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Fatty acid utilization of pikeperch (*Sander lucioperca* (Linnaeus, 1758)) larvae under starvation conditions during early development

Die Nutzung von Fettsäuren durch Larven des Zanders *Sander lucioperca* (Linnaeus, 1758) unter Hungerbedingungen während der Frühentwicklung

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Summary. The cultivation of pikeperch (*Sander lucioperca*) is gaining more and more interest from research and production, but essential questions concerning the physiology during its life cycle, such as required quantity and composition of fatty acids of the supplied feed, are still not answered satisfactorily. Therefore, an experiment was conducted to investigate the utilization of fatty acids as well as the morphological development of pikeperch during the first ten days after hatching. Larvae were maintained in a recirculating aquaculture system with continuous air supply. Life and formulated feed were regularly supplied to the two treatment groups but none of the feed was ingested. Daily samples were taken for morphological measurements as well as fatty acid analyses. Until the visible consumption of the yolk sac (day post hatch 6) somatic growth was recorded, together with a decrease in individual dry mass and total fatty acid concentrations of the larvae. After complete consumption of the yolk sac, total fatty acid concentrations of the larvae dropped significantly. Simultaneously, somatic growth stopped and until the depletion of the oil droplet (day post hatch 8) only the eye diameter and mouth opening increased further. Our experiment clearly demonstrates the initial utilization of internal resources for regular growth of pikeperch larvae, highlighting the importance of specific fatty acids, such as saturated fatty acids providing energy for the metabolism during the early larval development.

Key words: Pikeperch physiology, fatty acid composition, nutritional requirements, early life stages, morphological development

Zusammenfassung. Die Möglichkeiten zur Aquakultur des Zanders (*Sander lucioperca*) erfahren immer mehr Interesse aus Forschung und Produktion, aber wesentliche Fragen der Physiologie während seines Lebenszyklus, wie beispielweise die benötigte Menge sowie die Zusammensetzung der Fettsäuren des zugeführten Futters, können noch nicht befriedigend beantwortet werden. Daher wurde ein Experiment durchgeführt, welches die Nutzung der Fettsäuren sowie die morphologische Entwicklung von Zanderlarven in den ersten zehn Tagen nach Schlupf untersuchte. Die Larven wurden in einem rezirkulierenden Kreislaufsystem mit kontinuierlicher Luftzufuhr gehalten. Die beiden Futterarten, Lebendfutter und formuliertes Larvenfutter, wurden den beiden Behandlungsgruppen regelmäßig gefüttert, aber keines der angebotenen Futtermittel wurde von den Larven aufgenommen. Täglich wurden Proben für die morphologischen Messungen sowie für Fettsäureanalysen entnommen. Bis zur sichtbaren Aufzehrung des Dottersacks (Tag 6 nach Schlupf) wurde somatisches Wachstum zusammen mit einer Abnahme der individuellen Trockenmasse und der Gesamtfettsäurekonzentrationen der Larven festgestellt. Nach der vollständigen Aufzehrung des Dottersacks sanken die Gesamtfettsäurekonzentrationen der Larven signifikant ab. Gleichzeitig stoppte das somatische

Wachstum und bis zur Aufzehrung der Ölkugel (Tag 8 nach Schlupf) nahmen lediglich der Augendurchmesser und die Maulspalte weiter zu. Unser Experiment konnte deutlich die anfängliche Nutzung der internen Ressourcen für das regelmäßige Wachstum von Zanderlarven zeigen und unterstreicht die Bedeutung von bestimmten Fettsäuren, wie gesättigte Fettsäuren, welche Energie für den Stoffwechsel während der frühen Larvenentwicklung liefern.

Schlüsselwörter: Physiologie des Zanders, Nährstoffbedarf von Larven, Fettsäurezusammensetzung, morphologische Entwicklung

1. Introduction

Global aquaculture production of pikeperch *Sander lucioperca* (Linnaeus, 1758) often still depends on wild catches as broodstock for stocking the production systems. Pikeperch is well established as a by-product of carp production in ponds, but recently it gained attention as a promising candidate for aquaculture in monoculture (Ronyai 2007; Lund & Steenfeldt 2011). Since 1990, global pikeperch production in aquaculture systems increased to about 800 t in 2012, with some fluctuations in previous years (FAO 2017). Comparing the amount of produced and captured pikeperch in 2016, aquaculture still accounts only for a minor fraction, approximately 4.5% of globally available pikeperch on the market.

Pikeperch has a high potential for the European aquaculture business, due to its high price and market demand. Therefore, scientific efforts are made to optimize the productivity of pikeperch culture (Knaus et al. 2008; Knaus & Gallandt 2010; Bischoff et al. 2012). The year-around availability of larvae and fingerlings still remains a bottleneck, as it is a prerequisite to support the economic development of a sustainable pikeperch culture (Steenfeldt 2015). This issue becomes more obvious considering two critical phases during pikeperch larval development, which are directly after absorption of the yolk sac and later on during weaning to artificial feed. We assume that the major requirements for a successful establishment of exogenous feed uptake are a suitable biochemical composition, size and density of prey items. Failure in one of these factors leads to starvation, until the larvae reach the point of no return and die.

