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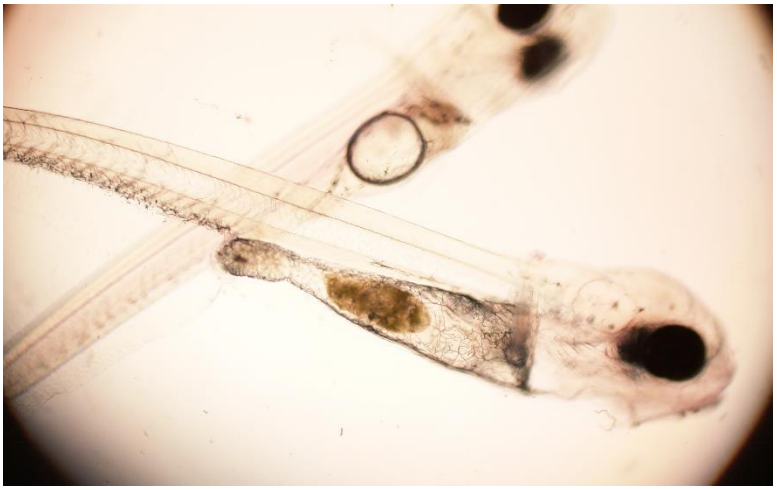
Improvement of *Sander lucioperca* (L., 1758) aquaculture during early life cycle stages in Recirculating Aquaculture Systems (RAS)



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I. List of abbreviation

FAO: Food and agriculture organisation

RAS: Recirculating aquaculture system

L: Linnaeus

EUMOFA: European market observatory for fisheries and aquaculture products

EU: European Union

LFA: Landesforschungsanstalt für Landwirtschaft und Fischerei

MV: Mecklenburg-Vorpommern

dph: Days post hatch

ANOVA: Analysis of Variance

Sp.: species

Ind.: individuals

E: experiment

Exp.: experiment

Syst.: system

B: *Brachionus plicatilis*

A: *Apocyclops panamensis*

Apo: *A. panamensis*

Art: *Artemia* sp.

ISO: *Isochrysis galbana*

NAN: *Nannochloropsis* sp.

N+I: *Nannochloropsis* sp. + *I. galbana*

SGR: Specific growth rate

DW: dry weight

UV: ultraviolet

C: carbon

Ca: calcium

P: potassium

CO_2: Carbon dioxide

°T: temperature

ATP: adenosine triphosphate

FAs: fatty acids

EFAs: essential fatty acids

FFAs: free fatty acids

HUFAs: Highly unsaturated fatty acids

PUFAs: Polyunsaturated fatty acids

n-3: omega 3 fatty acids

n-6: omega 6 fatty acids

MUFAs: Monounsaturated fatty acids

SFAs: Saturated fatty acids

DHA: Docosahexaenoic acid

EPA: Eicosapentaenoic acid

ARA: Arachidonic acid

SDA: Stearidonic acid

ETA: eicosatetraenoic acid

LA: Linoleic acid

ALA: Alpha-linolenic acid

PL: phospholipid

TG: triglycerides

PPAR: peroxisome proliferator-activated receptor

DNA: deoxyribonucleic acid

LPL: lipoprotein lipase

HL: hepatic lipase

LDH: lactate dehydrogenase

ROS: reactive oxygen species

ml: millilitre

h: hour

d: day

µm: micrometre

~ : approximately

%: percentage

Kg: kilogram

mg: milligram

µg: microgram

mm: millimetre

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1. Introduction and discussion

1.1. Abstract

The objective of the thesis was to enhance the feeding protocol for early developmental stages of pikeperch larvae (*Sander lucioperca* (L., 1758)) in recirculating aquaculture systems (RAS). This protocol needed to be species-specific and tailored to the larval development. The impact of two types of rotifers, a copepod, micro *Artemia*, *Artemia* sp., and microdiets, on pikeperch larvae during different larval stages was examined. Control markers included the fatty acid content, growth rate, and survival of the pikeperch larvae.

The survival rate of pikeperch larvae during the initial life stage was significantly improved by utilizing the live feed organism *Brachionus plicatilis* Mueller, 1786. By adjusting the daily feeding quantity of live feed organisms per pikeperch larva from day post hatching (dph) 4 to 10, survival rate of 93% was obtained with a live feed concentration of 6,300 individuals of *B. plicatilis* per liter and a daily live feed amount of 340 individuals per pikeperch larva. Further increases in live feed concentration or daily intake did not yield significant gains. Furthermore, survival rates exceeded 95% when modified feeding practices with the pseudo-green water technique were employed, using *Monoraphidium contortum* (Thuret) Komàrková-Legnerová, 1969 and *Brachionus calyciflorus* Pallas, 1766 in a freshwater RAS. During the second life stage from day 11 post-hatching to day 16, the use of live feed organisms *Artemia* sp. and *Apocyclops panamensis* (Marsh, 1913) also increased survival rates, with no noticeable difference between the two species.

Selection of live feed had a positive effect on pikeperch larval growth. Growth increased up to a live feed concentration of 6,300 individuals (*Brachionus plicatilis*) and a daily live feed amount of 340 individuals per pikeperch larva. Beyond these values, growth did not further improve. The specific growth rates achieved were higher when freshwater rotifers were used (5.0% day^{-1}). Improved growth was observed during the first 7 feeding days by employing the appropriate timing and live feed organism. From dph 11, *Apocyclops panamensis* demonstrated better growth than *Artemia* sp., indicating its suitability as a diet for pikeperch larviculture.

The fatty acid composition of pikeperch larvae served as a quality indicator for the nutrients provided by the diet. The proper timing and combination of live feed prey items positively affected the overall fatty acid content of the pikeperch larvae. Moreover, the chosen live feed organisms, *Brachionus* sp. and *Apocyclops panamensis*, had favorable effects on the fatty acid composition. Furthermore, it was demonstrated that not all fatty acids classified as essential needed to be available immediately after pikeperch larvae hatched. Some essential dietary fatty acids (EFAs), such as linoleic acid (LA) during

the first 7 days of feeding and docosahexaenoic acid (DHA) from dph 11, needed to be provided later through live feed until the larvae were capable of consuming DHA-rich microdiets.

Finally, as live feed influenced the lipid phenotypes of pikeperch, these phenotypes can serve as indicators to evaluate the nutritional status of the fish larvae. This thesis established a balanced pikeperch larval lipid phenotype of minimum 38.8% polyunsaturated fatty acids (PUFAs), which must be higher than monounsaturated fatty acids and than the saturated fatty acids. Moreover, 8.1 to 12.4% of the total fatty acids should be LA and 12.6 to 18.5% should be DHA with an omega-3/omega-6 ratio approximately 2.5.

1.2. Scope and structure

In order to enhance the feeding regimen and, consequently, the nutritional composition, survival, and growth rates of pikeperch, several live feed species for pikeperch larvae in a recirculating aquaculture system (RAS) were investigated with various methodologies in different life stages.

This cumulative dissertation contains 10 chapters. Chapter 1 gives an overview of the subject of the dissertation. The introduction starts with a general overview of RAS systems worldwide and their use for fish production from larvae to adults. Then, the trends in RAS aquaculture in Europe and Germany are described. The main bottlenecks for obtaining seed to start production and the newest methods in fish larviculture are presented. After the fish species *Sander lucioperca* studied in this thesis is introduced, a review of the characteristics of pikeperch aquaculture is presented, including nutrition in larval and juvenile rearing. Then, the importance of fatty acids is explained. A description of the fatty acids in the different feeds and the pikeperch larvae and juveniles fatty acid needs is given. Afterwards, the objectives of the thesis are given. Finally, in the discussion, the most relevant results are summarized and discussed with reference to the literature. In particular, the use of freshwater rotifers as natural feed during the first 10 days of larval life and its suitable quantity, the subsequent use of copepods, and the use of *Artemia* are discussed. Finally, we discuss the fatty acid dynamics in the larvae in the different periods, from dph 0 to 10 when fed with rotifers, *A. panamensis*, or *Artemia* sp., and from dph 11 to 16/18 when fed with *A. panamensis*, *Artemia* sp., or microdiets, and the effects of the microdiets on the fatty acid composition of the pikeperch juveniles.

Chapter 2 deals with the use of a freshwater rotifer, *Brachionus calyciflorus*, as a first live feed for pikeperch larvae by using the pseudo-green water technique with the alga *Monoraphidium contortum*. It is shown that larvae should be introduced into the system 5 days after establishing the *M. contortum* culture and 3 days before establishing the *B. calyciflorus* culture. It is discussed how this technique should be applied to achieve the highest survival and growth of the larvae. Moreover, *B. calyciflorus* fatty acid composition is suitable for pikeperch until dph 10.

Chapter 3 presents the results of two experiments. Different combinations and amounts of the rotifer *Brachionus plicatilis* and the copepod *Apocyclops panamensis* were analyzed, and their effects on the survival, growth, and fatty acid composition of the larvae up to post-hatching day 10 were studied. From the results obtained, it was established that the rotifer *B. plicatilis* is suitable in a certain amount per fish per day and that the copepod *A. panamensis* had no effect as part of the diet during this period. It was also shown that the linoleic acid in the diet is important at this stage and that the larvae, in its presence, are able to maintain a good level of other EFAs such as DHA.

Chapter 4 describes the use of *A. panamensis* as a diet for pikeperch larvae from dph 11 in two experiments. Larvae previously fed with rotifers grew better than those previously fed with *Artemia*. Subsequent feeding with *A. panamensis* reversed the poorer growth of larvae that ate *Artemia*. Better growth and FA composition in larvae were found when fed with *B. plicatilis* and then with *A. panamensis*. The need for dietary DHA for larvae during this period is shown.

Chapter 5 analyzes the effect of two microalgae, *Isochrysis galbana* Parke, 1949 and *Nannochloropsis* sp., on the development and fatty acid composition of the copepod *Apocyclops panamensis*. The copepod population developed better when the diet included *I. galbana*, which is rich in DHA. Furthermore, the higher amount of DHA in *I. galbana* was reflected in the fatty acid composition of the copepods, which was higher when the diet contained more *I. galbana*.

Chapter 6 presents the fatty acid dynamics in pikeperch larvae fed with *Artemia* and microdiets under commercial conditions. The objective of Chapter 6 was to determine the change in fatty acid composition of pikeperch larvae during early rearing and weaning. This study demonstrated that the lack of essential fatty acids in the diet caused deficiencies in larval fatty acid composition in the pikeperch during early development. Consequently, the biochemical composition of *Artemia* and microdiets was not sufficient for pikeperch larvae.

Finally, chapters 7 and 8 are declarations; chapter 9 is the acknowledgment section; and chapter 10 is the CV.

1.3. General introduction

1.3.1. Aquaculture worldwide

In 2020, the fisheries and aquaculture production for human consumption of fish and fish products was estimated at 214 million tonnes and seems to continue increasing by 2030 (FAO 2022).

The need for food production and protein sources increases due to the growing human population. Fish and fish products are known to provide essential nutrients for humans such as omega-3 and iodine, but they also represent a high percentage of the diet in developing countries (FAO 2022). The worldwide estimated fish consumption per capita has increased from 9 kg in 1990 to 20.2 kg per person in 2020. The highest growth sector in fish production since the early 1990s has been aquaculture (until 122.6 million tonnes in 2020), while capture fisheries have remained stable (FAO 2022). In order to support these trends in fish production and demand, sustainable aquaculture systems should be developed and optimized (Lekang 2020, Timmons et al. 2018). Aquaculture systems are currently being extensively used throughout the world to cultivate aquatic species (Duston and Liu 2020). Ponds, raceways, tanks, and cages make up the primary systems (Duston and Liu 2020). Regarding the level of production intensity within the system, the systems can be categorized as extensive, semi-intensive, and intensive, and according to the type of water supplied to the system, they can be divided into flow through (constant water replacement) and water reuse (reusing more than 90% of the water by recirculating aquaculture systems) (Lekang 2020, Timmons et al. 2018).

Intensive aquaculture in conventional production systems, however, may result in the discharge of suspended solids, nutrient and organic overload in waters, anoxic sediments, oxygen depletion, water quality degradation, changes in benthic communities in natural environments, eutrophication and habitat destruction, chemical discharge, parasite and disease introduction, escapees changing the biodiversity of natural populations, the introduction of invasive species, etc. (Timmons et al. 2018, Wik et al. 2009). It might lead to non-sustainable aquaculture. Moreover, there is a lack of space or limited access to the sea (Timmons et al. 2018). Hence, this global trend towards sustainable aquaculture systems requires the application of new technologies like RAS to increase production and the introduction of new species like *Sander lucioperca* (L., 1758) to diversify in order to meet the higher demand worldwide (Lekang 2020).

RAS is a technology to culture aquatic species that reuses more than 90% of the water by filtering it through mechanical and biological filters. Thus, RAS can solve some of the constraints in traditional aquaculture described above (Lekang 2020, Timmons et al. 2018). RAS is used for fish production because it minimizes the use of water thanks to its recirculation and treatment (Lekang 2020). It allows the control of the physico-chemical water conditions that are optimal for each fish species, such as temperature, pH, dissolved oxygen, etc. (Lekang 2020, Wik et al., 2009). Controlled conditions avoid and prevent parasites and allow treatments against diseases (Timmons et al. 2018). Ponds, for example, are more difficult to access since proper cleaning or treatments cannot be applied (Duston and Liu 2020).

Besides that, the RAS-controlled environment allows the non-use of antibiotics and medicines. RAS also avoids escapes and effluents that have the potential to damage the environment (Wik et al. 2009). Moreover, RAS promotes fish availability in regions that did not have access to some cultured species before at cheaper prices, improving human nutrition and food safety (Wik et al. 2009). Thanks to this developed technology, aquaculture enhances the production of fish for human consumption, reduces waste and loss, and increases fish health (FAO 2022, Lekang 2020). Another advantage of RAS use is that food production is close to markets or consumers (local production possibilities), which avoids excessive transport (CO_2 production), making the process more environmentally friendly, saving energy and time, and reducing costs (Wik et al. 2009). Nowadays, there is a higher demand for low carbon footprints that are reduced by using RAS since the RAS reduces energy, renews water, controls rearing conditions, and discharges waste (Timmons et al. 2018).

However, these systems demand substantial initial investment, high operating costs, and highly trained staff (Wik et al. 2009). Therefore, the RAS system has undergone numerous attempts to be made more effective, such as by merging the RAS with hydroponics to reuse nutrients (Dediu et al. 2012). Thus, aquaponics is a method of growing plants and aquatic animals where more than half of the nutrients required to promote proper plant development come from waste from feeding aquatic organisms (Palm et al. 2018). Another method for making the RAS system workable is to use co-cultured species (polyculture), which enables secondary species to use the waste products from the primary species (Knaus and Palm 2017, Metaxa et al. 2006). Another approach is the use of the green water technique (Papandroulakis et al. 2001). The green water technique is based on phytoplankton. This technique applies microalgae to intensive culture systems together with the zooplankton that will be used as live feed for fish and crustacean larviculture (Brown and Blackburn 2013). Green water appears to improve larval growth, survival, and feed intake when compared to clear water by simulating the natural conditions (Brown and Blackburn 2013, Planas and Cuhna 1999). Several factors could explain the positive effects of the green water technique. Microalgae in the water of a RAS may affect the microbiology of the system through interaction (Ramli 2018). Moreover, the turbidity of the water produced by the microalgae may modify larval feeding behavior (Koven et al. 2019). Besides that, microalgae may remove nitrogenous substances from the water, improving the quality of the water (Brown and Blackburn 2013). The enrichment of the live feed through the microalgae may improve the nutrition of the fish (Planas and Cunha 1999). Microalgae provide macro- and micronutrients to the zooplankton. Moreover, the green water technique provides rotifers with higher energy and protein content, indicating that these factors are crucial for the larvae to achieve high development and survival (Planas and Cuhna 1999). Nevertheless, the type of microalgae employed can also have an impact on the development and survival of fish larvae (Planas and Cuhna 1999). Although the green water technique has already been used (Bengtson et al. 1999, Nass et al. 1992, Van der Meeren et al. 2007), green water conditions are difficult to maintain. After 20–25 days, massive dead microalgae may produce high levels of nitrates and nitrites that are negative for the fish larvae and may produce high

larval mortality (Papandroulakis et al. 2001). The "pseudo-green" water technique can solve this problem. The "pseudo-green" water technique depends on adding phytoplankton and zooplankton to the larval rearing tanks on a regular basis instead of culturing plankton in the rearing tanks as in the green water technique (Papandroulakis et al. 2001). This thesis is the first attempt to use the pseudo-green water technique in pikeperch larvae fed with a freshwater rotifer, *B. calyciflorus*.

All these approaches in RAS might enhance the entire production cycle, from larviculture to adulthood. One of the growing uses of RAS is in hatcheries and nurseries for juveniles that were reared previously in tanks, raceways, or ponds (EUMOFA 2020). Cultured established fish species under well-known rearing conditions in captivity have higher survival rates and higher omega-3 (n-3) content than in nature, as shown in Sağglık et al. (2014) for gilthead seabream (*Sparus aurata* L., 1758) and sea bass (*Dicentrarchus labrax* (L., 1758)). For new fish species, these rearing conditions have to be further studied. As an example, the highest increase in RAS use is in salmon (*Salmo salar* L., 1758) smolt production, mainly due to the more fragile status of larvae and juveniles than adults in the grow-out phase. More environmental control is needed during these delicate stages, which can be accomplished by utilizing RAS technology. Afterwards, at a certain size, they are transferred to the normal grow-out environment, which can also be performed in RAS (EUMOFA 2020). The grow-out procedure depends on the fish species and shall be adapted to the life period, which is different from the larval and juvenile stages. Moreover, for the entire production of a fish species, it is essential to understand the factors regulating the life cycle and, thus, improve the rearing conditions and control the out-of-season reproduction. Fish reproduction is a highly variable trait between species, and therefore, there is a huge need to understand the male and female cycles, the reproduction strategy, as well as the natural environmental conditions under which the maturation of gonads and gametes take place to optimize the RAS (Song et al. 2019). For example, in high-temperature regions, gonad maturation occurs with increasing temperature and photoperiod. Under RAS conditions, the environmental conditions can be controlled, so out-of-season spawning can be performed. Nevertheless, reproduction has to be fully understood to perform an optimal protocol for reproduction because the quality and quantity of the larvae will depend on it (Song et al. 2019).

1.3.2. Aquaculture in Europe and in Germany

Aquaculture in Europe has increased in recent decades, reaching 1.37 million tonnes with a value of 4990 million euros in 2019. However, the European Union (EU) is one of the major importers of fish and fisheries products, with the main producers being Asia and Norway. In 2019, only 22% of the total fish production in the EU was from aquaculture (EUMOFA 2021a), with Norway being the main fish producer in aquaculture with salmon culture. In Europe, there was an increase in the value of aquaculture between 2010 and 2019, mainly due to the production of species such as salmon or sea bass and the price increase of other species like sea bream, not only because of higher demand but also because of bio-products. A special characteristic of European aquaculture is the specialization of the different

countries in different fish species production, like sea bream and sea bass in Greece, mussel (*Mytilus edulis* L., 1758) and turbot (*Scophthalmus maximus* (L., 1758)) in Spain, or mussel and rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) in Germany and Denmark (EUMOFA 2021a). Freshwater aquaculture represented 23% of EU production in 2018, with the main producers being France, Poland, and Italy, followed by Denmark, the Czech Republic, and Germany (EUMOFA 2021b). Nevertheless, freshwater aquaculture in the EU has had several constraints and has decreased since 2009, especially in trout and carp (*Cyprinus carpio* L., 1758) production. (EUMOFA 2021b). Freshwater aquaculture in the EU is done in extensive production in ponds, in intensive production in tanks and raceways, or in intensive production in recirculation systems (EUMOFA 2021b). In Europe, RAS has increased only in trout production, mainly in Denmark (EUMOFA 2021b).

In 2018, the EU produced 27.000 tonnes of fish in RAS, belonging 95% to freshwater species, and leading this sector were Denmark, the Netherlands, Germany, France, and Poland. The species produced in RAS are rainbow trout, North-African catfish (*Clarias gariepinus* (Burchell, 1822)), and European eel (*Anguilla anguilla* (L., 1758)). Although RAS production in the EU is still low, it is an emerging sector due to the interest in sustainable fish production (EUMOFA 2021b). This European trend towards RAS is reflected in the increasing number of enterprises in the EU, especially in Germany with 76 enterprises with commercially significant production in RAS, France with 52, Denmark with 34, the Netherlands with 27, and Poland with 24. This type of fish production is more diversified in Germany, with European eel, European catfish (*Silurus glanis* L., 1758), North-African catfish, sturgeons (*Acipenser* sp.), and pikeperch (EUMOFA 2021b).

Percid fish aquaculture comprises only 1% of the production in Europe. Traditional production in ponds is still common and performed together with other freshwater species, which makes the data difficult to analyze (Toner 2015). Nevertheless, some countries are increasing their percid production mainly using RAS, like Switzerland, Denmark, France, Ireland, and, recently, Germany.

Germany's per capita fish consumption was 12.8 kg in 2020, which is still significantly below the EU average (23.3 kg) (EUMOFA 2022). In terms of fish consumption, Germany is traditional, with only five species categories accounting for more than 70% of sales. Freshwater fish, sea fish, and shellfish are the major sources of fish product intake. Fish consumption is higher in northern Germany, but freshwater fish consumption is expected to be higher in the south and east, near traditional freshwater production areas (Toner 2015). In 2020, Germany produced approximately 18,600 tonnes of fish in nearly 2,300 aquaculture facilities, an increase of approximately 49 tonnes (+0.3 percent) over the previous year and nearly stable (Statistisches Bundesamt 2021).

A particular characteristic of Europe is that each European country has specialized in certain species (Mylonas et al. 2019). This makes European aquaculture highly diversified (Mylonas et al. 2019). The diversification of fish species cultured in aquaculture is essential for sustainability (Harache 2002). Diversification also has the potential to reduce imports since regional species supply the market, thus reducing transport for imports and the consequent CO_2 production (Harache 2002). Diversification may

also reduce the need for fishing (Harache, 2002). Moreover, higher fish culture diversity can help reduce the risk of disease spread and thus avoid huge economic losses (Harache 2002). Pikeperch are regarded as having Europe's greatest potential for diversifying inland aquaculture (Mylonas et al. 2019).

One of the main constraints in the diversification of species like pikeperch is the production of seed under controlled conditions since most of the mature fish for new aquaculture species are taken from the wild (Mylonas et al. 2019, Policar et al. 2019). Besides that, domestication is an important aspect of diversification. High-domesticated species are adapted to artificial conditions and have higher survival and growth rates than in nature. Breeding programs are necessary for domestication to select the traits needed for the culture of this specie to fill human needs and to avoid the negative consequences of inbreeding that will also determine the quality and quantity of the fish eggs, embryos, and finally, larvae (Mylonas et al. 2019, Policar et al. 2019). Domestication is a long-term activity that has the potential to improve growth, quality, resistance to disease, or digestion of formulated feeds (Duncan et al. 2013).

The broodstock management should keep the fish healthy, free from injuries and parasites, and allow the maximum growth and survival, which might be achieved by the use of RAS. These optimal environmental conditions influence the maturation of gonads, the quality of gametes, and, thus, embryonic and larval quality. The selection of breeders from well-managed and domesticated broodstock under artificial conditions is essential for seed production (Mylonas et al. 2019, Policar et al. 2019).

Controlled reproduction can also influence seed production. Fish have several reproduction strategies, and knowledge about reproduction will be crucial for the success of the production (Mylonas et al. 2019, Policar et al. 2019). By adjusting specific water factors, such as temperature, salinity, or pH, we can simulate natural spawning by recreating the conditions that lead to reproduction (Song et al. 2019). Another possibility is the use of hormones to stimulate spawning. A high fertilization rate will require proper management of the gametes produced during spawning (Policar et al. 2019). The improper temperature, water flow, oxygenation, or water composition during egg incubation may result in errors in embryonic development that compromise the reproduction (Policar et al. 2019). Thus, a proper protocol is needed.

Consequently, production of sufficient seed (larvae), especially in new and non-developed species like *Sander lucioperca*, is still a main constraint in aquaculture. Domestication (breeding programs), broodstock management, and controlled reproduction protocols will result in more stable egg and larva quality and quantity and thus enough seed for the aquaculture sector, particularly for new species (Policar et al. 2019). Moreover, rearing the larvae is one of the fundamental keys to the success of seed production.

1.3.3. Pikeperch (*Sander lucioperca* (L., 1758))

1.3.3.1. Biology

Pikeperch is a predatory species of freshwater environments that is a member of the Percidae family (Stepien and Haponski 2015). It is found in coastal brackish waters as well as lakes, rivers, and reservoirs. The fish is indigenous to Eastern Europe, although it is also common in Western Europe.

Pikeperch typically spawn between April and May. Pikeperch reproduction is governed by a seasonal cycle, which in freshwater fishes is influenced by changing environmental parameters including water temperature and photoperiod (LFA 2021). In pikeperch, gametogenesis starts as early as the days get shorter and the water temperature drops. The quality of the egg and its later viability are influenced by the amount of fat and yolk material that is kept in the oocytes during the subsequent vitellogenesis (LFA 2021). The hatching of the eggs depends on the temperature (in degrees, approximately 100°T). When they hatch, the larvae are around 5 mm in size. The larvae start actively feeding just before the yolk sac is consumed and after the mouth and intestinal opening have formed. Pikeperch start forming the swimming bladder around dph 4 at the same time that they resorb the yolk sac, open the mouth, and start exogenous feeding, and around dph 11 they close the pneumatic conduct (Demska-Zakes et al. 2003). Pikeperch larvae feed on zooplankton such as cladocerans, copepods, and *Daphnia* sp. Larvae must reach the zooplankton peak after hatching, and the zooplankton available at that time must have a suitable size for the small pikeperch larvae. The feeding condition of the larvae in the first few weeks determines the production of pikeperch in natural environments. Later, they also eat larger shore and benthic animals like lakefly (Diptera-Chironomidae) and mayfly (Ephemeroptera) larvae (LFA 2021). Since they are piscivores, they begin to eat other fish species. However, in the absence of other fish species of suitable size, pikeperch become cannibalistic (LFA 2021). Moreover, pikeperch contain high genetic diversity (Stepien and Haponski 2015), and thus, larvae have high variability in size and quality, which may enhance cannibalism. Moreover, another problem in the natural stocks is the presence of diseases or parasites, which have a detrimental impact on production or marketing. Toxic compounds or substances that enter the natural water cycle through human activities constitute another potential risk. Therefore, fish deaths are common in natural aquatic systems because of multiple combinations of environmental factors.

1.3.3.2. Pikeperch aquaculture

Pikeperch is a popular freshwater fish species that is highly valued for its firm and tasty flesh. As wild populations have declined and the current supply mainly depends on freshwater-caught fish from Kasachstan (LFA 2021). The demand for pikeperch has increased, leading to the development of aquaculture production systems to meet the demand for this fish (Mylonas et al. 2019). In Germany, pikeperch are present in numerous lakes and the Baltic Sea's coastal waters in Mecklenburg-Western Pomerania (MV) (LFA 2021). However, the population sizes are dwindling, which is reflected in decreased landings in the catch fisheries. In MV, pikeperch captures declined from 25.3 t in 2019

(Brämick 2019) to 18.2 t in 2021 (Brämick and Schiewe, 2021). Pikeperch are mostly raised as secondary fish in the carp pond sector, where the fish are typically sold as stock fish when they are young (LFA 2021). Pikeperch aquaculture production in recirculating aquaculture systems (RAS) has become more significant in recent years and is the only type of aquaculture that results in a significant increase in production due to high consumer demand and insufficient domestic production (LFA 2021). In fact, pikeperch production in aquaculture systems has increased in Germany from 57 tonnes in 2019 to 85 tonnes in 2021 (Fisch-Informationszentrum 2022). Nevertheless, a year-round supply of larvae or fingerlings, which requires egg production during out-of-season spawning and, thus, a RAS, is a fundamental requirement for the establishment of pikeperch in aquaculture.

1.3.3.2.1. Pikeperch larviculture

Larviculture consists of rearing fish in their early life cycle stages and requires specific culture techniques different from those in commercial hatcheries and grow-out procedures. Understanding the biological characteristics of fish larvae is necessary in order to create an optimal environment to meet the needs of the fish species during early development and bring a high quality and quantity of larvae to maturity to produce seed and a source of protein for human consumption, making the process more efficient and sustainable. Fish are exposed to high mortalities at the larval stages, which are particularly vulnerable. The main constraints in pikeperch larviculture encompass initial quality and size, cannibalism, deformations, and swim bladder inflation (Policar et al. 2019).

Poor understanding of the rearing conditions for breeders that produce reproduction failures can cause initial high mortality rates at hatching or at first feeding (Kestemont and Herrote 2015). The nutrition of breeders influences gamete quality. Most of the reserves of embryos come from the females' eggs, which come directly from their diets (Kestemont and Herrote 2015). As mentioned, egg and larval quality will depend not only on the breeders but also on the incubation environment. Broodstock management, including nutrition and the environmental changes to induce reproduction, may cause failures in embryonic development that are also affected by salinity and temperature and that can lead to larval deformities (Schrandinger and Zarski 2015).

Larval quality and quantity affect production efficiency. The larval quality influences the survival rate since the larvae are not able to feed for the first few days of life and they use their reserves from the yolk sac and the oil droplet. The properties of the eggs have a considerable amount of influence over the quality of the larvae and the entire process of larval rearing (Schaefer et al. 2019). At hatching, pikeperch larvae have a relatively small body size between 4.5 and 6 mm (LFA 2021). Generally, fish larvae are small, and they need a live feed of a suitable size to be ingested.

Additionally, they have a yolk sac and a primitive digestive system but no stomach (Kestemont and Herrote 2015, Xu et al. 2017). Larvae have a poorly developed digestive system at hatch with non-specialist cells and poor enzymatic production to digest proteins (Rønnestad et al. 2013), and thus, in

this development phase, the fatty acid and free amino acid supply is important to facilitate nutrient absorption.

Another constraint in predator fish larviculture is the initial size heterogeneity related to the initial larval quality variability that may enhance cannibalism. Cannibalism is a natural characteristic of some fish that serves to control the population. However, in aquaculture, cannibalism is an important constraint. In pikeperch aquaculture, individual differences resulting from maternal and paternal effects also play a role in this size variability (Policar et al. 2019). Stocking individuals of the same size and age and providing enough meal size might diminish this growth heterogeneity in larval rearing, reducing cannibalism. To reduce cannibalism, it is essential to have optimal rearing conditions, such as stocking density and light, and an adequate feeding protocol (Policar et al. 2019).

Finally, swim bladder inflation failure may occur. Larvae with an uninflated swim bladder consume more energy. Failure of the swim bladder may increase deformation occurrence in pikeperch and enhance cannibalism since some larvae grow slower due to difficulties in swimming and preying and spend more energy on it (Demska-Zakes et al. 2003). Lack of certain macro- and micronutrients might cause deformations in fish larvae. According to Steenfeldt et al. (2015), external parameters that affect swim bladder inflation in a production farm include water surface tension, enclosure depth, salinity, turbidity, light intensity, photoperiod, and even tank color. Thus, maintenance of the environmental conditions is critical during this period, including the right feeding, in order to obtain maximal survival and growth rates. Most larvae begin the switch to commercial dry food once their swim bladders have filled. Therefore, the methods used to rear pikeperch larvae include the use of recirculation technology and live feeds, which are adopted from previous research on marine larval rearing (Steenfeldt 2015). Nevertheless, some adaptations to the special characteristics of pikeperch larvae have taken place.

Larviculture can be done with different rearing systems. Rearing systems for larviculture can be indoor or outdoor. Outdoor systems are larger and have a lower cost, such as ponds or semi-intensive systems. Indoor systems use smaller tanks, are more expensive, and consume more energy. Nonetheless, the most common method is intensive production in RAS, with live feed gradually replaced by artificial feed. Adequate rearing conditions are essential and include the use of a high-quality diet (nutrition), the maintenance of the water temperature, dissolved oxygen, chemical composition, stocking density, etc. (environmental conditions), the procurement of tanks with enough space (rearing system), and the avoidance of stressful situations such as unnecessary handling or abrupt changes. This is done by including an appropriate system design for the RAS, which can use the green or pseudo-green water technique.

Appropriate feeding strategies have to be established since nutrition is an important factor in larviculture to improve larval quality (Hamre et al. 2013). Due to their suitable prey size and simple digestion, zooplankton species like *Artemia salina* (L., 1758), rotifers, or copepods can be used as feed for the larvae in the early life stages (Policar et al. 2019, Kestemont and Herrote 2015).

The use of live feed, which has replaced formulated diets in recent decades, has improved the feeding protocol. Nowadays, the use of live feeds as the first feeding in larviculture is widely used. Live feed includes organisms bellowing to the zooplankton such as *Artemia*, rotifers, copepods, cladocerans, etc. that are the natural food for fish larvae in the wild. These organisms are believed to have suitable size, behavior, digestibility, and nutritional composition for the fish larvae. Most of the fish larvae are visual predators, which feed selectively according to the prey's size, color, and motion (Buskey 2005).

The identification of particles by the fish as viable food items often depends on the zooplankton's motion patterns. In natural environments, zooplankton species have evolved adaptations to hide from potential predators in response to fish predation (Buskey 2005). Adequate movement will catch the attention of the fish larvae and allow them to catch it. However, fast movements of the live feeds avoid predation (Buskey 2005). The movement of the prey should be adequate for the fish species, but this also depends on the prey density in the water. This is in close relation to coevolution and the match-mismatch in natural environments, and thus, it should be taken into account for nutrition in larviculture.

Newly born fish larvae have a small mouth gap. Encounter enhancement between predator and prey is only worthwhile if the size of the prey is suitable for the fish larvae (Buskey 2005). Therefore, a suitable size of the live feed organism is essential for ingestion.

The digestive system of fish larvae is poorly developed. This results in a low absorption rate and, thus, limited energy and/or nutrient intake (Buskey 2005). Consequently, adequate digestibility of the prey is crucial for successful nutrition during early development. Another important characteristic of the live feeds is that they should include all the essential nutrients, such as fatty acids.

In pikeperch larviculture, highly saline *Artemia* and marine rotifers are commonly used. However, they can only survive in freshwater for a few hours. With the help of these organisms, there have been various attempts to increase the survival and growth rates of pikeperch. For example, Imentai et al. (2019a) used increased salinity to increase *B. plicatilis* survivability in the RAS and subsequently larval feeding and growth rates. However, the use of natural feed, such as freshwater rotifers like *B. calyciflorus*, might be more appropriate to increase production efficiency. Enriching the marine rotifers (*B. plicatilis*) with phytoplankton (*Chlorella vulgaris* Beijerinck, 1890) has improved the pikeperch's performance, according to Yanes-Roca et al. (2020). However, to our knowledge, neither green water nor pseudo-green water techniques have been applied to pikeperch larviculture. Since most of the live feeds are marine zooplankton, there is a need to establish a freshwater organism as the first live feed for pikeperch. Consequently, the green water or pseudo-green water technique has the potential to improve pikeperch larviculture by improving rearing conditions and larval nutrition. This thesis is the first attempt to use the freshwater rotifer *B. calyciflorus* with the pseudo-green water technique in pikeperch larviculture.

In order to maintain high enough ingestion rates to cover nutrient assimilation and catabolism, larvae should be fed feed that satisfies their nutritional needs and is readily available in sufficient amounts (Steenfeldt 2015). Therefore, an adequate feeding protocol includes feeding suitable live organisms at the right time of day and period of life and in the optimal amount to increase survival and growth rates.

Imentai et al. (2019b) have evaluated the minimum concentration of *B. plicatilis* needed for pikeperch during the first days of feeding. Although this was a first attempt to evaluate nutritional requirements, there is still a lack of a direct or indirect amount of feed that pikeperch larvae need per day. Both parameters should be considered with the stocking density to supply the optimal live feed quantity to improve survival and growth.

