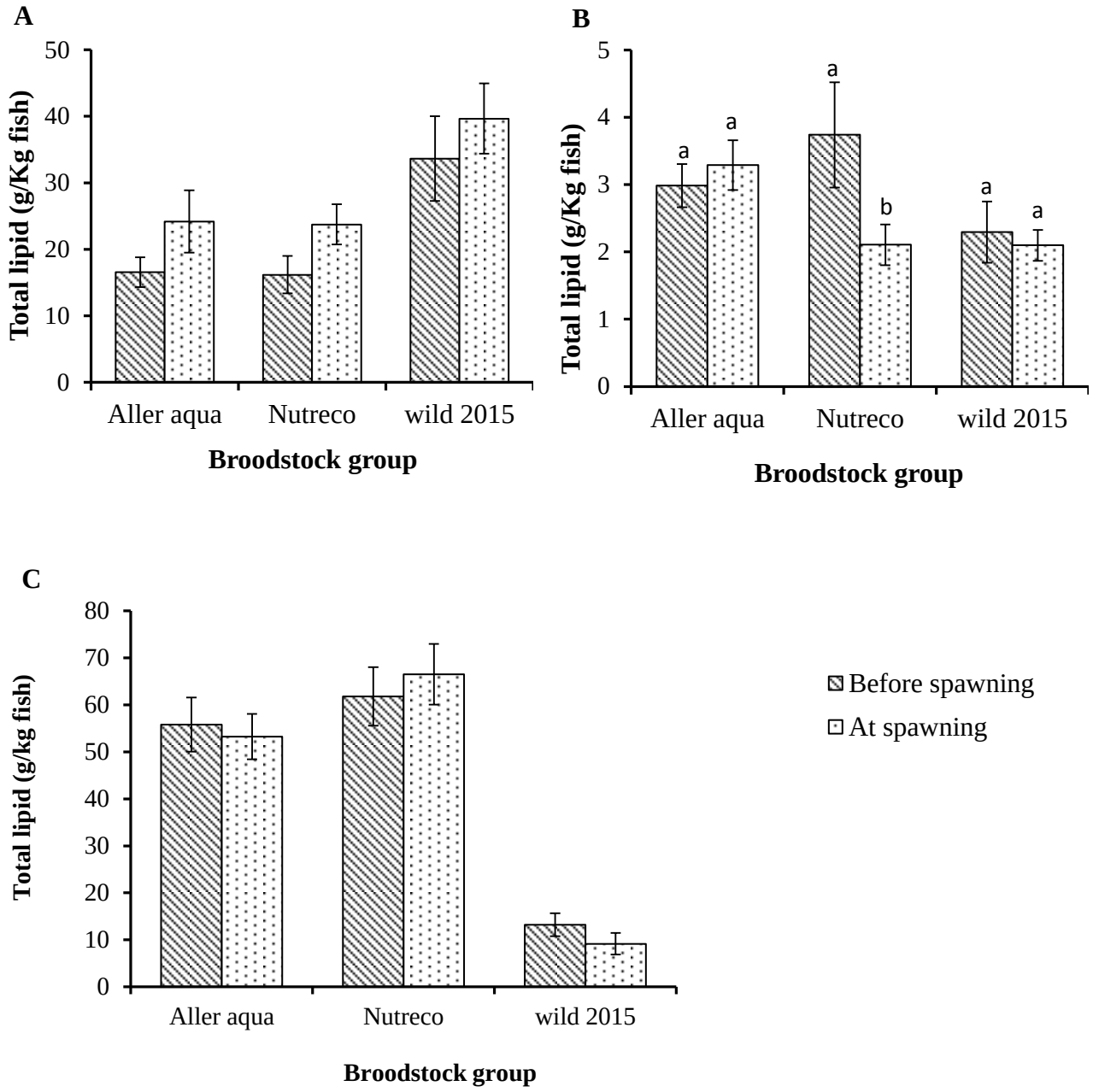


Figure 14: Total lipid content (mean± S.E, n=5) of gonad (A), Liver (B) and visceral fat (C) of pikeperch broodstock at spawning compared to one month before spawning period. Different letter within a group denotes significant difference ($P<0.05$,)

4.8 Fatty acid analysis

4.8.1 Fatty acid composition of pikeperch gonad

The top five most important FA found in the gonad differed considerably in each treatment one month before spawning period (Table 8). The percentage of EPA and DHA was significantly lower in wild 2015 broodstock while that of ARA was significantly higher compared to aller aqua and nutreco fed broodstock, resulting to 4-fold higher ratios of n-6/n-3 in wild 2015 compared to aller aqua and nutreco fed broodstock. The total FAME (mg/g DW) showed no significant difference between all broodstock experimental groups. No significant differences were found in the fatty acid composition of gonad between aller aqua and nutreco fed broodstocks.

Table 8: Summary of percentage fatty acid composition of pikeperch gonad from three different groups of broodstock one month before spawning period (n=5)

Lipid name	Aller aqua	Nutreco	Wild 2015
18:2(n-6); LA	6.38 ± 3.40^{a}	6.14 ± 1.49^{a}	8.53 ± 0.81^{b}
18:3(n-3); ALA	6.79 ± 2.97^{a}	7.82 ± 1.98^{a}	10.82 ± 1.13^{b}
20:4(n-6); ARA	0.71 ± 0.05^{a}	0.78 ± 0.06^{a}	2.85 ± 0.27^{b}
20:5(n-3); EPA	7.48 ± 1.36^{a}	6.86 ± 0.86^{a}	1.44 ± 0.18^{b}
22:6(n-3); DHA	20.62 ± 3.41^{a}	20.75 ± 2.30^{a}	9.17 ± 0.92^{b}
Total n-3	32.05 ± 4.68^{a}	31.58 ± 3.07^{a}	12.66 ± 1.29^{b}
Total n-6	7.35 ± 3.34^{a}	7.24 ± 1.62^{a}	13.71 ± 1.47^{b}
ARA:EPA	0.10 ± 0.02^{a}	0.12 ± 0.02^{a}	1.99 ± 0.15^{b}
ARA:DHA	0.03 ± 0.00^{a}	0.04 ± 0.01^{a}	0.31 ± 0.02^{b}
EPA:DHA	0.36 ± 0.02^{a}	0.33 ± 0.02^{a}	0.16 ± 0.01^{b}
n-6:n-3	0.25 ± 0.16^{a}	0.23 ± 0.07^{a}	1.10 ± 0.24^{b}
Total mg FAME/g DW	272.55 ± 13.20^{a}	277.20 ± 14.58^{a}	260.61 ± 12.75^{a}