In fishes the major energy substrates are lipids (Bell & Tocher 1989; Sargent 1995) and they are also crucial for larval development (Rainuzzo et al. 1997; Sargent et al. 1999). Normal growth and survival of fish strongly depend on the availability of n-3 and n-6 fatty acids in the diet. Initially required lipids for larval survival originate from their yolk sac reserve. After the consumption of the yolk sac necessary nutrients have to be obtained by exogenous feeding. In case exogenous feed uptake fails the missing fatty acids have to be re-synthesized from other tissues of the larval body. Fish have very limited abilities to synthesize these components de novo. Bioconversion capacity, i.e. the transformation of precursors into poly-unsaturated fatty acids (PUFAs), is known for some freshwater fish species. This has been demonstrated for rainbow trout (Buzzi et al. 1996), Japanese eel (Kanazawa et al. 1979) and for northern pike (Buzzi et al. 1997). Some marine fish species, such as gilthead sea bream (*Sparus aurata*) also seem to be able to transform precursors into PUFAs (Bischoff et al. 2009). Some of these fatty acids, e.g. docosahexaenoic acid (DHA, 20:5), eicosapentaenoic acid (EPA, 22:6) and arachidonic acid (ARA, 20:4), are dominant structural components in cell membranes (e.g. brain and retina) and precursors of physiologically very active molecules such as eicosanoids (Goetz et al. 1989a; Goetz et al. 1989b; Bell et al. 1992).

Data elucidating the nutritional requirements of percid fishes are available (Brown et al. 1996; Fiogbé et al. 1996; Kestemont et al. 1996; Kestemont et al. 2001) but only a part of these data are available for juveniles of pikeperch (Molnar et al. 2004; Molnar et al. 2006; Nyina-Wamwiza et al. 2005; Schulz et al. 2005; Schulz et al. 2006). Furthermore, studies highlighting the fatty acid profiles during the first days of pikeperch larval development are very limited.

Abi-Ayad et al. (2004) published one of these rare data sets concerning the fatty acid profiles of pikeperch larvae before 10 days post hatch (dph), cultured under feed-deprivation, which illustrates the actual fatty acid utilization. Unfortunately, this study is not able to answer all questions. The abiotic water parameters were missing. Therefore, the interpretation of the presented fatty acid results is hardly possible due to the temperature influence on metabolic processes. According to the authors, in starved larvae fatty acids were utilized as metabolic substrates until day 9 and at day 10 the fatty acid levels remained stable or, at least, increased in larval body. Furthermore, critical events during ontogeny of the larvae, such as the complete consumption of the yolk sac or the oil droplet were not considered. Consequently, additional evidence concerning the fatty acid utilization of pikeperch during their early life stages is strongly needed, e.g. for the refinement of species and size specific feeding regimes or for the development of proper starter feeds for weaning sensitive fish larvae, such as pikeperch.

The purpose of the present study was to analyze the fatty acid utilization of pikeperch larvae, additional to the record of morphological data, during the first ten days of larval development. We expected no feed uptake during the first days of larval development under yolk sac consumption, followed by subsequent uptake of *Artemia* nauplii or dry feeds, depending on the treatment group, providing the respective fatty acids. Unintentionally, no feed at all was ingested by the pikeperch larvae, which resulted in a starvation experiment instead of a feeding trial. According to the experimental design the larvae were observed over time with respect to changes in fatty acid profiles as well as morphological development.

2. Materials and Methods

2.1. Husbandry of pikeperch larvae

The intended experiment was in accordance with the guidelines and legislation in force, such as the Protection of Animals Act, and the permission for keeping fish larvae under farming conditions was granted according to the required regulations.

The pikeperch larvae used for the experiment were all from a single batch and they hatched at the end of May (natural spawning period). They were obtained one day post hatch (dph 1) from a commercial fish farm of the Institute for Fisheries of the State Research Centre for Agriculture and Fishery Mecklenburg-West Pomerania (LFA MV) in Hohen Wangelin. After arrival at the laboratory of the University of Rostock, the larvae were acclimatized (from 16 °C to approximately 19 °C) for about four hours, before they were randomly distributed into six individual rectangular 10 liter plastic tanks. These tanks were filled with 8 liters of freshwater, which were constant aerated. Each tank was part of a freshwater recirculation system, including a water treatment compartment, which consisted of a settling tank, a moving bed biofilter, aeration and temperature regulation. Each individual tank was equipped with an overflow device, where an 100 µm gauze prevented the escape of the larvae from the tank through the overflow device. The water to the individual aquaria was supplied via gravity from an elevated tank with a flow-through rate of 2 $L*h^{-1}$. This elevated tank was part of the recirculation system and constantly refilled.

Pikeperch larvae were stocked according to literature (Zienert & Heidrich 2005), reaching stocking densities between 100 and 200 $individuals*L^{-1}$. The experiment was performed under very low light intensities (< 10 Lux). This was ensured by shading devices at the windows and by a wooden frame equipped with a 0.5 mm thick plastic foil, which was only removed during feeding and cleaning intervals. Water temperature was set at 21 °C (± 1 °C) via a flow-through cooling device, which was installed within the recirculation system. Cleaning of the individual tanks was done once a day in the morning prior to the first feeding of the day by syphoning dead larvae and feed items from the bottom of the tanks.