However, rotifers are limited to the first few days of feeding. Larger live prey has to be provided to the pikeperch larvae to increase growth. Copepods have shown promising results for halibut larvae (*Hippoglossus hippoglossus* (L., 1758)) (Evjemo et al. 2003), for winter flounder larvae (*Pseudopleuronectes americanus* (Walbaum, 1792)) (Martinez-Silva et al. 2018), or for Atlantic cod (*Gadus morhua* L., 1758) (Karlsen et al. 2015). Nauplii of copepods have been found in the stomachs of pikeperch larvae (Peterka et al. 2003). Nevertheless, to our knowledge, only *Acartia tonsa* Dana, 1849 has been successfully used in combination with *Artemia* for pikeperch larvae (Jonathan 2020), where they reported a benefit between dph 15 and dph 18. Therefore, more species of copepods like cyclopoids, which have shown good results, must be studied as live feed for small fish larvae.

The dry food is gradually raised, while *Artemia* nauplii supply decreased from days 12–15 to days 19–22 after hatching, according to Kestemont and Henrotte (2015).

Increasing larval growth may enhance further growth since the fish may be able to swim faster, prey more, or ingest bigger prey (Feiner and Höök 2015). The presence of the appropriate nutrients avoids deformations (Kestemont et al. 2007). Moreover, enough feed reduces the formation of an oil layer at the surface, avoiding failure of swim bladder inflation, and, at the same time, provides enough energy to the fish larvae to swim to prey and/or break the oil layer. All this will increase survival, growth rate, and fish nutrient composition, bringing more larvae to commercial sizes and, therefore, optimizing production and sustainability. The development of protocols to reduce nutrient inputs (feed) that optimize fish growth and welfare and reduce the energy required for waste removal and nitrification/denitrification is essential for future sustainable production. This can be achieved by understanding the nutritional and energetic needs of the early larvae for the large-scale production of juvenile fish. Since the larvae catabolize a variety of energy substrates present in the yolk for development, mainly fatty acids, it is plausible to assume that the composition of the yolk sac covers the needs of the larvae during initial feeding, when they consume live feed species. However, it is known that these requirements fluctuate with growth.

1.3.3.2.2. Pikeperch juveniles

One of the key challenges in pikeperch aquaculture is the production of juveniles. In pikeperch aquaculture, juveniles are typically produced by spawning adult fish in captivity. As the larvae develop into juveniles, their nutritional requirements change. While live prey is suitable for the early stages, as the fish grow, their diet must be supplemented with other protein sources, such as fishmeal, soy protein, or insect meal. The completion of the metamorphosis in percid larvae occurs together with the stomach's

ability to function and pepsin activity, and it typically occurs 29 days after hatching at a total length of about 25 mm (Kovalev et al. 1976). At this point, pikeperch juveniles can be fed commercial feed. The feed should be of appropriate size, texture, and shape for the fish's mouth and digestive system (Steenfeldt 2015, Steenfeldt and Lund 2008). Overfeeding should be avoided as it can lead to digestive problems and water quality issues (Steenfeldt 2015). The feeding protocol for pikeperch juveniles should consist of several small meals per day with high-quality proteins, lipids, vitamins, and minerals.

However, pikeperch juveniles fed diets for rainbow trout showed an unbalanced diet composition, particularly in the lipid content (Schulz et al. 2005). Thus, to create diets that meet pikeperch nutritional needs, further research on the quantitative and qualitative nutritional composition is essential (Schulz et al. 2005). Lipid sources, such as fish oil or vegetable oil, are also important for proper growth and development (Schulz et al. 2005). In addition to diet, water quality is a critical factor in pikeperch production. Juvenile fish are sensitive to fluctuations in temperature, pH, and oxygen levels and must be kept in clean water with optimal conditions to ensure their survival and growth.

To address these challenges, advanced technologies have been developed in recent years, such as recirculating aquaculture systems and automated feeding systems, which allow for more precise control of water quality and feeding regimes (Peter et al. 2023). These technologies have helped to improve the survival rates and growth of pikeperch juveniles, making their production more sustainable and economically viable. By using advanced technologies and best practices, aquaculture producers can successfully produce healthy and high-quality pikeperch for the market.

However, pikeperch juveniles have a high metabolic rate and require a balanced diet to grow and develop properly. Therefore, their nutritional requirements need to be better understood to develop more precise feeding protocols. By providing adequate fatty acids, pikeperch juveniles can grow and develop properly, leading to a successful aquaculture operation.

1.3.3.3. Fatty acids

Fatty acids are essential nutrients for life, as are proteins and carbohydrates. Fatty acids have a polar carboxylic group on one side and a chain of nonpolar hydrocarbon bonds on the other, giving the molecule two ends that are hydrophobic and hydrophilic, respectively. They are divided into two categories: saturated and unsaturated fat, and they are often in the form of triglycerides, phospholipids or free fatty acids (Dey et al. 2022). The nutrient composition of fish flesh varies between species. Most animals have a high amount of saturated fatty acids (SFAs), which have no double bonds (only single C-C-bonds). Unsaturated fatty acids, on the other hand, contain one double bond between carbon atoms (monounsaturated fatty acids or MUFAs) or more than one double bond (polyunsaturated fatty acids or PUFAs). In general, fish have a high proportion of fatty acidsandh a high content of polyunsaturated fatty acids (PUFAs) in comparison to othermeatst like chicken, pork, or beef. The benefits of eating fish for humans are mainly related to the high content of PUFAs (Thomas et al. 2015).

Fatty acids play an important role as an energy store and in the chemical structure of cells. Fatty acids are the main source of energy. Through the ß-oxidation of the fatty acids, fish obtain adenosine triphosphate (ATP). SFAs and MUFAs are used for energy, although PUFAs may also be used. SFAs like palmitic acid (C16:0) and MUFAs like oleic acid (C18:1) are likely found in the liver to be used for energy. However, PUFAs are also needed for structural functions, and thus they are retained in the tissues.

1.3.3.3.1. Fatty acids in live feed

Artemia. *Artemia* sp. is a genus of aquatic crustaceans, also called brine shrimp. This primordial arthropod has a segmented body with leaf-like appendages. There are seven to nine species of *Artemia* sp. *Artemia* can be found worldwide in saltwater lakes and in the sea (Lavens and Sorgeloos 1996). The abundance of *Artemia* in aquaculture is a result of its capacity to create cysts, which are dormant eggs (Dhont et al. 2013). The largest known *Artemia* cyst collection is housed in the Laboratory of Aquaculture and *Artemia* Reference Center (ARC) at Ghent University. The nutritional composition in terms of fatty acids, amino acids, or vitamins depends not only on the species of *Artemia* but also on the strain (Lavens and Sorgeloos 1996). *Artemia* is still used in many hatcheries to rear fish larvae, despite some constraints such as the nutritional composition, the high price, the dependency on extraction from nature, or the large nauplii size for some fish larvae. *Artemia* is known to be unsuitable for some fish species (Bischoff et al. 2018). *Artemia* lacks DHA, which is considered the most important fatty acid for marine fish larvae (Tocher 2010). Different enrichment products have been developed to address these nutritional limitations, including microalgae, yeasts, bacteria, microencapsulated feeds, emulsified products, microparticulate products, or mixtures of these (Dhont et al. 2013, Kandathil Radhakrishnan et al. 2020). The use of such diets allows the modification of the content of total lipids, lipid classes, fatty acids, fatty acid ratios, protein, or ascorbic acid. Nevertheless, most commonly used fatty acid enrichments provide the zooplankton lipids in their neutral form, which may cause an imbalance between protein and lipids. On the other hand, microalgae provide lipids in the polar fraction, particularly high unsaturated fatty acids (HUFAs), and thus may be more adequate for enrichment (Dhont et al. 2013). *Artemia* nauplii are still the most commonly used first feed in commercial intensive pikeperch production (Kestemont and Herrote 2015).

Rotifers. Rotifers form part of the zooplankton in marine, brackish, and freshwater environments. The genus *Brachionus* is the most widely used group of rotifers in aquaculture. Rotifers can be cultured in high densities, have a high reproduction rate (life cycle, asexual reproduction), and are resistant to a wide range of abiotic factors such as salinity or temperature. Rotifers' size may be suitable for ingestion by fish during their early development stages, and it has been reported to improve initial feeding performance at larval stages (Planas and Cunha 1999). The nutritional composition depends on the microalgae diet since they are nonselective filter feeders (Laven and Sorgeloos 1996, Lubenz 1989).

This characteristic is used to enrich them before feeding them to the fish larvae, as done by Yanes-Roca et al. (2020). Rotifers enriched with yeast are known to have fewer fatty acids, especially PUFAs, than those fed with microalgae (Oie and Olsen 1997, Dhont et al. 2013). Moreover, rotifer fatty acid will depend on the microalgae's quantity and quality, as seen for *B. calyciflorus* (Kennari et al. 2008, Schälicke et al. 2019, 2020). Therefore, the use of the previously described "green water technique" may enrich the rotifers just before the fish larvae prey on them. Nowadays, the most commonly used marine rotifer for fish larval rearing is *B. plicatilis* (Hamasaki et al. 2009, Imentai et al. 2019a, 2019b, 2020, 2022, Pantazi et al. 2014, Rodriguez et al. 1997), and the freshwater rotifer is *B. calyciflorus* (Awaiss et al. 1992, 1996, Harzevili et al. 2003, Lim and Wong, 1997).

Copepods. Copepods are the major group of organisms forming the zooplankton and are the main link between primary production and higher levels of the food web (Lavens and Sorgeloos 1996). The life cycle of copepods is more complicated than the *Artemia* or rotifer life cycle (Lavens and Sorgeloos 1996), and thus, the culture needs more time to achieve high densities. The lack of some FA in rotifers and *Artemia* makes them dependent on fish oil, which is limited and has unstable prices (Nielsen et al. 2017). In copepod culture, the main constraint is economic feasibility due to the slower growth rates. Nevertheless, the culture of copepods has improved in recent years (Santhosh et al. 2018). These are the reasons to believe that copepods may fill the fish larvae's requirements better than *Artemia* or rotifers since they have more protein, n-3 fatty acids, polar lipids, vitamins, and nutrients (summarized in Hamre et al. 2013). Nevertheless, the nutrient composition of copepods may vary between species, microalgae diets, and/or environmental conditions. Phytoplankton is the basis of the aquatic food web. Despite accounting for only 1% of total plant biomass, they are thought to be the major contributors to global oxygen production as well as the primary source of essential fatty acids for the food chain. They are mainly used as feed for the production of bivalves, crustaceans, and some early fish stages. In relation to fish larviculture, they are used as a feed for the zooplankton that will be provided to the fish larvae, like *Artemia*, rotifers, or copepods (Brown and Blackburn 2013). In general, the lipid profile of microalgae will determine the lipid profile of live feed organisms. The fatty acid composition of each microalgae will depend on the species and environmental conditions. In general, microalgae have a high percentage of lipids. However, light intensity, photoperiod, temperature, pH, or nutrient limitation may affect the lipid composition. During the exponential phase, microalgae have a higher percentage of fatty acids, although in the stationary phase they accumulate fatty acids belonging to the SFAs group and the MUFAs group. Under nutrient limitation, PUFAs tend to decrease during the stationary phase (Brown and Blackburn 2013). PUFAs are considered the most important group in aquaculture. Docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6) seem to be essential for most of the fish species, as explained above. Thus, the nutritional requirements of copepods have to be evaluated before they are used as live feed for larviculture, as done for some copepod species (Drillet et al. 2006, Fahradian et al. 2014, Lee et al. 2006, Nielsen et al. 2019, Rahmati

et al. 2020, Rasdi et al. 2018, Rasdi and Qin 2018, Van Someren et al. 2020, Velasquez et al. 2001). The present thesis evaluates the effects of three different microalgae diets on population performance and the FA composition of the copepod *Apocyclops panamensis* for pikeperch larviculture.

1.3.3.3.2. Fatty acids requirements of pikeperch

The early life stages of freshwater fish are crucial for their growth and development, and the dietary fatty acid composition plays a significant role in these processes. Understanding the effects of dietary fatty acids on growth and fatty acid composition is essential for optimizing nutrition and ensuring the production of high-quality fish.

Within PUFAs, EPA, DHA, and ARA represent a high proportion of the lipids in fish (Dhont et al. 2013, Geay and Kestemont 2015, Hamza et al. 2015, Kestemont and Henrotte 2015, Tocher 2010). Nevertheless, more studies are needed to identify which fatty acids are relevant for the larvae and quantify them (Hamre et al. 2013). In terms of nutrients, fatty acids are crucial for pikeperch. Pikeperch has a high content of PUFAs, and it is supposed to need them in the diet. Moreover, pikeperch larvae use as energy SFAs, MUFAs, and PUFAs. However, the high content of PUFAs in the larvae indicated that they retained this group of FAs for growth. Pikeperch use LA as energy under starvation conditions, and later, other PUFAs such as alpha-linolenic acid (ALA), EPA, and DHA (Kestemont and Henrotte 2015). Besides that, an increase in polar lipids in the diet increased growth rate in the study of Hamza et al. (2008) and also showed earlier digestive structure development.

As already explained, *Artemia* lacks DHA, which is believed to be essential for pikeperch. Although it is possible to enrich *Artemia* to rear pikeperch larvae (Lund and Steenfeldt 2011), the use of *B. plicatilis* for pikeperch larviculture has increased in recent years (Imentai 2019, 2020a, 2020b, 2022, Yanes-Roca 2018, 2020). Rotifers are one of the main natural feeds for pikeperch larvae (Peterka et al. 2003), and they are supposed to meet the nutritional requirements. Even when they are enriched, *Artemia* and rotifers do not appear to adequately satisfy the nutritional needs of some fish species throughout their early life stages (Rasdi and Qin, 2016). Because of their high natural amounts of PUFAs, free amino acids, and antioxidant pigments, copepods are believed to have a higher nutritional value than rotifers and *Artemia* (Conceiço et al., 2010). Provision of a high omega-3 (n-3) PUFA content is a requirement for copepod production to maintain their high values of PUFAs. These HUFAs in copepods are stored in the polar lipid fraction (Brown and Black 2013). Fish seem to need lipids in the polar fraction as phospholipids for their cell membranes. In the case of the intestine, phospholipids in the diet might contribute to better absorption and transport of long-chain fatty acids through enhanced lipoprotein synthesis (Tocher et al. 2008). Phospholipid composition is high in copepods, and pikeperch larvae seem to improve their growth rate when the diet contains a higher amount of phospholipids (Hamza et al. 2008). Copepods also have higher amounts of PUFAs, in particular DHA, than rotifers and *Artemia* (Hamre et al. 2013). Furthermore, copepod nauplii may be able to fit into the mouth of a pikeperch (Kestemont and Henrotte 2015).

Fatty acids are essential nutrients for pikeperch juveniles, as they play important roles in growth, development, and health. Pikeperch juveniles require both omega-3 (n-3) and omega-6 (n-6) fatty acids in their diet, as they cannot synthesize these fatty acids on their own. Omega-3 fatty acids, such as EPA and DHA, are crucial for the development of the nervous system, immune system, and eyesight. Omega-6 fatty acids, such as LA and ARA, are important for the growth and maintenance of tissues. The optimal n-3/n-6 ratio in the diet of pikeperch juveniles is still under investigation.

1.4.Working hypotheses

Five different chapters (chapter 2 to 6) are included to study the following **hypotheses**:

(1) It is possible to establish new adequate first feed prey items for pikeperch aquaculture based on natural feeding sources.
(2) It is possible to establish a minimum amount and concentration of live feed per pikeperch larva.
(3) It is possible to use *A. panamensis* to rear pikeperch larvae.
(4) It is possible to obtain the copepod *A. panamensis* in high culture density and with an adequate fatty acid profile to be used as live feed in pikeperch larviculture.
(5) It is possible to use larval fatty acid composition to detect the malnutrition status of pikeperch larvae.
(6) It is possible to identify the best life feed organisms for pikeperch during the first life cycle stages by focusing on relevant fatty acids, detectable already during the early days of life.

The overall aim of this study was to gain new knowledge of a balanced and “in time” fatty acids composition for pikeperch larval development during early life cycle stages based on suitable live feed provision in terms of quality, quantity, duration and fatty acid supply, to improve pikeperch larviculture in recirculating aquaculture systems (RAS).

1.5. Discussion

The aim of this work was to analyse the effect of different live feed organisms on pikeperch larval survival, growth, and fatty acid composition and to identify areas for feeding improvement during the early life stages. For this purpose, several live feed organisms were applied as diet in different qualities, quantities, and at different larval periods. We studied two types of rotifers until dph 10, a saltwater rotifer, *B. plicatilis*, which is the most commonly used in aquaculture to replace *Artemia* nauplii in pikeperch larviculture, and the freshwater *B. calyciflorus*. Both have a suitable size for rearing larvae during the first days of exogenous feeding. (1) The use of the freshwater rotifer *B. calyciflorus* was applied under a pseudo-green water technique in order to improve the survival, growth, and nutrition of pikeperch larvae. However, pikeperch larvae are commonly reared with *B. plicatilis,* and thus, (2) *B. plicatilis* was studied in different quantities and densities to obtain the best larval performance. Furthermore, (3) the use of *A. panamensis* was evaluated from dph 11 and compared with the use of *Artemia* nauplii. (4) The copepod *A. panamensis* was studied to determine its nutritional composition under different microalgae diets. Finally, (5) the dynamics of the fatty acids in pikeperch larvae and juveniles were evaluated under the use of *Artemia* and microdiets at a commercial scale.

This thesis is the first attempt to apply the pseudo-green water technique in larval pikeperch rearing, to determine the minimum quantity of live feed per larva per day, and to apply exclusive feeding with copepods in pikeperch larviculture. Moreover, this thesis brings to light important information on timely and balanced nutrition during the early developmental stages of *S. lucioperca*. Four zooplankton species were considered as live feed, two different techniques, clear- and pseudo-green water, were used in a RAS, and different periods within the larval stage of pikeperch were evaluated.

1.5.1. Pikeperch larviculture

1.5.1.1. Rotifers as live feed

The use of rotifers has increased in recent years in fish larviculture due to the need to improve nutrition from the beginning of exogenous larval feeding. In pikeperch, the rotifer *Brachionus plicatilis* showed promising results (Imentai et al. 2019a, 2019b, Yanes-Roca et al. 2018, 2020). However, there is still room for improvement since survival rates must be optimized. In this section, the progress made in the feeding strategies with rotifers as the first live feed organism in Bischoff and Kubitz et al. (2022) and Ballesteros-Redondo et al. (2023a) is discussed. This section focuses on the implications of the use of rotifers for pikeperch survival and growth and on the implications of natural characteristics on feeding strategies, which include feed quality, quantity, and duration.

Feed quality. The feeding protocol for *S. lucioperca* larvae has been based on the methods developed for marine fish species (Steenfelt 2015). However, in pikeperch rearing facilities, the freshwater environment does not support the survival of *B. plicatilis* and *Artemia*, as they are native to saline

environments (Dhont et al. 2013). To stimulate the fish's prey response, the use of freshwater *B. calyciflorus* is more suitable, although freshwater rotifers are not commercially available as live prey to replace *Artemia* easily (Dhont et al. 2013). Considering the natural characteristics of pikeperch as a freshwater or brackish-water species, the use of freshwater rotifers, such as *B. calyciflorus*, is more appropriate. Additionally, due to co-evolution, *B. calyciflorus* may better fulfill the nutritional requirements of the fish larvae. As freshwater rotifers can introduce infections that affect fish and shrimp (Yan et al., 2007), the use of saltwater zooplankton remains common in freshwater aquaculture. Nevertheless, the presence of parasites does not always result in disease and can enhance the fish's immunity (Hennersdorf et al. 2016). Moreover, certain bacteria can benefit rotifers in the same environment by producing vitamin B12 (Rombaut et al. 1999). Furthermore, microalgae in larval rearing have demonstrated probiotic effects and the ability to autoregulate the microbial community (Cahu et al. 1998, Van der Meeren et al. 2007). Therefore, utilizing organisms from the same environment might be advantageous in larviculture, enabling techniques like green water or pseudo-green water. Consequently, in our study, we investigated *B. calyciflorus* as the initial live feed for pikeperch larviculture using the pseudo-green water technique for continuous feed supply.

After the use of *B. calyciflorus*, pikeperch survival rates of 94% in system I (experiment I) and 64–74% in system II and III (experiment II) were reached at dph 10 (see Table 2 in Bischoff and Kubitz et al. 2022). These survival rates were similar to those found for pikeperch larvae fed with the saltwater rotifer *B. plicatilis* (64-93.7%) in Ballesteros-Redondo et al. (2023a). However, the specific growth rate (SGR) reached the highest value (5% d^{-1}) (considering linear growth) with *B. calyciflorus* in the System I (experiment I) than with *B. plicatilis* (3% d^{-1}) (Ballesteros-Redondo et al. 2023). Consequently, our study demonstrated suitable survival and growth rates by using *B. calyciflorus*. Therefore, these data confirm **hypothesis 1** that *"it is possible to establish new adequate first feed prey items for pikeperch aquaculture based on natural feeding sources".*

Our study demonstrated the potential of using natural feed sources as a solution for improvement in fish larviculture. When there is a good match between feed (prey) and predator, the results were maximized (see figures 2 and 3 in Bischoff and Kubitz et al. 2022). This is of great importance for increasing the production efficiency of *S. lucioperca* in the future since there is a growing demand for this species in Germany. Improving production will improve the economic profits of the companies by producing pikeperch and bringing a high-quality, regionally produced species to the market, making their production more and more sustainable. Pikeperch larval rearing is still characterized by significant mortality rates during the early developmental phases. Bischoff and Kubitz et al. (2022) described a recently created culture system that enables the modern, sustainable farming of fish larvae while obtaining high survival and growth rates. Additionally, it demonstrated that the positive outcomes under the applied conditions were due to the adoption of the freshwater zooplankton organism *B. calyciflorus* rather than the typical saltwater species. The fatty acid readings demonstrate a healthy larval nutritional state (see 1.5.3).

Feed quantity. The saltwater *B. plicatilis* was given exclusively or in combinations with the copepod *A. panamensis* in different amounts of individuals (ind.) per larva per day. We evaluated different amounts of *B. plicatilis* per lava per day in two different experiments (see table 1 in Ballesteros-Redondo et al. 2023a). Our findings showed the highest growth rate with the diet B340+A60, when we fed larvae with 340 ind. of *B. plicatilis* and 60 ind. of *A. panamensis* per larva per day and there were 6.3 *B. plicatilis*/mL at each feeding time. Increasing the amount of *B. plicatilis* by more than 340 ind. per larva per day did not significantly increase the survival and/or growth rates. On the one hand, Ballesteros-Redondo et al. (2023a) illustrated the significance of taking into account live feed density and the amount of feed consumed per larva daily. Yanes-Roca et al. (2020) fed 13 ind./ml with a larval stocking density of 100 larvae per liter, which is a similar amount of feed per larva than in our study. However, they obtained lower growth and survival rates. Thus, our results indicated that a stocking density of 50 larvae per liter is adequate to rear pikeperch larvae. Our data showed that the larval stocking density of pikeperch should be considered for feeding, as shown by Król and Zielinski (2015) for perch (*Perca fluviatilis* L., 1758), Ramos et al. (2016) for leaf fish (*Monocirrhus polyacanthus* Heckel, 1840), and Zarski et al. (2011) for common barbel (*Barbus barbus* (L., 1758)), all of which used the same amount of feed per fish. Larval stocking density is therefore crucial for larval feeding. As long as larval survival and growth are not negatively impacted, high larval densities result in lower production costs (Steenfeldt 2015). Consequently, our study showed that the combination of the optimal feeding organism, feed density, feed quantity, and fish stocking density must be taken into account for successful pikeperch larviculture to improve the efficiency of the entire production (Ballesteros-Redondo et al. 2023a).

On the other hand, our results indicated a maximum ingestion rate, or satiety, in pikeperch larvae. Instead of looking for the "golden density" (Saraiva et al. 2022), this maximum feeding intake indicates an appropriate feed density in the water at each feeding time of 6 ind./ml in accordance with Imentai et al. (2019b) and Bischoff and Kubitz et al. (2022). Our minimum recommended amount of *B. plicatilis* per larva per day indicated an indirect measurement of the nutritional requirements of pikeperch larvae between dph 4 and 10 (see 1.5.3). Therefore, our study demonstrated **hypothesis 2:** "*It is possible to establish a minimum amount and concentration of live feed per pikeperch larva".* Ballesteros-Redondo et al. (2023a) showed that live feed for pikeperch larviculture has to be adequate in terms of quality and quantity, together with the fish stocking density, to maximize larval performance, reduce the production of dissolved and particulate nutritional wastes and feed losses, and consequently maintain a good nutritional profile of the larva for further development. Our data indicated a maximum amount of 340 *B. plicatilis* per pikeperch larva per day during the first days of exogenous feeding, at which a plateau of survival and growth rate is reached, which was also confirmed by the fatty acid profile of the pikeperch larvae. This pattern might be related to the match-mismatch of prey and predator in natural environments. This is significant because feeding above the minimum required amount of live feed per fish increases performance, reduces waste, and decreases costs. Therefore, Ballesteros-Redondo et al. (2023a) showed how environmental characteristics of the fish larvae might affect nutrition and, hence,

feeding strategies and stocking practices, which affect the larval growth rate of emergent species like *S. lucioperca*.

Ensuring an adequate supply of feed is crucial when pikeperch larvae begin feeding. This aligns with the match-mismatch theory, which says that fish reproduction corresponds to environmental conditions that optimize food availability for larvae (Cushing 1974). In aquatic ecosystems, light availability and temperatures increase during spring, influencing the production of microalgae, the primary producers. Unlike photoinhibition, primary producers engage in maximal photosynthesis. Subsequently, herbivorous zooplankton, following the phytoplankton bloom, experience population growth, coinciding with the emergence of larval fish. However, these larvae may encounter herbivorous zooplankton consuming late-blooming phytoplankton rather than early-blooming phytoplankton (Boerman et al. 2008). This autoregulation is a common feature in ecosystems and contributes to the characteristics of farmed fish through evolution. Incorporating these natural characteristics into future nutrition and feed research can optimize fish culture, enhance feeding strategies, and promote sustainability in fish production.

This match-mismatch pattern was observed in Bischoff and Kubitz et al. (2022). In system I (experiment I), the highest survival and growth were reached with a mean of 3 *B. calyciflorus*/ml from 4 to 10 dph, meaning 300 ind. per larva per day. These results were obtained when microalgae were stocked 5 days before and *B. calyciflorus* 3 days before the stocking of the pikeperch larvae. This amount of *B. calyciflorus* was similar to the recommended amount of *B. plicatilis* in Ballesteros-Redondo et al. (2023a), also confirming **hypothesis 2**. However, stocking the microalgae 10 days before stocking the larvae resulted in a poorer match between the needs of the larvae and the feed, leading to lower survival and growth rates (see systems II and III in experiment I in table 2 in Bischoff and Kubitz et al. 2022).

Phytoplankton nutrient levels vary according to nutrient availability, with the exponential phase considered optimal. A mismatch in feeding on late-bloom algae indicates their higher depletion of phosphorus and nitrogen, making them a lower-quality feed source for larval fish (Boerman et al. 2008). This mismatch drives pikeperch larvae to seek alternative prey, such as smaller larvae of other fish species. However, in RAS-based pikeperch culture, a mismatch can result in cannibalism due to their carnivorous nature. Therefore, utilizing these ecological characteristics becomes crucial to prevent larval cannibalism in larviculture.

This thesis underscored the significant implications of the match-mismatch theory for aquaculture feeding practices, specifically in larval fish culture and sustainability. It demonstrated how applying the principles of this theory can optimize feeding strategies, enhance fish larval growth and survival, and promote sustainable aquaculture practices. By aligning prey availability with larval nutritional requirements, aquaculture can address challenges in live feed production, minimize environmental impacts, and improve overall efficiency. By optimizing feeding efficiency and minimizing reliance on live prey, aquaculture operations can reduce environmental impacts associated with live feed production, including resource consumption and waste generation. While the match-mismatch theory shows promise

for improving fish larval culture and sustainability in aquaculture, several challenges remain, such as understanding the nutritional requirements of fish larvae during different developmental stages (see 1.5.3).

Duration. There is increasing scientific evidence that rotifers are necessary in the early days of pikeperch larval feeding (Imentai et al. 2019a, 2019b, 2020, 2022, Yanes-Roca et al. 2018, 2020). All these studies showed that feeding *B. plicatilis* followed by *Artemia* sp. improved survival and growth in pikeperch larvae. Nevertheless, the use of rotifers must be restricted to the first days of feeding to avoid significantly decreased enterocyte height in the anterior intestine. Thus, feeding pikeperch larvae rotifers for more than 12 dph may negatively influence their growth and intestinal development (Imentai et al. 2020).

1.5.1.2. Copepods as live feed

Fish larvae undergo various physiological changes during the early life cycle stages; hence, it is necessary to provide appropriate feeding throughout the entire larval time to meet the larval nutritional needs (Planas and Cunha 1999). Several studies have shown that pikeperch larvae need DHA on dph 20 (El Kertaoui et al. 2019, Lund et al. 2018). Although freshwater fish are known to have a certain capacity to elongate FAs, this desaturation or elongation of ALA into EPA or DHA consumes energy, and thus, feeding larvae differently with enough amounts of EPA or DHA seems to be more effective. Therefore, finding a natural source for DHA seems to be essential for pikeperch larviculture. Cyclopoids nauplii have been found in the stomach of pikeperch larvae (Peterka et al. 2004), and thus, they might have a suitable nutrient profile. Although *A. panamensis* is a saltwater copepod, it belongs to the cyclopoids, which have already shown a high potential for aquaculture (Pan et al. 2018). Thus, the use of *A. panamensis* for pikeperch was studied in the period 3–10 dph in Ballesteros-Redondo et al. (2023a) and from dph 11 in Ballesteros-Redondo et al. (2023c). *A. panamensis* was studied under different microalgae diets in Ballesteros-Redondo et al. (2023b). This section focuses on the implications of the use of copepods for pikeperch growth and feeding strategies.

Feed quality. Since we saw no advantages to using *A. panamensis* (Ballesteros-Redondo et al. 2023a), we hypothesized that larvae were not able to ingest *A. panamensis*. The quality of the feed includes the organism's behavior, its nutritional composition, and its size, which determine the likelihood of ingestion by the larvae. Ballesteros-Redondo et al. (2023b) measured the size of each *A. panamensis* stage. The average size of our *A. panamensis* population was 158.7 µm for nauplii, 372.4 µm for copepodites, and 637.2–676 µm for adults. However, our size measurements are within the range of a closely related species, *Apocyclops royi* (Lindberg, 1940) (78–250 m for nauplii and 260–997 m for copepodites and adults) (Jepsen et al. 2021). Therefore, *A. panamensis* nauplii were suitable as live feed

for pikeperch larviculture from the beginning of the exogenous feeding as they are similar in size to rotifers.

In a poster-published study, we analyzed daily the stomach contents, survival rate, and growth rate of the pikeperch larvae from 3 to 7 dph. Significant higher survival rates were found on dph 7 in larvae fed with *B. plicatilis* (56%) than with diets containing *A. panamensis* (~ 40%) (ANOVA, p = 0.07). The total body length did not show any significant difference between diets (ANOVA, p = 0.411). From dph 7, we observed feed in the stomach of the fish larvae, although we could not determine the amount when they fed on *B. plicatilis*. Conversely, larvae did not digest the copepods (Figure 1).

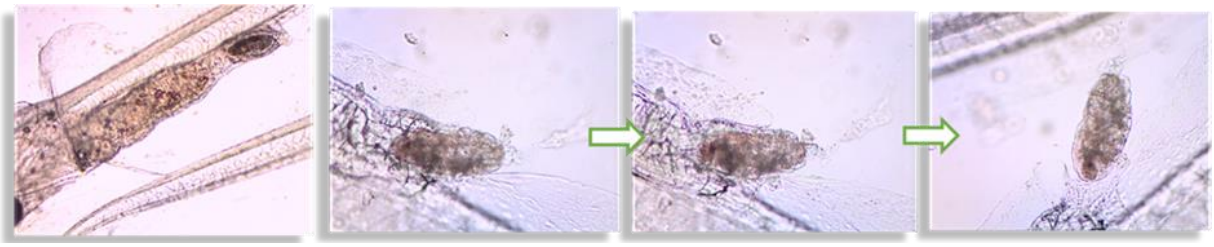

Figure 1. Serial photo shooting of one larva defecating the copepod on dph 7.

This demonstrated for the first time that pikeperch larvae can ingest *A. panamensis* on dph 7, but they were not able to digest them well. The difficulty in counting individuals in the stomach of larvae fed with *B. plicatilis* indicated that digestion took place. This might be related to the higher survival rates of the pikeperch, confirming previous studies (Yanes-Roca et al. 2018, Imentai et al. 2019, Imentai et al. 2020). Our studies showed important information about the ingestion and digestion of different live feeds by the pikeperch larvae.

Nevertheless, the exclusive use of *A. panamensis* as live feed for pikeperch larvae was studied from dph 11 (Ballesteros-Redondo et al. 2023c). In experiment 1 (E1), pikeperch larvae at dph 11 grew more with *B. plicatilis* (length of 6.18 mm in diets B+Apo300 and B+Apo600) than with *Artemia* sp. (length of 4.89 mm in diets Art+Apo300 and Art+Apo600) (see figure 2 in Ballesteros-Redondo et al. 2023c). After feeding all groups with *A. panamensis* on dph 18, diet B+Apo300 produced the longest size (7.13 mm). Despite the initial shorter size of pikeperch larvae fed with *Artemia* sp., the SGR of larvae previously fed with *Artemia* sp. was higher from dph 11 to dph 18, suggesting better larval development. According to this finding, larvae that had previously consumed *Artemia* sp. during the first 10 days were capable of compensating for reduced growth from dph 0 to dph 11 by consuming *A. panamensis* later on. However, B+Apo300 exhibited an even higher SGR (4.33% d^{-1}) than the SGR data in Ballesteros-Redondo et al. (2023a) and Imentai et al. (2019a). This suggests that, even though the growth was compensated, the initial feeding diet had an impact on the larva's long-term growth (Hou and Fuiman 2020). In experiment 2 (E2), *A. panamensis* increased larval growth compared to the application of *Artemia* sp. on dph 16 (Ballesteros-Redondo et al. 2023c). On dph 16, larvae fed *Artemia* sp. had a slightly better survival rate (93.0%) than larvae fed *A. panamensis* (87.9%). The SGR was substantially

higher in larvae fed *A. panamensis* (2.95% d^{-1}) than *Artemia* sp. (1.32% d^{-1}) on dph 16, and in comparison to Imentai et al. (2020), who fed pikeperch larvae various combinations of *B. plicatilis* and/or *Artemia* sp. Hence, when compared to *Artemia* sp., our findings on larval growth showed that *A. panamensis* had a distinctly positive impact on the growth of pikeperch larvae between dph 11 and dph 16 (Ballesteros-Redondo et al. 2023c). Consequently, chapter 4 confirmed **hypothesis 3,** which stated that "*it is possible to use A. panamensis to rear pikeperch larvae".* It must be considered that the better performance of pikeperch larvae with *A. panamensis* occurred during the five consecutive days after an initial 7-day *B. plicatilis* feeding. This suggested adequate timing and availability of both live feed organisms, making the larviculture of pikeperch more complex. The high cost of copepod production is another constraint to be considered by the pikeperch hatcheries. Therefore, the economic viability and production efficiency of the combined use of rotifers and *A. panamensis* must be further assessed. However, our data showed that they satisfied the fish's nutritional needs better than rotifers and *Artemia* (Conceiço et al. 2010). To fully realize the potential of copepods in aquaculture, there is a need to improve copepod culture techniques in practical, economic, and sustainable terms (see 1.5.2).