Data are expressed as mean ± SD. Value within row with different superscripts are significantly different ($P<0.05$, n=5)

At spawning period, the fatty acid composition of gonad showed no significant different between aller aqua and nutreco fed broodstock, whilst wild 2014 and 2015 broodstock differed significantly with aller aqua and nutreco fed broodstock. The total FAME (mg/g DW) showed no significant difference between all experimental broodstock groups. Broodstock caught from the wild had significantly more ARA (3.32%), amounting to about 4- fold increase over the 0.97% found in broodstock caught from the wild in 2014 and 0.80% found aller aqua and nutreco fed

broodstock and this, in conjugation with the significant lower amount of EPA and DHA, resulted in 6-fold and 4-fold higher ARA:EPA and ARA:DHA ratios respectively when compared to broodstock caught from the wild in 2014 and about 12-fold and 6-fold higher ARA:EPA and ARA:DHA ratios respectively when compared to broodstock fed commercial diet (Table 9).

Table 9: Summary of percentage fatty acid composition of pikeperch gonad from four different groups of broodstock at spawning period (n=5)

Lipid name	Aller aqua	Nutreco	Wild 2015	Wild 2014
18:2(n-6); LA	5.69 ± 3.05^{a}	6.03 ± 0.40^{a}	6.69 ± 1.08^{a}	6.31 ± 0.38^{a}
18:3(n-3); ALA	6.44 ± 2.92^{a}	7.97 ± 0.77^{ab}	8.85 ± 1.14^{ab}	9.41 ± 1.18^{b}
20:4(n-6); ARA	0.78 ± 0.07^{a}	0.80 ± 0.03^{a}	3.32 ± 0.69^{b}	0.97 ± 0.11^{a}
20:5(n-3); EPA	8.11 ± 1.45^{a}	7.15 ± 0.17^{a}	2.56 ± 0.73^{b}	4.87 ± 0.49^{c}
22:6(n-3); DHA	22.19 ± 3.65^{a}	20.61 ± 0.94^{a}	14.27 ± 3.65^{b}	16.52 ± 2.48^{b}
Total n-3	34.03 ± 5.00^{a}	31.63 ± 0.88^{a}	20.40 ± 5.00^{b}	24.75 ± 3.28^{b}
Total n-6	6.61 ± 2.92^{a}	7.16 ± 0.42^{a}	11.72 ± 1.03^{b}	7.37 ± 0.30^{a}
ARA:EPA	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}	1.34 ± 0.24^{b}	0.20 ± 0.01^{c}
ARA:DHA	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.24 ± 0.03^{b}	0.06 ± 0.01^{c}
EPA:DHA	0.37 ± 0.03^{a}	0.35 ± 0.02^{a}	0.18 ± 0.03^{b}	0.30 ± 0.03^{c}
n-6:n-3	0.21 ± 0.13^{a}	0.23 ± 0.02^{a}	0.61 ± 0.21^{b}	0.30 ± 0.04^{a}
Total mg FAME/g DW	285.76 ± 13.72^{a}	294.42 ± 20.92^{a}	296.39 ± 28.47^{a}	325.57 ± 60.30^{a}

Data are expressed as mean ± SD. Value within row with different superscripts are significantly different ($P<0.05$, n=5)

Significant difference in fatty acid composition of gonad at spawning compared to one month before spawning period was found in the broodstock caught from the wild in 2015, whereby the amount of EPA and DHA was almost double at spawning compared to a month before while the total n-6 HUFAs was significantly lower leading to decrease by half of the n-6/n-6 ratio. Broodstock fed commercial diet (aller aqua and nutreco) showed no significant difference in fatty acid composition at spawning compared to one month before spawning period.

4.8.2 Fatty acid composition of pikeperch liver

Table 10 shows the percentage fatty acid composition of liver from pikeperch broodstock one month before spawning period. The overall difference in fatty acid composition of the liver between aller aqua and nutreco fed broodstock was not significant. Broodstock caught from the wild had significantly lower total FAME 86.14± 34.21mg/g compared to 186.72 ± 94.67mg/g of aller aqua fed broodstock despite the high standard deviation, whilst no significant difference was found with 142.14 ± 57.69mg/g of nutreco fed broodstock. Broodstock caught from the wild had 4 times more ARA (7.38%) than aller aqua and nutreco fed broodstock and this, in conjugation with the significant lower amount of EPA 2.47%, resulted in about 18-folds higher ARA:EPA ratios respectively when compared to broodstock fed commercial diet (aller aqua and nutreco). No significant differences were observed in percentage DHA between all broodstock experimental groups. Significant higher percentage total n-6 and lower percentage total n-3 was found in broodstock caught from the wild in 2015 compared to aller aqua and nutreco fed broodstock.

Table 10: Summary of percentage fatty acid composition of pikeperch liver from three different groups of broodstock one month before spawning period

Lipid name	Aller aqua	Nutreco	Wild 2015
18:2(n-6); LA	5.79 ± 0.78^{a}	4.27 ± 0.87^{b}	3.88 ± 0.89^{a}
18:3(n-3); ALA	1.05 ± 0.19^{a}	0.89 ± 0.17^{a}	1.18 ± 0.27^{a}
20:4(n-6); ARA	1.55 ± 0.41^{a}	1.87 ± 0.42^{a}	7.38 ± 2.98^{b}
20:5(n-3); EPA	9.65 ± 0.67^{a}	10.70 ± 0.55^{a}	2.47 ± 1.21^{b}
22:6(n-3); DHA	19.01 ± 5.21^{a}	23.67 ± 3.68^{a}	20.08 ± 7.37^{a}
Total n-3	32.16 ± 5.46^{ab}	37.77 ± 3.45^{a}	25.32 ± 9.33^{b}
Total n-6	7.71 ± 1.05^{a}	6.54 ± 0.90^{a}	13.79 ± 3.77^{b}
ARA:EPA	0.16 ± 0.05^{a}	0.18 ± 0.04^{a}	3.32 ± 0.89^{b}
ARA:DHA	0.08 ± 0.01^{a}	0.08 ± 0.01^{a}	0.37 ± 0.08^{b}
EPA:DHA	0.55 ± 0.18^{a}	0.46 ± 0.09^{a}	0.12 ± 0.04^{b}
n-6:n-3	0.24 ± 0.04^{a}	0.17 ± 0.03^{a}	0.59 ± 0.17^{b}
Total mg FAME/g DW	186.72 ± 94.67^{a}	142.14 ± 57.69^{ab}	86.14 ± 34.21^{b}

Data are expressed as mean ± SD. Value within row with different superscripts are significantly different ($P<0.05$, n=5)

At spawning, broodstock caught from the wild in 2015 differed significantly in total FAME composition of the liver (138.63 ± 32.23mg/g) with that of broodstock caught from the wild 2014 (220.56 ± 39.50mg/g) and aller aqua fed broodstock (270.97 ± 159.93) (Table 11).