2.2. Feeding

The six tanks holding the pikeperch larvae were divided into two triplicate treatment groups. The first group was fed dry feed (Perla larva 6.0, grain size 100 – 200 µm) an amount of approximately 100 mg*day^{-1}, which was above the calculated feed requirements of the stocked larvae. The feed was applied automatically during 8 hours (09:00 – 17:00) one patch of feed every 30 minutes. The second group was fed with live *Artemia* nauplii (Sanders, Great Salt Lake Artemia Cysts, Utah, USA), which were used directly after hatch and were not enriched. *Artemia* cysts were decapsulated according to the procedure described by SORGELOOS et al. (1978). Hatching of decapsulated *Artemia* was performed in V-shaped hatching containers (PET) containing 1 liter water with a salinity of 30 psu under constant aeration. *Artemia* nauplii were applied four times a day (09:00, 11:30, 14:00, and 16:30). Before each feeding event the approximate amount of required *Artemia* nauplii were collected from the hatching containers, rinsed and suspended with freshwater. Cultured *Artemia* nauplii were not enriched or fed before use. Concentrations of *Artemia* nauplii within the holding tanks were between 2 – 4 individuals*mL^{-1}.

2.3. Experimental conditions

The abiotic parameters temperature [°C], oxygen saturation [%], oxygen content [mg*L^{-1}] and pH were recorded with a hand-held measuring device HQ40d (Hach Lange GmbH, Düsseldorf, Germany), the latter three parameters via individual probes. Temperature recordings were carried out simultaneously, in association with the pH-value or the oxygen probe. Triplicate measurements were performed every second day for each individual tank.

The dissolved nitrogen compounds ammonia (NH_3/NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) as well as orthophosphate (PO_4^{3-}) were analyzed with the spectral photometer DR3900 (Hach Lange GmbH, Düsseldorf, Germany) according to the protocol by Hach Lange (method no. for ammonia 8155, nitrite 8507, nitrate 8039 and orthophosphate 8048). All mentioned dissolved nutrients were analyzed daily in triplicate during the first three days of the experiment, from experimental day 4 (dph 4) until the end of the experiment triplicate analyses were performed every second day.

2.4. Morphometric measurements

All measurements were performed using the stereo-microscope SZX10 (Olympus, Hamburg, Germany) and the software package cellSens Dimension 1.6 (Olympus Soft Imaging Solutions, Münster, Germany).

15 fish larvae per sample were collected from the individual tanks and stored in 70% methanol until measurement, which was performed between 15 to 20 days after sampling. Average total length of fish larvae was measured by recording the longest distance between the tip of the head and the tip of the pectoral fin of a total of ten pikeperch larvae per sampling day. Specific growth rate (SGR) [%*d^{-1}] of the pikeperch larvae was calculated by adapting the formula from Jørgensen (1990):

$$SGR = (\ln(L_t/L_0)*t^{-1})*100 \quad (1)$$

L_t and L_0 represent the average length of the larvae at time t and time t = 0, the start of the experiment at dph 1.

Average yolk sac volume was recorded measuring the maximum length as well as the maximum width of the individual yolk sac and calculating the volume of an ellipsoid body:

$$\text{Yolk sac volume} = 4/3*\pi*L*W^2 \quad (2)$$

L represents the length and W the width of the yolk sac.

Average oil droplet volume was recorded measuring the largest diameter of the individual oil droplets and calculating the volume of a sphere:

$$\text{Oil droplet volume} = 4/3*\pi*r^3 \quad (3)$$

r represents the radius of the oil droplet.

Average eye diameter of the fish larvae was recorded by measuring the largest diameter of one eye of the individual larvae. For the measurement of the average mouth opening each individual larva had to be positioned dorso-ventrally under the stereo-microscope.

Measurements of the mouth openings were performed in case the mouth opening already existed, so the number of measurements varied for the first few days of the experiment.

2.5. Fatty acid analyses

For lipid analyses one sample consisting of 15 pikeperch larvae were collected daily from each individual culture tank. Immediately after sampling the larvae were stored individually in pre-combusted (12 h at 400 °C) glass vials, which were pre-cooled (-80 °C) before use, and stored at -80°C until analysis.

The larvae samples were lyophilized and transferred into pre-combusted and pre-weighed glass vials for dry weight measurement. Subsequently, the dry weight of the samples was divided by the corresponding number of fish larvae collected for the sample to receive the dry weight of individual larvae.

Lipid extraction was conducted as described by Peters et al. (2006) based on Folch et al. (1957) by homogenizing the tissue in a dichloromethane:methanol solution (2:1/v:v) for at least 24 h, followed by ultrasonic disruption (twice). Fatty acids were quantified by adding an internal standard (tricosanoic acid 23:0). Lipids were separated with aqueous KCl solution (0.88%) and centrifuged for 10 min with 1500 g at 2 °C. Fatty acids were derivatized to methyl esters (FAMEs) with 1 mL methanol containing 3% sulphuric acid and 0.25 mL hexane at 80 °C for 4 h. FAMEs were extracted with 2 mL aqua bidest. and hexane (3 x 1 mL) and analyzed by gas-liquid chromatography as described by Kattner & Fricke (1986) and modified by Peters et al. (2006). For fatty acid analyses a Hewlett-Packard gas chromatograph (HP 6890A) and an Agilent Technologies gas chromatograph (7890A) were used, which were equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness). Temperature-programming with helium as carrier was applied. Samples were injected using a hot split/splitless inlet (250 °C, split mode 1:20) or a programmable temperature vaporizer injector (Gerstel® CIS3). FAMEs were detected by flame ionization and identified by comparing the retention times of detected peaks with those derived from several standards of known composition.

2.6. Mortality

Mortality was determined according to the formula:

$$\text{Mortality} = (\text{calculated number of fish}_{t+1} / \text{calculated number of fish}_{t})*100 \quad (4)$$

t represents a sampling day during the experiment and t+1 represents the next day during the experiment. For each tank and each experimental day five homogenous samples were taken to calculate the average stocking density. After each sampling and counting the larvae were set back into the tanks.