Feed quantity. An attempt to introduce *A. panamensis* was made in order to increase survival and growth (Ballesteros-Redondo et al. 2023a). Diets with *A. panamensis* showed no advantages over those with *B. plicatilis*. Larvae fed with a higher amount of *A. panamensis* (diets B280+A120, B510+A90, or B420+A180) did not show higher survival or growth rates than larvae fed with less *A. panamensis* (diet B340+A60) (Ballesteros-Redondo et al. 2023a). Moreover, in E1 of Ballesteros-Redondo et al. (2023c *A. panamensis* was introduced on dph 11 to the larvae after feeding them with *B. plicatilis* or *Artemia* sp. in two different amounts, 300 and 600 ind. per larva per day. No benefits of using higher amounts of *A. panamensis* were identified in terms of survival or growth rates. Thus, the recommended amount might be considered 300 ind. of *A. panamensis* per larva per day from dph 11. These results are similar to the recommended amount of *B. plicatilis* during the first period (3–10 dph). Thus, a minimum feed amount with a maximum survival and growth rate will improve the efficiency of larval rearing, as discussed previously. Despite the shown benefits of *A. panamensis* as a diet for pikeperch larvae, there are still knowledge gaps that should be addressed, like investigating the long-term effects of copepod feeding on growth, development, and health of fish larvae, including the potential effects on later life stages, or assessing the economic feasibility of copepods.

Duration. In E1 of Ballesteros-Redondo et al. (2023c), survival rates were the highest for protocols with *B. plicatilis* and *A. panamensis*. However, the mortality drastically increased in all treatments from dph 16. This might indicate that *A. panamensis* should be best used until dph 16 (for a five-day feeding period) after being fed with *B. plicatilis* or *B. calyciflorus*.

1.5.2. Fatty acids in live feed

Manipulating environmental factors such as temperature, salinity, or feeding can enhance live feed reproduction, growth, and nutritional quality. Understanding the specific requirements of different zooplankton species and tailoring culture conditions accordingly can maximize their production potential. This section focuses on the implications of microalgae diets on the fatty profiles of rotifers and copepods.

1.5.2.1. Fatty acid of rotifers

As seen, rotifers were the best option as the first live feed organisms (Bischoff and Kubitz et al. 2022, Ballesteros-Redondo et al. 2023a). However, the fatty acid composition of the rotifers depends on the microalgae diet. Although Ferreira et al. (2017) showed that the quality of microalgae is the most important factor influencing the biochemical composition of the rotifers, the FA composition of the rotifers will also depend on the quantity. Kennari et al. (2008) found that at a higher concentration of microalgae, rotifers obtained a higher HUFA content. Schälicke et al. (2019) stated that quality is as important as quantity. Enough feed and energy but lack of some individual nutrients can negatively affect rotifers.

We observed that *B. plicatilis* had a higher LA while *B. calyciflorus* had a higher ALA. *B. plicatilis* was fed with *Nannochloropsis* sp. and fed to the larvae when they were not enriched (Ballesteros-Redondo et al. 2023a). *B. calyciflorus* was fed with *Monoraphidium contortum* and fed continuously to the larvae with a pseudo-green-water technique; thus, *B. calyciflorus* was enriched (Bischoff and Kubitz et al. 2022). Nevertheless, as the microalgae diet is not similar, it is difficult to compare the nutritional value between these fresh- and saltwater rotifers as live feed for pikeperch larvae. Schälicke et al. (2020) found out that C18 PUFAs seem to be more important for rotifers than C20 PUFAs. *M. contortum* also provided high ALA to *B. calyciflorus* in Schälicke et al. (2020) in comparison to *Nannochloropsis limnetica* Krienitz, 1998. In Kennari et al. (2008), the best results in *B. calyciflorus* culture were obtained after feeding with *Chlorella* sp., obtaining high ALA content only with a sufficient microalgae supply. However, the content of LA was higher than ALA with *Chlorella* sp. Yanes-Roca et al. (2020) used *Chlorella* sp. to feed *B. plicatilis* and obtained a similar profile of LA and ALA to that in *B. plicatilis* by Ballesteros-Redondo et al. (2023a). However, the best results in pikeperch larval survival and growth were with the rotifer *B. calyciflorus,* which had the highest n-3/n-6 (approximately 2) when fed with *M. contortum*. The next best larval survival and growth were found when feeding *B. plicatilis* with *Nannochloropsis* sp., which had a ratio n-3/n-6 around 1.66 (Ballesteros-Redondo et al. 2023a), and finally feeding *B. plicatilis* with *Chlorella* sp.*,* obtaining rotifers with a ratio n-3/n-6 around 1.53 (Yanes-Roca et al. 2020).

Marine organisms have more EPA and DHA than freshwater organisms, which have more ALA (Twinning et al. 2021). Therefore, *M. contortum* (a freshwater species) may have provided a better FA profile with high ALA to the freshwater rotifers *B. calyciflorus,* which resulted in a suitable n-3/n-6 ratio

of 2 for pikeperch larvae during this larval period and providing enough LA at the same time. Moreover, the pseudo-green water technique supplies microalgae in sufficient quantity. After some time, the high PUFA content in rotifer decreased (Kennari et al. 2008), and thus, pseudo-green water might be better for the supply of PUFAs in the food web. Thus, pseudo-green water provided high-quality fatty acids to *B. calyciflorus* eaten by the pikeperch larvae, yielding higher larval survival and growth (Bischoff and Kubitz et al. 2022) than clear water (Ballesteros-Redondo et al. 2023a). Therefore, our data showed that pikeperch larvae should be fed with rotifers with a n-3/n-6 ratio of approximately 2. All these data again confirm **hypothesis 1:** "*It is possible to establish new adequate first feed prey items for pikeperch aquaculture based on natural feeding sources".* However, the benefits might also come from the fact that the use of the pseudo-green water technique might improve light conditions (Heidemann et al. 2019) or the microbiome, as well as allowing the rotifers to remain swimming longer than if they were in saltwater. The reduced waste of food and its non-death and decomposition may also improve the water conditions in favor of the larvae.

1.5.2.2. Fatty acid of copepods

Although copepods have shown promising results for fish larvae (Barroso et al. 2013, Evjemo et al. 2003, Karlsen et al. 2015, Malzahn et al. 2022, Martinez-Silva et al. 2018), there are still many gaps in knowledge about the culture of these organisms. To fully realize the potential of copepods in aquaculture, there is a need to improve copepod culture techniques in practical, economical, and sustainable terms. Environmental conditions such as water parameters have been studied for several species (Cruz-Rosado et al. 2020, Jepsen et al. 2015). Nevertheless, they seem to be species-specific, and thus, optimal conditions, including feeding, must be studied for each species. According to the literature, since enrichment approaches are appropriate only for some copepods and since it is essential to include a high PUFA content in their feed (Rasdi and Qin 2016), microalgae diets must be applied. This is thus essential before feeding fish larvae with a certain copepod species. Therefore, exploring innovative culture techniques, such as copepod culture with microalgae or bacteria, is essential to enhance copepod nutritional quality and improve overall production efficiency.

The PUFAs in the microalgae diet influenced the PUFAs in *A. panamensis*. Higher n-3/n-6 ratios in microalgae increase n-3/n-6 ratios in copepods within PUFAs (Ballesteros-Redondo et al. 2023b). In E1, copepods given *Isochrysis galbana* (ISO) and a combination of *I. galbana* and *Nannochloropsis* sp. (N+I) had reduced PUFA levels. Nevertheless, the highest n-3/n-6 ratio was achieved in *A. panamensis* fed wtih ISO (2.20), which was similar to the ratio of the rotifers. In E2, the highest final densities were achieved by feeding *A. panamensis* with ISO and N+I also reaching the highest n-3/n-6 ratios. The diet ISO, which had a higher n-3/n-6 ratio (4) than *Nannochloropsis* sp. (3.6), showed the highest n-3/n-6 ratio in *A. panamensis* in both experiments (Ballesteros-Redondo et al. 2023b). When *A. royi* fed *I. galbana* instead of *Nannochloropsis oculata* (Droop) Hibberd, 1981, the same pattern was seen in Pan et al. 2018. Therefore, our results showed that the PUFAs in *A. panamensis* were influenced by the

PUFAs in the microalga diet. This was also supported by the concentrations of stearidonic acid (SDA), eicosatetraenoic acid (ETA, C20:4 n-3), and DHA. Copepods given ISO had a higher DHA content of 2.6% in E1 and 1.9% in E2. Tocher (2010) advocated for a minimum amount of 1–2.6% DHA in live feed for fish larvae, although our data showed lower DHA levels than for *A. royi* reported by Jepsen et al. (2021), Nielsen et al. (2019), and Pan et al. (2018). However, since DHA is linked to copepod fecundity (Kleppel et al. 2005), the DHA levels in our copepods were sufficient.

As a result, our findings showed that *A. panamensis* has the capacity to satisfy the DHA needs of fish larvae (Ballesteros-Redondo et al. 2023b). Although it is possible to culture *A. panamensis* in fertilized ponds (Phelps 2005, Sumiarsa and Phelps 2007), feeding *A. panamensis* with *I. galbana* resulted in a higher DHA/EPA ratio (2.5–2.9). The DHA/EPA ratio reported for species like *Acartia tonsa* (1.35) and *Tisbe holoturidae* Humes, 1957 (1.63) was lower than this result (Drillet et al. 2006). Our DHA/EPA ratio was in the range of 0.3–2.0, which is necessary for fish (Tocher 2010). *A. panamensis* might therefore satisfy the nutritional needs of fish larvae. Additionally, El Kertaoui et al. (2019) found that 3.5% of EPA+DHA and the calcium/potassium (Ca/P) ratio were the optimal dietary components for pikeperch larvae. Our study showed that *A. panamensis* fed ISO in E1 displayed a 3.5% EPA+DHA concentration (Ballesteros-Redondo et al. 2023b). Moreover, *A. panamensis* fed ISO in E1 showed also an n-3/n-6 ratio of approximately 2 as rotifers (see 1.5.2.1). Therefore, our results demonstrated that the fatty acid composition of *A. panamensis* was adequate to fulfill the nutritional needs of pikeperch larvae. Our results confirmed **hypothesis 4,** which stated, "it *is possible to obtain the copepod A. panamensis in high culture density and with an adequate fatty acid profile to be used as live feed in pikeperch larviculture´*.

Copepods are not regularly used in pikeperch larviculture; however, they might be considered adequate feed. *A. panamensis* can provide adequate and optimal feed after it has been fed with *I. galbana*. Traditional feeds such as dry feeds or *Artemia* nauplii have shown some limitations, in particular for small and sensitive larvae species. Consequently, the use of live feed organisms is expanding rapidly in aquaculture, especially copepods, which are becoming more important. Our data about this novel species in aquaculture indicated that *A. panamensis* size, density, population composition, and fatty acid profile are suitable, on the one hand, for efficient live feed cultures and, on the other hand, for feeding fish larvae. This is significant because it provides new relevant information for the proper management of *A. panamensis* culture, which might reduce the high costs that are the main constraint in copepod culture and lead to more sustainable production. Furthermore, best practices concerning nutrition produce high quality live feed, which has the potential to increase the survival and growth rates of the fish larvae.

Incorporating copepods into aquaculture systems requires practical and economically viable approaches. Strategies such as optimizing copepod production protocols have the potential to reduce production costs through efficient use of resources, and exploring potential value-added markets for copepods can contribute to their commercial viability. Developing sustainable copepod culture methods is crucial to minimizing environmental impacts and promoting long-term viability. This thesis proportions novel

information for the culture of *A. panamensis* with relevant information to develop large-scale production techniques.

1.5.3. Fatty acid in pikeperch larvae

In this final part of the discussion, we compared the fatty acid composition of larvae during different periods: from dph 0 to 10, we compared rotifers with *A. panamensis* and *Artemia* sp., and from dph 11 to 16/18, we compared *A. panamensis* and *Artemia* sp. Moreover, we discussed about proper weaning time between dph 16 and 25 based on the larval fatty acid composition. A comparison of all the FA dynamics under the different live feed organisms provided the information needed to establish the best feeding protocol and informs what the balanced fatty acid composition of the pikeperch larvae must be. Lipid profiles, or lipid phenotypes, are part of the fitness of organisms (Twinning et al. 2021). Changes in these physiological phenotypes might have been nutritionally programmed via live feed (Hou and Fuiman 2020). Therefore, lipid phenotypes might be used to evaluate the nutritional status of fish larvae.

1.5.3.1. Period dph 0- dph 11

The quality of the gametes of the parents influences the quality of the eggs and, therefore, of the larvae. It is important to consider that the higher the quality at hatching, the better the larvae might perform. In Bischoff and Kubitz et al. (2022), the larvae hatched with a total FA of 110.4 µg /mg dry weight (DW), which was one of the lowest values of all the studies included in this thesis. However, the SGR was one of the highest when we applied the right feeding protocol. This showed the importance of proper feeding and also confirmed that *B. calyciflorus* can be used as a natural feed source for pikeperch larviculture (**hypothesis 1**).

The use of rotifers seemed to be essential as the first feed source for pikeperch larvae (Bischoff and Kubitz et al. 2022, Ballesteros-Redondo et al. 2023a). PUFA content increased in pikeperch larvae fed with *B. plicatilis* and *A. panamensis* (diet B100+A100), particularly the DHA content and the DHA/EPA ratio (Ballesteros-Redondo et al. 2023a). *A. panamensis* was fed with *I. galbana*, which had a high content of DHA (Dustan et al. 1993, Roncarati et al. 2004), and thus, *A. panamensis* contained high levels of DHA (Figure 2). Higher DHA content in larvae fed with diet B100+A100 showed that *A. panamensis* was ingested and digested by pikeperch larvae during the trial, contrary to what was previously discussed. However, higher DHA content in the diet did not result in higher survival or better larval growth. These results were opposite to some studies that showed that pikeperch larvae need PUFAs and, in particular, DHA around dph 20 (Hamza et al. 2008, Hamza et al. 2015, Kestemont et al. 2007, Lund et al. 2012, Lund et al. 2014, Lund et al. 2018). Consequently, our study showed that at this larval stage, they did not need dietary DHA as long as they have reserves in the sac. This result supported the suitability of rotifers as first live feed and, thus, the suitability of their fatty acid composition for pikeperch larvae. Furthermore, other authors have shown that pikeperch need PUFAs like linoleic acid

(LA) (Yanes-Roca et al. 2020). *B. plicatilis* was fed with *Nannochloropsis* sp., which contains high LA, and thus, *B. plicatilis* fed on *Nannochloropsis* sp. contains high LA (Figure 2). Animals are not able to synthesize LA and ALA de novo. The amounts in the pikeperch larvae depend on their reserves from the yolk sac or on their diets. Ballesteros-Redondo et al. (2023a) showed that over a certain amount of rotifers per fish larvae per day, they did not perform better, and they did not get higher amounts of ALA or LA, showing a maximum in ingestion or digestion (see figure 3 in Ballesteros-Redondo et al. 2023a). Thus, with the fatty acid data, we confirmed the minimum feed to supply to the pikeperch larvae in this period. Our results showed that this minimum amount of *B. plicatilis* per larva and day filled the nutritional requirements of pikeperch larvae in terms of fatty acids since high survival and growth rates were achieved. Therefore, we established an indirect measurement of the fatty acid requirements for pikeperch larval rearing until dph 10, which kept the LA in the larvae at 8.8-12.4% of the total fatty acids (TFAs) (Ballesteros-Redondo et al. 2023a). Consequently, an optimal *B. plicatilis* concentration and density enabled the highest larval survival and growth rates and optimal fatty acid composition in the early life cycle stages of pikeperch (**hypothesis 2**). Morevoer, this percentage of LA in pikeperch larvae fed with *B. plicatilis* was similar to the LA percentage of pikeperch larvae fed with *B. calyciflorus* (Bischoff and Kubitz et al. 2022).

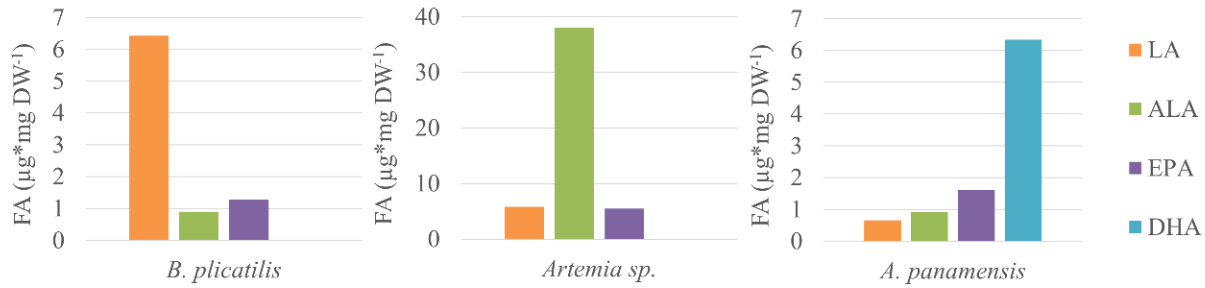

Figure 2. Main polyunsaturated fatty acids found in our experiment for *B. plicatilis* reared with *Nannochloropsis* sp. (left), *Artemia* sp. after hatch (middle), and *A. panamensis* cultured with *Isochrysis galbana* (right).

B. plicatilis is becoming a regular live feed, but no one is using it in the right and best concentrations, as can be seen by the higher mortalities in the literature. *Artemia* sp. is still employed since rotifers are too tiny and constrained for fast growth after dph 11 (Policar et al. 2019, Yanes-Roca et al. 2018). Although these authors considered the use of *Artemia* sp. adequate after *B. plicatilis*, Bischoff et al. (2018) showed that the exclusive use of *Artemia* resulted in the starvation of the pikeperch larvae. Nevertheless, Abi-Ayad et al. (2004) showed that *Artemia* together with microdiets containing DHA are suitable for pikeperch larvae. Besides, *Artemia* species are deficient in crucial fish nutrients, including docosahexaenoic acid (DHA) (Figure 2) (Hamre et al. 2013). Since it depends on fish oils to increase its levels of long-chain polyunsaturated fatty acids (LC-PUFAs), enriched *Artemia* is constrained (Nielsen et al. 2017). Although even enriched, they appear to meet the nutritional needs of the larvae

poorly since the fatty acids are in the neutral fraction (Rasdi and Qin 2016). Moreover, high levels of DHA have also been linked to a rise in malformations due to free-radical oxidation (Izquierdo et al. 2013).

In Bischoff et al. (2023), pikeperch larvae were fed with micro *Artemia* the first seven days. The absolute amounts of LA, ALA, and DHA per larva declined. The depletion of C18 PUFAs throughout the first seven days of larval development might be caused by low concentrations of LA and particularly ALA in micro *Artemia*. Moreover, EPA content increased. This proved that the larvae consumed micro *Artemia*. However, LA levels dropped below 6% and DHA levels below 2%. Our DHA findings on dph 7 were lower than those of Bischoff et al. (2018) on dph 10 during starvation of the pikeperch larvae (28.8%). These values are below larval DHA content of 12–15% on dph 10 in Bischoff and Kubitz et al. (2022) and Ballesteros-Redondo et al. (2023a), which were considered adequate due to the high survival and growth rates achieved. Therefore, these results showed the nutritional deficiencies caused by the *Artemia* feeding. In Bischoff et al. (2023), the pikeperch growth of the surviving larvae was comparable to Ballesteros-Redondo et al. (2023a). However, survival rates were lower (Schmidt and Kühn, 2014). Our results demonstrated **hypothesis 5**, which stated that *"it is possible to use larval fatty acid composition to detect the malnutrition status of pikeperch larvae"* with LA under 6% and DHA under 2%, an indication of poorly nourished pikeperch larvae. The sole use of *Artemia* as a live feed caused problems for larviculture and fish survival, including fatty acid transfer into the pikeperch. This might explain the still high mortality rates in hatcheries and require feed adjustments in the future.

Moreover, in Ballesteros-Redondo et al. (2023c), the applied *B. plicatilis* contained less total FAs and PUFAs per dry weight than *Artemia* sp. Hence, compared to pikeperch larvae fed *Artemia* sp., *B. plicatilis*-fed larvae had reduced total FA and PUFA levels. However, the larvae fed with *B. plicatilis*, grew more. As noted by Bischoff and Kubitz et al. (2022), Ballesteros-Redondo et al. (2023a), and Yanes-Roca et al. (2018), LA appears to be an important fatty acid in the diet of pikeperch. This was confirmed by the results of larval DHA on dph 10 in E1 when feeding *Artemia* sp., which kept an acceptable DHA level of 17%, but the LA was under 6% (Ballesteros-Redondo et al. 2023c).

Analyzing patterns of fatty acid preservation and loss is a technique to learn about the requirements for fatty acids in fish larvae. Therefore, the fatty acid composition of pikeperch larvae fed with *Artemia* sp. in Bischoff et al. (2018), Ballesteros-Redondo et al. (2023c) and Bischoff et al. (2023) and of larvae fed with *Brachionus* sp. in Ballesteros-Redondo et al. (2023a), Bischoff and Kubitz et al. (2022), and Ballesteros-Redondo et al. (2023c) was analysed together in terms of fatty acid content (µg per mg DW), percentage of increase or decrease during the trials, and in percentage of the TFAs. Any significant difference in fatty acid content or percentage increase or decrease was found in larvae fed different live feeds (*Brachionus* sp. vs. *Artemia* sp.). Moreover, the percentages of SFAs, MUFAs, and PUFAs in the larvae were not significantly different. SFAs were 18–36%, MUFAs 19–39%, and PUFAs 39–51% of the total fatty acid content.

In all these studies, PUFAs were the dominant and least variable group, independently of the feed or the growth rate, which indicated that this might be a stable parameter for the pikeperch larvae (Figure 3, Table 1). When fish starved, some polyunsaturated fatty acids were conserved (Bischoff et al. 2018). This allows fish the preservation of vital biological membranes. PUFAs maintain membrane structure and function and are precursors of bioactive compounds in vertebrates (Hou and Fuiman 2020). Less variability in our studies indicated that PUFAs were retained in the larval body. However, single PUFAs such as DHA have different functions. DHA is important in eye development and thus essential for visual predators like pikeperch (Bell et al. 1995). Consequently, the present thesis establishes a balanced minimum relative content of at least 39% PUFA and a 12.6% of DHA of the TFAs together with the percentage of LA, which seems to be adequate to maintain functions in pikeperch larvae. However, for somatic growth, larvae also need energy. SFAs and MUFAs are commonly used as energy in aquatic environments (Turchini et al. 2022). Our analysis indicated that SFAs and MUFAs were probably used upon availability by the larvae since no pattern was identify in relation to the live feed (Figure 3). Nevertheless, a significantly higher LA percentage was found in the larvae fed with rotifers ($p = 0.019$ Mann-Whitney) (Figure 3).

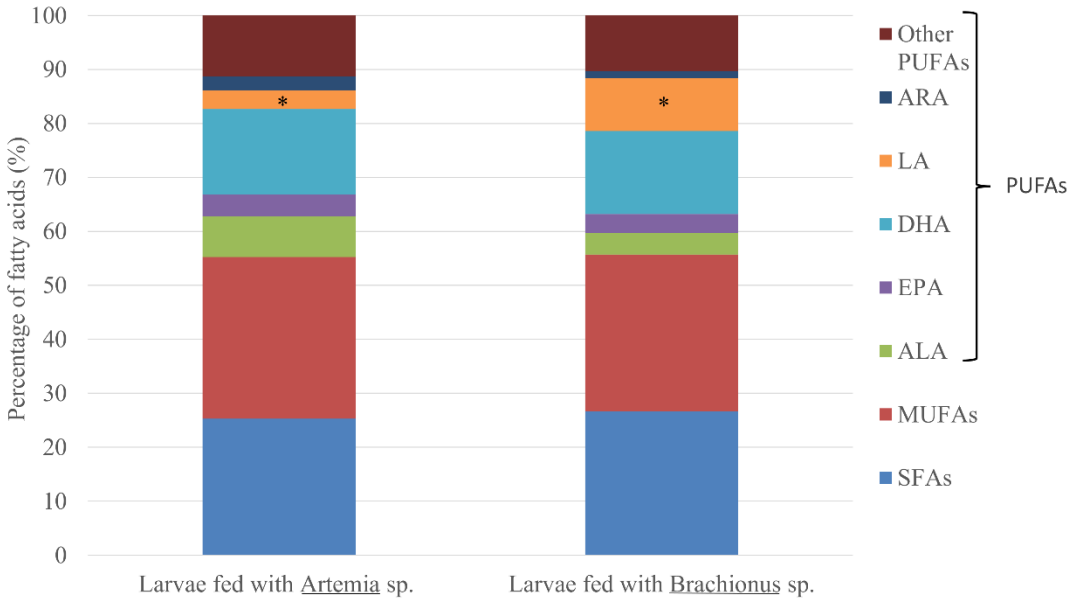

Figure 3. Percentage of fatty acids in larvae fed with *Artemia* sp. (mean based on Bischoff et al. (2018), Ballesteros-Redondo et al. (2023c) and Bischoff et al. (2023)) and with *Brachionus* (mean based on Ballesteros-Redondo et al. (2023a), Bischoff and Kubitz et al. (2022), and Ballesteros-Redondo et al. (2023c)). Significant differences are indicated with *.

As seen previously, *B. plicatilis* was characterized by high LA (Figure 2). As animals are not able to synthesize LA *de novo*, the differences in LA in pikeperch larvae should be related to the dietary LA of the rotifers. Yanes-Roca et al. (2018) have speculated that the benefits of the LA from *B. plicatilis* during

the first 12 days of life were due to the esterification of the dietary LA to obtain DHA. However, Reis et al. (2020) showed that larvae were not able to do this, at least on dph 20. Perez and Reis et al. (2022) showed that *B. plicatilis has a* low capacity to esterify DHA, but what they mainly do is esterify C18 PUFAs into phospholipids (PL). Hamza et al. (2008) showed that the growth rate of pikeperch was accelerated and the development of their digestive systems was seen earlier when dietary polar lipids were higher. By increasing lipoprotein synthesis, phospholipids in the diet may help to improve long-chain fatty acid absorption and transport (Tocher et al. 2008). Thus, if LA is in the form of PL, the advantages might be due to an improvement in lipid absorption and transfer in the intestine of pikeperch larvae. The cells of the intestine absorb dietary fat by the diffusion of monoglycerides and fatty acids (Izquierdo et al. 2000). Therefore, LA might be monoglycerides or free fatty acids (FFAs).

Hachero-Cruzado et al. (2020) observed higher lipid vacuoles in the intestines of sole (*Solea solea* (L., 1758)) larvae fed low-phospholipid (PL) diets compared to high-PL diets, indicating less effective lipid mobilization and transport when phospholipids are limited. Similarly, Imentai et al. (2020) associated increased hepatocyte vacuolation in pikeperch with higher lipid content in *Artemia*. However, lower accumulation in pikeperch larvae fed with *B. plicatilis* suggests more efficient lipid mobilization facilitated by phospholipids, specifically linoleic acid (LA) in the form of PL. Wang et al. (2022) demonstrated that phospholipids promote lipid oxidation, supporting the hypothesis that LA in the form of PL enhances the oxidation of yolk sac reserves, leading to better larval growth. Ballesteros-Redondo et al. (2023c) observed a decrease in almost all fatty acids (FAs) in pikeperch, indicating that LA increases beta-oxidation, thereby improving yolk sac reserve mobilization. Contrarily, the slower-growing larvae (after feeding *Artemia* sp.) may simply consume energy (carbohydrates and SFA) without developing, increasing their proportion of MUFAs and PUFAs as a result of a less suitable diet (Ballesteros-Redondo et al. 2023c).

This hypothesis is also supported by the fact that the micro *Artemia* and *Artemia* contained LA (Figure 2) (Bischoff et al. 2023). *Artemia* is known to have a lower amount of PL (Brown and Blackburn, 2013). Larvae fed with micro *Artemia* and *Artemia* increased their content in SFAs and MUFAs (Bischoff et al. 2023). This shows that the benefits of LA might be because LA is in the form of PL. Therefore, rotifer-LA is important; it could enhance the oxidation of yolk sac fatty acids and benefit larval growth. Other studies have shown the benefits of LA in the larval stages of other fish species. Jiao et al. (2020) recently demonstrated that a higher LA content in the tilapia (*Oreochromis niloticus* (L., 1758)) diet improved growth compared to a diet based on fish oils. They found that LA was not more abundant than ALA in tilapia, but LA produced more expression of fatty acid transporters. They also observed that LA was more efficiently oxidized in hepatocytes than ALA. Leaver et al. (2006) demonstrated that after LA feeding, salmon liver triacylglycerol significantly decreased along with an overall drop in total body lipid. They saw an increase of the acyl-CoA oxidase by LA via a PPAR-dependent mechanism, which meant a large rise in liver oxidation. Consequently, our data indicate that LA might have caused more oxidation, which suggests that it improves growth, showing on the one hand that this minimal amount

of *B. plicatilis* covered the needs of the larvae and that the larvae improved their growth with the addition of LA. Moreover, Hou and Fuiman (2020) observed that vegetable-based diets (assuming more LA and ALA) as the first feeding produced faster growth and higher retention of EPA and DHA in salmon.

The same occurred when feeding *B. plicatilis* to pikeperch larvae, increasing retention of DHA. Ballesteros-Redondo et al. (2023a) showed that pikeperch larvae appear to retain DHA during this period with high-LA-*B. plicatilis*, oppositely to rich-EPA-micro-*Artemia* (Bischoff et al. 2023). Larvae fed the high-ALA-*Artemia* also retained DHA, even higher than when feeding *B. plicatilis* (Ballesteros-Redondo et al. 2023c). DHA is part of the important omega-3 group. Bischoff and Kubitz et al. (2022) showed that the n-3/n-6 ratio of 2:1 reflects the natural ratio for pikeperch larvae and may indicate that *B. calyciflorus* meets the requirements of pikeperch in relation to these PUFAs. In fact, this ratio was approximately 2:1 for *B. plicatilis* and more than 3:1 for *Artemia* sp. (Ballesteros-Redondo et al. 2023c). However, higher omega-3 levels in the larvae did not correspond to the best larval performance in these studies. The ratio n-3/n-6 was not significantly higher when feeding *Artemia* or micro-*Artemia* (3.0-3.7), which are higher in ALA or EPA, than when feeding on rotifers (1-2.5) (Ballesteros-Redondo et al. 2023a, Bischoff and Kubitz et al. 2022, Bischoff et al. 2023). Freshwater fish species, in contrast to the majority of marine fish species, have significant needs for both n-3 and n-6 PUFAs, as previously reported by Sargent et al. (2002). Henrotte et al. (2010) found that in perch breeders, an n-3/n-6 ratio of between 0.8 and 2.5 was ideal for the success of sexual reproduction, while a higher proportion of n-3 PUFAs reduced fertilization and spawning rate, and too high a concentration of n-6 PUFAs, on the other hand, can result in inflammation and diseases in fish (Tocher et al. 2003). Omega-3 and omega-6 fatty acids connect to the PPAR transcription factor in several steps. Both together form a complex that directly binds to DNA. Thus, fatty acids directly affect gene expression. The breakdown of omega-6 fatty acids such as LA promotes inflammation due to higher oxidation. Higher oxidation increases reactive oxygen species (ROS) that produce cell damage. However, Luo et al. (2012) showed an increase in lipoprotein lipase (LPL) and hepatic lipase (HL) and low ALA, EPA, and DHA when feeding LA. These enzymes are involved in glycolysis and lipid metabolism in the liver. This indicated higher lipid utilization (oxidation). Moreover, the authors demonstrated a decrease in antioxidant activity, which indicates indirectly that less ROS were formed when feeding LA. This study showed an antioxidant effect of dietary LA.

After feeding pikeperch larvae with rotifers and *Artemia*, Imentai et al. (2022) found that digestive enzymes such as lipase and trypsin do not increase with more use of *B. plicatilis*. In this study, larvae that grew larger produced more enzymes. Enzyme production seemed to be more related to larval development than to diet, not forgetting that diet influences larval development. In fact, Izquierdo et al. (2000) showed that larvae fed a prepared diet experienced a delayed improvement in their ability to absorb fat. Izquierdo et al. (2000) showed that increasing dietary phosphatidylcholine (a type of PL) levels increased lipid absorption, but the latter improved hepatic lipid consumption. Since triglycerides are hydrolyzed by lipoprotein lipase, the fact that Imentai et al. (2022) did not find higher lipase activity

and/or expression might mean that the diet does not provide lipids in the form of triglycerides. This reinforces the hypothesis that the LA is in the form of PL.

Moreover, *Brachionus* provides a low-lipid diet. Rotifers are only 12% lipids and more than 50% proteins and have fewer lipids than *Artemia* (Kandathil Radhakrishnan et al. 2020).

Kennedy et al. (2006) found that higher oxidation due to LA only happened when the diet was low in lipids. When there were many PUFAs, the opposite happened. In addition, they found that it did not influence oxidation in the muscle; this only happens in the liver.

This might indicate that the diet with rotifers provides enough LA to increase the oxidation of the reserves but does not provide too many lipids, which have the potential to increase ROS production and its consequent damage. Although the effect of LA on the oxidation process of pikeperch larvae has to be proven in future studies, our data indicated that the diet with *B. calyciflorus* and *B. plicatilis* was balanced and drived the larvae into a metabolic homeostasis between oxidation and anti-oxidation processes. Besides the effect of LA, omega-3 FAs, such as EPA and DHA, on the other hand, have anti-inflammatory capabilities. LA might be producing oxidation, and the omega-3 FAs are sufficient to provoke the antioxidant effect, reaching a metabolic homeostasis that we suppose benefits larval development. Consequently, our data demonstrated that dietary LA from rotifers made the n-3/n-6 ratio lower than when feeding *Artemia* sp., suggesting a metabolic match with n-3/n-6 ratio of approx. 2, 8–12% of LA, 12.6 to 18.5% of DHA and 39% of PUFAs in pikeperch larvae (Table 1). These results were obtained by feeding the larvae from dph 3 until dph 10 with rotifers. Consequently, our studies demonstrated **hypothesis 6:** *"it is possible to identify the best live feed organisms for pikeperch during the first life cycle stages by focusing on relevant fatty acids, detectable already during the early days of life".*

The present thesis establishes a healthy fatty acid profile for the first feeding period of pikeperch larvae, which improves survival and growth rates (Table 1).

Table 1. Fatty acid composition of pikeperch larvae and juveniles on the different periods considered in this thesis.

Dph	Diet	SFA/MUFA/PUFA	PUFAs	n-3/n-6	LA	DHA
On dph 10	*B. calyciflorus*	1.7 / 1 / 2.5	47.4%	2.4	8.1%	15.2%
On dph 10	*B. plicatilis*	1 / 1.4-1.6 / 1.6-2	38.8-46.2%	2.5	8.8-12.4%	12.6-18.5%
On dph 16	*A. panamensis*	1 / 1.5-2 / 2.3-4.1	48-58%	1.6-3.2	7.3-10.9%	25%
On dph 25	*Artemia* + microdiets	1.2 / 1 / 1.6	42%	2.6	8.2%	16.7%
On dph 41	Microdiets	1/1.1/1.5	41.2%	1.8	13.9%	15.7%
Dph 56	Microdiets	1/ 1.5/ 1.3	36.9%	1.2	14.7%	10.4%

1.5.3.2. Period dph 11- dph 18

As seen, DHA might be relevant in the period of dph 3–10 as part of the n-3 PUFAs. However, Lund et al. (2012) showed the effects of DHA-deficient diets during the larval stage (7–29 dph) on the neural development of juvenile pikeperch. Therefore, dietary DHA seems to be essential for pikeperch larvae, at least from dph 10.

Bischoff et al. (2023) showed a deficiency of DHA in pikeperch larvae. Larvae were fed micro *Artemia*, *Artemia* sp., and microdiets in this period (dph 7–13). The increasing amount of ALA in the larvae showed that the larvae were feeding *Artemia* sp, which is rich in ALA. The conversion from C18 PUFAs to their higher homologous n-3 and n-6 HUFAs happens in other freshwater species. Despite *Artemia* sp. being rich in ALA, there was a decrease in DHA in the larvae, which indicated that the larvae did not synthesize DHA from ALA. This is in accordance with Reis et al. (2020), who recently demonstrated that pikeperch larvae on dph 20 did not synthesize DHA themselves. Moreover, Tan et al. (2009) have shown that the increase in ALA produced an increase in lactate dehydrogenase (LDH), which is a marker for tissue damage in fish. Therefore, larvae with increasing ALA and decreasing DHA cannot be considered to have a balanced fatty acid profile. This highlights the importance of considering more than one fatty acid at a time. ALA and DHA are both omega-3 FAs, which made the ratio n-3/n-6 stay stable; however, the DHA was more relevant for the pikeperch larvae. Consequently, these results confirmed **hypothesis 5:** fatty acids can be used to detect malnutrition in pikeperch larvae.