Significantly higher percentage of ARA was found in broodstock caught from the wild in 2015. Percentage DHA was found to be significantly lower in the broodstock caught from the wild in 2014 compared to that caught in 2015, while EPA showed no significant difference between wild broodstocks. The ratios of n-6: n-3 was significantly higher in wild broodstocks than commercial diet fed broodstock.

A significant increase in total FAME was observed at spawning in the wild 2015 broodstock compared to a month before, particularly in EPA. The EPA: DHA ratio increased almost 3-fold.

Table 11: Summary of percentage fatty acid composition of pikeperch liver from four different groups of broodstock at spawning period (n=5)

Lipid name	Aller aqua	Nutreco	Wild 2015	Wild 2014
18:2(n-6); LA	5.99 ± 1.12^{a}	4.36 ± 0.76^{b}	4.26 ± 1.31^{b}	6.08 ± 0.73^{a}
18:3(n-3); ALA	1.09 ± 0.28^{a}	0.92 ± 0.13^{a}	0.99 ± 0.61^{a}	0.86 ± 0.09^{a}
20:4(n-6); ARA	1.30 ± 0.15^{a}	1.79 ± 0.19^{b}	4.34 ± 0.81^{c}	1.59 ± 0.10^{b}
20:5(n-3); EPA	10.69 ± 3.37^{a}	9.39 ± 1.02^{ab}	7.27 ± 1.78^{b}	6.98 ± 0.52^{b}
22:6(n-3); DHA	19.41 ± 3.09^{ab}	22.41 ± 3.20^{a}	21.63 ± 4.76^{a}	16.66 ± 1.03^{b}
Total n-3	33.63 ± 2.03^{a}	35.58 ± 4.25^{a}	32.14 ± 6.32^{ab}	27.09 ± 0.91^{b}
Total n-6	7.76 ± 1.14^{ac}	6.58 ± 0.58^{a}	9.61 ± 1.37^{b}	8.19 ± 0.61^{c}
ARA:EPA	0.14 ± 0.06^{a}	$0.19 \pm 0,02^{a}$	0.62 ± 0.17^{b}	0.23 ± 0.03^{a}
ARA:DHA	0.07 ± 0.00^{a}	0.08 ± 0.01^{b}	0.20 ± 0.03^{c}	0.10 ± 0.00^{d}
EPA:DHA	0.58 ± 0.27^{a}	0.42 ± 0.04^{a}	0.34 ± 0.05^{a}	0.42 ± 0.05^{a}
n-6:n-3	0.23 ± 0.04^{ab}	0.19 ± 0.03^{a}	0.31 ± 0.11^{b}	0.30 ± 0.03^{b}
Total mg FAME/g DW	270.97 ± 159.93^{a}	109.96 ± 16.70^{b}	138.63 ± 32.23^{b}	220.56 ± 39.50^{a}

Data are expressed as mean ± SD. Value within row with different superscripts are significantly different ($P<0.05$, n=5)

4.8.3 Fatty acid composition of pikeperch visceral fat

Pikeperch broodstocks differed considerably in their FAME composition of visceral fat one month before spawning period (Table 12). The percentage of EPA and DHA was significantly lower in wild 2015 broodstock while that of ARA and n6/n3 ratio was significantly higher compared to aller aqua and nutreco fed broodstock. The total FAME (mg/g DW) showed significant difference between wild broodstock and aller aqua fed broodstock. Apart from total FAME, no significant differences were found in the fatty acid composition of visceral fat between aller aqua and nutreco fed broodstocks.

Table 12: Summary of percentage fatty acid composition of pikeperch visceral fat from three groups of pikeperch broodstock one month before spawning period (n=5)

Lipid name	Aller aqua	Nutreco	Wild 2015
18:2(n-6); LA	7.08 ± 1.63[a]	6.67 ± 0.66[a]	10.13 ± 1.16[b]
18:3(n-3); ALA	1.42 ± 0.30[a]	1.42 ± 0.14[a]	4.38 ± 0.74[b]
20:4(n-6); ARA	0.80 ± 0.06[a]	0.81 ± 0.04[a]	2.66 ± 0.86[b]
20:5(n-3); EPA	9.91 ± 0.44[a]	10.24 ± 0.10[a]	1.57 ± 0.51[b]
22:6(n-3); DHA	9.52 ± 0.49[a]	10.07 ± 0.32[a]	2.67 ± 0.83[b]
Sum n-3	22.61 ± 0.78[a]	23.50 ± 0.53[a]	5.95 ± 1.41[b]
Sum n-6	8.55 ± 1.60[a]	8.23 ± 0.75[a]	14.38 ± 2.03[b]
ARA:EPA	0.08 ± 0.00[a]	0.08 ± 0.00[a]	1.71 ± 0.17[b]
ARA:DHA	0.08 ± 0.01[a]	0.08 ± 0.01[a]	1.03 ± 0.32[b]
EPA:DHA	1.04 ± 0.04[a]	1.02 ± 0.02[a]	0.60 ± 0.16[b]
n-6:n-3	0.38 ± 0.08[a]	0.35 ± 0.03[a]	2.51 ± 0.54[b]
Total mg FAME/g DW	824.77 ± 31.85[a]	922.11 ± 23.89[b]	922.24 ± 58.92[b]

Data are expressed as mean ± SD. Value within row with different superscripts are significantly different ($P<0.05$, n=5)