2.7. Statistical analyses

All statistical analyses were performed by applying the software IBM SPSS Statistics, version 20. Normal distribution was tested by the Kolmogorov-Smirnov test and homogeneity of variance by Levene's test. To analyze differences between means either a t-test or an Analysis of Variance (ANOVA) was performed followed by the Tukey-Kramer method, in case significant results were obtained. Significance level α was set to 0.05 for all statistical analyses.

3. Results

Larvae were constantly offered *Artemia* nauplii during the entire experimental period. They tried to catch the nauplii, but failed to ingest them. This could be observed either directly after feeding or during the analysis of the larvae under a stereo-microscope, where no *Artemia* nauplii were detectable in their guts. Larvae also swam towards the dry feed, but showed not much interest to ingest it. Therefore, during subsequent analyses no feed items could be detected in the intestinal tract of all sampled pikeperch larvae.

Husbandry conditions with respect to abiotic parameters were adequate during the experimental period (tab. 1). No significant differences

Tab. 1: Recorded abiotic conditions during the experimental period (n=30) and concentrations of dissolved nitrogen and phosphorous compounds [mg*L^{-1}] in the holding tanks with the pikeperch larvae (n=33)
Tab. 1: Die abiotischen Bedingungen während des Versuchszeitraums (n = 30) und ermittelte Konzentrationen der gelösten Stickstoff- und Phosphorverbindungen in [mg * L^{-1}] in den Versuchsbecken der Zanderlarven (n = 33)

Parameter	Mean value	Standard deviation (SD)
Oxygen concentration [mg*mL^{-1}]	8.5	0.5
Oxygen saturation [%]	96.3	4.3
Temperature [°C]	21.6	1.6
Salinity	0.8	1.2
pH value	8.36	0.08
Ammonia (NH_3/NH_4^+) [mg*L^{-1}]	0.1	0.1
Nitrite (NO_2^-) [mg*L^{-1}]	0.2	0.2
Nitrate (NO_3^-) [mg*L^{-1}]	8.1	2.1
Orthophosphate (PO_4^{3-}) [mg*L^{-1}]	3.6	0.6

for the husbandry conditions were recorded between the different experimental tanks and therefore the abiotic data were pooled for further analysis.

Concentrations of dissolved nitrogen and phosphorous compounds were within the range suggested for pikeperch larvae (tab. 1), despite the unconsumed *Artemia* and dry feed. Significant differences between the experimental tanks regarding the dissolved nutrient compounds were not detectable. Therefore, the data for the dissolved nutrients were pooled.

The average total length of larvae at stocking (dph 1) was 4693 ± 303 µm (fig. 1), which was statistically smaller compared to all other lengths ($p < 0.05$). Until dph 6 larvae grew to a total length of 5870 ± 238 µm. After that, growth stagnated. Until the end of the experiment, no further growth and consequently no significant differences could be detected for the total

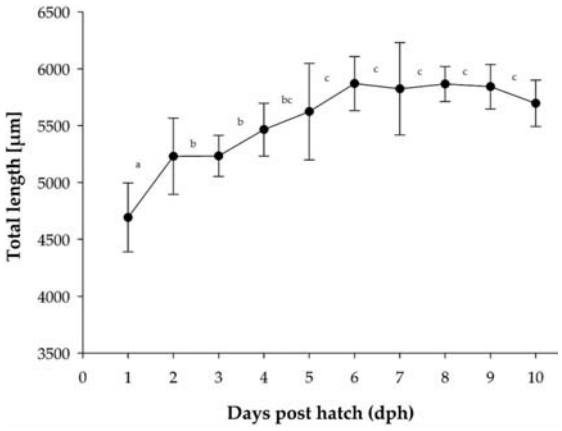

Fig. 1: Total length of pikeperch larvae (mean ± SD, n≥15; length variations between days marked with different annotations indicate significant differences).
Abb. 1: Gesamtlänge der Zanderlarven (Mittelwert ± Standardabweichung, n ≥ 15; Längenabweichungen zwischen den Tagen, die mit verschiedenen Buchstaben versehen sind, weisen auf signifikante Unterschiede hin).

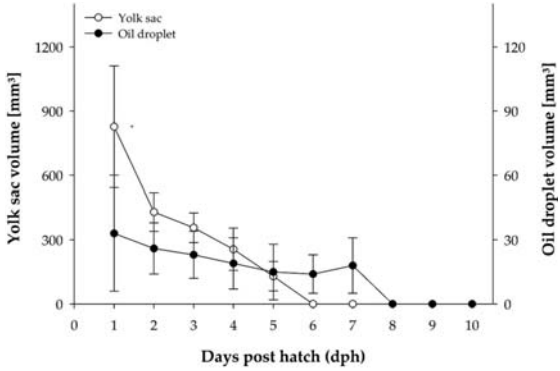

Fig. 2: Yolk sac and oil droplet volume of the pikeperch larvae (mean ± SD, n≥4; asterisk indicates a significant difference between experimental day 1 and all other experimental days for the yolk sac volume).
Abb. 2: Dottersack- und Öltröpfchenvolumen der Zanderlarven (Mittelwert ± SD, n ≥ 4; der Stern weist auf einen signifikanten Unterschied zwischen Versuchstag 1 und allen anderen Versuchstagen für das Dottersackvolumen hin).