The microdiets were either eaten or digested by the larvae since they did not increase their DHA content, which is present in the microdiets (Bischoff et al. 2023). However, the inclusion of DHA in the diet through the use of the copepod *A. panamensis* showed advantages. Larvae fed with *A. panamensis* raised their DHA levels (Ballesteros-Redondo et al. 2023c). *A. panamensis* distinguished itself from *B. plicatilis* and *Artemia* sp. by having a higher DHA content (Figure 2). Therefore, our data demonstrate that pikeperch larvae are able to ingest and digest *A. panamensis* and, consequently, were able to utilize the supplied nutrients. This confirmed the possibility of rearing pikeperch larvae from dph 11 until dph 18 with this copepod (**hypothesis 3**). Besides, the larvae previously fed with *Artemia* sp. were able to recover after a lower initial growth when feeding *A. panamensis* (Ballesteros-Redondo et al. 2023c). The fatty acid profile of the larvae after feeding *A. panamensis* was similar regardless of whether they previously fed *B. plicatilis* or *Artemia* sp. (Ballesteros-Redondo et al. 2023c). The cephalic index of juvenile pikeperch decreased irreversibly as a result of dietary DHA deprivation during the larval stage (Hou and Fuiman 2020). The authors showed that the fatty acid content of the brain recovered over time. However, the restoration of DHA in the brain through nutrition does not always imply the repair of brain functions. The lack of DHA during larval rearing might lead to later problems since early nutrients might program the larvae for further development (Malzahn et al. 2022). Therefore, the fatty acid profile of the larvae must be considered along with other markers such as growth and survival rates.

The DHA content of the pikeperch larvae fed with *A. panamensis* rose in comparison with larvae fed *Artemia* sp. (Ballesteros-Redondo et al. 2023c). Our results showed that pikeperch larvae fed with *A.*

panamensis have superior fatty acid content than those fed with *Artemia* sp. Moreover, the larvae fed with *Artemia* sp. raised their EPA concentration less than the larvae fed with *A. panamensis*, despite the fact that *Artemia* sp. had greater EPA. Moreover, copepods are known to have more fatty acids in the form of PL than *Artemia* sp. (Brown and Blackburn 2013). Phospholipids might increase the production of lipoproteins, which promote long-chain fatty acid absorption and transport (Tocher et al. 2008). It might be because copepods have more phospholipids than *Artemia* spp. that the pikeperch larvae in our study incorporated EPA more effectively. This demonstrated how much more effectively the larvae incorporated these nutrients when they fed *A. panamensis*. As a result, our data showed that using *A. panamensis* instead of *Artemia* sp. improved pikeperch larviculture. Besides, *A. panamensis* might be used in this period to supply the DHA that was not taken by the larvae from the microdiets (Bischoff et al. 2023). This is relevant for aquaculture since nutrition during the larval stage can have a long-lasting impact (Hou and Fuiman 2020).

As fish larvae grow, they require more energy. Both groups of fatty acids (SFAs and MUFAs) are used through ß-oxidation to obtain ATP. The total FA concentrations, SFAs, and MUFAs decreased more in larvae fed with *A. panamensis* between dph 11 and dph 16, coinciding with higher growth (Ballesteros-Redondo et al. 2023c). This suggests that the pikeperch larvae used these groups of FAs for growth. However, PUFAs decreased more in larvae fed with *Artemia* sp., which had a higher content of PUFAs than *A. panamensis* (Ballesteros-Redondo et al. 2023c). This allows the conclusion that the PUFA profile of *Artemia* sp. lacks important single fatty acids and that the FA provided by *A. panamensis* was optimally used. Consequently, Ballesteros-Redondo et al. (2023c) confirmed **hypothesis 3**. Our data indicate that *A. panamensis* fulfills the nutritional requirements of the pikeperch larvae better than *Artemia* sp. after the first feeding with *B. plicatilis*. Our study shows that high DHA content in *A. panamensis* provided essential fatty acids to pikeperch larvae and, consequently, the dietary need for this essential fatty acid from day 11 post-hatch in pikeperch. Moreover, comparing larval fatty acid composition of pikeperch between period 0-10 dph and 11-16, a pattern was identified. Pikeperch larvae kept SFAs and MUFAs lower than PUFAs, being PUFAs at least 39% of the TFAs, probably because they used these fatty acids for energy production (Table 1).

Our studies demonstrated that, also in this period, it is possible to identify the best live feed organisms for *S. lucioperca* during the first life cycle stages by focusing on certain relevant fatty acids (**hypothesis 6**). A diet with a more favorable fatty acid composition promotes faster growth. This is significant because suitable and "in-time" live feed increases performance, reduces feed waste, and decreases costs. Our research improves the feeding strategy, and thus, it has the potential to optimize fish cultivation. Therefore, our work showed how new developments in the nutrition of fish larviculture can contribute to improving the farming of emergent species like *S. lucioperca*. Improved feeding protocols increase production, which reduces costs and mitigates the environmental impact, thus achieving sustainability goals.

Increasing DHA also increases the DHA/EPA ratio. Luo et al. (2018) showed that with increasing levels of DHA/EPA ratios from 0.73 to 2.33, Siberian sturgeon (*Acipenser baerii* Brandt, 1869) larval growth was enhanced but kept stable (dph 30). They hypothesized that higher DHA/EPA will favor the larvae to keep a higher amount of DHA over EPA and then over MUFAs and SFAs, and this will be beneficial for early development. DHA is known to have an antioxidant effect, while the oxidation of MUFAs and SFAs might produce ROS. As our data indicated a suitable growth rate, we conclude that the fatty acid profile of the larvae shows a healthy status with SFAs/MUFAs/PUFAs 1/1.5-2/2.3-4.1, PUFAs 48-58%, LA 7.3-10.9%, DHA 25%, and a n-3/n-6 ratio of 1.6–3.2, which shows that the omega-3 PUFA, DHA, is the essential FA in this period of larval development (Ballesteros -Redondo et al. 2023c).

The present thesis showed a change in the fatty acid-related dietary requirements of pikeperch larvae around dph 8–13. Our studies show the necessity of DHA in the diet for better larval growth and that a decrease in this fatty acid below 6% (Bischoff et al. 2023) leads to an unbalanced fatty acid composition, confirming **hypothesis 5**. Therefore, it is important to know the nutritional needs of larvae, which may change until adulthood.

1.5.3.3. Period dph 16- dph 25

A. panamensis was only suitable until dph 16 since the survival rate decreased substantially later on (Ballesteros-Redondo et al. 2023c). In Bischoff et al. (2023), *Artemia* sp. and microdiets were used from dph 11 until dph 20. As seen at dph 13, we demonstrated that larvae are not able to feed on microdiets. Between dph 13 and dph 25, an increase in DHA and a decrease in ALA were found. The decrease in ALA might be caused by the removal of *Artemia* sp. (dph 20) from the diet. However, the increase in DHA indicated that at a certain point between dph 13 and dph 25, the larvae started feeding on microdiets. As *Artemia* sp. was removed at dph 20 without negatively affecting growth rate, we conclude that weaning can be performed around dph 20, as demonstrated by Kestemont et al. (2007).

In this period, we obtained a similar larval fatty acid composition for pikeperch with SFAs and MUFAs lower than PUFAs, being PUFAs 42.0%, LA 8.2%, DHA 16.7% and n-3/n-6 ratio 2.6 (Table 1, Figure 4) than in the periods before. These data confirmed that *Artemia* sp. and microdiets might be used in this period for a successful weaning.

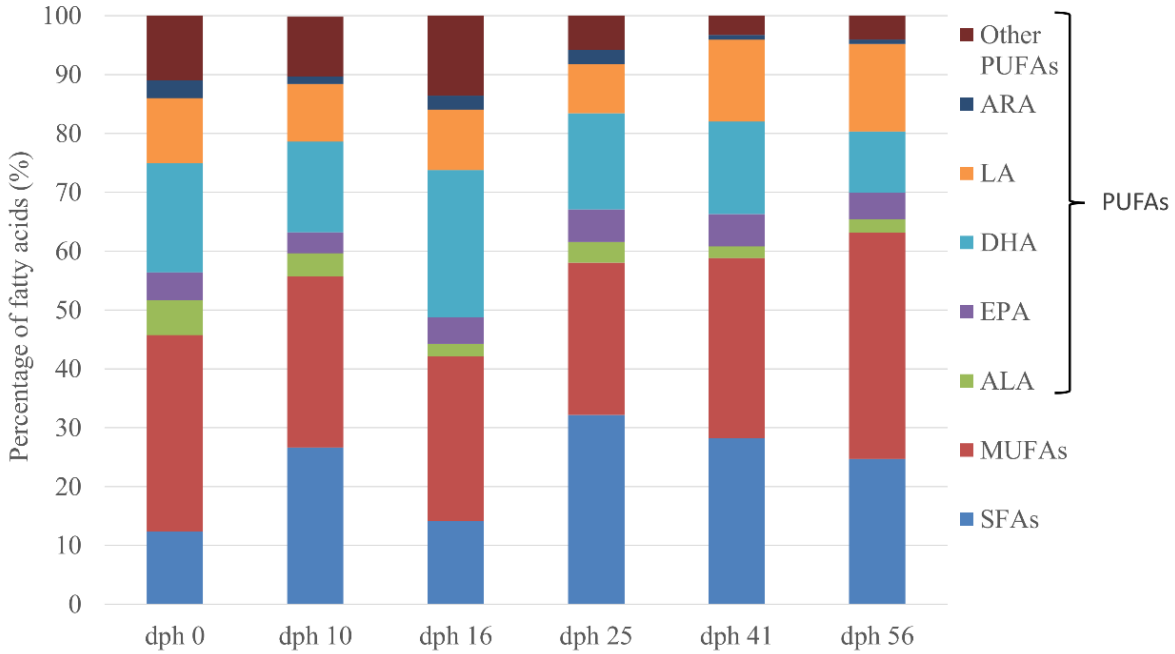

Figure 4. Percentage of larval fatty acids on dph 0 (mean calculated with all the studies of this thesis), on dph 10 (mean calculated after feeding *Brachionus* sp. in Ballesteros-Redondo et al. (2023a), Bischoff and Kubitz et al. (2022), and Ballesteros-Redondo et al. (2023c)), on dph 16 after feeding with *A. panamensis* and, dph 25, dph 41 and dph 56 after feeding with microdiets.

1.5.4. Fatty acid in pikeperch juveniles

Studies comparing the effects of dietary fatty acids on growth performance in juvenile fish have shown species-specific variations. Dietary fatty acids significantly influence the fatty acid composition of pikeperch juveniles (Bischoff et al. 2023). The fatty acid profiles of juvenile freshwater fish were reflective of their dietary intake. In Bischoff et al. (2023), pikeperch juveniles were only given microdiets. Pikeperch juveniles contained large amounts of fatty acids, particularly DHA, which is a dietary component in high abundance. However, pikeperch only contained trace amounts of ALA and ARA, which were present in small amounts in the diet. If these fatty acids were present in greater quantities in the diet, we expected that pikeperch to accumulate them, and their initial level at hatch might serve as a form of reference. In fact, DHA and EPA in the juveniles fed microdiets increased until dph 41 and stayed stable until dph 56, reaching similar levels as in the yolk sac. The dietary fatty acid composition has profound health implications for juvenile fish. Omega-3 fatty acids, particularly EPA and DHA, play a crucial role in immune system development, reducing inflammation, and promoting cardiovascular health. The presence of these fatty acids in the diet can improve the nutritional quality of the fish, making them more desirable to consumers. However, the optimal levels and ratios of other dietary fatty acids have to be considered. Our data showed low dietary ARA yielded low ARA content in the juveniles. Although growth rates were high, metabolic homeostasis was not assured. A decrease

in ARA might yield to an unbalanced n-3/n-6 ratio. A balanced n-3/n-6 ratio in pikeperch is important to avoid deformations, according to El Kertaoui et al. (2019), who demonstrated a substantial interaction between EPA + DHA, and ARA in pikeperch. Microdiets seemed to fill these nutritional requirements until dph 41. On dph 41, SFAs and MUFAs lower than PUFAs, being PUFAs 41.2%, LA 13.9%, DHA 15.7% and n-3/n-6 ratio 1.8 (Table 1, Figure 4), which is the same pattern found for the pikeperch larval stage. However, on dph 56, PUFAs were lower than MUFAs, being PUFAs 36.9%, LA 14.7%, DHA 10.4% and n-3/n-6 ratio 1.2 (Table 1, Figure 4). From dph 25, there was a decrease in PUFAs, an increase in LA, a decrease in DHA and thus, a decrease in n-3/n-6 ratio. These data showed that the microdiets did not provide suitable PUFAs to the juveniles. Although any disturbance in the juvenile growth was found in Bischoff et al. (2023), the lack of adequate and sufficient PUFAs could be impairing the development of juvenile pikeperch. By including vegetables oils in the diet as done by Schulz et al. (2005), pikeperch juveniles might increase their levels of DHA and EPA. However, pikeperch larvae showed a reduced use of these vegetables oils (Schulz et al. 2005). Therefore, it is still necessary to better understand their nutritional requirements for the reformulation of specific microdiets for juvenile pikeperch.

1.6.Conclusion and further research

The present thesis shows that a crucial step toward the effective culture of the pikeperch larvae is the adaptation of the live feed organisms to the age and subsequently to the developmental stage of the pikeperch larvae. Due to the wide range of sizes of the utilized organisms, a change in live feed organisms was required. With a size range of between 150 and 200 µm, *Brachionus* spp. provided an ideal first feeding for the freshly hatched pikeperch larvae. Moreover, this thesis demonstrates that the use of natural freshwater feed organisms has advantages over saltwater rotifers. Larvae fed *B. calyciflorus* with an adequate protocol to match feed and larval development showed the best results, even when the initial quality of the larvae was lower. The use of natural feeds has the potential to reduce waste and water degradation in larviculture. Additionally, *Brachionus plicatilis* was able to supply the pikeperch larvae with enough fatty acids, applying an adequate amount per larva, and, most likely, other nutrients, including proteins and vitamins. Nevertheless, as the pikeperch larvae grow, rotifers with a small size range are no longer able to provide enough energy and nutrients, and the cost:benefit ratio for the pikeperch larvae to catch the life feed decreased starting around day 8 after hatching. Therefore, a change to a larger live feed organism should be performed at the latest on day 10 after hatching. The copepod *Apocyclops panamensis* was used in this instance. This copepod has a size range of 80 to 600 µm depending on the developmental stage of the nauplii, through the copepodite stages to the adults, and is thus ideally suited as a follow-up diet. *A. panamensis* demonstrated its suitability for intensive cultures and was thus made accessible in the necessary numbers at the requisite time. *A. panamensis* has also shown that its fatty acid composition is adequate. In comparison with *Artemia* sp., larvae fed with this copepod reached higher growth rates. Our data have shown the larval need for dietary DHA from

dph 11. Based on our studies, the feeding regime was successfully adjusted to the needs of the pikeperch larvae during their early developmental stages, resulting in good survival and growth rates, an adequate dietary supply of total fatty acids, and a balanced larval fatty acid composition. This thesis highlights the importance of dietary PUFAs in pikeperch rearing, specifically LA from dph 4 until dph 11, and DHA from dph 11 onward. Consequently, this thesis provides relevant information about the balanced and "in time" fatty acid composition of the pikeperch larvae and its future use as a health parameter.

Our findings are in agreement with the literature; however, the full potential of the pikeperch larviculture has not yet been fully realized. Consequently, further investigation using the learned information has to be performed.

Moreover, the study of a single nutrient is limited in larviculture (dose studies). Rotifer LA is part of a dietary matrix formed by several macronutrients and micronutrients. Since live feed organisms constitute a matrix of nutrients, the study of the fatty acids should be performed together with proteins and carbohydrates, as well as other micronutrients such as vitamins and antioxidants, as they may have a synergistic effect on the larvae. Furthermore, future studies should include additional types of fatty acids (FFAs, PL, or triglycerids (TG)) or proteins (free amino acids (FAAs)), which might influence larval nutrition. Characterizing the exact composition of rotifers might allow us to find out about other formulated synthetic diets, and consequently, his multi-nutrient approach should be considered in fish nutrition in the future.

Further study of the effect of LA on adult pikeperch might open the door to feeds of vegetable origin richer in LA and more ecologically and economically sustainable than fish oils.

Finally, the use of freshwater organisms in this thesis has been demonstrated to be superior to saltwater. However, this needs to be further developed by finding organisms such as freshwater copepods to continue the feeding protocol in the pseudo-green water technique.

The use of live feed in freshwater fish larviculture presents several challenges, including the high cost and limited availability of live feed, the need for specialized culture systems, and the risk of introducing pathogens and parasites to the fish larvae. To overcome these challenges, research efforts should focus on developing cost-effective and sustainable techniques, including the implementation of the match-mismatch theory in aquaculture feeding practices, while also considering the broader ecological context. Understanding the potential impacts on natural prey populations and the associated food web dynamics is crucial to ensuring the long-term sustainability of aquaculture operations.

Furthermore, there is a need for a better understanding of the specific fatty acid requirements and metabolic adaptations of freshwater fish species during their early life stages. By elucidating the mechanisms by which freshwater fish species utilize and metabolize fatty acids, it will be possible to formulate more tailored diets that meet their specific nutritional needs. This knowledge can also contribute to the development of improved larviculture protocols and the optimization of fish health and growth. In the case of pikeperch, future studies should verify the effect of LA and DHA on pikeperch during its early development. The nutritional status of the larvae should be checked by analyses of

digestion (digestive enzymes and their gene expression), genes related to the nutritional status (PPAR, lipoproteins), and genes related to growth (growth hormone receptor GHR and insulin growth factor IGF, or BMP4 and BMP7) (Schaefer et al. 2021). Identifying key genes and regulatory pathways involved in fatty acid synthesis, uptake, and utilization will enable targeted genetic selection and breeding programs to enhance the ability of freshwater fish species to utilize dietary fatty acids effectively. Advancements in nutrigenomics, the study of how nutrients interact with genes, can provide valuable insights into the molecular mechanisms underlying the effects of dietary fatty acids on growth and fatty acid metabolism. Integrating nutrigenomics approaches with functional feed development can lead to the formulation of customized feeds that optimize growth performance, health, and fatty acid composition in juvenile fish. This interdisciplinary approach holds promise for enhancing aquaculture practices and producing nutritionally superior fish products.

In conclusion, the use of live feed in freshwater fish larviculture holds promise as a nutritionally rich food source. However, there is a need for further research to understand the specific fatty acid requirements and metabolic adaptations of different freshwater fish species, develop cost-effective feeding strategies, and ensure sustainable production practices. By addressing these challenges, the aquaculture industry can optimize the nutrition and growth of freshwater fish larvae and juveniles while minimizing environmental impacts.

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2. The effect of *Brachionus calyciflorus* (Rotifera) on larviculture and fatty acid composition of pikeperch (*Sander lucioperca* (L.)) cultured under pseudo-green water conditions

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The Effect of *Brachionus calyciflorus* (Rotifera) on Larviculture and Fatty Acid Composition of Pikeperch (*Sander lucioperca* (L.)) Cultured under Pseudo-Green Water Conditions

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Abstract: A new cultivation system with the chlorophyte *Monoraphidium contortum* combined with a self-sustaining culture of the freshwater rotifer *Brachionus calyciflorus* was applied for *Sander lucioperca* (L.) larviculture. Survival, morphometrics, as well as fatty acid composition of pikeperch larvae were analyzed after a ten-day feeding period. By using the pseudo-green water technique with improved aeration and water movement at the surface, survival rates reached up to 94%, with a total larval length of 8.1 ± 0.3 mm and a specific length growth rate of up to 4.1% day^{-1} for *S. lucioperca*. The biochemical composition of *B. calyciflorus* and especially its contents in C18 PUFAs and suitable n-3/n-6 ratios met the nutritional requirements of pikeperch larvae. The high abundance of highly unsaturated fatty acids (HUFAs) in the diet appeared to be less important in the first feeding due to a possible retention of essential fatty acids, which originate from the yolk sac reserves, at adequate levels. Exponential growth of microalgae and zooplankton under the applied conditions was most effective when stocking *M. contortum* five days and *B. calyciflorus* three days before adding the fish larvae. Appropriate timing and sufficient live feed density allowed a successful integration of *B. calyciflorus* into pikeperch larviculture. We hypothesize that feeding pikeperch larvae with a self-sustaining *Brachionus*-culture under pseudo-green water conditions with minor disruptions during larviculture will improve survival and growth. This system is a first step towards pikeperch larviculture inside recirculated aquaculture systems (RAS) under continuous feed supply with live feed within the same aquaculture unit.

Keywords: pikeperch *Sander lucioperca*; pseudo-green water technique; *Brachionus calyciflorus*; survival; growth; fatty acid composition

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1. Introduction

The cultivation of pikeperch (*Sander lucioperca* (L.)), a high-priced commercial fish species, is gaining more and more attention [1–6]. In order to reach ecological and economical sustainability, an improvement of larviculture conditions is required, as the production of fry is still the major bottleneck in pikeperch aquaculture [6–10]. In order to improve pikeperch culture in terms of improved survival rates and hence better resource utilization, alternative live feed as well as improved culture techniques for intensive larval rearing seem necessary. To reduce water consumption and enhance control of rearing parameters,

recirculating aquaculture systems (RAS) were already applied for intensive larval rearing in Europe [11].

Lund and Steenfeldt (2011) [10] recorded higher survival rates, but a high output of pikeperch larvae relies on the use of cost-intensive *Artemia* nauplii and the inevitable need for *Artemia* enrichment with highly unsaturated fatty acids (HUFAs). As for the routine use of *Artemia* nauplii in marine aquaculture [12], this is also common in larval rearing of pikeperch [8]. However, the specific nutritional needs of pikeperch larvae are little understood [10], and it is assumed that *Artemia* nauplii cannot entirely cover the nutritional needs of freshwater species [11]. Periodical supplementation of live feeds and the short survival of *Artemia* nauplii in freshwater further reduce water quality [10].

As an alternative, the freshwater rotifer *Brachionus calyciflorus* was established as another live feed candidate for larval development of freshwater fish [13]. Especially in the rearing of fish with small and sensitive larvae, rotifers might increase larval survival and performance [13,14]. Awaiss et al. (1996) [15] reported improved survival, growth, and food conversion rates in larvae of perch (*Perca fluviatilis*) and gudgeon (*Gobio gobio*) fed with *B. calyciflorus* compared to dry or mixed feeds. However, unlike the mass cultivation of the marine rotifer *Brachionus plicatilis*, the commercial scale production of freshwater rotifers for larviculture so far is less established [11,16,17], and freshwater rotifers were only occasionally used in freshwater larval rearing [14]. This is despite the fact that freshwater rotifers do not lose their motility compared to saltwater rotifers in freshwater [6,18]. This sustained motility acts as a visual stimulus for the larvae and thus encourages them to forage. For the use of *Brachionus plicatilis*, it was further argued that a transfer of diseases and parasites should be avoided through the change of environment from saltwater to freshwater. However, based on Hennersdorf et al. (2016) [19], we argue that an early and low exposure to parasites might activate and improve the immune system of the fish larvae that consequently can react more quickly to future stressors. Furthermore, the plankton cultures used as feed are usually isolated cultures that have been cultivated over several life cycles without exchange with the natural environment. As a result, the risk of containing disease-causing pathogens or parasites and transferring them to the fish larvae is significantly reduced [20].

The positive effect of phytoplankton on larval rearing was previously established for Atlantic cod (*Gadus morhua*), turbot (*Scophthalmus maximus*), Atlantic halibut (*Hippoglossus hippoglossus*), European sea bass (*Dicentrarcus labrax*), striped mullet (*Mugil cephalus*), and summer flounder (*Paralichthys dentatus*) [21–26]. Beneficial effects may be due to the reduction of metabolites from fish and zooplankton [27]. Stimulating effects on the feeding behavior of European sea bass larvae (*Dicentrarchus labrax*) as well as digestive functions were described by Cahu et al. (1998) [22]. Additionally, the nutritional value of rotifers can be maintained through "green water" after addition of microalgae to the fish larval rearing tanks [24,28]. Reitan et al. (1993) [24] already reported increased biomass production, constant total lipid contents, as well as enhanced reproduction activity of rotifers in rearing systems of marine fish larvae supplied with microalgae. Papandroulakis et al. (2001) [29] developed a "pseudo-green" water technique based on a periodic supplementation of phyto- and zooplankton, combining the advantages of "clear water" and "green water". This method was already successfully applied in sea bream larviculture [29].

Due to the high abundance in the natural nursery grounds of pikeperch in Mecklenburg-Western Pomerania [30,31] and high reproduction and growth rates under laboratory conditions, the freshwater rotifer *B. calyciflorus* Pallas, 1766 (Gilbert, 1967) was applied as a live feed organism to establish the self-sustained zooplankton culture with high live feed density.

The aim of the present study was the establishment of a newly designed fish larviculture system to increase pikeperch larvae survival under aquaculture conditions. We hypothesize that feeding pikeperch larvae with a self-sustaining *Brachionus*-culture under pseudo-green water conditions with minor disruptions during larviculture will improve

survival and growth. The benefits of *B. calyciflorus* for pikeperch larviculture and an optimized run of the applied system are discussed.

2. Materials and Methods

2.1. Recirculating Aquaculture System for Pikeperch Larviculture

Three culture systems were assembled and used as replicates. Two independent, timely, separated experiments were carried out at the laboratory for Aquaculture and Sea-ranching, University of Rostock, from 17–27 May 2014 (experiment I) and 28 October–6 November 2014 (experiment II) (Table 1). The reason why two separate experiments were performed is that the reproducibility of the operating principle should be tested. Each culture system (total capacity of 90 L) consisted of two separated compartments, a larvae culture tank (filled with 30 L water), and a reservoir with 40 L, resulting, with the additional volume in the hoses and pipes, in a total volume of approximately 75 L per RAS during both experiments. In each system, the water circulated from the reservoir via a submersible pump, which did not damage *B. calyciflorus* during pumping, to the larvae culture tank (Figure 1). The water returned via an overflow from the larval culture tank, which was covered with a 200 µm mesh to hold back the fish larvae but allowed *Monoraphidium contortum* and *B. calyciflorus* to pass and circulate in the complete experimental system. Thus, a uniform *Monoraphidium contortum* and *B. calyciflorus* concentration could be assumed in all parts of the experimental system. The reservoir allowed *B. calyciflorus* reproduction and growth, nutrient uptake through microalgae, and water aeration.

Table 1. Experimental design for the two independent trials.

	Experiment	
	I	**II**
Duration	7–27 May 2014	28 October–6 November 2014
Experimental start	dph 0	dph 0
Experimental end	dph 10	dph 10
No. of replicates	3	3
System identification	I–III	I–III
Components	Culture tank, reservoir, pump	Culture tank, reservoir, pump
Stocking of *M. contortum*	10 days prior to start *	5 days prior to start
Stocking of *B. calyciflorus*	3 days prior to start	3 days prior to start
Stocking of *S. lucioperca* larvae	This was the start	This was the start

* System I: 5 days prior to start.

2.2. Microalgae and Zooplankton Culture

The chlorophycean *Monoraphidium contortum* (Thuret) Komàrková-Legnerová (1969) (strain 47.80, obtained from SAG Culture Collection of Algae Göttingen, Göttingen, Germany) was grown in batch culture, inoculated in Erlenmeyer flasks of increasing volumes and finally cultured in 80 L carboys. For algal growth, 3 psu F/2-medium [32] was continuously added daily. The chosen light cycle was 16L:8D. For mass culture in the facilities of the University of Rostock, *M. contortum* was cultured with the same medium at room temperature (~20 °C) and the same light conditions and constantly aerated.

For the first experiment, each culture system was initially filled with a 75 L mixture of algae suspension (approximately 6.0×10^5 cells mL^{-1}), F/2 medium, and oxygen-saturated tap water ten days before starting the fish larvae experiment and continuously supplied with a set amount of F/2-medium ("fed-batch-process"). The algal cell density in the experimental systems was maintained at the initial level during the experiments by adding a continuous supply from an external phytoplankton-chemostat system, which was connected to the individual culture units as additional phytoplankton supply. The

maintenance of this cell density was indirectly controlled by absorption measurements (Hach-Lange, DR 3900, Düsseldorf, Germany) at a wavelength of 665 nm.

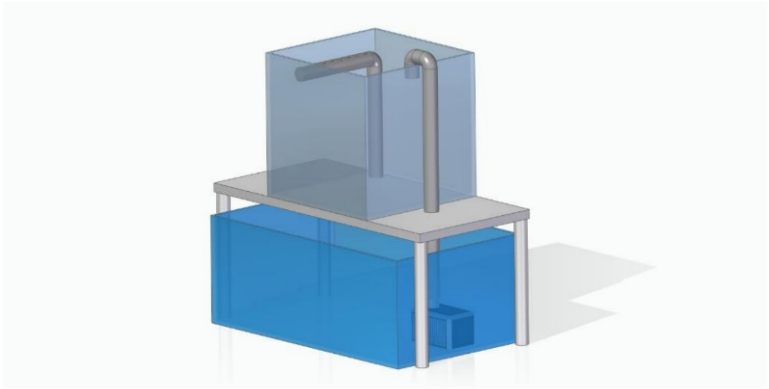

Figure 1. Scheme of the recirculating aquaculture systems with a 30 L larvae culture tank and a 40 L reservoir. The water circulated via an overflow from the larval rearing tank and returned via a submersible pump.

B. calyciflorus was obtained from Plankton-Zoo (www.plankton-zoo.de, Munich, Germany; accessed on 15 June 2013) and cultured as batch system in a 120 L cylindrical-conical polyethylene tank (PE) with daily addition of 1 to 2 L suspension of the chlorophyte *M. contortum* (including 3 psu F/2 medium). Culture was maintained at a salinity of 2.5 psu. No aeration or additional light was applied. Mixed size stages were fed ad libitum with *M. contortum* before inserting them into the experimental tanks.

Mixed stages of *B. calyciflorus* were stocked to each culture unit's reservoir three days before adding the fish larvae. *Brachionus* density was maintained at 5 Ind mL^{-1} in the whole experimental system and an amount of 500 individuals as feed for each stocked fish larva per day was adjusted inside the tank. Therefore, each larvae culture tank was continuously supplied from the reservoir by the water circulation and from an additional external *Brachionus* culture tank to meet the nutritional requirements set above. This was necessary due to the insufficient reproduction rate of *B. calyciflorus* within the reservoir caused by the limited volume and the culture conditions that deviate from the optimal conditions of a monoculture. The externally added daily volume of the *Brachionus* culture depended on the density within the larvae culture tank. The daily addition was approximately 200 mL per system, which was thus the daily make-up of water. For quantification of the microlagae and *B. calyciflorus* growth in each culture system, five replicate samples of 5 mL were sampled daily and filtered through a 63 µm mesh. The filtrate was either transferred to a cuvette for photometric measurements or fixed with Lugol's solution for counting algae cells in a Fuchs–Rosenthal counting chamber (Marienfeld, Lauda-Königshofen, Germany) under a stereo light microscope (BX 53 Olympus). The extinction of the microalgae was determined with Hach–Lange photometer (DR 3900) at 665 nm. The retained *B. calyciflorus* on the mesh was fixed with Lugol's solution and was counted using a stereo light microscope (SZX10 Olympus, Hamburg, Germany).

2.3. Pikeperch Larval Culture

Pikeperch larvae were obtained from a commercial fisherman in Hohen Sprenz (Germany, Mecklenburg-Western Pomerania, 53°54′47″ N; 12°11′49″ E) and from the research facility Hohen Wangelin of the Institute for Fisheries of the State Research Centre Mecklenburg-West Pomerania. Fish used as broodstock for pikeperch experiments, originating from wild catches in Lake Hohen Sprenz and Lake Müritz, had a length range of 60

to 75 cm and were assumed to spawn repeatedly. The breeders were reared in net cages in Lake Hohen Sprenz and were fed a natural diet (site-specific lake fish). Since the fisherman manages Lake Hohen Sprenz, he also regularly stocks commercially important species. This also includes pikeperch. When the spawning temperature of approximately 16 °C was reached, the breeders were transferred to tanks, and these tanks were equipped with spawning substrate to which the females could attach the eggs. After spawning, the eggs were removed from the tanks, deglutinated, and transferred to incubators for hatching. Fish used as broodstock for pikeperch experiments from Hohen Wangelin were offspring of the system and were also repeated spawners.

The larvae hatched at a water temperature of 16 °C, and directly thereafter (dph = 0), they were transferred to the laboratory facility of the University of Rostock via car in a specialized fish transport box. We applied a temperature increase during several hours to acclimate the larvae to laboratory conditions. Fish larvae were randomly distributed into our three setups for both individual experiments.

Pikeperch larvae were stocked at lower densities compared to Szkudlarek and Zakęś (2007) [2] to ensure a high live feed concentration per fish larvae and to minimize interspecific competition. The initial larval stocking density was 10 Ind L^{-1} inside the units, which resulted in a total larval number of 300 individuals per culture tank.

The average culture conditions (±SD) were 19.3 ± 0.9 °C temperature, a salinity of 0.4 ± 0.2 psu, oxygen saturation of 100.9 ± 7.1%, pH of 6.9 ± 0.2, and a redox potential of 159.4 ± 14.8 mV. The average dissolved nutrients were 0.12 ± 0.15 mg L^{-1} NH_4^+, 0.071 ± 0.085 mg L^{-1} NO_2^-, 4.6 ± 3.1 mg L^{-1} NO_3^-, and 0.77 ± 0.42 mg L^{-1} PO_4^{3-}. Larval rearing was performed under daylight with a photo period of 11:13 h (L:D). In the systems, pikeperch larvae were reared with the pseudo-green water technique [29] by adding daily phytoplankton and zooplankton to the culture tanks (see above). The survival rate of the pikeperch larvae was determined at the end of each experiment (dph 10) by individual counting of all remaining larvae.

Fish larvae were sampled for morphological analyses from the individual tanks and stored in 70% ethanol until measurement approximately 15 to 20 days after sampling. At the start of the experiment, 45 larvae were initially sampled from the transport boxes. At the end of the feeding period of experiment I, 38 larvae from system I, 22 larvae from system II, and 14 larvae from system III were sampled, according to the survival rates of the replicates. For experiment II, the final samples for the morphological analyses consisted of 10 individual larvae per unit. The total length of the fish larvae was measured by using a stereo light microscope (SZX10 Olympus, Hamburg, Germany) connected to a UC30 digital camera (Olympus, Hamburg, Germany) and the software package cellSens Dimension 1.6 (Olympus Soft Imaging Solutions, Hamburg, Germany). Therefore, fish larvae were individually placed under the stereo light microscope, and the longest distance between the tip of the head and the tip of the tail was recorded.

The specific growth rate (SGR) [% d^{-1}] of the pikeperch larvae was calculated, with the total length of the larvae according to Jørgensen (1990) [33], applying the formula

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

where L_t and L_o represent the total length of the larvae at time t and time t = 0.

2.4. Sampling of Fish Larvae for Fatty Acid Analyses

In order to investigate the supply of pikeperch larvae with highly unsaturated fatty acids (HUFAs) during exclusive feeding with *B. calyciflorus* under pseudo-green water conditions, fatty acid analyses of pikeperch larvae were conducted. Five fish larvae samples were taken at the beginning of the experiment (dph 0) and 4 samples at the end (dph 10). One sample encompassed between 10 and 25 individuals per tank, depending on the individual body mass of the larvae, to collect sufficient material for the fatty acid extraction. The larvae were collected and killed in accordance with the applicable laws and regulations, and the larvae for fatty acid analyses were shock frozen immediately after death.

The detection of larval dry mass and fatty acid analyses were carried out for larvae of replicate I of the first experiment because microalgae and zooplankton did not develop as expected in the other replicates, and therefore, high mortality rates of the fish larvae were observed during experiment I. Due to limited capacities concerning the fatty acid analyses and freezer storage problems, no samples were taken from the second experiment.