At spawning, fatty acid composition of the visceral fat from commercial diet feed (aller aqua and nutreco) broodstock showed no significant difference between them. The total FAME of visceral fat showed no significant difference among all experimental broodstock groups. Broodstock caught from the wild had significantly more ARA (1.82%), amounting to over two times the 0.74% found in broodstock caught from the wild in 2014, 0.86% found aller aqua and 0.83% found in nutreco fed broodstock. Higher ARA in conjugation with the significant lower amount of EPA and DHA in wild 2015 broodstock, resulted in about 3-fold and 4-fold higher ARA:EPA and ARA:DHA ratios respectively when compared to broodstock caught from the wild in 2014 and about 6-fold and 5-fold higher ARA:EPA and ARA:DHA ratios respectively when compared to broodstock fed commercial diet (Table 13). Percentage total n-3 was significantly lower while total n-6 was significantly higher in wild 2015 broodstock visceral fat compared to aller aqua and nutreco fed broodstock.

Table 13: Summary of percentage fatty acid composition of pikeperch visceral fat from three groups of pikeperch broodstock at spawning period (n=5)

Lipid name	Aller aqua	Nutreco	Wild 2015	Wild 2014
18:2(n-6); LA	7.28 ± 1.84^{ab}	6.35 ± 0.33^{a}	7.83 ± 1.23^{ab}	8.30 ± 0.43^{b}
18:3(n-3); ALA	1.41 ± 0.31^{a}	1.35 ± 0.04^{a}	1.87 ± 1.15^{a}	1.26 ± 0.13^{a}
20:4(n-6); ARA	0.86 ± 0.08^{a}	0.83 ± 0.05^{a}	1.82 ± 0.58^{b}	0.74 ± 0.15^{a}
20:5(n-3); EPA	9.77 ± 1.35^{a}	10.31 ± 0.25^{a}	5.17 ± 2.44^{b}	5.64 ± 0.16^{b}
22:6(n-3); DHA	9.75 ± 1.33^{a}	9.93 ± 0.56^{a}	5.51 ± 1.64^{b}	6.82 ± 0.28^{a}
Sum n-3	22.68 ± 2.69^{a}	23.32 ± 0.83^{a}	13.46 ± 4.58^{b}	15.40 ± 0.52^{b}
Sum n-6	8.71 ± 1.76^{ab}	7.84 ± 0.35^{a}	10.75 ± 2.23^{c}	9.69 ± 0.62^{bc}
ARA/EPA	0.09 ± 0.00^{a}	0.08 ± 0.01^{a}	0.47 ± 0.35^{b}	0.13 ± 0.03^{a}
ARA/DHA	0.09 ± 0.01^{a}	0.08 ± 0.01^{a}	0.39 ± 0.22^{b}	0.10 ± 0.02^{a}
EPA/DHA	1.00 ± 0.04^{a}	1.04 ± 0.05^{a}	0.90 ± 0.19^{ab}	0.83 ± 0.03^{b}
n-6/n-3	0.40 ± 0.14^{a}	0.34 ± 0.02^{a}	0.95 ± 0.58^{b}	0.63 ± 0.06^{ab}
Total mg FAME/g DW	895.52 ± 15.98^{a}	907.44 ± 24.20^{a}	832.94 ± 102.45^{a}	887.40 ± 19.68^{a}

Data are expressed as mean ± SD. Value within row with different superscripts are significantly different ($P<0.05$)

Fatty acid composition of visceral fat at spawning compared to one month before spawning period was considerably different in broodstock caught from the wild in 2015, whereby at spawning the amount of EPA and DHA was three times the amount a month before (Table 20). Total n-3 HUFAs was significantly higher while the total n-6 HUFAs was significantly low at spawning than one month before spawning in the broodstock caught from the wild in 2015. Significant decrease in LA, ALA, ARA, ARA:EPA, ARA:DHA and n-6:n-3 levels at spawning was observed in wild 2015 broodstock. Broodstock fed commercial diet (aller aqua and nutreco) showed no significant difference in fatty acid composition at spawning compared to one month before spawning period.

4.9 Diet composition

Aller aqua had the lowest content of total lipids (17,05%) and protein (55,3%) content, compared to Nutreco and goldfish (Table 14). The experimental diets also differed in their fatty acid composition. Nutreco pellets had about 2 times the percentage of EPA compare to goldfish (diet for wild 2014 broodstock), whilst aller aqua pellets had double amount of DHA compared to nutreco and goldfish. Total FAME was found higher in goldfish than pellet feeds.

Table 14: Summary of protein, total lipid and fatty acid composition of experimental fish diets

	FAME (mg/g DW)			AREA (%)		
Lipid name	Aller aqua	Nutreco	Goldfish	Aller aqua	Nutreco	Goldfish
18:2(n-6); LA	19.92	14.30	16.48	14.27	8.04	7.51
18:3(n-3); ALA	4.23	3.55	3.25	3.03	2.00	1.48
20:4(n-6); ARA	0.67	1.52	2.06	0.48	0.86	0.94
20:5(n-3); EPA	13.36	18.41	17.93	9.57	10.36	8.17
22:6(n-3); DHA	10.30	20.70	22.91	7.37	11.64	10.44
ARA/EPA	0.05	0.08	0.11	0.05	0.08	0.12
ARA/DHA	0.07	0.07	0.09	0.07	0.07	0.09
EPA/DHA	1.30	0.89	0.79	1.30	0.89	0.78
n-6/n-3	0.77	0.38	0.38	0.77	0.38	0.38
Total mg FAME/g DW	139.63	177.81	219.40			
Total Lipid (% DW)	17.05	22.69	26.01			
Protein (%)	55.30	59.12	-			

B: PIKEPERCH LARVAE

4.10 Larval length and weight analysis

The total length of larvae from wild 2015 broodstock was significantly higher compared to length of larvae from the wild 2014 broodstock. Both wet weight and dry weight of larvae from wild 2014 broodstock was significantly higher than the larvae from wild 2015broodstock (Figure 15)