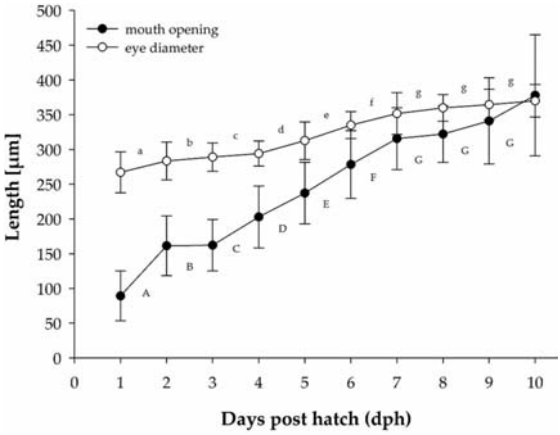

Fig. 3: Length of mouth opening and eye diameter recorded for pikeperch larvae during the experiment (mean ± SD, n≥16; size variations between experimental days marked with different annotations indicate significant differences).
Abb. 3: Maulspaltenöffnung und Augendurchmesser der Zanderlarven während des Experiments (Mittelwert ± Standardabweichung, n ≥ 16; Größenunterschiede zwischen den mit unterschiedlichen Buchstaben markierten Versuchstagen weisen auf signifikante Unterschiede hin).

lengths of the larvae ($p > 0.05$). Between the dph 1 and 6 the average specific growth rate (SGR) was 4.5 ± 4.0%*d^{-1} and between dph 7 and 10 it was -0.8 ± 1.4%*d^{-1}.

The yolk sac volume decreased significantly during the experiment ($p < 0.05$) (fig. 2), until reaching the last recordable volume at dph 5. At dph 6 no visible remains of the yolk sac could be detected. The oil droplet lasted two days longer than the yolk sac (fig. 2), but decreased also significantly during the course of the experiment ($p < 0.05$). At dph 8 no oil droplet could be detected in any of the investigated pikeperch larvae.

The lengths of the mouth openings as well as the diameters of the eyes are given in figure 3. At dph 1 only three larvae with an existing mouth opening could be recorded. During the next days this number increased and at dph 3 all pikeperch larvae had an open mouth.

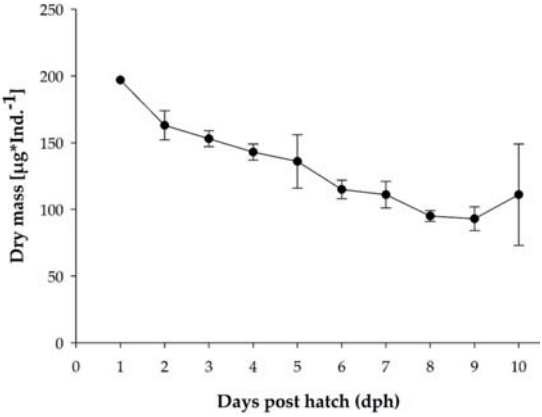

Fig. 4: Dry mass of pikeperch larvae (mean ± SD, n≥5; no annotations are given but significant differences over time could be observed).
Abb. 4: Trockenmasse der Zanderlarven (Mittelwert ± SD, n ≥ 5; es wurde in der Grafik nicht dargestellt, aber es sind signifikante Unterschiede im zeitlichen Verlauf des Versuchs beobachtet worden).

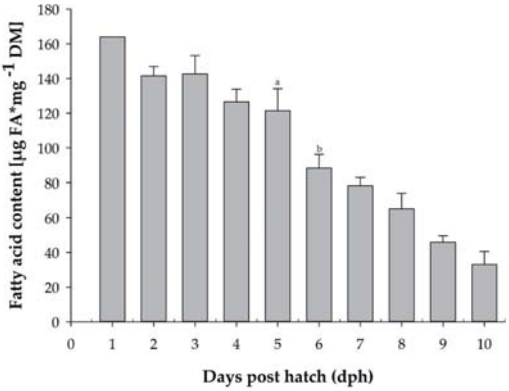

Fig. 5: Total fatty acid contents (means ± SD, on a dry mass basis; n≥3, except experimental day 1, where n=1; different annotations for experimental days 5 and 6 indicate significant difference for consecutive days).
Abb. 5: Gesamtgehalt der Fettsäuren (Mittelwerte ± Standardabweichung, bezogen auf die Trockenmasse; n≥3, mit Ausnahme des Versuchstages 1, an dem n = 1; verschiedene Buchstaben für die Versuchstage 5 und 6 weisen auf signifikante Unterschiede für die beiden aufeinanderfolgende Tage hin).

The size increased steadily until the end of the experiment and reaching a final length of 378 ± 87 µm. Increases in the mouth opening were significant for consecutive days until dph 7 ($p < 0.05$), after dph 8 no further significant increase was recorded ($p > 0.05$).

The mean diameter of the eyes of the pikeperch larvae also increased steadily (fig. 3) until the end of the experimental period. Until dph 7 the eyes grew significantly ($p < 0.05$).

Dry mass data of the larvae are presented in figure 4. During the experiment the larval dry mass decreased, reaching its minimum at dph 9. The observed decrease in dry mass over time (comparing initial and final dry mass) was statistically significant ($p < 0.05$).