Samples were taken, and fatty acid analyses were conducted according to the method by Windisch and Fink (2018) [34]. Lipid extraction was conducted by immersing homogenized tissue in a dichloromethane: methanol mixture (2:1/*v*:*v*) for at least 12 h. Thereafter, fish samples were sonicated and centrifuged for 5 min (4500× *g*). After taking up the lipid phase quantitatively, the solvent was evaporated to dryness under a stream of nitrogen gas (5.0 purity grade) at 40 °C. In the following, fatty acids were trans-esterified with 5 mL of 3 N methanolic HCl at 70 °C for 20 min to their fatty acid methyl ester (FAME) derivatives [35]. FAMEs were extracted with 2 × 2 mL iso-hexane and after evaporation of the samples under a stream of nitrogen gas were finally dissolved in 100 µL isohexane. For gas chromatographic analyses, 1 µL of the sample solution were measured on a 6890 N GC System (Agilent Technologies. Waldbronn, Germany) equipped with a DB-225 capillary column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness). Temperature programming with helium (5.0 purity grade) as carrier was applied (flow rate of 1.5 mL min^{-1}). Samples were injected using a programmable temperature vaporizer injector (solvent vent mode) using the following temperature program: injector and FID temperatures 200 °C, initial oven temperature 60 °C for 1 min, followed by a 20 °C min^{-1} temperature ramp to 150 °C, then 7 °C min^{-1} to 220 °C, followed by a final 14 min at 220 °C. FAMEs were detected by flame ionization and identified by comparing the retention times of detected peaks with those of reference compounds and quantified using two internal standards (tricosanic acid methyl ester, 23:0 ME and nonadecanic acid, 19:0 ME) and previously established calibration functions for each individual FAME.

2.5. Statistical Analyses

Statistical analyses were performed by using IBM SPSS Statistics, Version 22. For the test of normal distribution, the Shapiro–Wilk test was applied. To test the homogeneity of variance the Levene's test was used. To analyze differences between means an analysis of variance (ANOVA) was performed. In case significant results were obtained, post hoc tests followed either Tukey–Kramer (with variance homogeneity) or Dunnett T3 post hoc tests (without variance homogeneity). To analyze differences between two groups, a *t*-test was applied. As non-parametric tests, either a Mann–Whitney U-test or a Kruskal–Wallis analysis of variance (ANOVA) was chosen. All significance levels α were set to 0.05.

3. Results

3.1. Growth of Monoraphidium Contortum and Brachionus Calyciflorus in the Culture Units

Each culture unit of the first experiment was inoculated with *M. contortum* 10 days before starting the fish larval experiment at dph 0. Due to a yellowish discoloration of the culture water in system I, a complete water exchange was conducted five days before stocking the fish larvae. At the beginning of the fish larvae experiment, the highest cell density of 2.5 ± 0.5 × 10^6 cells mL^{-1} of *M. contortum* could be detected in replicate III, while in replicate systems I and II, lower algal cell densities of 2.2 ± 0.1 × 10^5 cells mL^{-1} and 3.2 ± 0.3 × 10^5 cells mL^{-1} were observed (Figure 2). After stocking pikeperch larvae, the green water of replicates II and III also turned yellow-brown one day post hatch, but a complete water exchange was no longer possible to avoid additional stress on the pikeperch larvae and to maintain comparability. For experiment II, the three individual culture units were inoculated with microalgae five days prior to stocking of the fish larvae at dph 0.

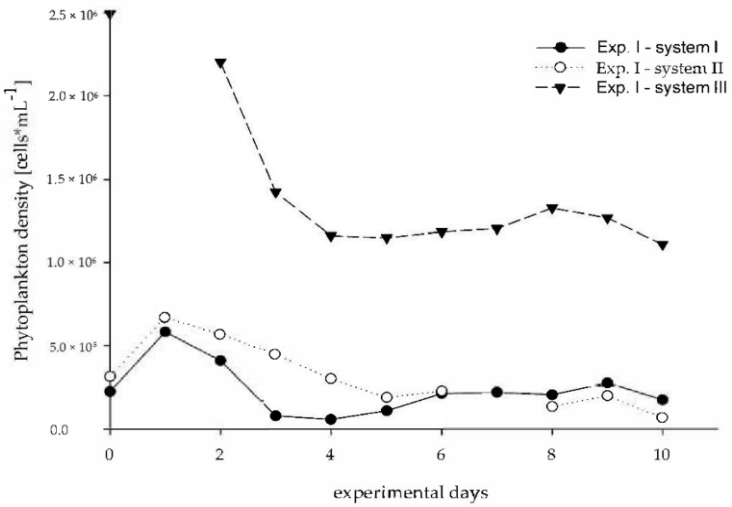

Figure 2. Density of *Monoraphidium contortum*, displayed as daily means (±SD in cells mL^{-1}) for each culture system during the pikeperch larval rearing of experiment I (day post hatch 0–10) (n = 5 per sampling).

Mixed stages of *B. calyciflorus* were stocked three days before starting experiment I. After three days, at the beginning of the fish larval experiment I, system I reached a live feed density of 2.8 ± 0.3 Ind mL^{-1}, while a density of 0.2 ± 0.1 Ind mL^{-1} and 0.0 Ind mL^{-1} of *B. calyciflorus* was detected in samples taken from systems III and II, respectively (Figure 3). Despite external addition of *B. calyciflorus*, an increase in its concentrations was only detected in replicate I, while the mean densities in systems II and III were close to zero (0.1 ± 0.2 Ind. mL^{-1} and 0.1 ± 0.1 Ind. mL^{-1}, respectively). In system I, *M. contortum* as well as *B. calyciflorus* grew exponentially, with a peak of *M. contortum* at day one post hatch (5.8 ± 0.2 × 10^5 cells mL^{-1}) and the highest *B. calyciflorus* density at day four post hatch (12.7 ± 0.5 Ind mL^{-1}).

During experiment II, all cultures of *M. contortum* developed as expected and according to the conversion with the absorption data exceeded cell densities above 1.2 ± 0.5 × 10^6 cells mL^{-1}, which, on the basis of previous experience from other plankton experiments, has been assumed to be sufficient to support a good zooplankton growth. The initial *B. calyciflorus* densities of the three individual systems were 0.03, 0.08, and 0.06 Ind. mL^{-1} (Figure 4). Due to these low zooplankton concentrations, additional feeding was required at experimental day 2. *B. calyciflorus* concentration peaked in the systems at 7.9 Ind. mL^{-1}, 33.4 Ind. mL^{-1}, and 4.2 Ind. mL^{-1} for the three individual culture units (Figure 2) at experimental days 8, 9, and 8, respectively. Comparing the concentration of *B. calyciflorus* within the three separate systems, it is noticeable that there is a clear variability between system II and the other two systems. This manifests itself in concentrations of over 3o Ind. mL^{-1} in system II compared to concentrations of about 6–8 Ind. mL^{-1}.

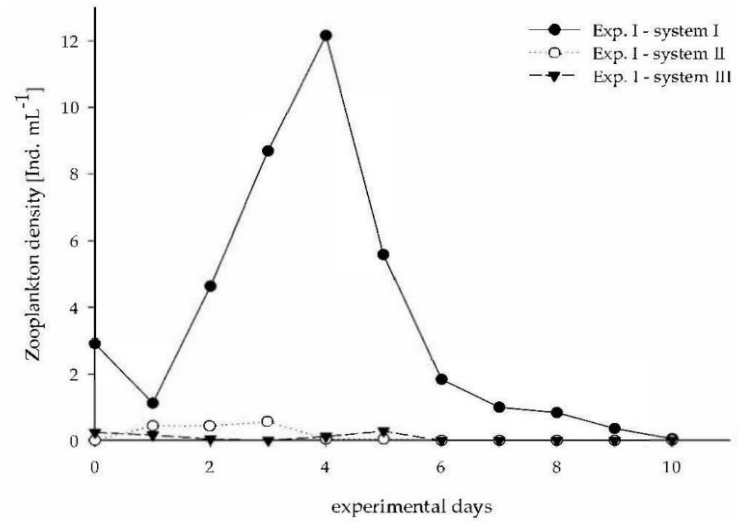

Figure 3. Density of *Brachionus calyciflorus*, displayed as daily means (±SD in Ind. mL^{-1}) for each culture system during pikeperch larval rearing of experiment I (day post hatch 0–10) (n = 5 per sampling).

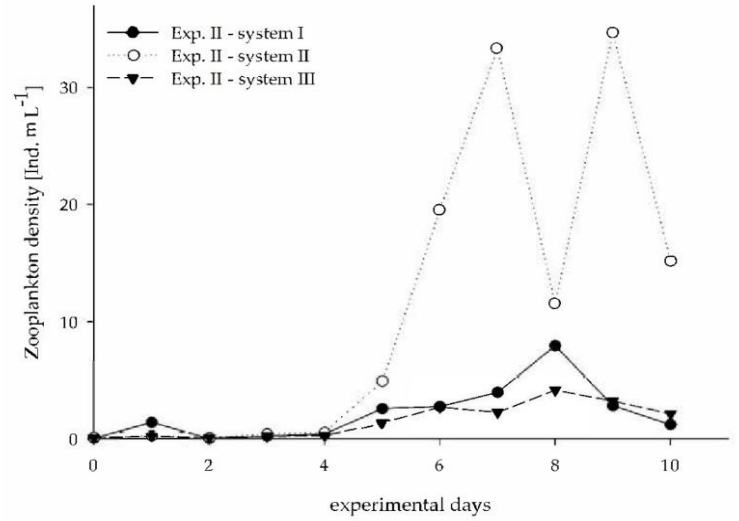

Figure 4. Density of *Brachionus calyciflorus*, displayed as daily means (±SD in Ind. mL^{-1}) for each culture system during pikeperch larval rearing of experiment II (day post hatch 0–10) (n = 5 per sampling).

3.2. Survival, Growth, and Total Fatty Acid Content of Pikeperch Larvae

After a ten-day feeding period with *B. calyciflorus* under pseudo-green water conditions in experiment I, the highest survival rate of 94% was observed in system I, whereas the survival rates in systems II and III were considerably lower (Table 2). The lowest larval survival of 5% was observed in system III, followed by a survival rate of 7% in system II. For experiment II, survival rates ranged from 25% (system I) to 64% (system II) and 74% (system III) (Table 2).

Table 2. Morphometric characteristics and survival of pikeperch larvae at dph 0 and dph 10 (Experiment I: initial sample: $n = 45$; ten days post hatch: system I, $n = 38$; system II, $n = 22$; system III, $n = 14$. Experiment II: initial and final $n = 10$). Superscript capitals indicate statistically different groups in terms of size after hatching and at the end of the experiment. Superscript lower-case letters indicate statistical differences between the three treatments at the end of the experiment.

	Total Length (mm)	Specific Growth Rate—SGR (% d^{-1})	Survival Rate (%)
Experiment I:			
After hatch	5.4 ± 0.1^{A}		
Culture system I	8.1 ± 0.3^{Ba}	4.1	94
Culture system II	6.6 ± 0.6^{Bb}	2.0	7
Culture system III	5.9 ± 0.1^{Bb}	0.9	5
Experiment II:			
After hatch	5.1 ± 0.2^{C}		
Culture system I	6.0 ± 0.2^{Dc}	1.7	25
Culture system II	6.8 ± 0.3^{Dd}	2.9	64
Culture system III	6.4 ± 0.2^{Dcd}	2.3	74

In each replicate of the first experiment, a significant (Kruskal–Wallis, $p < 0.001$ and $p = 0.019$) increase of the total length (TL) of the pikeperch larvae was detected during the first ten days, and the TL of pikeperch larvae in system I was significantly (Kruskal–Wallis, $p < 0.001$ and $p = 0.013$) higher compared to larvae of the other two culture units (Table 2). The larval dry mass increased significantly (ANOVA, $p < 0.001$), while the sum of total fatty acids (TFAs) as well as the amount of fatty acids per unit dry weight decreased significantly (ANOVA, $p < 0.001$) within the first ten days (Table 3). Due to low survival rates, fatty acid profiles as well as dry masses (DM) could not be determined for larvae of systems II and III.

Table 3. Total fatty acids (µg mg^{-1} DM) and individual larval dry mass (µg) of pikeperch larvae at dph 0 and dph 10 from system I (initial sample: $n = 5$; ten days post hatch: replicate I, $n = 4$). Superscript lower-case letters indicate statistical differences between larval hatching and the end of the experiment. The letters are to be read for the columns of the table, respectively.

	Total Length (mm)	Dry Mass (µg)	Total Fatty Acids (µg mg^{-1} DM)
Days post hatch: 0	5.4 ± 0.1^{a}	113.8 ± 7.0^{c}	110.4 ± 2.2^{e}
Days post hatch: 10	8.1 ± 0.3^{b}	270.1 ± 3.5^{d}	39.0 ± 0.9^{f}

3.3. Fatty Acid Profile of Pikeperch Larvae at Hatching and at 10 dph

Within the PUFAs, DHA was the most abundant fatty acid in yolk-sac larvae, followed by the two n-6 PUFAs, linoleic acid, and ARA. The content of the n-3 PUFAs α-linolenic acid and EPA was lower at hatching, but due to a high DHA level, the sum of n-3 PUFAs was higher than n-6 PUFAs in newly hatched pikeperch larvae (Table 4). According to the measured concentrations, in descending order, docosahexaenoic (22:6 n-3; DHA), oleic (18:1 n-9), palmitoleic (16:1 n-7), palmitic (16:0), linoleic (18:2 n-6), arachidonic (20:4 n-6; ARA), eicosapentaenoic (20:5 n-3; EPA), α-linolenic (18:3 n-3), and vaccenic (18:1 n-7) acid were the dominant (i.e., concentrations ≥ 5 µg mg^{-1} DM) fatty acids in newly hatched larvae (Table 5).

Table 4. Initial and final fatty acid ratios obtained from the pikeperch larvae from system I of the experiment I.

Fatty Acid Ratios	dph 0	dph 10
n-3/n-6	2.0:1.0	2.4:1.0
DHA/EPA	3.7:1.0	5.1:1.0
ARA/EPA	1.1:1.0	1.5:1.0
18:3n-3/18:2n-6	1.0:1.7	1.0:1.1

Table 5. Dominant fatty acids (means ± SD in μg mg^{-1} DM) of pikeperch larvae at days post hatch 0 (≥5 μg mg^{-1} DM) and at days post hatch 10 (≥1 μg mg^{-1} DM) as well as percentages of the corresponding initial content (initial sample: $n = 5$; after ten days post hatch: system I, $n = 4$). A "−" indicates a significant decrease in the fatty acid concentration, whereas a "+" indicates a significant increase in the fatty acid concentration. Data are organized in descending quantities.

Larvae at Days Post Hatch 0			Larvae at Days Post Hatch 10			
	Fatty Acids	μg mg^{-1} DM		Fatty Acids	μg mg^{-1} DM	% of Initial Content
1.	22:6n-3	20.5 ± 1.7	1.	16:0	7.9 ± 0.6 *−	81
2.	18:1n-9	18.6 ± 0.6	2.	22:6n-3	5.9 ± 0.1 *−	29
3.	16:1n-7	12.1 ± 0.4	3.	18:1n-9	4.9 ± 0.3 *−	26
4.	16:0	9.8 ± 0.5	4.	18:0	4.9 ± 0.1 *+	121
5.	18:2n-6	8.9 ± 0.1	5.	18:2n-6	3.2 ± 0.1 *−	36
6.	20:4n-6	6.0 ± 0.3	6.	18:3n-3	3.0 ± 0.1 *−	58
7.	20:5n-3	5.6 ± 0.4	7.	20:4n-6	1.7 ± 0.0 *−	28
8.	18:3n-3	5.2 ± 0.1	8.	20:5n-3	1.2 ± 0.0 *−	21
9.	18:1n-7	5.2 ± 0.2	9.			

* Significantly different contents compared to the initial value.

Compared to newly hatched larvae, there was an increasing n-3/n-6 ratio, DHA/EPA ratio, ARA/EPA ratio, and α-linolenic acid:linoleic acid ratio after ten days of feeding on *B. calyciflorus* (experiment I, system I). Each ratio was significantly (*t*-test, $p < 0.001$ and $p = 0.001$) different compared to the ratios observed for newly hatched larvae.

Due to a general decrease of fatty acid contents (per dry matter) during the run of the experiment, the term dominant fatty acid was adjusted for fatty acids recorded with concentrations ≥1 μg mg^{-1} DM. Consequently, ten days post hatch, palmitic acid (16:0) was the dominant (≥1 μg mg^{-1} DM) fatty acid, followed by DHA, oleic acid (18:1 n-9), stearic acid (18:0), α-linoleic acid, linolenic acid, ARA, and EPA. For the dominant fatty acids, in relation to dry mass, the level of each PUFA as well as of palmitic acid decreased significantly (ANOVA, $p < 0.001$, and Kruskal–Wallis $p = 0.003$) within the first ten days, while a significant increase (ANOVA, $p < 0.001$) of stearic acid per dry mass was detected. DHA remained the most abundant and linoleic acid the second-most abundant PUFA ten days post hatch. In terms of C18 PUFAs, the α-linolenic acid/linoleic acid ratio was nearly balanced ten days post hatch contrary to newly hatched larvae. Further, the proportion of DHA as well as of ARA increased in relation to EPA compared to newly hatched larvae.

Among MUFAs, each of the dominant MUFAs in newly hatched larvae was utilized significantly (ANOVA $p < 0.001$ and Kruskal–Wallis, $p = 0.007$). While the final content of oleic acid was still high ten days post hatch, the level of palmitoleic acid and vaccenic acid was lower than 1 μg mg^{-1} DM, and only 4.1% and 18.2%, respectively, of the initial contents were detected at the end of the experimental period.

4. Discussion

The overall idea of the experimental setup was separation of the fish larval rearing and the cultivation unit of *B. calyciflorus* into different compartments of one single RAS under constantly circulating water. The reservoir should enable undisturbed *B. calyciflorus*

reproduction, thereby achieving a self-sustained live feed culture, which should serve also as a feed reservoir for the fish larvae. Additionally, control of the culture conditions inside the culture system, without intervention into the larval rearing tank, was possible in the reservoir. The green water with *Monoraphidium contortum* ensured water turbidity and enhanced the quality of the culture water through nutrient extraction by microalgae, and the primary production served as food for the freshwater rotifer *Brachionus calyciflorus*.

It was demonstrated that the newly developed culture system for larviculture of pikeperch achieved survival rates that were above average by using *B. calyciflorus* as first live feed (between 5 and 94% at 10 dph). The maximum survival rate of 94% exceeds most of the published rates for pikeperch and perch larvae [2,14,36–38]. The only known exception is the study of Lund and Steenfeldt (2011) [10], where at a temperature of 18 °C and a larval stocking density of 28 Ind L^{-1}, no mortality was observed until days post hatch 16. A final survival rate of 91% was detected 21 days post hatch. However, in our experiments, the survival rates between the triplicates varied, and 3 of the 6 systems had much lower survival rates, in the range of 5–25%. This may be a consequence of the small system size that causes variation between replicates. Other influencing factors in addition to the small system volume could also have been the action by several employees or the different locations of the test systems although each system was individually equipped with lighting and a water and air pump. Nevertheless, it demonstrates that very high survival rates can be achieved under the conditions we describe.

The average total length and the SGR of pikeperch larvae after a culture period of ten days in system I of experiment I were 8.1 mm and 4.1% day^{-1} and in system II of experiment II reached 6.8 mm and 2.9% day^{-1} (Table 2) and was comparable to larval lengths observed in Szkudlarek and Zakęś (2007) [2] 11 days post hatch and even higher than total lengths observed in pikeperch larvae 12 days post hatch fed different enriched *Artemia* [39] or 11 days post hatch fed with *Brachionus plicatilis* at different salinities [18]. Imentai et al. (2019) [18] found that at a salinity of 2 psu, the total length was about 5.75 mm and the SGR 10.0 ± 5.4% day^{-1}, which is not consistent with our data, which show, for example, a length of 8.1 ± 0.3 mm at dph 10 and a specific growth rate of 4.1% day^{-1}. Consequently, our data demonstrate an advantage of using the freshwater rotifer, *B. calyciflorus*, in comparison to saltwater live feed, such as *B. plicatilis* and *Artemia* spec. Applying *B. plicatilis* in freshwater, it is noticeable that they die after a relatively short time and are no longer available as feed. Consequently, there is less feed available. Compensating this effect of lower feed quantity with increased feed portions, leads to a deterioration in water quality, as dead *B. plicatilis* sink to the bottom and are quickly decomposed. This aspect, which does not occur with *B. calyciflorus*, was interpreted by us as an advantage of *B. calyciflorus* over *B. plicatilis*.

Artemia eggs that have not been sieved and are therefore not offered as micro-artemia cannot be ingested by the pikeperch larvae in the first days of external feeding due to their size, as the mouth opening of the pikeperch larvae is simply smaller than he *Artemia* nauplii. This fact was also interpreted as an advantage of *B. calyciflorus,* which can be ingested be the pikeperch larvae. The use of micro-artemia mitigates the size argument, but *Artemia* spec. does not fully meet the nutritional requirements of pikeperch larvae. This was also considered an advantage for *B. calyciflorus* due to the fact that B. calyciflorus can be ingested and digested by the pikeperch larvae.

However, our observed growth clearly depended on the timing of the zooplankton peak that could be achieved in the utilized setup. The observed length of 5.1–6.8 mm during experiment II was lower compared to the best-achieved values in experiment I (8.1 mm), which reached the *B. calyciflorus* peak about 4–5 days post hatch. In experiment II, the peak was reached not before days 8 or 9 post hatch, which was too late for an optimal feed supply and resulted in a reduced growth. It should be mentioned here that the biology of *B. calyciflorus* played an important role here. During both experiments, no account was taken of egg-bearing females when determining the concentration of *B. calyciflorus*, so the sudden increase in system II during experiment II can be attributed to the hatching of existing eggs. The rapid decrease can then be attributed to the feeding of the pikeperch larvae.

Unfortunately, due to the late Brachionus peak, this increased feed availability could not be used for increased growth rates. Yet, such variations should not be over-interpreted as long as the concentrations do not fall below a critical threshold.

B. calyciflorus is known to multiply its population within few days. Rico-Martínez and Dodson (1992) [40] demonstrated that it increased from 25–104 Ind. mL^{-1} in 2 days at 30 °C with 5×10^6 cells mL^{-1} of *Chlorella vulgaris*. Consequently, our freshwater rotifer with specimen numbers of 2.8 and 12.7 Ind. mL^{-1} after 4 days in experiment I–system I had similar population growth rates with less feed concentration (*M. contortum* 5.8×10^5 cells mL^{-1}), demonstrating that *B. calyciflorus* grew well during our experiment I. Moreover, the peak in *B. calyciflorus* abundance matched with the start of exogenous feeding, which is known to be around 5 dph for pikeperch larvae [6]. On the other hand, a lack of enough adequate microalgae for *B. calyciflorus* occurred during the first experiment in systems II and III, resulting in an inadequate live feed density for pikeperch larvae. During the second experiment, maximum *B. calyciflorus* densities appeared at the middle or the end of the experimental period. This delay reduced survival and growth rate in comparison to experiment I–system I. According to Bischoff et al. (2018) [5], the delay in zooplankton production should not exceed 160 to 170 day-degrees, when all internal reserves must be compensated to avoid increased mortality rates.

M. contortum in experiment I–system I reached a density of $5.8 \pm 0.2 \times 10^5$ cells mL^{-1} at 1 dph. The microalgae at the beginning had no nutrient limitation and was at the exponential phase when *B. calyciflorus* where stocked. Preliminary tests and other experiments also clearly demonstrated that *M. contortum* can be considered a suitable feeding alga for the establishment of an artificial feed chain for pikeperch larvae. Further manuscripts to prove this statement are in preparation. Own studies showed that the availability of nutrients in relation to the N:P ratio can enhance the production of PUFAs by the microalgae, which was supported by literature [41,42]. Although this is species-specific, the phosphate limitations that occur at the stationary phase reduce the PUFAs production, being the best N:P ratio for *Monoraphidium* 0.153:1 to 1.53:1 [43–45]. Our microalgae culture in system I was at the appropriate phase and nutrient profile in the first experiment, when we stocked the *B. calyciflorus*. As a consequence, *B. calyciflorus* grew exponentially and thus allowed for a high *B. calyciflorus* density at the start of the exogenous feeding of pikeperch larvae. Therefore, our study demonstrates that stocking of *Monoraphidium contortum* in such small-scale systems should be done, considering the size of the culture system and the initial stocking density. In our case, this means it should not commence earlier than 3 days before the stocking of *B. calyciflorus* and five days before stocking the fish larvae. These results were confirmed by the second experiment, where all three individual microalgae cultures were similar. The results indicate that the synchronization of *M. contortum* and *B. calyciflorus* growth is essential to increase survival and growth rates of pikeperch larvae by using life feed.

Individual dry mass of pikeperch larvae during the first experiment (system I) increased significantly after ten days post hatch. The dry mass was comparable to dry weights of pikeperch larvae after eight days post hatch in the study of Lund and Steenfeldt (2011) [10] and lower compared to dry weights observed 12 days post hatch in the study of Lund et al. (2012) [39]. Differences in growth might be explained by different culture conditions, different times of measurement, and the use of *Artemia* in the study of Lund et al. (2012) [39] from day 4 post hatch onwards with a higher biomass gain compared to rotifers. Total fatty acids (TFAs) per individual larvae as well as per mg of dry mass decreased significantly, which was also observed in several studies of other fish species [7,46–49]. Bischoff et al. (2018) [5] reported a decrease in fatty acids to about 20% of the original concentration, which was even lower compared to the present study, which reached a fraction of 35%. This can be explained by the yolk sac consumption during the first days before the start of the exogenous feeding. In perch, after feeding with *B. calyciflorus*, the TFAs was 24.2 % [15]. Therefore, we assume our results of about 35% of the original fraction or 39 $\mu g\ mg^{-1}$ DM as an adequate content in TFAs.

In relation to the PUFAs, our data of n-3/n-6 ratio in pikeperch larvae before and after feeding *B. calyciflorus* are similar to n-3/n-6 ratios that could be observed in pikeperch eggs and larvae from wild, mature breeders [10]. Therefore, our results of n-3/n-6 (2:1) were assumed to reflect the natural ratio for pikeperch larvae and may indicate that *B. calyciflorus* meets the requirements of pikeperch in relation to these PUFAs. DHA was the second most abundant fatty acid and the most abundant PUFA in larvae ten days post hatch, and 29% of the initial content could be recovered in larvae. Similar observations could be made for ARA, for which 28% of the initial content could be detected in larvae after ten days feeding *B. calyciflorus*, and the final content was higher compared to the final EPA level which was 21% of the initial content. As a result, the DHA/EPA ratio was also considerably different in pikeperch at 10 dph, but the EPA/ARA ratio remained similar. The fatty acid profile of the *B. calyciflorus* culture at different conditions [15,50,51] could explain the high levels of DHA that were found in Awaiis et al. (1996) [15] for perch as well as in our experiment. Furthermore, other studies observed that in starved pikeperch larvae, a minimum of 30% of the initial DHA content that was retained in larvae after ten days without exogenous feeding [5,46]. Therefore, DHA and consequently the HUFA ratios may be less affected by the diet and could be regulated through selective retention of specific HUFAs from larval reserves. Hence, we assume from our results that (dietary) enrichments of n-3 HUFAs are not crucial in the early development of pikeperch larvae, contrary to other studies in pikeperch FA requirements [52]. Nonetheless, such a statement must be backed up by scientific experiments and data. Therefore, further experiments have been and are being conducted, and the data already obtained are currently being prepared for further publications that will provide a more accurate picture of the importance of the dynamics of fatty acids in the early development of pikeperch larvae.

Both C18 PUFAs linoleic acid and α-linolenic acid were well present in pikeperch larvae after the ten-day feeding period with *B. calyciflorus*, and the final content of both C18 fatty acids was higher compared to, e.g., ARA and EPA. It is well-established that C18 PUFAs are highly abundant in freshwater ecosystems [53–55] and that there is a general demand in freshwater and diadromous fish for C18 PUFAs [56,57]. Due to significantly higher levels of C18 PUFAs in the eggs of wild compared to the eggs of cultivated pikeperch breeders in the study of Khemis et al. (2014) [58], a significant role of C18 PUFAs and a natural demand can be assumed in pikeperch. Despite this natural demand, observations during previous studies (unpublished data) provided evidence that the content in α-linolenic and linoleic acid as well as their ratio in fish larvae are highly associated with the diet of pikeperch breeders [58] and with the content of larval first feeds [10,14,15].

Different studies have tried to understand the interaction of microalgae, *B. calyciflorus*, and fish larvae in terms of fatty acids [15,50,51]. In Kennari et al. (2008) [51], *B. calyciflorus* had a α-linolenic/linoleic ratio of approximately 1:1 with less α-linolenic acid compared to the respective diet (*Chlorella*) and the linoleic acid content similar to the microalgae. This suggests that *B. calyciflorus* also needs and uses α-linolenic acid for growth. Furthermore, in our experiment, due to the water supplementation and thus also nutrient supply, microalgae were supposed to be not limited in nutrients, producing more n-3 and providing enough α-linolenic acid to rotifers, as was also observed by Jensen and Verschoor (2004) [50], where *B. calyciflorus* had a α-linolenic/linoleic acid ratio of 2.3:1 when feeding on microalgae without nutrient limitation. The α-linolenic acid was in a similar range as the applied microalgae diet, and compared to linoleic, it showed twice the amount. This means that, even if rotifers used α-linolenic acid to live, the supplied amount was definitively enough and further pointed out the importance of the microalgae diet for the FAs content of the fish [59]. In our system, rotifers should have filled the needs of pikeperch in terms of their α-linolenic/linoleic acid ratio of 1:1.1. This is in contrast to previous observations of Bischoff et al. (2018) [5] for starved larvae (1:3). This could also explain the higher levels of DHA since the pikeperch larvae could have converted α-linolenic acids into DHA, which was previously discussed [15] and is a known process in freshwater fishes [60]. Moreover, a diet rich in α-linolenic and poor in HUFAs, as *B. calyciflorus* [15,50,51], seem to enhance the

ß-oxidation [61]. However, one study showed no evidence of this process in pikeperch [52] and thus reinforces our hypothesis that pikeperch larvae retain HUFAs. Nevertheless, we demonstrate that *B. calyciflorus* fills the nutritional requirements of pikeperch larvae producing a balanced α-linolenic acid/linoleic acid ratio (1:1) that might be optimal in pikeperch during early development.

5. Conclusions

Our study confirmed that the pseudo-green water technique in RAS is adequate for pikeperch larviculture, promoting high survival and growth rates by the use of an adequate timing/matching of *M. contortum* and *B. calyciflorus* with the start of exogenous feeding. Moreover, our results indicate that *B. calyciflorus* is an adequate live feed for the first 10 days post hatch of pikeperch larvae, and the interaction with the microalgae plays an important role. The microalgae culture conditions determine the fatty acid composition of *B. calyciflorus* as a diet for pikeperch, and thus, a good timing of microalgae and zooplankton results in a high live feed density with an adequate nutrient profile at the start of larval feeding. In the absence of algal nutrient limitation, the biochemical composition of *B. calyciflorus* and especially their content in C18 PUFAs and suitable n-3/n-6 and α-linolenic acid/linoleic acid ratios seems to meet the nutritional requirements of pikeperch larvae. Furthermore, the dietary HUFA composition seems to be less important for pikeperch during the first ten days after hatching.

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3. Pikeperch larviculture (*Sander lucioperca* [L., 1758]) with *Brachionus plicatilis* (Mueller, 1786) (Rotifera) and *Apocyclops panamensis* (Marsh, 1913) (Copepoda)

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Pikeperch larviculture (*Sander lucioperca* [L., 1758]) with *Brachionus plicatilis* (Mueller, 1786) (Rotifera) and *Apocyclops panamensis* (Marsh, 1913) (Copepoda)

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Abstract

The effect of live feed diets containing *Brachionus plicatilis* (B) and *Apocyclops panamensis* (A) was investigated on pikeperch larval survival, growth, and fatty acid composition in two experiments (E1 and E2). Up to six different diets were provided to the larvae, in which the letters of the diet names represent the live feed organisms as mentioned above and the subsequent number of feed organisms per larva and day. In E1, start cultures with 35 larvae/L were supplied with two diets (B200 and B100 + A100) 7 days until 10 days post hatch (dph). In E2, 50 larvae/L were fed six diets, B280 + A120, B340 + A60, B400, B420 + A180, B510 + A90, and B600. The highest survival and specific growth rates (SGR) occurred in B200 (E1). In E2, B340 + A60 performed best. A trend of increasing survival rates was found in E1 from 68.9% (B100 + A100) to 71.6% B200 and in E2 from 64.0% (B280 + A120) to 93.7% (B340 + A60). Larval SGR tended to increase with higher shares of dietary *B. plicatilis* in both experiments. The polyunsaturated fatty acid, linoleic acid (LA), followed the same pattern as survival and SGR increasing until a plateau was reached, indirectly indicating a feeding threshold. *B. plicatilis*

provided the LA to pikeperch larvae, which seemed to play an important role in this feeding period. Consequently, a live feed diet should contain at least 340 *B. plicatilis* ind. larva^{-1} day^{-1} and 6.3 *B. plicatilis* per milliliter until 10 dph to improve pikeperch larviculture.

KEYWORDS
copepods, fatty acid composition, first live feed, pikeperch larvae, rotifers

1 | INTRODUCTION

Increasing global fish consumption (FAO, 2020) is leading to greater interest in new aquaculture fish species. Production of several new emerging species, such as pikeperch (*Sander lucioperca* [L. 1758]) is increasing, with a consumption of 3074 tons in 2020 (FAO, 2023). However, the production costs are still high because of the lack of a stable production volume (Policar et al., 2019). To achieve economically sustainable production, newly cultured fish species require improvements in broodstock management, controlled reproduction, and larviculture (Steenfeldt, 2015). The main bottleneck in larviculture is the initial status of the offspring and is determined by the egg quality, which directly affects the hatching rate and larval quality (Kestemon & Henrotte, 2015; Schaefer et al., 2018).

Under consideration of recent improvements in pikeperch larviculture as done in Szczepkowski et al. (2011), Ljubobratović et al. (2015), Yanes-Roca et al. (2018), Bischoff et al. (2022) or Imentai et al. (2022), successful rearing practices involve live feed that covers the nutritional requirements. The most widespread practice so far is the use of *Artemia* sp. as the first exogenous feed (summarized in Policar et al., 2019; Steenfeldt, 2015), but its exclusive use is still debated (Bischoff et al., 2018). Alternative diets such as the marine rotifer *Brachionus plicatilis* (Müller, 1786) are commonly used as the first live feed source for species like gilthead seabream, *Sparus aurata* (Pantazis et al., 2014; Rodríguez et al., 1997) or greater amberjack, *Seriola dumerili* (Hamasaki et al., 2009), mainly because of their small size, high culture densities, and fast reproduction (Hagiwara & Marcial, 2019; Lubzens et al., 1989). Although these marine rotifers are not part of the natural diet of pikeperch larvae (Peterka et al., 2003), they were used as diets in recent years (Imentai et al., 2022; Yanes-Roca et al., 2020). Pikeperch larvae have a small mouth opening, approximately 85–90 µm at dph 3 (Bischoff et al., 2018) and hence they need an appropriate live feed size for first exogenous feeding. *B. plicatilis* has the advantage of being smaller (100–340 µm) than *Artemia* nauplii (400–500 µm at hatch) (Lavens & Sorgeloos, 1996). Moreover, *B. plicatilis* is easily enriched with commercial products as shown by Eryalçın (2018). This offers advantages over *Artemia*, which become even lager during enrichment, and over copepods, which have to be fed with microalgae. Consequently, during the first 10 days the size and nutritional profile of *B. plicatilis* seem to fit the requirements of pikeperch larvae better than *Artemia* sp., increasing survival and growth rates (Imentai et al. 2019a, 2019b, 2020; Yanes-Roca et al., 2018, 2020).