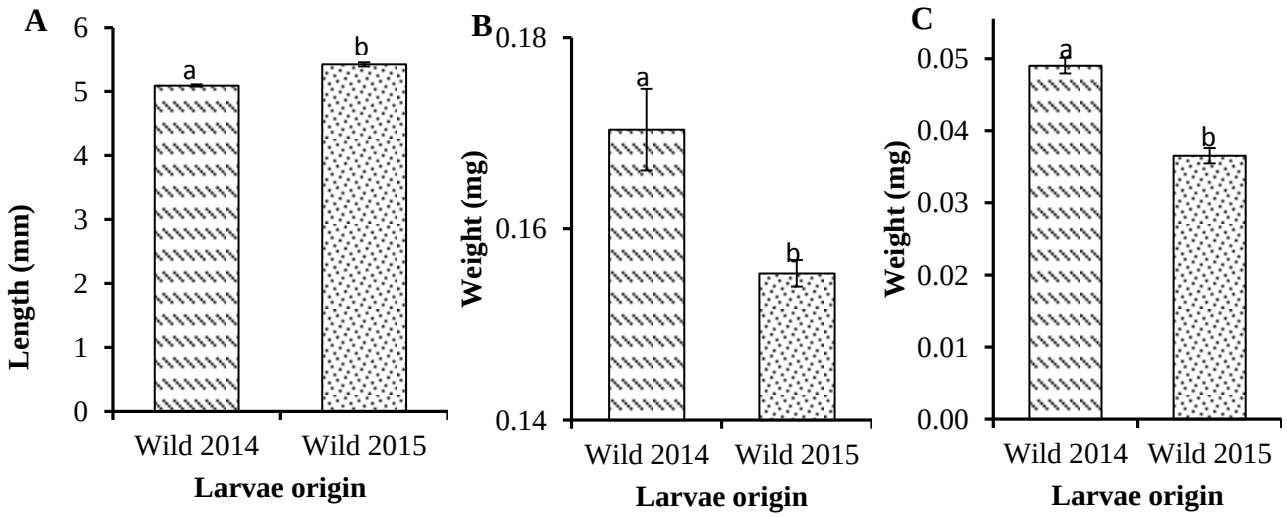


Figure 15: Length (A), wet weight (B) and dry weight (C) of larvae from broodstock caught from the wild in 2014 and 2015

4.4 Pikeperch larval stress test

4.4.1 Starvation stress test

For four days, percentage survival in both groups of larvae showed no significant difference. On the fifth day the larvae from the 2014 wild broodstock showed significantly higher survival in synthetic freshwater than larvae from 2015 wild broodstock. From day 6 to day 10 high percentage survival was observed in the larvae from broodstock caught from the wild in 2014 than larvae from broodstock caught from the wild in 2015. At the end of the experiment (day 10) larvae from wild broodstock caught in 2014 was significantly higher in both synthetic freshwater (57%) and rearing water (40%) compared to 33% and 2% respectively for larvae from 2015 wild broodstock (Table 15). Larvae survival of both groups was better in synthetic freshwater than that of rearing water.

Table 15: Survival (%) of pikeperch larvae from two groups of wild broodstock after starvation stress test

	Larvae survival (%)			
	Rearing water		Synthetic freshwater	
Time (day)	Wild-2014 larvae	Wild-2015 larvae	Wild-2014 larvae	Wild-2015 larvae
1	100^{a}	100^{a}	100^{a}	100^{a}
2	97 ± 6^{a}	98 ± 3^{a}	97 ± 6^{a}	100^{a}
3	93 ± 8^{a}	98 ± 3^{a}	97 ± 6^{a}	93 ± 8^{a}
4	80 ± 5^{a}	87 ± 6^{a}	97 ± 6^{a}	90 ± 10^{a}
5	67 ± 8^{b}	70 ± 5^{b}	95 ± 5^{a}	60 ± 17^{b}
6	50 ± 13^{b}	33 ± 16^{b}	95 ± 5^{a}	48 ± 13^{b}
7	45 ± 18^{b}	2 ± 3^{c}	93 ± 8^{a}	48 ± 13^{b}
8	42 ± 21^{b}	2 ± 3^{c}	87 ± 10^{a}	42 ± 10^{b}
9	42 ± 21^{b}	2 ± 3^{c}	78 ± 16^{a}	40 ± 10^{b}
10	40 ± 18^{ab}	2 ± 3^{c}	57 ± 25^{a}	33 ± 10^{b}

Note: Data are shown as mean ± SD. Value within row with different superscripts are significantly different ($P<0.05$)

4.4.2 Osmotic stress test

In about 72h, larvae from both experimental groups were death at 15‰, 20‰ and 25‰. Based on cumulative mortality, no significant difference was observed between the two groups of larvae in both control (Rearing water and synthetic freshwater) and at the salinity of 5‰, 15‰

and 25‰. At the salinity 5‰, 10‰ and 25‰ larvae from the 2015 wild broodstock showed to be more sensitive to osmotic stress than larvae from 2014 wild broodstock although the differences in cumulative stress index values was only significant at 10‰. At the salinity of 20‰ larvae from 2014 wild broodstock showed significant high sensitivity to osmotic stress than the larvae from 2015 wild broodstock (Table 16)

Table 16: Cumulative stress index of pikeperch larvae from two broodstock groups

	Cumulative stress index	
Salinity ($g.l^{-1}$)	**Wild 2014-Larvae**	**Wild 2015-Larvae**
Control- rearing water (0)	60 ± 0^{a}	56 ± 0^{a}
Control- synthetic freshwater (0)	36 ± 0^{a}	29 ± 0^{a}
5	21 ± 9^{a}	27 ± 6^{a}
10	24 ± 8^{a}	41 ± 10^{b}
15	86 ± 1^{a}	83 ± 2^{a}
20	106 ± 2^{a}	94 ± 1^{b}
25	120 ± 0^{a}	124 ± 1^{a}

Note: Data are shown as mean ± SD Value within row of the same salinity with different superscripts are significantly different ($P<0.05$)

4.5 Fatty acid and total lipid composition of pikeperch larvae

Larvae of wild 2015 broodstock had higher amount of total lipid (31%) than larvae of wild 2014 broodstock (29%). Total FAME (mg/g DW) was found to be higher in larvae of wild 2014 broodstock than that of larvae from wild 2015 broodstock. The amount of EPA and DHA was higher in larvae of 2014 wild broodstock while ARA was higher in the larvae of 2015 wild broodstock. Total n-3 HUFAs was high in larvae of 2014 wild broodstock while total n-6 HUFAs was higher in larvae of 2015 wild broodstock (Table 17).