Fatty acid data of the larvae are given in figure 5 and table 2. The total fatty acid (TFA) content decreased throughout the experiment. Most of these differences between consecu-

Tab. 2: Fatty acid dynamics in pikeperch larvae during the experiment (mean ± SD in µg FA*mg^{-1}, on a dry mass basis). (Experimental day 1: n=1 (15 pooled fish larvae), due to technical difficulties during the storage of the samples; all other days: n≥3 (3 x 15 pooled fish larvae).)
Tab. 2: Fettsäuredynamik in den Zanderlarven während des Experiments (Mittelwert ± Standardabweichung in µg FS * mg^{-1}, bezogen auf die Trockenmasse). (Versuchstag 1: n = 1, d.h. 15 Fischlarven zu einer Probe gepoolt; aufgrund technischer Schwierigkeiten während der Lagerung der Proben; alle anderen Tage: n ≥ 3, d.h. mindestens 3 x 15 Fischlarven, gepoolt.)

	Experimental days					
Fatty acids	**1**	**3**	**5**	**6**	**8**	**10**
14:0	1.5	1.3 ± 0.8	0.9 ± 0.1	0.6 ± 0.2	0.4 ± 0.0	0.2 ± 0.1
16:0	13.5	14.7 ± 1.0	16.9 ± 2.1	12.8 ± 1.3	12.6 ± 0.4	7.6 ± 2.5
18:0	4.1	4.8 ± 0.1	5.1 ± 0.7	4.6 ± 0.2	5.4 ± 0.2	3.9 ± 0.9
∑ SFAs	19.5	21.1 ± 1.7	22.9 ± 2.8	18.0 ± 1.6	18.4 ± 0.4	11.8 ± 3.4
16:1(n-9)	5.0	3.9 ± 0.1	3.1 ± 0.2	2.0 ± 0.3	1.4 ± 0.3	0.5 ± 0.3
16:1(n-7)	29.8	24.2 ± 1.5	19.0 ± 1.6	12.0 ± 1.9	6.1 ± 1.2	1.3 ± 0.7
16:1(n-5)	1.4	1.1 ± 0.0	0.8 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.0 ± 0.1
18:1(n-9)	31.5	27.8 ± 1.3	25.4 ± 2.4	18.5 ± 1.9	9.8 ± 6.5	3.0 ± 2.8
18:1(n-7)	9.2	7.9 ± 0.3	6.9 ± 0.6	4.8 ± 0.5	3.2 ± 0.3	1.3 ± 0.5
18:1(n-5)	0.4	0.3 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
20:1(n-9)	0.7	1.4 ± 1.9	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.2 ± 0.1
24:1(n-9)	0.5	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
∑ MUFAs	78.9	67.9 ± 6.5	56.6 ± 5.1	38.8 ± 4.5	21.7 ± 8.1	6.8 ± 2.0
16:2(n-4)	0.6	0.5 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
16:3(n-4)	2.0	1.6 ± 0.1	1.5 ± 0.2	1.0 ± 0.1	0.6 ± 0.1	0.2 ± 0.1
16:4(n-1)	1.2	0.7 ± 0.4	0.7 ± 0.1	0.7 ± 0.7	0.3 ± 0.2	0.1 ± 0.1
18:2(n-6)	6.7	5.5 ± 0.3	4.8 ± 0.7	3.4 ± 0.3	1.9 ± 0.2	0.6 ± 0.2
18:3(n-6)	0.6	0.5 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
18:3(n-3)	6.0	4.7 ± 0.1	3.6 ± 0.4	2.3 ± 0.2	1.0 ± 0.2	0.2 ± 0.1
20:4(n-6)	6.1	5.3 ± 0.3	4.8 ± 0.6	3.9 ± 0.2	3.8 ± 0.1	2.4 ± 0.6
20:3(n-3)	0.5	0.4 ± 0.0	0.2 ± 0.2	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
20:4(n-3)	1.6	1.3 ± 0.0	0.8 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.0 ± 0.1
20:5(n-3)	6.6	5.9 ± 2.2	3.8 ± 0.5	2.4 ± 0.2	1.6 ± 0.1	0.7 ± 0.2
22:5(n-3)	4.4	3.3 ± 0.2	2.3 ± 0.4	1.7 ± 0.2	1.0 ± 0.2	0.5 ± 0.1
22:6(n-3)	28.1	23.2 ± 0.6	19.3 ± 2.3	15.3 ± 1.0	14.5 ± 0.6	9.5 ± 2.5
∑ PUFAs	65.6	53.7 ± 2.8	41.8 ± 5.0	31.4 ± 2.2	25.1 ± 1.3	14.5 ± 3.7
∑ TFAs	164.0	142.6 ± 10.6	121.3 ± 12.8	88.2 ± 8.0	65.2 ± 8.9	33.0 ± 7.5
∑ C18 PUFAs	13.9	11.0 ± 0.5	8.4 ± 1.0	5.7 ± 0.5	2.9 ± 0.4	0.9 ± 0.3
∑ C20+22 PUFAs	48.0	39.8 ± 2.7	31.2 ± 3.8	24.0 ± 1.5	21.4 ± 1.0	13.3 ± 3.3
∑ (n-3)	47.7	39.1 ± 2.7	30.0 ± 3.5	22.2 ± 1.6	18.4 ± 0.9	11.0 ± 2.9
∑ (n-6)	14.1	11.7 ± 0.6	9.6 ± 1.3	7.5 ± 0.4	5.9 ± 0.2	3.2 ± 0.7
(n-3/n-6)	3.4	3.3 ± 0.2	3.1 ± 0.1	3.0 ± 0.1	3.1 ± 0.0	3.4 ± 0.3
DHA/EPA	4.3	4.2 ± 0.9	5.0 ± 0.4	6.3 ± 0.2	9.3 ± 0.7	12.8 ± 0.1
C18/C20+22	0.3	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

tive experimental days were not significant (fig. 5). The only exception was between dph 5 and 6, when TFA dropped significantly ($p < 0.05$). In terms of TFAs an initial mean content of 164.0 $\mu g*mg^{-1}$ DM was determined for the larvae. At dph 10 a TFA content of 33.0 ± 7.5 $\mu g*mg^{-1}$ DM was retained in larval bodies. A net depletion of 130 $\mu g*mg^{-1}$ DM and a final portion of 20% of the initial content could be detected under feed deficiency. The observed decrease in TFAs over time (comparing initial and final TFAs) was statistically significant ($p < 0.05$).