Although the nutritional requirements of pikeperch larvae have been studied (Hamza et al., 2008; Hamza et al., 2012; Kestemont et al., 2007; Lund et al., 2012, 2014, 2018), there is still a need to find alternative and additional live feed organisms for larviculture. Another zooplankton group gaining interest are copepods (Ajiboye et al., 2010). Copepods constitute the majority of the zooplankton; they are the main food source for fish larvae in marine ecosystems and the most important connection between primary production and the food web (Lavens & Sorgeloos, 1996). Copepods seem to fulfill the nutritional requirements of fish larvae in nature because of their life history traits, biochemical composition as well as swimming behavior, and are suitable for commercial aquaculture (Drillet et al., 2011). The cyclopoid copepod *Apocyclops panamensis* (Marsh 1913) is such a promising candidate (Cruz-Rosado et al., 2020; Phelps et al., 2005).

Optimization of the feeding protocols would make larviculture more sustainable and economically efficient. Furthermore, ingestion and digestion capacity and nutrient requirements may shift during larval development. Consequently, further information on the optimal feeding period and feed organism including specific amounts and/or concentration is necessary. To assess the potential success of larval rearing, the fatty acid dynamics during early larval development can be studied (e.g., Bischoff et al., 2018). The aim of this study was to investigate the effects of different live feed diets containing the rotifer *B. plicatilis* and the copepod *A. panamensis* in different amounts on larval survival, growth, and fatty acid composition during the first 10 days of larval development. An optimized live feed feeding protocol for early pikeperch larvae is discussed.

2 | MATERIALS AND METHODS

2.1 | Microalgae and rotifer culture

Microalgae species used *Nannochloropsis* sp. and *Isochrysis galbana* were obtained from Aquacopa GmbH (Jabel, Germany) and were cultivated at the facilities of the University of Rostock. Five liters of each algae culture were mixed with 5 L of saltwater made of aqua dest. and super soluble of synthetic sea salt AquaMedic GmbH (Germany) at 30 g/L. For *Nannochloropsis* sp., 5 mL of Guillard's medium (F/2) (Guillard, 1975) was added and for *I. galbana*, 5 mL of Walnes medium (Walne, 1970) as indicated by Aquacopa GmbH.

B. plicatilis was also obtained from Aquacopa GmbH (Jabel, Germany), cultured at the facilities of Rostock University in 200 L zooplankton reactor at 23°C with a light cycle of 18:6 (L:D) and salinity of 30 g/L and fed with *Nannochloropsis* sp. once a day. During the pikeperch experiments and prior to harvest, the zooplankton, the density of *B. plicatilis* culture was determined each day by counting the number of individuals for representative subsamples. *Nannochloropsis* sp. was added to the rotifer culture after the daily harvest to avoid enrichment.

2.2 | Copepod culture

A. panamensis was provided by Aquacopa GmbH (Jabel, Germany) and cultured in a 200 L zooplankton reactor at 23°C with a light cycle of 18:6 (L:D) and salinity of 30 g/L. We fed them each 4–5 days with 0.5–1.0 $\times$ 10^6 cells of *I. galbana* per milliliter.

During the pikeperch experiment, the density of *A. panamensis* culture was determined each day by counting the number of individuals for representative subsamples.

2.3 | Fish husbandry

In March 2020, fertilized pikeperch eggs were transported under cooling (≤10°C) from Prague, Czech Republic, to Rostock and transferred to the University of Rostock, Germany. Upon arrival, the temperature was slowly raised to 12°C and the eggs were transferred into an incubator. The water temperature was gradually increased over the next 48 h until it reached 15°C. Five days after transfer to the incubator, pikeperch larvae hatched, and were stocked into the tanks at day after hatch (dph) 1 (6 days after transfer). Pikeperch larvae were maintained in a RAS, including water treatment (mechanical and biological filtration as well as UV light treatment) under a light regime of 16L:8D, constant temperature, and oxygen concentration. Six 60-L tanks, filled with 43 L, were operated in parallel, arranged in two groups with 35 pikeperch larvae per liter at dph 4. A second experiment took place in July 2020. Pikeperch larvae were provided by the State Institute of Fisheries in Hohen Wangelin (Mecklenburg-Western Pomerania, Germany) at dph 3. Larvae were transported at 15°C to the lab facilities of the University of Rostock and were

directly stocked into the experimental tanks at 16°C. Pikeperch larvae were maintained in the same RAS as during the E1 using three of the previous tanks divided into floating subunits of 1 L each to establish a stocking density of 50 larvae per liter and, thus, to increase the concentration of feed inside the water. In both experiments, the physico-chemical water parameters temperature, oxygen, pH, ammonia (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and phosphate (PO_4^{3-}) were monitored daily. In addition, we performed daily siphoning of the bottom and removal of the dirt, dust, and lipid layer at the water surface.

2.4 | Diets and experimental protocols

Two independent experiments with *S. lucioperca* larvae took place in 2020 in order to improve the feeding protocol during the first 10 days post hatch (dph), studying larval survival, growth, and fatty acid composition. Different diets containing the marine rotifer *B. plicatilis* and the marine copepod *A. panamensis* were tested. In E1, two different diets containing *B. plicatilis* (abbr. B) and *A. panamensis* (abbr. A) in different amounts per fish larva were applied (B200 ind. $larva^{-1}$ day^{-1} and B100 ind. $larva^{-1}$ day^{-1} + A100 ind. $larva^{-1}$ day^{-1}) (Table 1). In E2, six different diets were tested, three with an intermediate quantity of live feed organisms B280 + A120, B340 + A60, and B400 and another three with high amounts B420 + A180, B510 + A90, and B600 (Table 1). Based on the daily stocking density of the larvae per experimental unit, the volume to be fed was then calculated using the previously determined zooplankton density, measured, and then fed.

In both experiments, pikeperch larvae were fed three times per day (9:00 a.m., 12:00 a.m., and 3:00 p.m.), starting the experiment at 4 dph, feeding the larvae for 7 days, including dph 10.

2.5 | Larval performance

Survival rates were calculated from dph 4, when the feeding started, by counting recorded dead larvae from siphoning every day as follows:

TABLE 1 Experimental diets containing different quantities and densities of *Brachionus plicatilis* and *Apocyclops panamensis* (live feed organism) used in both experiments

Diet	Experiment	Feed quantity	Feed composition	Live feed organisms per fish per day	Live feed organisms per milliliter
B100 + A100	E1	Low	*B. plicatilis* 50% *A. panamensis* 50%	200	2.3
B200	E1	Low	*B. plicatilis* 100%	200	2.3
B280 + A120	E2	Intermediate	*B. plicatilis* 70% *A. panamensis* 30%	400	6.3
B340 + A60	E2	Intermediate	*B. plicatilis* 85% *A. panamensis* 15%	400	6.3
B400	E2	Intermediate	*B. plicatilis* 100%	400	6.3
B420 + A180	E2	High	*B. plicatilis* 70% *A. panamensis* 30%	600	9.5
B510 + A90	E2	High	*B. plicatilis* 85% *A. panamensis* 15%	600	9.5
B600	E2	High	*B. plicatilis* 100%	600	9.5

$$\text{Survival}\,(\%) = [(N_i - \text{TD}) \times 100]/N_i, \quad (1)$$

where N_i is the initial number of larvae and TD is the total number of dead larvae found.

The experiment with pikeperch larvae had to be approved by the authorities; in our case, the Mecklenburg-Western Pomerania State Office for Agriculture, Food Safety and Fisheries, based in Rostock. According to the animal experiment permit issued, 7 individual larvae could be taken from each replicate of each experimental group on each sampling day.

Samples were taken at 1 dph (E1) and 3 dph (E2), and at 11 dph (E1 and E2) after the last feeding day (dph 10), cooled down first until 12°C and then until 0°C to anesthetize them. Pictures for measurements were taken, and larvae were killed by cutting the spinal cord and frozen for fatty acid analyses.

The total body length as well as yolk sac and oil droplet sizes were measured under a stereo light microscope (SZX10 Olympus, Hamburg, Germany) connected to a UC30 digital camera (Olympus, Hamburg, Germany) and the software package cellSens Dimension 1.6 (Olympus Soft Imaging Solutions, Hamburg, Germany). Yolk sac volume and oil droplet volume were calculated according to Bischoff et al. (2018). Finally, we calculated the specific growth rate (SGR) [%/day] as follows:

$$\text{SGR} = \{[(L_t - L_o)/L_o]/t\}100, \quad (2)$$

where L_t and L_o represent the average length of the larvae at time t and time $t = 0$. This formula was applied assuming linear growth for this life stage because of an own unpublished data set.

2.6 | Fatty acid analysis

Fatty acid analyses were performed at Greifswald University, Laboratory of Animal Ecology. The samples were freeze-dried, weighed, and transferred to extraction tubes. Fatty acid extraction started with the addition of 2:1 (vol:vol) dichloromethane:methanol and nonadecanoic acid methyl ester as internal standard according to Wacker et al. (2016). After storage for 24 h at −20°C, samples were placed in the ultrasonic bath for 5 s and the solvent was transferred to a new tube. Dichloromethane:methanol solution was added again into the sample tube and introduced into the ultrasonic bath and finally, the solvent was transferred to the same new tube. The solvent was evaporated at 40°C under a stream of nitrogen. The dried sample was resuspended in 4 mL 3 mol/L methanolic HCl (Sigma-Aldrich Chemie, Taufkirchen, Germany) and subsequently incubated under nitrogen for 20 min at 60°C in a sealed vial to transesterify fatty acids into fatty acid methyl esters (FAMEs). After cooling down, FAMEs were extracted three times: For each extraction, 3 mL isohexane was added to the tubes with the methanolic HCl, for 5 s vortexed, and let the two phases settle, the vortex step was repeated twice. The isohexane phase was collected in a new vial. This fraction was evaporated to dryness under nitrogen at 40°C and resuspended in a volume of 50–100 µL isohexane. The FAMEs were analyzed by gas chromatography (6890N, Agilent Technologies, Böblingen, Germany) and helium as carrier gas according to Wacker and Weithoff (2009). FAMEs were separated on a 50% cyanopropyl-phenyl methyl-polysiloxane column (Agilent Technologies J&W DB-225, 30 m × 0.25 mm × 0.25 µm) applying a temperature gradient; 60°C for 1 min, increasing at 20°C/min until 150°C, 10°C/min until 220°C and then held for 13.75 min. FAMEs were detected using a flame ionization detector (FID) at 250°C and quantified using multipoint standard calibration curves with HP chemstation (Agilent Technologies, Böblingen, Germany). For confirmation, mass spectra were recorded with a gas chromatograph-mass spectrometer (Pegasus 4D GC-TOFMS, LECO Instruments, Mönchengladbach, Germany).

2.7 | Statistical analysis

Statistical analyses were performed using the software IBM SPSS Statistics, version 27. The Shapiro–Wilk test was used to determine the normal distribution. To analyze differences between means in E1, an analysis of variance (ANOVA) was applied when normality was proven. Without normality, a nonparametric *t*-test for independent samples was applied. For E2, a two-way-ANOVA was performed. When variables in E2 did not follow the assumptions for the two-way ANOVA, variables were analyzed per feed quantity (intermediate and high) as previously explained for E1. All significance levels α were set to 0.05 (Table S1).

3 | RESULTS

3.1 | Fish husbandry

During the first experiment, larvae were reared at a temperature of 15.9 ± 0.7°C, oxygen concentration of 98.5 ± 0.7% and pH = 8.4 ± 0.6, ammonia 0.01 ± 0.01 mg/L, nitrite 0.01 ± 0.01 mg/L, nitrate 1.5 ± 0.3 mg/L, and phosphate 0.22 ± 0.06 mg/L. During the second experiment, larvae were reared at a temperature of 16.3 ± 0.5°C, oxygen concentration of 101.9 ± 1.2% and pH = 8.1 ± 0.2, ammonia 0.70 ± 0.01 mg/L, nitrite 0.00 ± 0.01 mg/L, nitrate 7.9 ± 1.3 mg/L, and phosphate 0.12 ± 0.06 mg/L.

3.2 | Larval performance

For the first experiment, the average survival rate of the mix of *B. plicatilis* and *A. panamensis* reached 68.9% at dph 11, while the exclusively *B. plicatilis* fed larvae showed a final survival rate of 71.6% (Figure 1). There was no significant difference between the two treatments (ANOVA $p = 0.538$) (Table S1). During the second experiment, the lowest survival rate was 64.0% with the diet B280 + A120, while diets B340 + A60 and B400 showed higher survival rates of 93.7% and 88.8% respectively. For high-quantity diets, the highest observed survival rate of 94.4% with the diet B510 + A90 (Figure 1) was achieved. Statistically significant differences were found in E2 only for intermediate quantity diets (ANOVA $p = 0.011$) (Table S1).

In the first experiment, at 1 dph, larvae had a total length of 4.7 ± 0.25 mm, with a yolk sac volume of 0.26 mm^3. During the first experiment, the growth of the larvae fed with B100 + A100 reached a final length of 5.08 ± 0.38 mm while those larvae fed exclusively with *B. plicatilis* (B200) reached 5.33 ± 0.35 mm. In E2, larvae at dph 3 had a total length of 4.06 ± 0.17 mm with a yolk sac volume of 0.43 mm^3. The treatment that achieved the shortest length was B280 + A120 with 4.3 ± 0.58 mm while the highest larval length of 4.9 ± 0.31 mm was for B340 + A60.

In E1, the treatment with the lowest SGR (0.8%/day) was found for B100 + A100 while the highest SGR (1.2%/day) was found for B200. No significant differences were found in SGR (ANOVA $p = 2.69$) (Table S1). During E2, diet B280 + A120 had the lowest SGR (0.8%/day) and diet B340 + A60 had the highest SGR (3.0%/day) (Figure 1). There were significant differences in feed quality (or composition) and in the interaction between feed quantity and quality (Table S1).

3.3 | Fatty acid composition

The initial fatty acid contents of pikeperch larvae in E1 showed a mean total fatty acid (FA) content of 243.1 μg/mg dry weight (DW) and in E2 of 273.9 μg/mg DW, and in both, the most concentrated group was the polyunsaturated fatty acids (PUFAs) (Table S2).

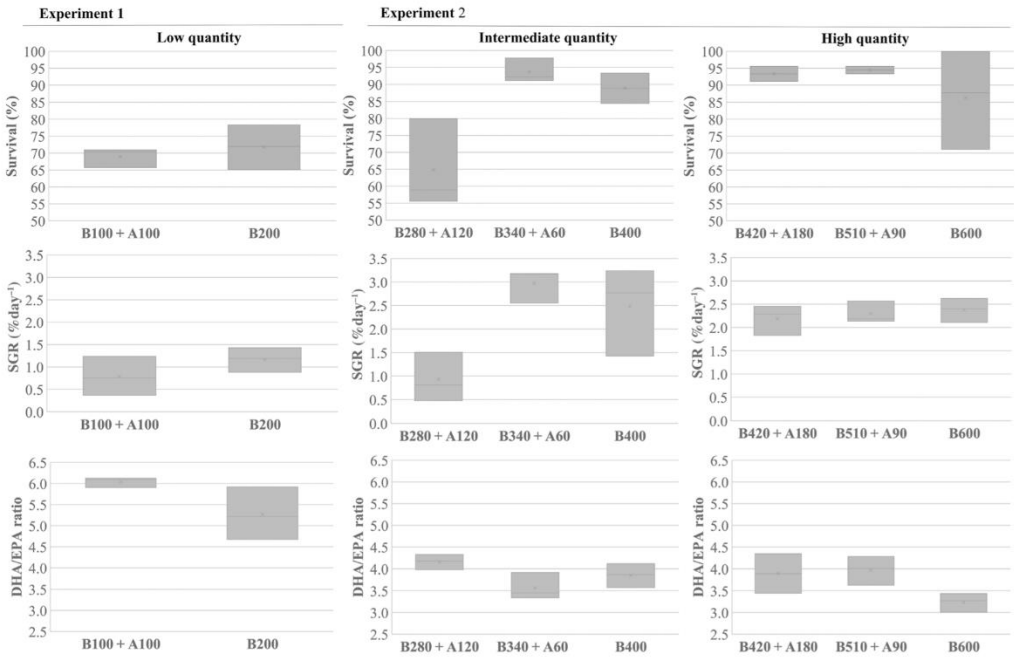

FIGURE 1 Larval survival (%), SGR (%/day), and DHA/EPA ratio at 11 dph for E1 (low quantity) and E2 (intermediate quantity: B280 + A120, B340 + A60 and B400 and high quantity: B420 + A180, B510 + A90 and B600) ($N = 3$). Boxes represent quarters and × the mean values.

After the feeding period, larvae in E1 contained a total fatty acid (FA) content of 93.4 μg/mg in B100 + A100 and 86.6 μg/mg in diet B200. In E2, B280 + A120 showed the lowest total FA concentration (108.9 μg/mg) and the highest was in B600 (171.9 μg/mg) at dph 11 (Table S3). Nonsignificant difference was found in total FA content during both experiments, and further comparisons among fatty acids were performed on the basis of their percentages (Table S1).

Saturated fatty acids (SFAs) in E1 were 31.4% in B100 + A100 and 29.6% in B200, with no significant differences (Table S1). Significant differences were found in the percentage of monounsaturated fatty acids (MUFAs) (ANOVA $p = 0.002$, Table S1), being 18.7% in B100 + A100 and 25.6% in B200 (Figure 2). PUFAs were the most concentrated fatty acid group, being 49.6% for B100 + A100 and 44.8% B200 (nonsignificant, Table S1). Within PUFAs, significant differences were found in n-6 and n-3 percentage, being for B100 + A100 11.0% and 38.5%, respectively, and for B200 13.6% and 31.2% respectively (Figure 2, Table S1). Moreover, within n-6, linoleic acid (LA, C18:2 n-6) was lower in B100 + A100 (4.9%) than in B200 (7.2%), while arachidonic acid (ARA, C20:4 n-6) was higher in B100 + A100 (2.4%) than in B200 (2.1%). Within the n-3, docosahexaenoic acid (DHA, C22:6 n-3) was higher in B100 + A100 (29.7%) than in B200 (22.4%) (Figure 3) with significant differences (Table S1). Moreover, B100 + A100 had higher eicosapentaenoic acid (EPA, C20:5 n-3) and DHA/EPA although no significant differences were found for both variables (Table S1).

In E2, SFAs showed significant differences in the interaction of feed quality and quantity (Table S1). For intermediate quantity diets, the percentage of SFAs decreased when more *B. plicatilis* was provided in the diet. For high-quantity diets, SFAs increased with an increasing amount of *B. plicatilis* (Figure 2). The MUFAs percentage was similar for all the diets. PUFAs showed significant differences in the interaction of feed quality and quantity (Table S1). For intermediate quantity diets, PUFAs increased with an increasing amount of *B. plicatilis*, while for high-quantity diets, PUFAs decreased. Within PUFAs, the n-6 PUFAs were not different, while n-3 PUFAs showed the same pattern than

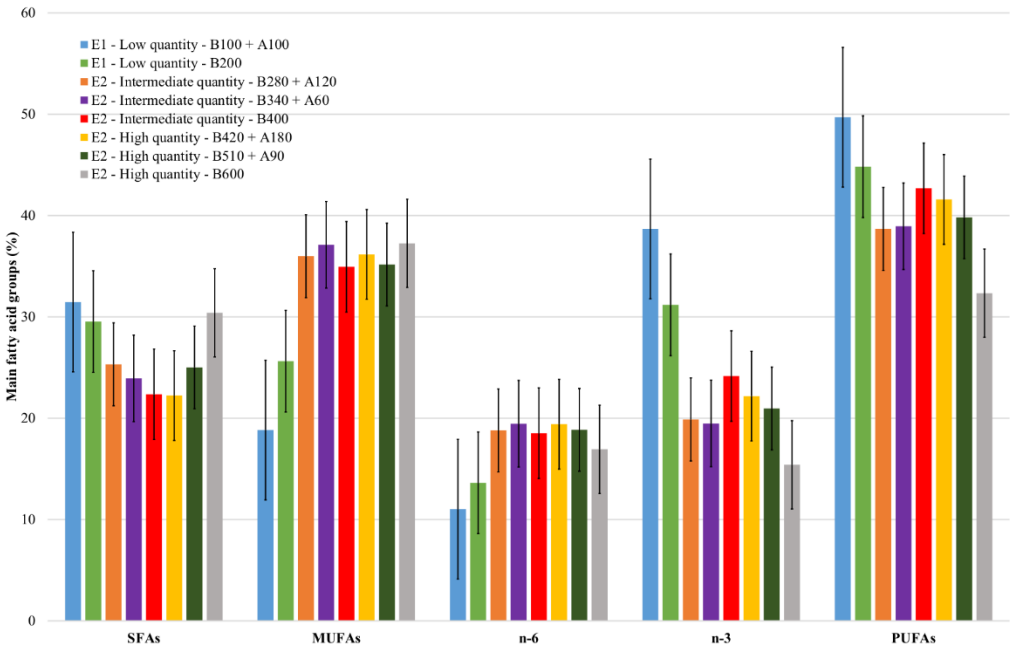

FIGURE 2 Main fatty acid group percentage of the total fatty acid content in pikeperch larvae at dph 11 after all the different diet regimes.

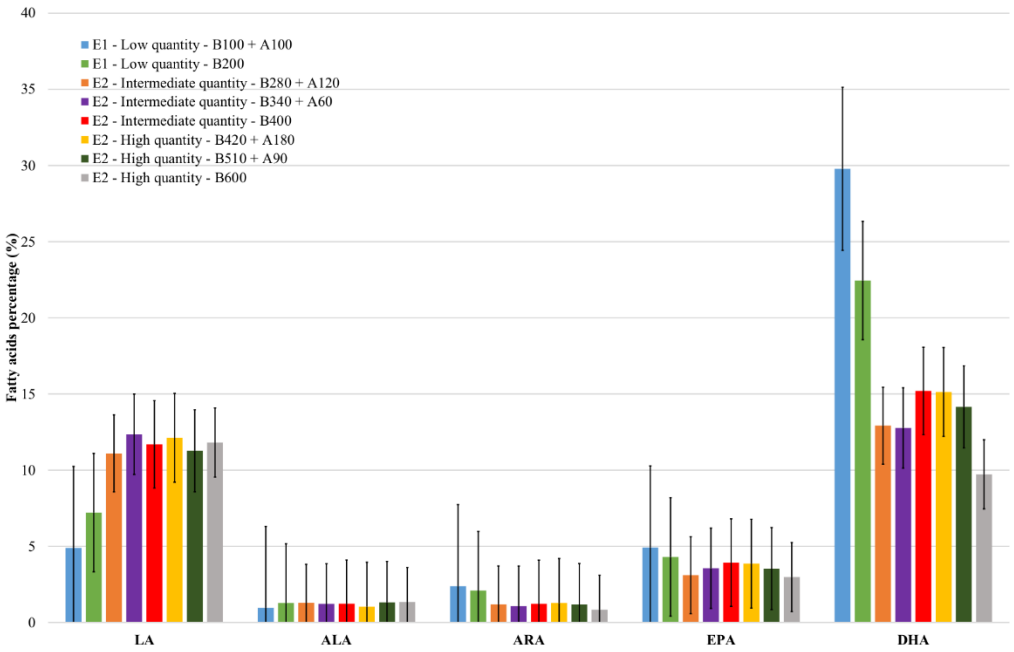

FIGURE 3 Single important polyunsaturated fatty acids percentage of the total fatty acid content in pikeperch larvae at dph 11 after all the different diet regimes.

PUFAs. LA, a specific n-6 PUFA, increased at low feed quantity while at intermediate and high quantities remained similar (Figure 3; Table S1). However, ARA, another n-6 PUFA, decreased at low and high feed quantities while at intermediate quantity ARA increased (Figure 3; Table S1).

Within the n-3 PUFAs, DHA and EPA showed the same pattern as ARA (Figure 3; Table S1).

4 | DISCUSSION

4.1 | Larval performance

Larviculture of *S. lucioperca* in an experimental RAS with live feed reached very high survival rates of 86.7%–94.4% until 11 dph, especially under the presence of more than 340 *B. plicatilis* larva^{-1} day^{-1}. This seems to be an optimal amount of feed organisms, combining *B. plicatilis* as a good prey item with an adequate number of live feed organisms per fish. Successful larval rearing of *S. lucioperca* requires both best suitable life feed combined with an optimal larval stocking density and feed concentration.

Survival rates of 64.0% and 94.4% in E1 and E2 were higher than those by Bischoff et al. (2018) when using *Artemia* spec. as the first live feed. This demonstrates that the rotifer *B. plicatilis* is a better first live feed during the first life cycle stage of pikeperch larvae, which is also supported by Yanes-Roca et al. (2018). In Imentai et al. (2019a), the highest larval survival rate, which was reached by using *B. plicatilis* at a concentration of 10 ind./mL, was 65.3% at dph 11, similar to our lowest survival rates in B280 + A120 (E2). Furthermore, Imentai et al. (2019b) observed survival rates of 78.9%–89.4% at different concentrations of *B. plicatilis* between 2–20 rotifers per milliliter, which is comparable to our results in diets B400 and B600 (E2) but not higher than the highest survival rates of 94.4% in diet B510 + A90, 93.7% in B340 + A60, and 93.3% B420 + A180 (E2). A trend is seen to increase the survival rates from 68.9% (B100 + A100) to 71.6% B200 (E1) and from 64.0% (B280 + A120) to 94.4% (B510 + A90) (E2). This suggests that diets containing a minimum of 340 *B. plicatilis* larva^{-1} day^{-1} may better suit the requirements of pikeperch larvae, decreasing the mortality rate to a minimum. Moreover, the slight differences in survival rates between the diets B200 and B100 + A100 in E1 and between B400 and B600 with the other diets in E2 (except B280 + A120) showed no clear influence of *A. panamensis* in larval survival at this point, indicating that *B. plicatilis* and its concentration was mainly responsible for the observed differences in survival during this life stage.

During the first experiment, the longest larval length of 5.33 mm was achieved at dph 11 when each larva had available 200 *B. plicatilis* per day (B200). Larvae fed with diet B100 + A100 had a length of 5.08 mm at dph 11, significantly different in comparison with B200. Even though both diets had the same density of live feed in the water and the same amount of live feed organisms per larvae and per day, the fish larvae exclusively fed with *B. plicatilis* had better growth. This also indicates that pikeperch larvae needed more *B. plicatilis* and that *A. panamensis* seemed to be a less adequate live feed organism during the first 7 days of feeding. In E2, the results showed a total body length of between 4.3 and 4.9 mm at dph 11. The larvae were smaller (4.06 mm) at 3 dph in E2 than at 1 dph in E1. Consequently, the total length at dph 11 was also lower than in E1 and, in both experiments, lower compared with earlier studies (Bischoff et al., 2022; Imentai et al. 2019a; Imentai et al., 2020; Yanes-Roca et al., 2018, 2020). This is a result of the initial status of the larvae, which was not identical, showing the importance of the different larval quality at hatch (Policar et al., 2019). In the treatment B340 + A60, the highest total body length of 4.9 mm was reached, while the diet B280 + A120 had the lowest growth (4.3 mm), although the same amount of feed per larvae per day was given. Again, the amount of *B. plicatilis* was more relevant during this period of life than *A. panamensis*. A similar pattern was seen for the diets B420 + A180, B510 + A90, and B600, with lengths at dph 11 of 4.5, 4.4, and 4.7 mm respectively, all containing the same amount of feed per fish per day and the same feed density. This demonstrates that *A. panamensis* did not provide any advantages over *B. plicatilis* during the early development of *S. lucioperca* larvae and emphasizes the importance of the latter prey for the first exogenous feeding period. We suggest that this is

a matter of prey item size, because Bischoff et al. (2018) demonstrated that larger sized *Artemia* spec. was not ingested and led to the early death of the pikeperch larvae.

According to Molnár et al. (2004) and Ramos et al. (2016), the appropriate live feed concentration is of importance to allow the fish larvae to develop the capacity to prey. Imentai et al. (2019b) showed that the density of *B. plicatilis* in *S. lucioperca* larviculture should be between 6 and 20 individuals per milliliter, which is consistent with our results in the diet B340 + A60 with a concentration of 6.3 *B. plicatilis* per milliliter at each feeding time. Our results do not show any better development with higher live feed density and demonstrate the importance of considering live feed density in combination with the amount of feed per larvae per day or with the larval stocking density. This is in agreement with Król and Zieliński (2015) for *Perca fluviatilis*, Ramos et al. (2016) for leaf fish, *Monocirrhus polyacanthus*, and Żarski et al. (2011) for common barbel, *Barbus barbus*, who used the same amount of feed per fish, but it is not consistent with Szkudlarek and Zakęś (2007) for pikeperch and Bein and Ribi (1994) for *Perca fluviatilis*, who used the same amount of feed at all stocking densities. As a result, larval stocking density is very important for larval nutrition. High larvae densities can reduce the cost of production as long as survival and growth are not negatively affected (Steenfeldt, 2015). For this reason, the combination of feed density, feed quantity, and fish stocking density must be considered for successful pikeperch larviculture.

The growth rates of the larvae applying the diets B100 + A100 (0.7%/day), B200 (1.2%/day) in E1 and B280 + A120 (0.8%/day) in E2 were the lowest and contained the lowest number of *B. plicatilis*. The growth rates were for the rest of the diets: B340 + A60 (3.0%/day), B400 (2.3%/day), B420 + A180 (2.2%/day), B510 + A90 (2.3%/day) and B600 (2.4%/day). Imentai et al. (2019a) found at a salinity of 2‰, a mean length of 5.2 mm at dph 4 and 5.7 mm at dph 11. Because of different SGR calculations, the data was difficult to compare directly. Nevertheless, under our calculation, the SGR in Imentai et al. (2019a) would be around 1.37%/day, which was higher than in E1 but lower than in E2. Our fish performed better during E2 (except diet B280 + A120), with the highest SGR of 3.0%/day in larvae fed with B340 + A60. However, our data also indicate that the use of the rotifer higher than 340 *B. plicatilis* larva^{-1} day^{-1} did not further increase the growth rate. Consequently, we suggest an optimal growth rate when feeding pikeperch larvae with at least 340 individuals of *B. plicatilis* larva^{-1} day^{-1}.

4.2 | Fatty acid composition

The total FA composition of the pikeperch larvae in both experiments decreased until 11 dph, which is common during the early development of pikeperch larvae while using the yolk sac reserves. The decrease in E1 for B100 + A100 was 61.4% and 64.3% for B200. In E2, the decrease was 85.4% for B280 + A120, 60.0% for B340 + A60, 60.3% for B400, 57.7% for B420 + A180, 62.5% for B510 + A90, and 68.1% for B600. However, our total FA data in both experiments were higher at dph 11 compared with the concentrations found at dph 6 in Bischoff et al. (2018), when the pikeperch larvae had consumed all the yolk sac reserves and started starving. Consequently, the larvae must have fed on *B. plicatilis* and increased its fatty acid reserves, together with the observed survival and growth rates, demonstrating that our larvae were in good condition during both experiments.

During E1, the larvae fed with B100 + A100 had a higher content of SFA than B200. The percentage of SFAs found in E1 was similar to the percentage found in Yanes-Roca et al. (2020) when feeding *B. plicatilis* with *Nannochloropsis* sp. at a stocking density of 100 larvae/L and 10–14 *B. plicatilis* per milliliter, providing approximately 140 *B. plicatilis* larva^{-1} day^{-1} with a survival rate of around 55%. During E2, the SFAs were the lowest in B280 + A120 and the highest in B600. However, the percentage of SFAs over the total FA ranged from 22.2% to 30.2% in E2, lower than in E1 with lower survival and growth rates could be observed.

During E1, MUFAs were higher in B200 than in B100 + A100, possibly resulting from a better supply with *B. plicatilis*. MUFAs were between 18.7% and 25.6% of the total FA concentration, in correspondence to the results of Yanes-Roca et al. (2020). During E2, the MUFAs ranged between 34.8% and 37.2% of the total FA concentration,

higher than observed by Yanes-Roca et al. (2020). The MUFA concentrations were also higher than those measured by Bischoff et al. (2018) at dph 6.

In E1, larvae fed with B100 + A100 had a higher content of PUFAs, and in particular in DHA and the ratio DHA/EPA (E1). The higher amount of DHA in B100 + A100 can be explained by the diet of *A. panamensis* that fed on *I. galbana*, containing higher amounts of DHA than *Nannochloropsis* sp. (Dunstan et al., 1993; Roncarati et al., 2004). This indicates that pikeperch larvae are able to ingest and digest *A. panamensis* during the experiment, and even so, higher DHA content in the diet showed neither higher survival nor better larval development. Although PUFAs were higher for B100 + A100, ALA and LA were higher in B200 during E1, suggesting that ALA and LA are more important for the development of the pikeperch larvae during the first days of exogenous feeding. During E2, PUFAs were between 32.2% and 42.6%. The higher LA content in larvae fed with a higher amount of *B. plicatilis* can be explained by the higher amount of LA in rotifers that have been fed with *Nannochloropsis* sp. (Eryalçın, 2019; Yanes-Roca et al., 2020). Moreover, rotifers fed with microalgae seem to have more adequate fatty acid profile, higher PUFAs, than those fed with yeast (Dhont et al., 2013). LA (and the n-6 group) follows the same pattern as survival and SGR, which increased until a maximum. From this maximum, the plateau in survival, SGR, and LA may indicate the larvae are not able to ingest/digest all the available rotifers.

Finally, the PUFA concentrations of the pikeperch larvae in both experiments decreased until 11 dph, however, with LA, ALA, and EPA above the starvation limit observed by Bischoff et al. (2018). In Bischoff et al. (2018), DHA in starved larvae at dph 10 was 9.5 µg/mg DW. Interestingly, this value was higher than in B600 (E2) at dph 11, where we still achieved good survival and growth rates. It seems as if DHA is retained but not fully consumed by the fish larvae. Moreover, larvae fed with at least 340 *B. plicatilis* per larvae per day maintained an appropriate fatty acid profile of PUFAs slightly higher than MUFAs and both higher than SFAs, also reflected by high survival and growth rates. This constant fatty acid pattern must be maintained during the early life cycle stage of *S. lucioperca* by feeding, meaning that an adverse fatty acid profile in the larvae automatically leads to lower growth and survival. Therefore, our data demonstrate that LA seems to be more important in the diet of pikeperch larvae (freshwater fish) than DHA during this early life time period. We therefore support Tocher (2010) who demonstrated that *B. plicatilis* is an appropriate live feed for pikeperch larvae until dph 11. Consequently, *B. plicatilis* provided the appropriate nutrients to the pikeperch larvae, being an adequate live feed organism for this period, and the quantity of 340 *B. plicatilis* per larvae per day with a density of at least 6.3 *B. plicatilis* per milliliter was the optimal amount of prey organisms for an economical and sustainable rearing process (Steenfeldt, 2015).

5 | CONCLUSION

The best possible growth and survival of pikeperch larvae during the first 10 days post hatch can be achieved with at least 340 individuals of *B. plicatilis* per larvae per day at a concentration of 6.3 *B. plicatilis* per milliliter. With the stocking density of 47 pikeperch larvae/L and solely *B. plicatilis* as the first live feed organism, the highest survival rates of 93.7% and SGR of 3.0%/day can be achieved. *A. panamensis* is considered not suitable as life feed during the first stages of larval development, which require prey of adequate, small size and at the optimal density to provide the required feed quantity per larvae per day for successful larviculture.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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4. Growth and fatty acid composition of pikeperch (*Sander lucioperca* [L., 1758]) larvae under altered feeding protocol including the copepod *Apocyclops panamensis* (Marsh, 1913)

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Growth and fatty acid composition of pikeperch (*Sander lucioperca* L., 1758) larvae under altered feeding protocol including the copepod *Apocyclops panamensis* (Marsh, 1913)

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Alternative live feeds for small and sensitive fish early life stages such as pikeperch (*Sander lucioperca* L., 1758) can improve the larval quantity, quality and performance in aquaculture. Therefore, this study evaluated the cyclopoid copepod *Apocyclops panamensis* (Marsh, 1913) as live feed for pikeperch larviculture from day 11 post hatch (dph) in two independent experiments. In both experiments, pikeperch larvae had the highest specific growth rate (SGR) when they fed on *Brachionus plicatilis* until dph 11 and *A. panamensis* until dph 16–18. SGR was related to a decrease in total fatty acids (FAs), saturated FAs and monounsaturated FAs in pikeperch larvae, indicating their use as energy for growth. Within the polyunsaturated FAs, docosahexaenoic acid (DHA) increased in larvae fed with *A. panamensis* and coincided with the highest SGR suggesting that DHA is accumulated in larvae as structural FA. Our study demonstrated a suitable pikeperch larval fatty acid composition for growth after feeding *A. panamensis* compared with *Artemia* sp. from dph 11 until dph 16 and previously fed with *B. plicatilis*. Moreover, it highlighted the importance of the dietary PUFAs in pikeperch rearing, specifically of linoleic acid (LA) from dph 4 until dph 11 and of DHA from dph 11 onwards.