Table 17: FAME and total lipid composition of pikeperch larvae from wild 2014 and 2015 broodstock (n=1)

		FAME (mg/g DW)		AREA (%)	
Common name	Lipid name	Wild 2014-Larvae	Wild 2015-Larvae	Wild 2014-Larvae	Wild 2015-larvae
Palmitic acid (PA)	16:00	20.59	18.71	5.96	6.74
Palmitoleic acid (POA)	16:1(n-7)	19.44	17.73	5.63	6.38
Vaccenic acid (VA)	18:1(n-7)	6.64	8.61	1.92	3.10
Oleic acid (OA)	18:1(n-9)	47.11	36.41	13.63	13.11
Linolenic acid (LA)	18:2(n-6)	19.14	20.90	5.54	7.52
α-Linoleric acid (ALA)	18:3(n-3)	31.64	24.96	9.15	8.99
Arachidonic acid (ARA)	20:4(n-6)	3.99	9.08	1.15	3.27
Eicosapentanoic acid (EPA)	20:5(n-3)	18.53	5.94	5.36	2.14
Docosapentaenoic acid (DPA)	22:5(n-3)	7.76	4.50	2.24	1.62
Docosahexaenoic acid (DHA)	22:6(n-3)	64.27	34.40	18.59	12.38
Total n-3		95.90	47.10	27.74	16.96
Total n-6		24.42	34.60	7.06	12.46
ARA:EPA		0.22	1.53	0.22	1.53
ARA:DHA		0.06	0.26	0.06	0.26
EPA:DHA		0.29	0.17	0.29	0.17
n-6/n-3		0.25	0.73	0.25	0.73
Total mg FAME/g DW		345.67	277.75		
Total lipid (%)		29.03	31.19		

5. DISCUSSION

Despite relative scarcity of work on pikeperch broodstock nutrition, the nutritional status of broodstock is known to have a profound effect on the reproductive performance and quality of offspring (Izquierdo *et al*., 2001). Thus the difference in nutrition status between wild and cultured pikeperch is obvious and attributes to the variation in quality and quantity of pikeperch eggs and larvae.

The gonadosomatic index(GSI) as unambiguous indicator of reproductive status (Hermelink *et al.,* 2011) was found significantly lower in farmed broodstock fed nutreco and aller aqua pelleted feeds than wild 2015 broodstock. Similar trend was revealed at spawning period (one month after fist sampling) where the mean GSI of the wild 2015 broodstock (12.50 ± 2.91%) almost doubled that of farmed broodstock fed nutreco and aller aqua commercial dry feeds (7.68 ± 3.23% & 7.57 ± 2.02% respectively), this corresponded to the report by Zakes *et al.*, (2012) in farmed fish and Lappalainen *et al*., (2003) in wild fish. Further increase in mean GSI in all experimental pikeperch broodstock at spawning compared to one month before spawning, indicates gonad development taking into account that wild females only shortly before spawning reported to reach a GSI of 7–15% (Hermelink *et al*., 2011). Also, these results relates to the findings by Nandikeswari & Anandan, (2013) and Lappalainen *et al*. (2003) where the GSI was found to increase with the maturation of fish and to reach its maximum at the peak period of maturity. Mean HIS in the present study showed no significant differences between farmed and wild broodstock, while VIS showed significant difference between wild and farmed broodstocks, this relate to the reports by Sulistyo *et al*. (2000) for wild Eurasian perch (*Perca fluviatilis* L.) and by Migaud *et al*. (2002) for farmed perch where the VIS of wild broodstock were lower compared to that of farmed fish perch. The increase in mean GSI and decrease in mean HIS and VSI at spawning period is probably related to the mobility of the hepatopancreas reserve to the gonad and the transfer of vitellogenin (VTG) from the liver and visceral fat to the ovaries which was found to occur at the final stage of ovarian development (Castile & Lawrence, 1989). Wild 2014 broodstock showed superior development of gonads than wild 2015 broodstock, which correspond to the report by Khemis *et al*., (2014). This could be due to the fact that wild 2015 broodstock were not fed until spawning period, hence the use of reserved energy was not only for gonadal development but also for their metabolism which resulted to the significant decrease

in mean body weight, gonad weight, liver weight and visceral fat weight at spawning compare to one month before.

In wild caught fish, mature unfertilized eggs are reported to reach diameter between 700 and 850 μm, whereas fertilized ones are between 1000 and 1670 μm (Schlumberger and Proteau, 1996; Lappalainen *et al*., 2003). In our experiment, oocytes diameter for wild and farmed broodstocks was within the range reported by these authors, although both groups of wild broodstock (wild 2014 and 2015) had significantly bigger oocytes than that of aller aqua and nutreco fed broodstock. According to Craig (2000), temperature increase in spring synchronizes spawning in Eurasian perch which relates to pikeperch. However, in our study, ovarian development was not synchronous among experimental broodstock, where broodstock from the wild were more mature than farmed broodstock at both sampling time (at spawning and one month before spawning), which correspond to the report by Wang *et al*., (2009). Oocyte maturation stages in broodstock were similar for the female when eggs was taken from the life broodstock using catheter or when was taken direct from the gonad which was also confermed by histological analysis of the oocyte. This indicates that any method among the three mentioned can be used to assess the maturation stage of pikeperch oocyte. Strong correlation obtained between maturation stage and oocyte diameter suggest that the equation obtained ($y= 121.38x + 380.3$) can be used to determine maturation stage by only measuring the oocyte diameter.