Table 2 presents all fatty acids separated into groups according to their level of saturation. The sum of all saturated fatty acids (SFAs) remained rather stable during most time of the experiment but decreased significantly at the end of the experimental period ($p < 0.05$). During the course of the experiment total monounsaturated fatty acid content (MUFAs) significantly declined ($p < 0.05$). At the end of the experiment the sum of MUFAs was lower than the sum of SFAs. Polyunsaturated fatty acids (PUFAs) also declined significantly during the course of the experiment ($p < 0.05$). Until dph 7 the sum of PUFAs was lower than that of the MUFAs but higher than that of the SFAs. At dph 8 this situation switched and the PUFAs showed the highest remaining concentrations until the end of the experiment.

In newly hatched larvae MUFAs (48.1%) were the dominant fatty acids (78.9 $\mu g*mg^{-1}$ DM), followed by PUFAs (40.0%) and SFAs (11.9%). MUFAs were the most intensely utilized fatty acids under feed deficiency. 10 days post hatch, total MUFAs were reduced to 9% of the initial MUFA content. In terms of SFAs and PUFAs a ratio of 60% and 22% of the initial content was retained.

Mortality during the first two days post hatch ranged between 10% and 15%. During the following days mortality increased resulting in a cumulative mortality of 90% after dph 5. Subsequently, mortality rates decreased and reached 100% one day after the final sampling at dph 10.

4. Discussion

Husbandry conditions with respect to abiotic parameters were adequate during the experimental period, set within the range described as optimal (Zienert & Heidrich 2005) for pikeperch larvae, except for the pH value. This was slightly above the optimal range but still in the range of tolerability. Due to the fact, that the abiotic conditions for the culture of the larvae were appropriate, normal behavior as well as adequate survival and development of the larvae was expected. However, none of the food items offered to the larvae were ingested by them, neither *Artemia* nauplii, nor the commercial dry feed. This is contrast to Ostaszewska et al (2005) and Ostaszewska & Boruta (2006) who stated that either *Artemia* nauplii alone or dry feed alone could be an adequate starting feed for pike-perch larvae. Therefore, the present study elucidates growth and utilization of fatty acids in pikeperch larvae during the first ten days of their development under starvation conditions and not as intended during a feeding trial.

Somatic growth occurred, as long as the yolk sac provided sufficient nutritional resources. However, after absorption of these reserves somatic growth stagnated and the larvae showed signs of starvation. The supplied feed, live as well as dry feed, was apparently not ingested or not adequately utilized. For *Artemia* the comparison of the nauplii size (data not presented) and the mouth opening revealed that the *Artemia* nauplii were too big for the larvae to ingest. For dry food, we can only guess why the larvae did not take it. The taste of dry feed could be a reason. Another reason could be the lack of movement, which did not stimulate larvae to hunt and feed, or the lack of tactile stimulus that prevents the larvae from ingesting the dry food. Hence, this study represents a starvation rather than a feeding experiment (consequently, without control group). Observations during the experiment revealed that at least some larvae made an effort to catch feed items. Hence, the offered *Artemia* strain was not sufficiently used as food for these pikeperch larvae due to the size of the instar I and II. Most of the pikeperch lar-

vae died between dph 3 and 5, when most of the yolk sac reserves were already consumed under the prevalent conditions. According to Peters et al. (2015) starvation resistance for small larvae is in the range of two to three days without feed. During the ten days of the experiment a constant decrease of larval dry mass as well as fatty acid content was observed, reflecting the observations that no feed was consumed. Obviously, the decrease in dry mass is due to the constant catabolism, primarily of lipids and proteins, by the larvae (Ferron & Leggett 1994). Nevertheless, further reasons such as elevated concentrations of heavy metals (copper and zinc) might be a problem for sensitive fish larvae. Zinc and copper are vital trace elements (Watanabe et al. 1997) but after exceeding certain concentrations they become toxic (Moor & Ramamurthy 1984). Kuz'mina & Ushakova (2007) revealed that elevated copper and zinc concentrations reduced the casein- and hemoglobinlytic activities of proteinases in the intestine mucosa of pikeperch. So Kuz'mina & Ushakova (2007) showed that copper and zinc are able to affect the digestive processes of fish. According to the regional water supplier (Eurawasser 2018) the used tab water contains no copper but a report of the state government (Landtag Mecklenburg-Vorpommern 2012) revealed that approximately 7% of all tested water samples revealed copper concentrations above the legal limits. For zinc no legal regulations concerning legal limits exist. Furthermore, the lab building used for the experiment was build decades ago and it might possible that there are still copper pipes in use. Therefore, it cannot be excluded that copper and/or zinc might have negatively influenced the performance of the pikeperch larvae during the experiment, which could have caused the high mortality rates. Another reason for the elevated mortality rate might be the severe temperature increase during acclimatization and the first days of the experiment. In contrast to this assumption is the fact that pikeperch is adapted to warm water conditions and therefore, we assume that the temperature increase had a direct influence on the metabolic processes but not on the mortality.