The use of live feed for small and sensitive fish larvae has increased in aquaculture. Nevertheless, the sole use of *Artemia* spp. must be reconsidered since it does not fulfil the nutritional requirements of some fish species like pikeperch (*Sander lucioperca* (L., 1758))[1]. Rotifers such as *Brachionus plicatilis* (Mueller, 1786)[2] and *B. calyciflorus* Pallas, 1766[3] have been successfully introduced to pikeperch larviculture. *B. plicatilis* in combination with *Artemia* sp.[2] or the exclusive diet with *B. plicatilis*[4,5] seemed to be adequate until day post hatch (dph) 10. However, the use should be limited to this period, avoiding negative effects on growth and intestinal development[5]. Fish larvae afford many physiological changes in the early life cycle stages and thus, a suitable feed must be supplied along all larval stages to fulfil their nutritional needs[6]. Beyond dph 11, rotifers are too small and limit fast growth therefore, *Artemia* sp. is still in use[2,7]. However, *Artemia* lacks important nutrients for fish like docosahexaenoic acid (DHA)[8]. Enriched *Artemia* is limited since it depends on fish oils to rise their long chain polyunsaturated fatty acid (LC-PUFA) levels[9] and even enriched, they seem to fulfil poorly the larval nutritional requirements[10]. El Kertaoui et al.[11], Hamza et al.[12], Hamza et al.[13] and Lund et al.[14] have shown that phospholipids and LC-PUFAs such as DHA are essential for pikeperch larvae at later larval stage (dph 17 to 34) since they may nutritionally program the fish for further development[11,15]. Thus, there is still the need to find the optimal live feed for pikeperch larviculture after dph 10 beyond the application of rotifers.

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Freshwater copepods are part of the natural diet of pikeperch larvae and thus, might fulfil the nutritional requirements. They have a higher nutritional value than rotifers and *Artemia* spp. due to their high natural amounts of PUFAs, free amino acids and antioxidant pigments[16]. For these reasons, the use of copepods in aquaculture has increased. Copepods are popular for ornamental fishes[17] and have shown promising results for halibut larvae (*Hippoglossus hippoglossus*)[18], winter flounder larvae (*Pseudopleuronectes americanus*)[19], Atlantic cod (*Gadus morhua*)[20], fat snook (*Centropomus parallelus*)[21] and ballan wrasse (*Labrus bergylta*)[15]. Despite some copepods having high-PUFA content with low-PUFA diets[22,23] for some copepods, it is essential to provide high-PUFA diets since enrichment techniques are not appropriate[10].

Ballesteros-Redondo et al.[24] evaluated the potential of *Apocyclops panamensis* (Marsh, 1913) as live feed for larviculture. When *A. panamensis* was fed with *Isochrysis galbana* at 0.5–1 10^5 cells mL^{-1} per day, copepod culture seemed to be adequate in terms of their fatty acid composition (1.8–2.6% of DHA and DHA/EPA ratio of 2.5–2.9) to rear fish larvae[25]. However, *A. panamensis* had no advantage for pikeperch larvae between dph 4 -10 in comparison to *B. plicatilis*[4]. Peterka et al.[24] found nauplii of cyclopoid copepods in the stomach of pikeperch larvae, and El Kertaoui et al.[11] reported a need of 3.5% of EPA + DHA for pikeperch larvae, which coincides with the fatty acid composition of *A. panamensis*[24]. The authors hypothesized that *A. panamensis* is an adequate live feed organism as a second live feed organism following the application of rotifers and improving the fatty acid composition. The present study evaluates the effect of *A. panamensis* on pikeperch larval growth and fatty acids composition between dph 11–18 after fed with *B. plicatilis* or *Artemia* sp. from dph 4 to dph 10 and compares it with the use of *Artemia* sp. between dph 11–16.

Methods

Live feed

Zooplankton as well as microalgae were obtained from Aquacopa GmbH, Jabel, Germany, and were cultivated at the facilities of the University of Rostock. According to Ferreira et al.[27], *Brachionus plicatilis* (Müller 1786) was fed with *Nannochloropsis* sp. and, according to Ballesteros-Redondo et al.[24], *Apocyclops panamensis* was fed with *Isochrysis galbana*. *Artemia* eggs (ArtemioPur, JBL GmbH & Co. KG, Germany) were hatched and a maximum of 24 h old Artemia nauplii was fed to the larvae. The density of each zooplankton culture was measured daily to harvest the amount needed to feed the pikeperch larvae (see experimental diets below). Besides that, three samples of *B. plicatilis* (67,500 individuals per sample) and *A. panamensis* (210,000 individuals per sample) were taken 24 h after the last supply of microalgae. Furthermore, three samples of recently hatched *Artemia* sp. were collected (40,000 individual per sample). To collect the individuals of each zooplankton organism, each culture was filtered through a 50 µm net, the organisms were collected with a minimum of water content in glass vials for subsequent lyophilisation. Afterward, samples were weighed and an amount between 1.3 and 1.9 mg dry weight (DW) of each sample was taken for fatty acid analyses. One mg DW for *B. plicatilis* corresponded to 1048 ± 186 individuals (ind.), for *Artemia* sp. 296 ± 36 ind. and for *A. panamensis* 4369 ± 533.

Experimental set-up

Two independent experiments took place in October 2020 and March 2022.

The first experiment (E1) was performed with fertilized pikeperch eggs from INAGRO, Belgium, transported cooled (< 10 °C) and brought to the experimental facilities of the University of Rostock, Germany. Upon arrival, the temperature was slowly raised and at a water temperature of 12 °C the eggs were transferred to an incubator. Within the next 48 h, the water temperature was continuously increased until 14 °C was reached. Three days after the transfer to the incubator, pikeperch larvae hatched. Larvae hatched within 24 h were stocked in 43 L tanks at 16 °C. Pikeperch larvae were maintained in a recirculating aquaculture system (RAS), including water treatment (mechanical and biological filtration as well as UV light treatment) under a light regime of 16L:8D, salinity of 0ppt, constant temperature and oxygen concentration. Two tanks with 43 L contained larvae at a density of 50 individuals per litre. While one tank was fed with *B. plicatilis* according to Ballesteros-Redondo et al.[4], the other tank was fed with *Artemia* sp. (ArtemioPur, JBL GmbH & Co. KG, Germany). Both were fed from 4–10 dph three times per day (09:00, 12:00 and 15:00). Sixteen floating sub-units of 1 L were operated in parallel in two further 43 L tanks in the same recirculation system, arranged in four groups (each 4 replicates). On dph 10 after the last feeding time, larvae were stocked into the sub-units. Eight sub-units contained larvae fed previously with *B. plicatilis* and the other 8 sub-units larvae fed previously with *Artemia* sp. Four sub-units each were stocked with 25 larvae L^{-1}, which were fed with 600 *A. panamensis* ind. $larva^{-1}$ day^{-1}, and the other sub-units with 50 larvae L^{-1} and fed with 300 ind. $larva^{-1}$ day^{-1} from dph 11 until dph 18 (Fig. 1).

For the second experiment (E2) pikeperch larvae were obtained directly from the Pikeperch facility of the Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries in Hohen Wangelin (Mecklenburg-Western Pomerania, Germany). Larvae were transported at 15°C to the experimental facilities of the University of Rostock at an age of 0dph and stocked into the experimental tanks at 16°C. Pikeperch larvae were maintained in the same RAS as during the first experiment. Larvae were stocked at a density of 50 larvae L^{-1} and fed 340 *B. plicatilis* $larva^{-1}$ day^{-1} from dph 4 until dph 10. After last feeding at dph10, again 50 larvae L^{-1} were stocked in 6 floating sub-units. As mortality increased during the first experiment from dph 16, in the second experiment the larvae were fed from dph 11 until dph 16 in two groups, one with *A. panamensis* and the other with *Artemia* sp. at 340 ind. $larva^{-1}$ day^{-1} (Fig. 1). The period from dph 0–10 was analysed in E1 to monitor the effect of the first feeding period on the second period (dph 11–18) and in E2 to have the reference to compare with E1.

Data collection and analyses

The physicochemical water parameters temperature, oxygen, and pH were monitored daily. Water samples were taken daily for subsequent analyses of dissolved nitrogen compounds ammonium (NH_4^+), nitrite (NO_2^-), nitrate

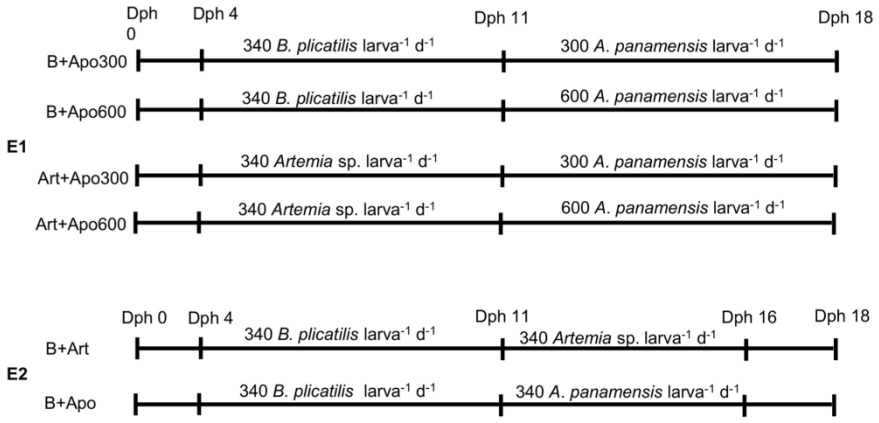

Figure 1. Feeding protocols applied in E1 (above) and E2 (below).

(NO_3^-), orthophosphate (PO_4^{3-}) using an auto-analyser (Gallery Automated Photometric Analyzer Thermo Fisher Scientific, Waltham, MA, USA). In E1, larvae were reared at a temperature of 16.4 ± 0.4 °C, oxygen saturation of 103.8 ± 1.2% and pH = 8.7 ± 0.1, ammonium 0.58 ± 0.02 mg L^{-1}, nitrite 0.07 ± 0.02 mg L^{-1}, nitrate 11.7 ± 1.5 mg L^{-1}, and phosphate 0.47 ± 0.29 mg L^{-1}. In E2, larvae were reared at a temperature of 16.5 ± 0.5 °C, oxygen saturation of 105.7 ± 1.3% and pH = 8.3 ± 0.3, ammonium 0.39 ± 0.07 mg L^{-1}, nitrite 0.35 ± 0.38 mg L^{-1}, nitrate 20.9 ± 1.4 mg L^{-1}, and phosphate 2.70 ± 0.48 mg L^{-1}.

In addition, we performed daily siphoning of the bottom and removal of the dirt, dust and lipid layer at the water surface. Survival rate (E1 N = 4 and E2 N = 3) was calculated from dph 11, when the feeding with *A. panamensis* started, by counting recorded dead larvae from siphoning every day as follows:

$$Survival(\%) = [(N_i - T_D)100]/N_i \quad (1)$$

where N_i is the initial number of larvae and T_D the total number of dead larvae found, cumulated over the experimental days.

All methods were carried out in accordance with German guidelines and regulations.

The experiments with pikeperch larvae were conducted within the German Animal Welfare Act guidelines and were approved by the authorities, in our case the Mecklenburg-Western Pomerania State Office for Agriculture, Food Safety and Fisheries, based in Rostock. The authors complied with the ARRIVE guidelines. According to the animal experiment permit issued (permission number 7221.3–1.1–051/19), in E1, 30 larvae were taken at random at dph 0 and dph 11 in each treatment. At dph 18, 7 larvae from each replicate were taken. In E2, 30 larvae were taken at random at dph 0, 4 and 11. At dph 16, 7 larvae from each replicate were taken. In both experiments, larvae were first cooled down until 12 °C, and subsequently until 0 °C to anesthetize them. Pictures for measurements were taken and larvae were killed by cutting the spinal cord and frozen for fatty acid analyses.

The total body length as well as yolk sac and oil droplet sizes were measured in both experiments under a stereo light microscope (SZX10 Olympus, Hamburg, Germany) connected to a UC30 digital camera (Olympus, Hamburg, Germany) and the software package cellSens Dimension 1.6 (Olympus Soft Imaging Solutions, Hamburg, Germany). Yolk sac volume and oil droplet volume were calculated according to Bischoff et al. (2018). Finally, we calculated the specific growth rate (SGR) [% d^{-1}] (E1 N = 4, E2 N = 3) as follows assuming linear growth[4,28]:

$$SGR = \{[(L_t - L_o)/L_o]/t\}100 \quad (2)$$

where L_t and Lo represent the average length of the larvae at time t and time t = 0.

Fatty acid analyses of zooplankton as well as pikeperch larvae were performed at Greifswald University, in the Laboratory of Animal Ecology. The freeze-dried samples were transferred to extraction tubes, and dichloromethane:methanol (2:1, v:v) and nonadecanoic acid methyl ester as an internal standard was added to the samples. After ultrasonic treatment for > 5 s samples were kept under a nitrogen atmosphere at -25 °C until further analysis, which was done according to Wacker et al.[29]. Fatty acids were transesterified into fatty acid methyl esters (FAMEs) with methanolic HCl (Sigma-Aldrich Chemie, Taufkirchen, Germany)[30,31] and FAMEs were analysed by gas chromatography (6890N, Agilent Technologies, Böblingen, Germany) with helium as carrier gas[32]. For verification, mass spectra were recorded using a gas chromatograph-mass spectrometer (Pegasus 4D GC-TOFMS, LECO Instruments, Mönchengladbach, Germany).

Statistical analyses were performed by using the software IBM SPSS Statistics, Version 27. Normal distribution was tested using the Shapiro–Wilk Test. To analyse differences between means, Analysis of Variance (one-way ANOVA) or t-Test for independent samples was applied when normality was proven. Without normality,

the Kruskal–Wallis or Mann–Whitney U test was applied. All significance levels α were set to 0.05. Data was reported as mean ± s.d.

Results

Live feed fatty acid composition

B. plicatilis had a total fatty acid (FA) content of 56.94 µg mg^{-1} DW consisting of 29.25% saturated fatty acids (SFAs), 48.50% monounsaturated fatty acids (MUFAs) and 22.23% polyunsaturated fatty acids (PUFAs). The SFA palmitic acid (16:0), the MUFA oleic acid (18:1) and the PUFA linoleic acid (LA, 18:2) were the most abundant single FA of each group (Table 1). *Artemia* sp. had the highest total FA concentration of 122.42 µg mg^{-1} DW, 18.00% SFAs, 37.4% MUFAs and 44.50% PUFAs. The SFA 16:0, the MUFA 18:1 and the PUFA linolenic acid (ALA, 18:3) were the most abundant single FA of each group (Table 1). *A. panamensis* had the lowest total FA concentration of 22.28 µg mg^{-1} DW, 40.00% SFAs, 8.90% MUFAs and 50.90% PUFAs. The SFA 16:0, the MUFA 18:1 and the PUFA docosahexaenoic acid (DHA, 22:6) were the most abundant single FA of each group (Table 1).

Pikeperch larvae survival

In E1, the survival rate until dph 16 was above 50% for all diets, for B + Apo300 72.0 ± 6.3%, B + Apo600 82.0 ± 7.7%, Art + Apo300 66.5 ± 6.8%, and Art + Apo600 59.0 ± 19.1%, and decreased until dph 18 to 7.5 ± 5.5% in B + Apo300, to 44.0 ± 3.3% in B + Apo600, to 32.0 ± 11.8% in Art + Apo300 and to 38.0 ± 10.1% in Art + Apo600. There were no significant differences in survival (Kruskal–Wallis $p = 0.224$, N = 4). In E2, the survival rate until dph 16 was 94 ± 2.8% for larvae fed with *Artemia* sp. and 87.9 ± 8.8% for B + Apo. No significant difference was found (t-Test $p = 0.175$, N = 3).

C:D	*B. plicatilis*		*Artemia* sp.		*A. panamensis*	
14:0	**1.87^{a}**	**± 0.10**	**0.32^{b}**	**± 0.08**	**0.32^{b}**	**± 0.17**
15:0	0.12	± 0.04	0.01	± 0.03	0.01	± 0.01
16:0	10.49^{a}	± 0.68	13.15^{a}	± 1.46	5.63^{b}	± 1.00
18:0	2.74^{a}	± 0.36	7.29^{b}	± 1.01	2.52^{a}	± 0.21
20:0	0.06	± 0.10	0.07	± 0.06	0.04	± 0.07
22:0	1.39	± 0.24	1.39	± 0.64	0.45	± 0.20
16:1 sum	9.43	± 0.49	6.53	± 0.64	0.75	± 0.19
18:1 sum	16.50^{a}	± 1.75	37.91^{b}	± 3.18	1.21^{c}	± 0.60
20:1 sum	1.64	± 0.20	1.32	± 0.63	0.01	± 0.02
22:1	0.05	± 0.09	0.00	± 0.00	0.01	± 0.02
18:2 n-6	6.43^{a}	± 0.14	5.83^{a}	± 0.72	0.66^{b}	± 0.23
18:3 n-6	0.15	± 0.17	0.00	± 0.00	0.00	± 0.00
20:2 n-6	0.34	± 0.35	0.00	± 0.00	0.00	± 0.00
20:3 n-6	2.00	± 0.25	0.17	± 0.29	0.01	± 0.02
16:3 n-3	0.72	± 0.09	0.78	± 0.13	0.88	± 0.10
16:4 n-3	0.66^{a}	± 0.16	0.96^{b}	± 0.08	0.45^{a}	± 0.07
18:3 n-3	0.90^{a}	± 0.19	38.04^{b}	± 4.04	0.91^{a}	± 0.43
18:4 n-3	0.02^{a*}	± 0.04	2.55^{b*}	± 0.23	0.22^{ab*}	± 0.10
20:3 n-3	0.00^{a*}	± 0.00	0.40^{b*}	± 0.32	0.00^{a*}	± 0.00
20:4 n-3	0.02	± 0.04	0.23	± 0.22	0.01	± 0.02
20:5 n-3	1.28^{a}	± 0.19	5.48^{b}	± 0.76	1.61^{a}	± 0.30
22:5 n-3	0.15	± 0.26	0.00	± 0.00	0.26	± 0.22
22:6 n-3	0.00^{a}	± 0.00	0.00^{a}	± 0.00	6.33^{b}	± 0.77
SFA	16.66^{a}	± 0.80	22.22^{b}	± 2.21	8.96^{c}	± 1.27
MUFA	27.62^{a}	± 2.06	45.76^{b}	± 3.05	1.98^{c}	± 0.79
n-6	8.91^{a}	± 0.81	6.00^{b}	± 0.71	0.67^{c}	± 0.25
n-3	3.75^{a}	± 0.69	48.45^{b}	± 5.24	10.66^{a}	± 1.78
PUFA	12.66^{a}	± 1.48	54.45^{b}	± 5.94	11.34^{a}	± 2.03
DHA/EPA	0.00^{a}	± 0.00	0.00^{a}	± 0.00	3.97^{b}	± 0.29
Total FA	56.94^{a}	± 1.43	122.42^{b}	± 11.10	22.28^{c}	± 4.00

Table 1. Fatty acids contents of zooplankton in [µg mg^{-1} DW, mean ± s.d. "C" defines the number of carbon atoms and "D" the number of double bonds in the carbon chain. The superscript a, b and c represent significant differences after ANOVA or Kruskal–Wallis test (*) (N = 3). Data reported as mean ± s.d.

Total body length and SGR

In E1, larvae at dph 1 had a yolk sac volume of 2.05 ± 0.75 mm^3 and a length of 4.11 ± 0.28 mm. After the feeding protocol with *B. plicatilis,* the larvae reached a length of 6.18 ± 0.58 mm at dph 11 while after being fed with *Artemia* sp. the larvae were 4.89 ± 0.78 mm long. The SGR in this period was 5.04 ± 1.42% d^{-1} for larvae fed *B. plicatilis* while the larvae fed with *Artemia* sp. reached 1.90 ± 1.89% d^{-1}. At dph 18 larval total body length for B + Apo300 was 7.13 ± 0.71 mm, for B + Apo600 6.87 ± 0.16 mm. Total body length reached 6.67 ± 0.10 mm for Art + Apo300 and 6.74 ± 0.10 mm for Art + Apo600. There was no significant difference in length at the end of the experiment (ANOVA $p = 0.092$, N = 4) (Fig. 2). After the 7 days of exclusive feeding *A. panamensis*, SGR for B + Apo300 was 1.50 ± 0.20% d^{-1}, for B + Apo600 1.12 ± 0.26% d^{-1}, for Art + Apo300 3.64 ± 0.20% d^{-1} and for Art + Apo600 3.79 ± 0.19% d^{-1}. Statistical differences were found between larvae previously fed with *B. plicatilis* and fed with *Artemia* sp. (ANOVA $p < 0.001$, N = 4). The SGR for the complete period (from 0 to 18 dph) was not significantly different among the groups B + Apo300 (4.33 ± 0.17% d^{-1}), B + Apo600 (3.96 ± 0.23% d^{-1}), Art + Apo300 (3.67 ± 0.14% d^{-1}) and Art + Apo600 (3.77 ± 0.14% d^{-1}) (ANOVA $p = 0.092$, N = 4).

In E2, larvae at dph 0 had a yolk sac volume of 0.84 ± 0.20 mm^3 and a length of 5.17 ± 0.44 mm. After the feeding protocol with *B. plicatilis,* the larvae at dph 11 were 5.47 ± 0.35 mm and the SGR in this period was 0.52 ± 1.12% d^{-1}. At dph 16 larval total body length for B + Apo was 6.31 ± 0.11 mm and significantly longer than 5.86 ± 0.24 mm for B + Art (Fig. 2, t-test $p < 0.001$, N = 3). After this 5-day feeding period with *A. panamensis* and *Artemia* sp., SGR for B + Apo was higher compared to the treatment B + Art (2.94 ± 0.4% d^{-1} and 1.32 ± 0.86% d^{-1}, respectively, t-test $p = 0.012$, N = 3). This difference in the SGR was present for the complete period (from 0–16 dph), with 1.42 ± 0.14% d^{-1} for B + Apo and 0.85 ± 0.30% d^{-1} for B + Art (t-test $p = 0.002$, N = 3).

Fatty acids composition

In E1, larvae at dph 0 had a total FA content of 221.9 µg mg^{-1} DW, consisting of 10.54% SFAs, 40.02% MUFAs and 49.44% PUFAs. At dph 11, larvae fed with *B. plicatilis* had a total FA content of 138.6 µg mg^{-1} DW, consisting of 22.72% SFAs, 31.02% MUFAs and 46.25% PUFAs. Larvae fed with *Artemia* sp. (Art) had 190.9 µg mg^{-1} DW, with 17.86% SFAs, 31.48% MUFAs and 50.65% PUFAs. There was a significant difference in PUFAs in the larvae (t-Test $p = 0.017$) (Fig. 3). Both diets resulted in a similar composition of larval SFAs. Regarding MUFAS, larvae showed no significant difference in the content of palmitoleic acid (16:1). However, there was a significant difference in oleic acid content being lower in larvae fed *B. plicatilis* (t-Test $p = 0.016$) (SI- Table 1).

Larvae fed with *B. plicatilis* had also lower concentration of PUFAs (Fig. 3) (t-Test $p = 0.016$). In particular omega-3 (n-3) were lower (t-Test $p = 0.007$), for example ALA (t-Test $p < 0.001$), stearidonic acid (SDA, 18:4n-3) (t-Test $p = 0.005$), eicosatrienoic acid (ETE, 20:3 n-3) (t-Test $p = 0.036$), eicosatetraenoic acid (ETA, 20:4 n-3) (t-Test $p = 0.011$) and eicosapentaenoic acid (EPA, 20:5 n-3) (t-Test $p = 0.041$). Moreover, larvae showed no significant difference in DHA and in LA (Fig. 4), eicosadienoic acid (20:2 n-6), dihomo-gamma-linolenic acid (DGLA, 20:3 n-6) and DHA/EPA ratio. At dph 18, after the diet with *A. panamensis*, the feeding protocol B + Apo led to a total larval FA of 130.0 µg mg^{-1} DW, 21.07% SFAs, 30.92% MUFAs and 48.0% PUFAs. After feeding with Art + Apo, the total FA was 151.0 µg mg^{-1} DW with 18.67% SFAs, 24.11% MUFAs and 57.22% PUFAs (Fig. 3).

In E2, larvae at dph 0 had a total FA of 216.0 µg mg^{-1} DW, consisting of 15.83% SFAs, 27.69% MUFAs and 56.48% PUFAs. At dph 4 before feeding, larvae had a total FA of 273.2 µg mg^{-1} DW, 14.65% SFAs, 23.83% MUFAs and 61.53% PUFAs (Fig. 3). At dph 11, after feeding *B. plicatilis*, larvae had 244.9 µg mg^{-1} DW with 34.58% SFAs, 24.50% MUFAs and 40.90% PUFAs. At dph 16, after feeding protocol B + Art larvae showed a total FA of 183.4 µg mg^{-1} DW with 20.45% SFAs, 29.39% MUFAs and 50.10% PUFAs (Fig. 3). After B + Apo, the total FA was 171.5 µg mg^{-1} DW with 14.11% SFAs, 27.99% MUFAs and 57.90% PUFAs. There were no significant

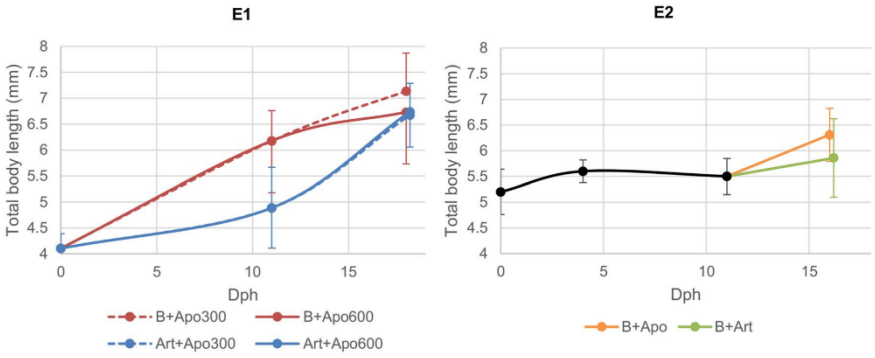

Figure 2. Total body length ± s.d. (mm) at the different days post hatch (dph) in experiment 1 (E1) and in experiment 2 (E2) with the different feeding protocols. Lines until dph 11 represent initial diet and different colour are different live feeds. Bifurcation at dph 11 shows change in diet. From dph 11, different colours are different live feeds and, in E1, continues or discontinuous lines are different quantities of feed.

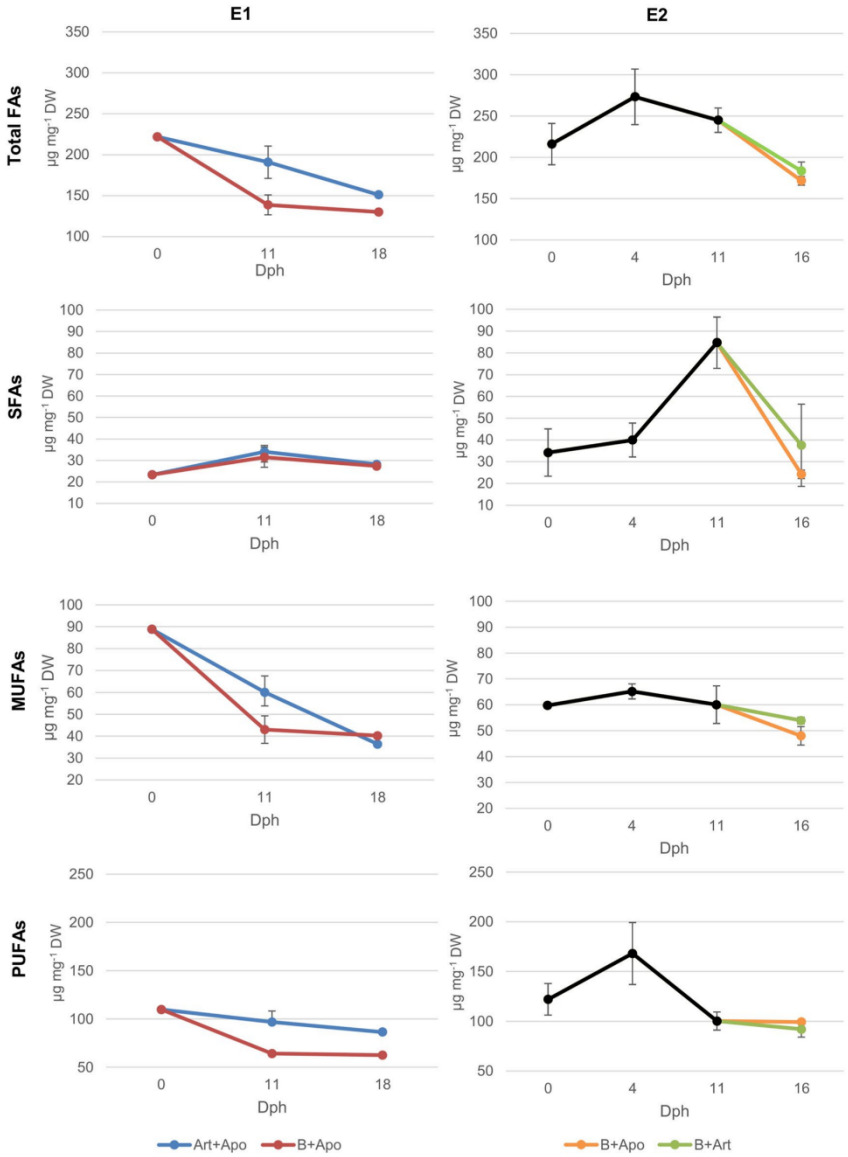

Figure 3. Fatty acids main group's dynamics during both experiments (E1 and E2) under different feeding protocols (mean ± s.d.). For E2, black line until dph 11 is *B. plicatilis* diet. Bifurcation at dph 11 shows the change in diet.

differences in SFAs and in particular C16:0 and stearic acid (18:0). Within the MUFAS, larvae fed B + Art diet showed significantly higher content of 18:1 (Mann–Whitney $p = 0.05$).

Larvae fed with B + Art had a lower concentration of PUFAs particularly in DGLA (Mann–Whitney $p = 0.05$), arachidonic acid (ARA, 20:4 n-6) (Mann–Whitney $p = 0.05$), docosapentaenoic acid (DPA, 22:5n-6) (t-Test $p = 0.007$) and EPA (Mann–Whitney $p = 0.05$) but a higher content of ALA (t-Test $p = 0.034$) (Fig. 4) and SDA

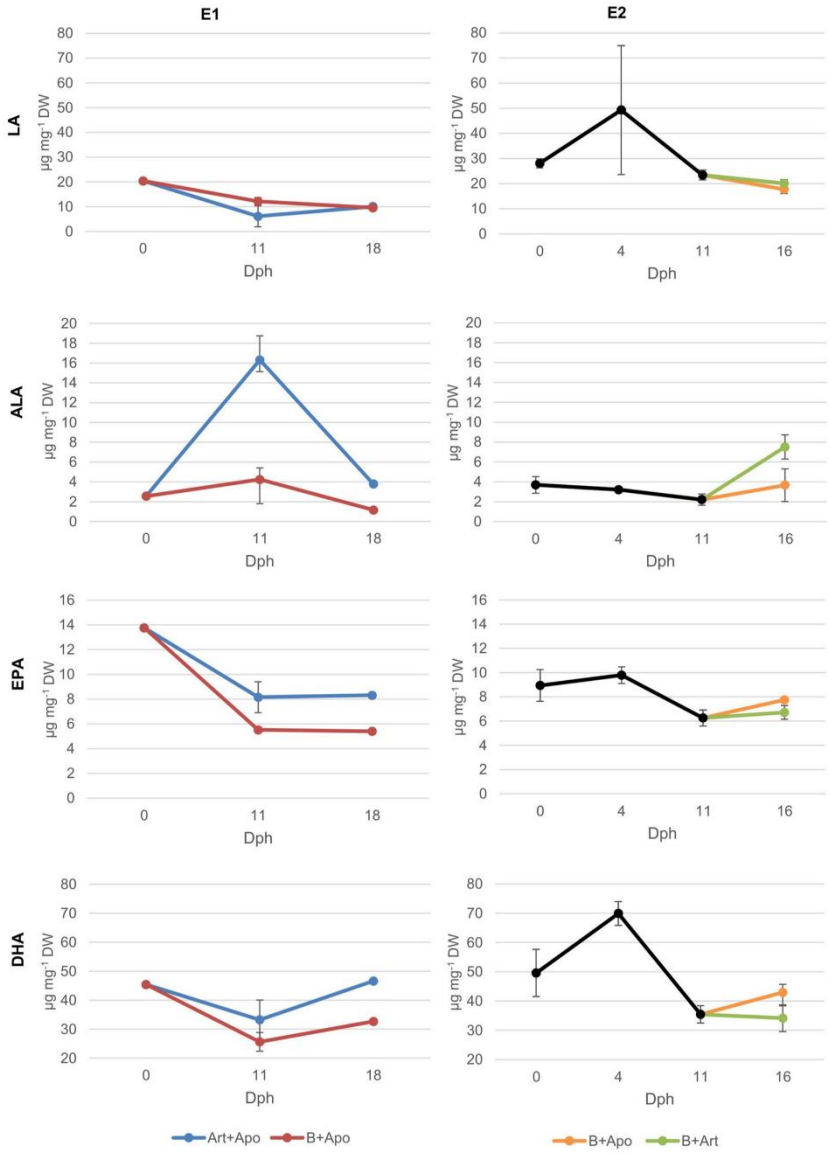

Figure 4. Important PUFAs dynamics during both experiments (E1 and E2) under different feeding protocols (mean ± s.d.). For E2, black line until dph 11 is B. *plicatilis* diet. Bifurcation at dph 11 shows the change in diet.

(t-Test $p = 0.038$). Moreover, larvae showed no significant difference in DHA content and in DHA/EPA ratio (SI- Table 2).

Discussion

Our study showed that *A. panamensis* is suitable as live feed for the rearing pikeperch larvae from dph 11 until dph 16. According to Ballesteros-Redondo et al.[4], *B. plicatilis* was a suitable live feed for pikeperch larvae from dph 4 until dph 10. In the present study (E1), pikeperch larvae fed initially with *B. plicatilis* and followed by *A.*

panamensis (B + Apo300 and B + Apo600) grew more (length of 6.18 mm on dph 11) than larvae fed initially with *Artemia* sp. (Art + Apo300 and Art + Apo600; length of 4.89 mm). Yanes-Roca et al.[2] reported the same result on dph 11. Moreover, larvae fed first with *B. plicatilis* reached a higher SGR (5.04% d^{-1}) from dph 0 to dph 11 compared with *Artemia* sp. (1.9% d^{-1}). These SGRs of both treatments were higher than in Imentai et al.[33] (1.37% d^{-1}) and in experiment 1 of Ballesteros-Redondo et al.[4]. Our SGR of the larvae first fed with *B. plicatilis* and subsequently with *A. panamensis* exceeded the so far highest SGR of 3.0% d^{-1} when feeding solely 340 *B. plicatilis* per larva per day[4]. Consequently, the pikeperch larvae in the present study performed best in comparison to earlier studies and life feed combinations during this early life cycle stage.