Dietary lipid and essential fatty acid content are among major nutritional factors that have been found to significantly affect reproductive performance in fish (Furuita *et al*., 2000 2002; Henrotte *et al*., 2010). In the present study wild broodstock 2014 and 2015 had higher amount of total lipid (39.64 ± 11.18g/kg fish and 35.18 ± 15.41g/kg fish respectively) compared to that of aller aqua and nutreco fed broodstock (24.19 ± 10.49g/kg and 23.75 ± 6.78g/kg), although the differences were not significant. Total lipid content of gonad from wild 2014 broodstock fed with goldfish indicates that goldfish diet was enriched in lipid content in comparison to aller aqua and nutreco pellet diet. This was further confirmed by lipid analysis of feed where goldfish (diet for wild 2014 broodstock) contained 26% TL which was higher compared to 18% and 22% found in aller aqua and nutreco respectively. Lipid content in the gonad of the all experimental broodstock at spawning was found higher at spawning compared to one month before. This indicates that the gonads were more mature at spawning since ovarian accumulation of lipid content associates with the storage of the nutrient and energy reserve for the embryo

development and early larval stages. Harel *et al*., (1994) have shown that the kinetics of lipid deposition in the reproductive tissues of the gilthead seabream (*Sparus aurata*) reached equilibrium with the dietary composition within 15 days of feeding on any given diet, but this species continues to eat during sexual maturation, while pikeperch tends to decrease its food intake during sexual maturation, and the energy and nutrients needed for ovarian growth are partially taken from its body reserves. This was found true in our experiment since increase in total lipid content of gonad was accompanied with the decrease in mean total lipid of the liver and visceral fat for experimental broodstock.

Although fecundity does not always equate to reproductive success, it can provide an objective measure of reproductive effort (Moyle and Cech, 2000) and egg quality (Izquierdo *et al*., 2001). In the present study, both absolute and relative fecundity obtained were within the range of reported values for pikeperch, *Sander lucioperca* (Lappalainen *et al*., 2003). No significant differences in fecundity were noted between wild broodstock and hatchery broodstock both at spawning and one month before spawning period. However, fecundity within a group was noted higher at spawning period compared to one month before spawning reflecting further maturation of eggs. High fecundity at spawning could be associated with increased amount of dietary essential fatty acids (EFA). For example, fecundity in gilthead seabream (*Sparus aurata)* was found to significantly increase with an increase in dietary n-3 HUFA polyunsaturated fatty acids with 20 or more carbon atoms (Fernandez-Palacios *et al*., 1995), while reproductive performance of Nile tilapia (*Oreochromis niloticus*) show that the performance was much higher in fish fed a basal diet supplemented with soybean oil high in n-6 fatty acids (Watanabe, 1982). Excess dietary n-3 HUFAs has been also reported to have a negative effect on reproductive performance of fish. For example, high levels of dietary n-3 HUFA reduced the total amount of eggs produced by gilthead seabream broodstock despite an increase in egg n-3 HUFA concentration (Fernandez-Palacios et al., 1995). This could be due to the fact that high dietary n-3 HUFA levels could affect the brain–pituitary–gonad endocrine axis since both EPA and DHA have been found to reduce in vitro the steroidogenic action of gonadotropin in the ovary of teleost fish (Mercure and Van Der Kraak, 1996). Spawning quality of pikeperch, which is a limiting factor for successful fish production, is directly affected by the ratio of DHA/EPA/ARA of broodstock diets, as suggested by Bell *et al*. (1997) and Bruce *et al*. (1999) on other teleost species. In the present study, all females broodstock fed pelleted feed (aller aqua and nutreco) failed to spawn

despite administration of spawning hormone while wild broodstock spawned naturally by the manipulation of photoperiod. Similar result were obtained by Wang *et al*., (2009) where pikeperch broodstock fed dry diet fail to spawn while those fed forage fish and mixture of dry diet and forage fish spawned by hormonal injection. This suggests that the composition of commercial feed developed for sturgeon (aller aqua) and seabass (nutreco) breeders is not suitable for pikeperch broodstocks, probably due to the FA composition of these diets. Moreover, the current study investigated FA composition of gonad, liver and visceral fat from experimental broodstock in relation to their diet. It was found that total FAME (mg/g DW) did not differ statically among experimental broodstock despite higher amount of total FAME in wild 2014 diet (goldfish) compared to other diet (aller aqua and nutreco). However, the ratios of DHA/EPA/ARA differed in the egg and diet of experimental broodstock, where aller aqua, nutreco and goldfish diet contained the ratio of 7/10/0.5, 12/10/1 and 10/8/1 respectively, while eggs from broodstock fed these diets had ratios of 22/8/2, 21/7/7 and 7/5/1 respectively.

It has been shown that EPA plays an important role in the biological activity of eicosanoids derived from ARA. Sargent *et al*, (2002), Henrotte *et al*, (2010) and also demonstrated that the eicosanoid production is influenced by the cellular ratio of EPA/ARA. ARA is the chief precursor of the eicosanoids, including the generation of series II prostaglandins (PGs) and EPA competitively interferes with eicosanoid production from ARA, because both series II and series III PGs are catalysed by the same cyclooxygenase and lipoxygenase. In the present study, aller aqua and nutreco diets contained very small amount of ARA (0.48% and 0.86% of total FA respectively), and the highest level of EPA (9.57% and 10.36% of total FA respectively), which led to the low ratio of ARA/EPA in the tested diets (0.04 and 0.08 respectively), and consequently in the eggs (0.10 ± 0.01 and 0.11 ± 0.01 respectively), compared to ARA/EPA ratio of 0.12 in diet and 0.20 ± 0.01 in eggs of goldfish fed wild 2014 broodstock and 1.34 ± 0.24 in eggs of wild 2015 broodstock. Overall, wild broodstocks (caught in 2014 and 2015) had high ARA/EPA ratios than that of broodstock fed aller aqua and nutreco feed. This corresponds to the values reported by Suloma & Ogata, (2012). Therefore, it can be supposed that the high level of EPA and low level of ARA in aller aqua and nutreco diet than goldfish reduced the production of ARA-derived, series II PGs, implicated in the maturation and ovulation processes. In spawners with up to 6 months of vitellogenesis, broodstock must be fed a good-quality diet for several months before the spawning season to improve their reproductive performance.