The observed growth of the pikeperch larvae during the first days was not uniform, i.e., the average daily length increments showed variations. After a strong increase in total length during the first days (dph 1-2), growth stagnated (dph 2-3). Following dph 3, growth rates were constant for the next three days (dph 3-6). After dph 6 and the complete depletion of the yolk sac, growth stagnated again. A short phase of growth stagnation, two or three days after hatch, is regularly observed for pikeperch larvae (C. Kühn, personal communication 2013). However, despite a stagnating larval length, our data show a constant increase of the eye diameter as well as the mouth opening until dph 10. Apparently, with decreasing nutritional reserves, the pikeperch larvae stop investing into somatic growth and the metabolism channels energy towards the development of physiologically relevant body parts for predation, such as eyes and mouth. This strategy would help to increase the success rate of the larvae to find and ingest suitable prey items.

In the present study TFAs were continuously reduced in pikeperch larvae throughout the experiment. Under feed deprivation 80% of the initially available TFAs were utilized. This consumption was detected with the onset of larval development, which is in accordance to results observed in Eurasian perch larvae (*Perca fluviatilis*) (Abi-Ayad et al. 2004). Rapid growth in fish larvae and juvenile fishes to avoid predation apparently requires high amounts of energy via fatty acid catabolism (Peters et al. 2015; Olsen et al. 1999). Pikeperch larvae did not utilize all groups of fatty acids (saturated, mono- and polyunsaturated FA) in equal portions for growth during their early development. The amount of SFAs, which normally provide energy for the metabolism (Abi-Ayad et al. 2004), remained rather stable during most of the experimental period. Although desaturation of PUFAs or MUFAs cannot be excluded, as it was described by Ortega & Mourente (2010). Still, at the end of the experiment all SFAs declined to about half of their initial level.

MUFAs exhibited the strongest change during the experimental period. Starting with

the highest concentration, these fatty acids are consistently utilized by the larvae. According to Guler et al. (2007) and Osman et al. (2001) oleic acid (OA, 18:1(n-9)) and palmitoleic acid (POA, 16:1(n-7)) are the two major MUFAs in the muscle tissue of pikeperch. The complete utilization of the yolk sac leads to a significant decline in MUFAs between dph 5 and 6. Apparently, the yolk sac is an important reservoir of these fatty acids, maintaining the regular metabolism and catabolism as long as possible, as stated by Ferron & Leggett (1994). After yolk sac depletion, when MUFAs have to be catabolized from other tissues by the starving pikeperch larvae, this process is soon detrimental, resulting in increased mortality rates around dph 5.

The prevalent group of PUFAs also undergoes pronounced changes with a significant decline during the experiment. At the end of the experimental period this group comprises the highest portion of the three groups of fatty acids. PUFAs represent major components of bio-membranes and have important functions within cells, e.g. related to neural processes (Lund et al. 2012). Hence, the different groups of fatty acids serve specific purposes during the early ontogeny of pikeperch larvae and especially PUFAs are crucial components for the successful development of the early larvae (Bell et al. 1995). It is noteworthy that the essential PUFAs linoleic acid (LLA, 18:2), α-linolenic acid (ALA, 18:3), arachidonic acid (ARA, 20:4), eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) also declined during the experimental phase. However, the amounts of these specific fatty acids remained at a level above 0.2 µg FA*mg^{-1} (DW) and did not disappear completely during the course of the experiment, while other fatty acids disappeared. Especially EPA and DHA as major structural components of bio-membranes cannot be depleted as much as other fatty acids without lethal effects and for instance, the concentrations of LLA (18:2) and ALA (18:3) were declining faster than EPA (20:5) or DHA (22:6). Accordingly, several other fatty acids, such as 16:2(n-4), 18:3(n-6), 18:4(n-3), 20:1(n-7) and 20:3(n-3), were no longer detectable after the complete depletion of the yolk sac.

In summary, the findings of this experiment confirm that during the critical first days after hatch pikeperch larvae can rely on sufficient internal resources for normal initial growth and development. They increase in length, and invest simultaneously in the development of their visual capacity and the ability to capture prey organisms. After yolk sac depletion, the development of organs crucial for prey capture and ingestion, such as eyes and mouth opening, are prioritized over somatic growth. Unsuccessful ingestion of food items eventually leads to the point of no return and when the pikeperch larvae are depleted in their reserves they starve to death. According to our data, this point is finally reached four days after consumption of the yolk sac and two days after the disappearance of the oil droplet under the prevalent conditions. The deteriorating condition of the larvae is clearly reflected in the fatty acid data.

According to the literature (Ostaszewska et al. 2005; Ostaszewska & Boruta 2006) the chosen feeding regimes should have allowed pikeperch larvae to grow and survive. However, the offered feed items turned out to be unsuitable, as the larvae could not ingest and/or utilize the feed.

For small-sized fish larvae such as pikeperch, perfect timing with matching prey items is crucial during their initial exogenous feeding phase. Feeding success strongly relies on the size, amount and biochemical composition of the available prey. Successful pikeperch larvae must learn to capture prey until the end of the yolk sac stage, which in our study was at dph 6. Hence, there is only a very short time period until the point of no return is reached, and during this critical initial phase the pikeperch larvae need to catch prey as soon as possible, ideally two or three days post hatch, when yolk sac and oil droplet are still available as buffer to provide metabolic energy.

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