In the present study, relatively low ratios EPA/DHA was found in the gonads of wild 2015 broodstock (0.18 ± 0.03) compare to that of aller aqua, nutreco and goldfish fed broodstock (0.37 ± 0.03, 0.35 ± 0.02 and0.30 ± 0.03 respectively) . Similar findings on relative proportions of DHA and EPA have been reported in fish eggs (Suloma & Ogata, 2012). Therefore, DHA must be superior to EPA in the broodstocks diets for this species. Our results also demonstrate a relatively low concentration of the other essential PUFA linoleic acid (LA) and α-linolenic acid (ALA) in gonad samples of experimental broodstock at spawning compared to one month before. This could be due to capacity of freshwater fish to convert these fatty acids to the higher homologues such as EPA, DHA and ARA, hence absolute amounts of LA and LNA in the flesh and gonads fish will decrease, while that of EPA, DHA and ARA increases (Sargent *et al*., 1999). Total n-3 HUFAs in the gonad, liver and visceral fat of all experimental broodstock was higher than n-6 HUFAs except for wild 2015 gonad and visceral fat for samples corrected one month before spawning. At spawning period, the ratios of n-6/n-3 found to be significantly higher for wild 2015 broodstock (0.61 ± 0.21) compared to that of aller aqua, nutreco and goldfish fed broodstock (0.21 ± 0.13, 0.23 ± 0.02 and 0.30 ± 0.04 respectively). Since both n-3 and n-6 HUFAs are critical during organogenesis in embryos and larvae, a balanced ratio of n-6/n-3 is required in broodstock diet for optimum reproductive success of fish (Bell *et al*., 1997).

Larval quality has been reported to be affected by broodstock nutritional status (Wang *et al*., 2008: Henrotte *et al*., 2010). Bell *et al*. (1997) and Furuita *et al*. (2007) showed that the ratio of EPA to ARA may be a critical nutritional factor for embryo and larval development and survival. For example, Japanese eel (*Anguilla japonica*) fed a diet with an EPA/ARA ratio of 15 resulted in low egg production and hatching rates, together with an increase in abnormal larvae percentage (Furuita *et al*., 2007). DHA plays an important role in the embryonic development as well as in early larval stage. In this study, larval quality was investigated by performing osmotic and starvation stress test on 1dph larvae from group of wild 2014 and wild 2015 broodstock. The cumulative stress index and survival performed on larvae showed that larvae from wild 2015 broodstock were more sensitive to starvation and osmotic stress than those from wild 2014 broodstock. Since low stress index was observed on wild 2014 larval and high percentage survival than wild 2015 larvae.Thus indicate low larval quality for wild 2015 larvae. This is similar to the findings by Khemis *et al*. (2013) where larvae from captive wild female fed chopped marine fish for one year performed better than larvae from females caught directly from the wild. This difference in larval quality can be associated with several factors including the

size and FA composition of the egg. Egg size has been documented to positively correlate with the size of newly hatched fish larvae (Gisbert *et al*., 2000; Kennedy *et al*., 2007). According to Schlumberger and Proteau (1996), larvae from small eggs (800–1000µm diameter) show low viability after hatching. For both wild 2015 and wild 2014 female, the obtained eggs were within this range and thus could not be considered of good quality in terms of larvae potential viability. However, wild 2014 had big egg (866 ± 120µm) compare to that of wild 2015 broodstock (844 ± 11.18µm). Larger eggs were reported to associate with larger larval energetic reserves (Bang *et al*., 2006; Donelson *et al*., 2008).

In Eurasian perch, differences in larvae robustness were observed regarding the fatty acids (FA) profile in the eggs, which was related to the dietary FA composition of breeders (Henrotte *et al*. 2010). Similar observations was found for pikeperch eggs and larvae in the present study, where total FAME in wild 2014 gonad and larvae (325.57 ± 60.30mg/g DW and 345.67mg/g DW respectively) was higher than that of wild 2015 broodstock (2015 (296.39 ± 28.47mg/g DW and 277.75mg/g DW respectively). Thus, the composition of FA in egg was reflected in larvae, considering the different diets previously consumed by spawning females. Moreover, the PUFA consisted mainly of fatty acids from n-6 series, the eggs from wild 2015 broodstocks, while contrarily, the PUFA were mainly from n-3 series in the eggs from wild 2014 broodstock. It is interesting to note that levels of DHA and EPA in the eggs were highly comparable to that of larvae from corresponding broodstock. HUFA rates were higher with the DHA being the most important component and EPA showing more than a 2-fold increase in egg and larvae from wild 2014 compared to eggs and larvae from wild 2015 females, while the latter had high ARA. These differences significantly affected the ratios (n-3/n-6), (DHA/EPA), and all the other FA specific indicators. In contrast, the study conducted by Akhter. (2014 unpublished) on the quality of larvae from wild 2014 and hatchery broodstock fed goldfish showed that larvae from wild 2014 broodstock (unfed after capture from the wild until spawning) performed better than larvae from hatchery broodstock fed same goldfish used as diet of wild 2014 in this experiment. This suggests that, larval quality is not affected only by nutritional status of broodstock but also can be affected by origin and genetic variation of broodstock. Fontaine *et al*. (2008) has drawn attention to the lower quality of reproduction in farmed perch bred under controlled conditions.

These authors add that, to address lower reproduction in farmed perch, it is necessary to improve the diet by adding prey fish and a larger proportion of natural ingredients to the feed.

6. CONCLUSIONS AND RECOMMENDATION

In conclusion, it is evident that nutritional status of broodstock has effect on the reproductive quality due to clear difference in reproductive performance of pikeperch females from wild and hatchery origin, but also difference in broodstock of the same origin fed different diet. Morpho-anatomic parameters performed on broodstock (GSI, HIS, VSI, fecundity & oocyte maturation) clearly show that, wild broodstocks were sexually more mature than hatchery pikeperch at the same spawning period. Fatty acid composition of diet has a marked influence on the quality of the egg spawned by broodstock, showing that the ratio of EPA/DHA present in the egg correlated direct that present in larvae which was important in early larval development. Moreover, reduced fecundity and spawning failure in hatchery broodstock was associated with excess egg composition of EPA, this alone should not be used as a criterion to assess the egg quality of broodstock. Furthermore, the ratio EPA/ARA determines the quality of reproduction to a huge extent by interrupting the production series II PGs, implicated in the maturation and ovulation processes. Therefore, it is necessary to take into consideration not only the individual levels of HUFAs but attention should be paid to the DHA/EPA/AA ratio in broodstock diet for improving egg and larval quality. The results suggest that commercial diet developed for sturgeon (aller aqua) and seabass (nutreco) breeders, is clearly not suitable for pikeperch broodstocks, suggesting that the development of diets for breeders must be more species-specific. In addition, by measuring the egg size the hatchery manager can estimate the maturation stage by using the equation obtained on correlation of oocyte diameter and maturation stage. More studies need to be conducted to determine the minimum and maximum value of DHA/EPA/ARA ratio and needed for pikeperch broodstock diets.

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