With the increasing growth, energy and nutrient requirements of the pikeperch larvae from dph 11 onwards, larvae fed with the copepod *A. panamensis* had the highest survival rates of 72% and 82% until dph 16 (E1) for protocols B + Apo300 and B + Apo600, respectively. However, the mortality drastically increased from dph 16. This might indicate that *A. panamensis* should be best used until dph 16 (for a five-days feeding period). However, the mortality increase might be caused by the start of cannibalism[34]. On dph 18, after feeding *A. panamensis*, the longest size was achieved in protocol B + Apo300 (7.13 mm). Nevertheless, larval length is higher in other studies[2,33]. Difference in breeders, genetics or initial larval quality makes the larval length on dph 18 difficult to compare. Thus, future studies should include more parameters such as initial fatty acid composition and larval weight[28]. Despite this, the SGR from dph 11 until dph 18 was lower for B + Apo300 and B + Apo600 compared with Art + Apo300 and Art + Apo600, which suggests a better larval development despite the initial supply with *Artemia* sp. This result indicated that larvae previously fed with *Artemia* sp. during the first 10 days were able to compensate a slower growth from dph 0 to dph 11 by feeding with *A. panamensis* afterwards. Nevertheless, B + Apo300 had a high SGR of 4.33% d^{-1} (dph 11–18), even higher than SGR data by Ballesteros-Redondo et al.[4] and Imentai et al.[35]. Therefore, *A. panamensis* supplied an adequate level of energy and nutrient supply for the pikeperch larvae and was consequently well suitable to obtain an adequate larval development, independent of the feeding protocol B + Apo300, B + Apo600, Art + Apo300, and Art + Apo600. Nevertheless, our data showed the importance of including the growth data for the different live feeds since similar results with different initial live feeds might make the growth process of the larvae up.

Despite a suitable *B. plicatilis* supply and adequate water quality in E2, the larval growth was low during the first few days. On dph 11, the total body length (5.47 mm) was still similar to the initial length (5.17 mm). The total FA contents until dph 11 almost did not decrease as in normally developing larvae or in starving larvae[1]. The larvae did not consume their FA reserves (fed on *Brachionus*) while the growth and survival rates were still low. However, from dph 11 onwards, the use of *A. panamensis* significantly improved larval performance compared to the use of *Artemia* sp. On dph 16, the survival rate for B + Art was slightly higher (94.0%) than in B + Apo (87.9%), the latter similar to B + Apo600 in E1 (82%). These survival rates were higher than in Imentai et al.[5], who reported survival rates of 35 -68% on dph 16, and Yanes-Roca et al.[2], who reported survival rates of 35–75% on dph 21. However, our calculated survival rates only considered dph 11 onwards to study the effect of *A. panamensis* as a live feed. Therefore, larval survival and growth rates should be reported at the change of live feed organism. On dph 16, the larvae fed with B + Art were smaller compared with B + Apo and thus, the SGR was significantly higher in B + Apo (2.95% d^{-1}) compared with B + Art (1.32% d^{-1}). The SGR for B + Apo was also higher compared with Imentai et al.[5], who fed pikeperch larvae with different combinations of *B. plicatilis* and/or *Artemia* sp. They reported the maximum SGR of 2.41% d^{-1} between dph 11–17 (according to our own calculations). Consequently, our larval growth data demonstrate that *A. panamensis* had a distinctly positive effect on the growth of pikeperch larvae between dph 11 and dph 16 in comparison to *Artemia* sp. as live feed.

The applied *B. plicatilis* had lower total FAs and PUFAs contents than *Artemia* sp. per dry weight (Table 1). Consequently, the pikeperch larvae fed with *B. plicatilis* showed a lower amount in total FAs and PUFA contents than the larvae fed with *Artemia* sp. However, the highest SGR was found for the larvae fed with *B. plicatilis*, which especially contained a higher amount of LA than *Artemia* sp. (Table 1). LA seems to be a highly relevant FA in the diet for pikeperch, as suggested by Ballesteros-Redondo et al.[4], Bischoff and Kubitz et al.[3] and Yanes-Roca et al.[2]. Yanes-Roca et al.[36] stated that pikeperch larvae might have the capacity to desaturate and elongate fatty acids with 18 carbons like LA to obtain DHA during the first 12 days of life. However, Reis et al.[37] demonstrated that pikeperch larvae cannot biosynthesize DHA at dph 20. Recently, Perez and Reis et al.[38] have shown that *B. plicatilis* esterifies C18 PUFAs into phospholipids. An increase in dietary polar lipids increased the growth rate of pikeperch and showed earlier digestive structure development[12]. Phospholipids in the diet might contribute to a better absorption and transport of long-chain fatty acids through enhanced lipoprotein synthesis[40]. This is supported by the fact that total FAs, MUFAs, PUFAs, EPA and DHA contents are lower in larvae fed with *B. plicatilis* which, at the same time, had the highest SGR, demonstrating that all these groups of FAs might have been used for growth and that the LA possibly as polar lipid plays a crucial role during these first days of larval development. Our results showed the importance of including the study of the lipids in form of neutral and polar lipids. Thereby growing larvae use up their larval storages from the yolk sac. With all their PUFA lipid storages, and by growing and increasing their body weight and by producing and accumulating non-lipid biomass, the relative content of PUFA decreases. In contrast, the slower-growing larvae (after feeding *Artemia* sp.) might just use up energy (carbohydrates and SFA) without growing due to a less appropriate diet thus, increasing their proportion of MUFAs and PUFAs. This suggests that *B. plicatilis* might have a suitable fatty acid composition in the adequate form of polar lipids for pikeperch larvae.

From dph 11 to dph 18 (E1), there was a higher decrease in PUFAs in larvae fed with more PUFAs Art + Apo (10.7%) than fed with B + Apo (2.7%). The use of *B. plicatilis* might have improved the absorption of the LC-PUFAs by the larvae. We therefore suggest that the first live feed might affect the future success of the larvae development although this effect was not shown by an improved SGR based on the larval length. This result highlighted the importance of measuring the survival and larval growth when changing live feed organisms. Moreover, including larval weight in future studies might allow us to detect the effect of the first live feed on

the larval growth. There was an increase in DHA for all treatments in E1 after feeding *A. panamensis*. *A. panamensis* is characterised by its higher content of DHA in comparison with *B. plicatilis* and *Artemia* sp. (Table 1). This higher DHA content has already been reported in copepods[41] and in particular for *Apocyclops* species (for *A. royi*[23,42,43] and for *A. panamensis*[24]). Therefore, our data demonstrate that pikeperch larvae could ingest and digest *A. panamensis*, and consequently could utilize the supplied nutrients. This underlined the possibility of rearing pikeperch larvae from dph 11 until dph 18 with this marine copepod species. However, since saltwater copepods do not survive long in freshwater, freshwater copepod species should be studied since they survive longer and might supply suitable nutrient composition to the freshwater fish species[3].

A. panamensis also seemed to fulfill the nutritional requirements of the pikeperch larvae after first feeding on *B. plicatilis* better than feeding *Artemia* sp. In E2, the total FA concentrations, SFAs and MUFAs decreased more in B + Apo between dph 11 and dph 16, coinciding with a higher growth. When fish larvae grow, they require more energy. Both groups of fatty acids are used through the ß-oxidation to obtain adenosine triphosphate (ATP). This suggests that the pikeperch larvae used these groups of FAs as energy for growth. However, PUFAs decreased more in larvae fed with *Artemia* sp., which had a higher content of PUFAs than *A. panamensis* in our study (Table 1). This allows the conclusion that the PUFAs profile of *Artemia* sp. lacks important single fatty acids and that the FAs provided through *A. panamensis* were used. Although the total PUFAs decreased for both protocols, DHA only decreased in larvae fed with *Artemia* sp. as also shown by Yanes-Roca et al.[36]. The pikeperch larvae fed with *A. panamensis* instead increased their DHA content. While some FAs might have been used as energy for growth, in larvae fed with *A. panamensis* DHA accumulated, which is an important component used in fish retina[39]. Consequently, we demonstrate a better pikeperch larval fatty acid composition after feeding with *A. panamensis* compared with *Artemia* sp. Although *Artemia* sp. has more EPA, the larvae fed with B + Art increased their EPA content less than the larvae fed with B + Apo. This shows that the incorporation of these nutrients is more efficient when feeding *A. panamensis*. As mentioned before, phospholipids may enhance lipoprotein synthesis that improves absorption and transport of long-chain fatty acids[40]. Higher content of phospholipids in copepods compared to *Artemia* spp.[41] might explain a better incorporation of EPA by the pikeperch larvae in our study. Consequently, our data demonstrate an improvement in pikeperch larviculture by the use of *A. panamensis* compared to *Artemia* sp.

Our pikeperch larvae kept during the experiments a minimum amount of 120 µg total FAs, 20 µg SFAs, 30 -40 µg MUFAs, 60 µg PUFAs, 4 µg EPA and 20 µg DHA per mg DW, and is definitively higher than those reported in starving larvae[1]. Furthermore, the content of PUFAs in our experiments was higher than those reported by Ballesteros-Redondo et al.[4] and by Bischoff and Kubitz et al.[3], which also reported lower SGR although the initial PUFAs contents were higher. Consequently, our study highlights the importance of the dietary PUFAs in pikeperch rearing, specifically of LA, from dph 4 until dph 11 and of DHA from dph 11.

It must be considered that the better performance of pikeperch larvae with *A. panamensis* occurred during the 5 consecutive days after an initial 10 days *B. plicatilis* feeding. This suggests an adequate timing and availability of both live feed organisms, making larviculture of pikeperch more complex. The high cost of copepod production is another constrain to be considered by the pikeperch hatcheries. Therefore, the economic viability and production efficiency of the combined *Brachionus* sp. and *A. panamensis* use must be further assessed.

Nevertheless, a more favourable dietary fatty acid composition will allow fish larvae to reach higher growth rates and thus allow the larvae to feed earlier and with less effort on bigger prey such as small fish. These other fish as prey items will then perfectly fit the nutritional requirements of the fish.

Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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Author contributions
L.B.R. performed the experiments and prepared the first draft of the manuscript. L.B.R., H.W.P., H.B. and A.A.B. designed the study. H.W.P., H.B. and A.A.B. obtained the funding. L.B.R. and A.W. performed the extraction and identification of the fatty acid data. L.B.R., M.S., T.R. and A.W. analysed the data. All authors further developed and reviewed the manuscript.

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Competing interests
I declare that the authors have no competing interests as defined by Nature Research, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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5. Effect of microalgae diets on population performance and fatty acids composition of *Apocyclops panamensis* (Marsh, 1913) (Cyclopoida, Copepoda)

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Effect of microalgae diets on population performance and fatty acids composition of *Apocyclops panamensis* (Marsh, 1913) (Cyclopoida, Copepoda)

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ABSTRACT

The microalgae effect on population performance and fatty acid composition of *Apocyclops panamensis* was studied in two independent experiments (E), with starting densities of 4,000 (E1) and 8,000 nauplii L^{-1} (E2). In both experiments, copepods were fed once a day for 20 days in 0.4 L triplicates with a single-species diet containing *Isochrysis galbana* at 1 10^5 cells mL^{-1} day^{-1} (ISO), a two-species diet containing *Nannochloropsis* sp. at 1 10^5 cells mL^{-1} day^{-1} and *I. galbana* at 0.5 10^5 cells mL^{-1} day^{-1} (N + I), and a single-species diet containing *Nannochloropsis* sp. at 2 10^5 cells mL^{-1} day^{-1} (NAN). In E1, the highest final total animal density was reached with diet ISO (11,000 ind. L^{-1}) and N + I (14,000 ind. L^{-1}). In E2, the highest final densities were also found with ISO (8,000 ind. L^{-1}) and N + I (6,000 ind. L^{-1}). At day 20, 90 % of the copepods belonged to nauplii and copepodite stages, except for NAN in E2. In E1, the number of eggs per females was higher when the diet included *I. galbana* in both experiments. Thus, copepods fed with diets containing *I. galbana* had the best performance. Exclusive feeding with *Nannochloropsis* sp. yielded copepods with higher omega-6 fatty acids (n-6) percentage, while those fed with *I. galbana* had a higher omega 3 (n-3)/n-6 ratio. The docosahexaenoic acid (DHA) content and the DHA/EPA ratio were higher in copepods fed exclusively *I. galbana*. Our data indicate that *I. galbana* provides a suitable nutritional profile for *A. panamensis* with 0.5–1.0 10^5 *I. galbana* cells mL^{-1} day^{-1}, reaching high densities of 6,000–14,000 copepods L^{-1}, a 1.8–2.6 % of DHA and a DHA/EPA ratio of 1.6–2.9. We conclude that *A. panamensis* has the potential for intensive cultivation and therefore is suitable as live feed for finfish larviculture requiring a fatty acid profile rich diet in omega-3 fatty acids and DHA.

1. Introduction

Due to the widespread use of *Artemia* and rotifers for rearing fish larvae, less attention has been paid to live feed alternatives such as copepods, which might better fulfill the larval nutritional requirements. Reproductive r-strategy in fishes results in small larvae with different quality that might explain high mortality rates. Thus, the best suitable live feed during the early life cycles stages is essential, providing the required nutrients for larvae development.

Several factors must be considered in order to find an accurate live feed organism for the larvae, such as swimming behavior or size (Bruno et al., 2018) and the nutritional value or biochemical composition. Therefore, also copepods are gaining more attention (Ajiboye et al., 2010). They are abundant in nature and an important link in the food web. They seem to fulfill the nutritional requirements of many fish larvae due to their life history traits, biochemical composition and swimming behavior and thus, copepods might be appropriate for commercial aquaculture (Drillet et al., 2011). They are common prey items (Peterka et al., 2003) and have a higher nutritional value than rotifers and *Artemia* because of their high natural amounts of polyunsaturated

Abbreviation: E1, experiment 1; E2, experiment 2; ISO, *Isochrysis galbana*; N + I, *Nannochloropsis* sp. plus *I. galbana*; NAN, *Nannochloropsis* sp.; n-6, omega 6 fatty acids; n-3, omega 3 fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

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fatty acids (PUFAs), free amino acids and antioxidant pigments (Conceição et al., 2010). PUFAs and in particular docosahexaenoic acid (DHA) are essential for fish larvae such as pikeperch (El Kertaoui et al., 2019; Hamza et al., 2008; and Hamza, and Lund et al., 2015, 2018) and most fresh- and saltwater fishes (Tocher, 2010). So far, the use of copepods in fish nutrition is common in ornamental fish production (Hill et al., 2020). In the aquaculture sector, copepods are only occasionally used for seafood. However, first studies have demonstrated the potential of copepods in larviculture. Evjemo et al. (2003) showed a stable high DHA contents in halibut larvae (*Hippoglossus hippoglossus*) fed with copepods in comparison to *Artemia*. A similar pattern was found for winter flounder larvae (*Pseudopleuronectes americanus*) when feeding copepod nauplii in comparison to enriched rotifers (Martínez-Silva et al., 2018). Karlsen et al. (2015) showed that copepods might enhance growth in Atlantic cod (*Gadus morhua*) larvae due to a higher content in protein and taurine than rotifers and *Artemia*.

Although some copepods can biosynthesize PUFAs from yeast or other diets that are low in PUFAs (Nielsen et al., 2019), most of the copepods need a supply of PUFAs from the diet (Rasdi and Qin, 2018). Most of the formulated diets are not adequate for copepods thus, they should be fed with microalgae. Therefore, an adequate microalgae management as copepod diets is necessary. Optimized nutrition for the copepods will later fulfill the nutritional requirements of the fish larvae (Kleppel et al., 2015). PUFAs may be delivered to the fish by feeding the copepods PUFA-rich microalgae, such as the two high-quality algae species we have chosen for our experiments, *Isochrysis galbana* and *Nannochloropsis* sp., which were also previously used successfully for copepods (Rasdi and Qin, 2018) and mussels (Wacker et al., 2002). *Nannochloropsis* sp. is rich in eicosapentaenoic acid (EPA) and has higher omega-3 (n-3) PUFAs during the logarithmic phase (Roncarati et al., 2004). *I. galbana* has high n-3 PUFAs in the logarithmic and stationary phase (Roncarati et al., 2004). Thus, both microalgae species might be used to sufficiently provide PUFAs to the copepods.

Different microalgae can serve as diet for cyclopoid copepods (Drillet et al., 2006; Farhadian et al., 2014; Lee et al., 2006; Nielsen et al., 2019; Rahmati et al., 2020; Rasdi et al., 2018; Van Someren et al., 2020; Velásquez et al., 2001). Rasdi and Qin (2018) showed that copepod culture depends on the microalgae species, which is species-specific and requires studies of the nutritional requirements for each copepod species. A copepod species considered of high potential for intensive culture is *Apocyclops panamensis* (Phelps et al., 2005; Cruz-Rosado et al., 2020). Its fatty acids profile has been studied under fertilized pond conditions (Phelps et al., 2005; Sumiarsa and Phelps, 2007). In both studies, copepods obtained a DHA/EPA ratio approximately between 1.6 and 2.4 and a n-3/n-6 ratio of 10.8–28.2, which clearly indicates that *A. panamensis* has proportionate high n-3 fatty acids, in particular DHA. Although Phelps et al. (2005) obtained a high-density culture by using *I. galbana*, to our knowledge, there is a lack of information about the fatty acid profile of *A. panamensis* under different microalgae diets. Because *I. galbana* and *Nannochloropsis* sp. have high levels of n-3 fatty acids, we hypothesize that both algae have a positive effect on the population dynamics and structure, reproduction, size and fatty acid composition of *Apocyclops panamensis* that subsequently can be used for finfish larviculture.

2. Material and methods

2.1. Apocyclops panamensis experiments

Two independent experiments (E1, E2) were performed. *A. panamensis* was provided by Aquacopa GmbH (Jabel, Germany) and cultured at the facilities of Rostock University in a 200 L zooplankton reactor at 23 °C with a light cycle of 18:6 (L:D) and a salinity of 30 g L^{-1} (super soluble of synthetic sea salt AquaMedic GmbH, Germany added to aqua dest.). They were fed every 4–5 days with 0.5–1.0 × 10^6 cells of *Isochrysis galbana* per mL. Five days after the last feeding, the above-described original culture was filtered several times through a net of 100 μm to remove adults and copepodites, and through a net of 50 μm to isolate the nauplii (size approx. 80 μm). In order to investigate the potential effect of different initial stocking densities on population performance, we collected the nauplii in new saltwater and adjusted their density to 4,000 and 8,000 nauplii L^{-1} in E1 and E2, respectively. In both experiments, nine individual 1 L glass bottles, which were kept open for air exchange, were filled with 0.4 L of saltwater as in Pan et al. (2018) with the above-mentioned nauplii densities. Nauplii were 24 h in the new saltwater without feeding until the experiments started. Copepods were kept under the same conditions than the pre-culture during the experiments, 23 °C, light:dark cycle of 18:6 and at a salinity of 30 g L^{-1}.

The copepods were fed for 20 days in triplicates with the single-species diet containing *I. galbana* at 1 × 10^5 cells mL^{-1} day^{-1} (ISO), a two-species diet containing *Nannochloropsis* sp. at 1 × 10^5 cells mL^{-1} day^{-1} and *I. galbana* at 0.5 × 10^5 cells mL^{-1} day^{-1} (N + I), and a single-species diet containing *Nannochloropsis* sp. at 2 × 10^5 cells mL^{-1} day^{-1} (NAN).

2.2. Microalgae cultures

The microalgae species were selected by their availability at the market and well-studied background on providing long-chain fatty acids. Both species were cultured at Rostock University (see Appendix). Both microalgae culture densities were established by counting microalgae cells in a Fuchs-Rosenthal counting chamber (Marienfeld, Lauda-Königshofen, Germany) under a stereo light microscope (BX 53 Olympus). Microalgae density was determined daily in order to calculate the volume needed to be added into the copepod cultures to obtain the above given food concentrations. Feeding concentration for ISO was based upon Pan et al. (2018) which coincided with Aquacopa feeding recommendation. Since cells of *Nannochloropsis* sp. are smaller in volume and dry weight than *I. galbana* but the total fatty acid per dry weight is similar (Patil et al., 2007), we provided NAN in double density to supply the copepods with similar amounts of total fatty acids. Furthermore, both microalgae provide different amounts of HUFAs (DHA and EPA). As both alga species differed in their contents in DHA and EPA, by mixing both algae in different proportions, allowed to formulate additional diet mixtures with intermediate DHA/EPA ratios. Here, we used a N + I mixture with an intermediate DHA/EPA ratio of 0.3 between both single-species microalgae diets. Microalgae were harvested at the end of their exponential growth phase, and provided to the copepods. The duration of the exponential growth phase was determined in our preceding algae culture experiment (Appendix).

2.3. Data acquisition and calculations

We analyzed the effect of the diets on mortality, total copepod density, density of each life stage, population structure (percentage of nauplii, copepodites, males and females), size per stage, reproduction (number of ovigerous females, eggs per females and ratio male to female (M:F)), and the fatty acids composition of *A. panamensis*. For this purpose, the copepod cultures were homogenized and then samples of 20 mL were taken with a pipette each second day. Samples were fixed with 2 % Lugol. By using a stereo light microscope (SZX10 Olympus, Hamburg, Germany) connected to a UC30 digital camera (Olympus, Hamburg, Germany), individuals (ind.) were counted and classified into nauplii, copepodite, adult male or female, as well as all females carrying eggs (ovigerous females).

2.3.1. Water parameters

Water samples from the copepod cultures were taken for subsequent analysis of dissolved nitrogen compounds ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), orthophosphate (PO_4^{3-}) using an auto-analyzer (Gallery Automated Photometric Analyzer Thermo Fisher Scientific, Waltham, MA, USA) in order to document water conditions.

2.3.2. Mortality

Instantaneous rates of mortality (Z, d^{-1}) were calculated as an average over the entire experiment for the total population, according to Drillet et al. (2008) and taking into account the removed samples.

2.3.3. Density and percentages

Densities of all animals and of each stage were calculated dividing the respective counts by the culture volume. For calculation of percentages of each stage counts of each stage were divided by the total count of all stages and multiplied per 100.

2.3.4. Size

Total body length of nauplii and prosome of copepodites and adults were measured by using the software package cellSens Dimension 1.6 (Olympus Soft Imaging Solutions, Hamburg, Germany). The size of each stage was measured at day 20.

2.3.5. Reproduction parameters

The sex of adults was determined by using the appendages modification of males (males need to grasp females during pairing) as an indicator. Moreover, the females were clearly bigger than the males. On day 20 of both experiments, the number of eggs were recorded in subsamples of 5 ovigerous females per replicate. In E2, one replicate fed with NAN consisted of only 3 ovigerous females. The ratio M:F was expressed as follows: 1:1 means 50 % males and 50 % females, 1:0 means 100 % males and 0:1 means 100 % females.

2.3.6. Fatty acid sampling and analyses

Samples for fatty acids were collected on day 20. Samples were obtained before the daily feeding to avoid direct enrichment. However, copepods were not starved in order to establish their nutritional potential as live feed for fish larvae. Copepod cultures (mixed stages) were carefully mixed and a subsample of 20 mL was filtered through a 50 μm mesh for each replicate. Specific numbers and stages of copepods present in each fatty acid sample can be calculated by using the respective proportions of stages and the final densities. After filtration, copepods were transferred into new saltwater and subsequently filtered on a precombusted Whatman® glass microfiber filters 47 mm diameter. These filters were lyophilized and stored at − 20 °C for further processing.

Fatty acid analyses were carried out at University of Greifswald, Laboratory of Animal Ecology. The samples were freeze-dried, and transferred to extraction tubes prior to analysis. According to Wacker et al. (2016), fatty acid extraction began with addition of 2:1 (v:v) dichloromethane: methanol and nonadecanoic acid methyl ester as an internal standard. The samples were kept at − 20 °C for 24 h. After that, the samples were placed in an ultrasonic bath for 15 s before the extract was transferred into a fresh tube. New dichloromethane:methanol solution was added to the sample tube, placed in the ultrasonic bath, and the extract was then again transferred to the respective tube. The collected extracts per sample were evaporated under a stream of nitrogen at 40 °C.

To transesterify fatty acids into fatty acid methyl esters (FAMEs), the dried sample was resuspended in 4 mL of 3 mol L^{-1} methanolic HCl (Sigma-Aldrich Chemie, Taufkirchen, Germany) and incubated under nitrogen atmosphere in a sealed vial at 60 °C for 20 min. FAMEs were extracted from the methanolic HCl solution once the sample had cooled down: Therefore, 3 mL isohexane was added to the tubes with the methanolic HCl, vortexed for 5 s, and the two phases were allowed to settle; the vortex step was repeated twice, always allowing the phases to settle in-between. This isohexane-extraction step was done 3-times, and the respective isohexane phases were collected in a new vial. After being evaporated to dryness under N_2 at 40 °C the fraction was resuspended in a volume of 100 mL isohexane, and transferred to a micro inlay glass vessel. The previous glass vessel was washed twice with new isohexane, and added to the solvent in the small glass vessel. In the end the volume was adjusted to 100 μL. Gas chromatography (6890N, Agilent Technologies, Böblingen, Germany) was used to analyze the FAMEs, with helium as the carrier gas, according to Wacker and Weithoff (2009). FAMEs were separated using a 50 % cyanopropyl-phenyl methyl--polysiloxane column (Agilent Technologies J&W DB-225, 30 m × 0.25 mm × 0.25 m) with a temperature gradient of 60 °C for 1 min, 20 °C min^{-1} until 150 °C, 10 °C min^{-1} until 220 °C, and then maintained for 13.75 min. FAME were measured with flame ionization detection (FID) at 250 °C using multipoint standard calibration curves (software: HP chemstation (Agilent Technologies, Böblingen, Germany). For confirmation, a gas chromatograph-mass spectrometer (Pegasus 4D GC-TOFMS, LECO Instruments, Mönchengladbach, Germany) was used to record mass spectra. Finally, fatty acids percentages were calculated over the sum of all fatty acids detected and quantified in each sample.

2.4. Statistics

All statistical analyses were performed applying the software IBM SPSS Statistics, Version 27. Normal distribution was checked by means of the Shapiro-Wilk Test. Data of both experiments were analyzed separately to detect potential differences among water parameters and densities, mortality, percentages, size, reproduction parameters and fatty acids of animals on day 20. Percentage data were transformed using the arcsin square root. One-way Analysis of Variance (ANOVA) followed by Tukey posthoc test was conducted if assumptions of normality were met, otherwise a Kruskal-Wallis test was performed. All significance levels α were set to 0.05. Closer statistical details are reported in Table A5 in the Supplementary material file. Data was reported as mean ± s.d.

3. Results

3.1. Water parameters

In E1, copepods were reared at concentrations of ammonium 2.3 ± 1.2 mg L^{-1}, nitrite 0.16 ± 0.2 mg L^{-1}, nitrate 11.2 ± 9.2 mg L^{-1}, and phosphate 1.2 ± 1.0 mg L^{-1} (Table A2). Significant differences were only found in nitrite between the diets (Kruskal-Wallis $p < 0.01$) on day 20.

In E2, copepods were reared at concentrations of ammonium 0.3 ± 0.2 mg L^{-1}, nitrite 1.70 ± 3.70 mg L^{-1}, nitrate 5.9 ± 2.7 mg L^{-1}, and phosphate 0.5 ± 0.9 mg L^{-1} (Table A2). Significant differences were found in nitrate (Kruskal-Wallis $p = 0.014$ $n = 3$) and nitrite (Kruskal-Wallis $p < 0.01$).

3.2. Mortality

In E1, the mortality among the different diet treatments (ISO diet: 0.195 ± 0.054 d^{-1}, N + I diet: 0.146 ± 0.039 d^{-1}, and NAN diet: 0.208 ± 0.065 d^{-1}) was not significantly different ($p = 0.725$, Kruskal-Wallis). In contrast, in E2, mean mortality rates differed significantly between animals on the ISO diet (0.188 ± 0.045 d^{-1}) and the NAN diet (0.280 ± 0.038 d^{-1}) ($p = 0.016$, Kruskal-Wallis pairwise comparison), while there was no difference to the N + I diet (0.219 ± 0.044 d^{-1}) (Table A5).

3.3. Population dynamics and structure

In E1, there was a decrease in total density of nauplii down to approximately 1,000 nauplii L^{-1} during the first 2 days. Afterwards the density stayed until day 6 when numbers of copepodites started to increase. On day 8, first adult males and females were found, and after day 12 there was a rapid increase in the densities of total numbers and nauplii, in particular. At the end of the experiment, the total density reached 11,000 copepods L^{-1} for the ISO diet, 14,000 for N + I diet and 8,000 for the NAN diet (Fig. 1). No significant differences were found between the three different microalgae diets for any density variable (Table A4).

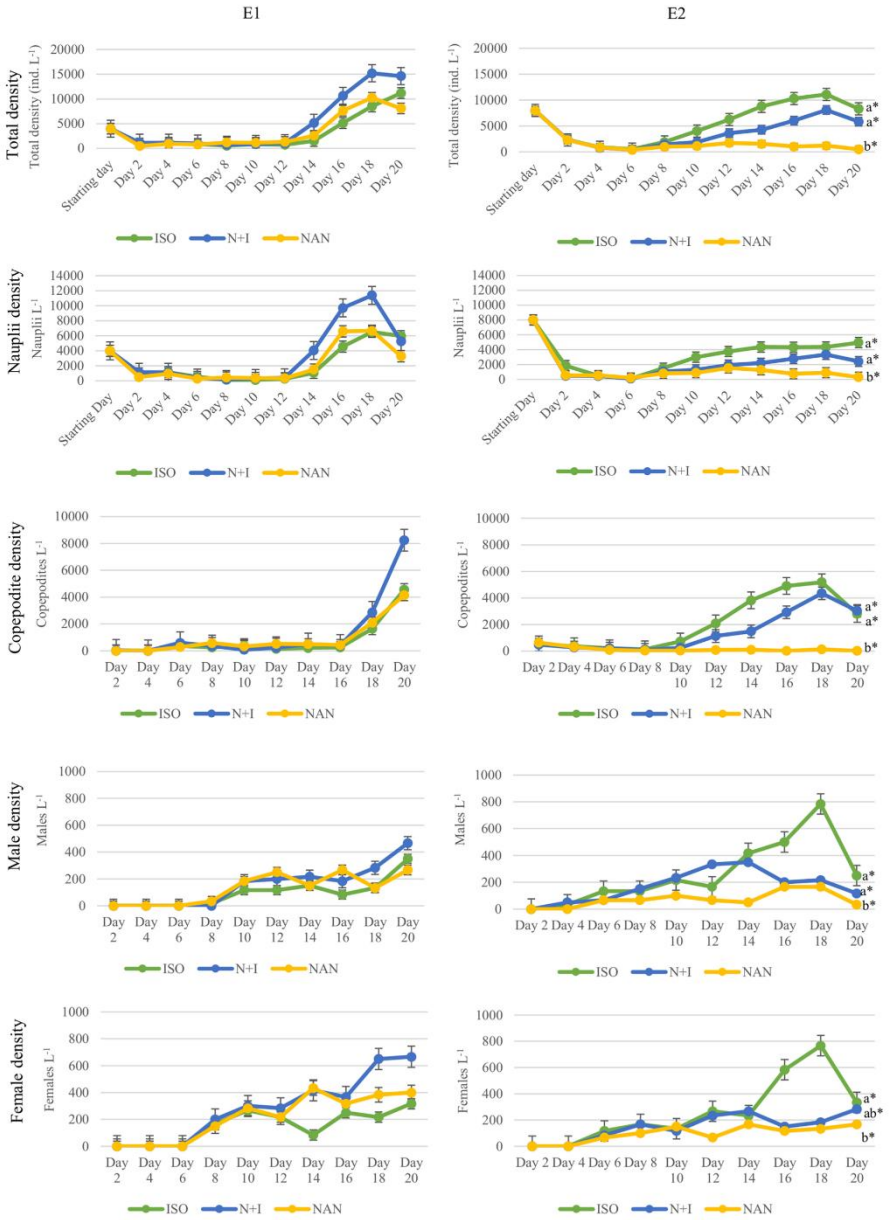

Fig. 1. Population dynamics during E1 (left) and E2 (right) under each feeding regime. From top to bottom: total density, nauplii density, copepodite density, male density and female density (N = 3). Points represent the mean at each sampling day and bars are the standard deviation. Different letters (a, b) indicate significant differences on day 20. Symbol * indicates that Kruskall-Wallis test was applied.

In E2, we observed a decrease in total density down to approximately 1,000 nauplii L^{-1} during the first 4 days. Like in E1 the density stayed, and increased on day 6 when number of adults increased. On days 8 and 10, respectively, nauplii and copepodite densities increased. The latter was not the case for copepodites on the NAN diet. Only the ISO diet led to a peak in the density of adults on day 18. Final total density was 8,000 ind. L^{-1} for the ISO, 6,000 for N + I and 1,000 for NAN diets (Fig. 1).

The densities of total numbers, nauplii, copepodites between the

three microalgae diets differed significantly (for total density, nauplii, copepodite and male density: Kruskal-Wallis $p < 0.01$, and for females density Kruskal-Wallis, $p = 0.013$).

In E1, the percentages of each stage on day 10 and 20 developed differently (Fig. 2). On day 10, the proportion of nauplii in the population fed with ISO was lower (15.1 %) than in the other treatments (N + I 30.8 % and NAN 44.3 %) whilst the proportion of copepodites was higher in ISO diets (45.2 %) than in N + I (6.8 %) and NAN diets (20.4 %). On day 20, the proportions of nauplii reached 33.8–53 %, of copepodites 41–58.5 %, of males 3.1–3.5 % and of females 2.8–4.9 %. On day 20 of the experiment significant differences among feeding trials were found for nauplii (ANOVA $p = 0.038$) and for copepodites (ANOVA $p = 0.029$).

In E2, on day 10, the proportion of nauplii was approx. 70 % in all the treatments while the copepodites proportion fed with ISO was higher (17.7 %) than in N + I (13.4 %) and in NAN (2.9 %). On day 20, the proportion of nauplii was similar between diets ISO (59.1 %) and NAN (56.3 %) but lower in diet N + I (41.1 %). The percentage of copepodites was higher in the diets ISO (33.7 %) and N + I (51.9 %) than in NAN (5.6 %) (Fig. 2). There were higher percentages of nauplii but significant lower percentages of copepodites in the NAN diet compared to the other diets on day 20 ((Kruskal-Wallis $p < 0.001$) and also differences in female density (ANOVA $p = 0.031$).

3.4. Size

In both experiments, we found differences in animal size assessed on day 20. In E1, the size of nauplii averaged across all treatments was 158.7 ± 7.9 μm. Copepodites measured 372.4 ± 12.5 μm, males 637.2 ± 14.6 μm and females 676 ± 16.4 μm. However, copepodites in the N + I diet were significant smaller (ANOVA $p = 0.047$) (Table A5).

In E2, the mean size of nauplii across all diet treatments was 143.4 ± 10.4 μm; copepodites measured 367.7 ± 23.9 μm, males 628.9 ± 33.4 μm and females 707.4 ± 23.3 μm. Females fed ISO were significantly larger than females from the other two dietary treatments (ANOVA $p < 0.001$). Males length was significant different among diets (ANOVA $p = 0.003$) (Table A3).

3.5. Reproduction parameters

In E1 the first ovigerous female was found on day 6 in treatment ISO, day 8 in N + I and day 12 in NAN. The number of ovigerous females depending on the diet were not significantly different (Kruskal-Wallis $p = 0.565$). The number of eggs per female was 18.0 ± 2.8 for ISO, 24.0 ± 12.7 for N + I and 19.6 ± 4.3 for NAN without significant differences (ANOVA $p = 0.75$), due to some females lack one egg sac. The ratio M:F was 0.5:1. for all the diets and showed no significant differences among the different diet treatments (Kruskal-Wallis $p = 0.672$).

In E2, the first ovigerous female was found on day 4 in treatment ISO and day 6 in N + I and in NAN. Significant differences were found in number of ovigerous females being higher in diet ISO compared with the other diets (Kruskal-Wallis. $p < 0.01$). There were also significant differences in number of eggs per female (18.3 ± 4.4 in ISO, 16.7 ± 3.7 with N + I and 14.7 ± 3.5 in NAN) (Kruskal-Wallis $p = 0.016$) and ratio M:F (1:1 for ISO and N + I and 0.7:1 for NAN) (Kruskal-Wallis $p = 0.016$) (Table A5).

3.6. Fatty acid composition

During E1, fatty acid profiles showed that the saturated fatty acids (SFAs) were higher in copepods fed with ISO (27.1 %) compared with the other two diets (Fig. 3, Table A4). Monounsaturated fatty acids

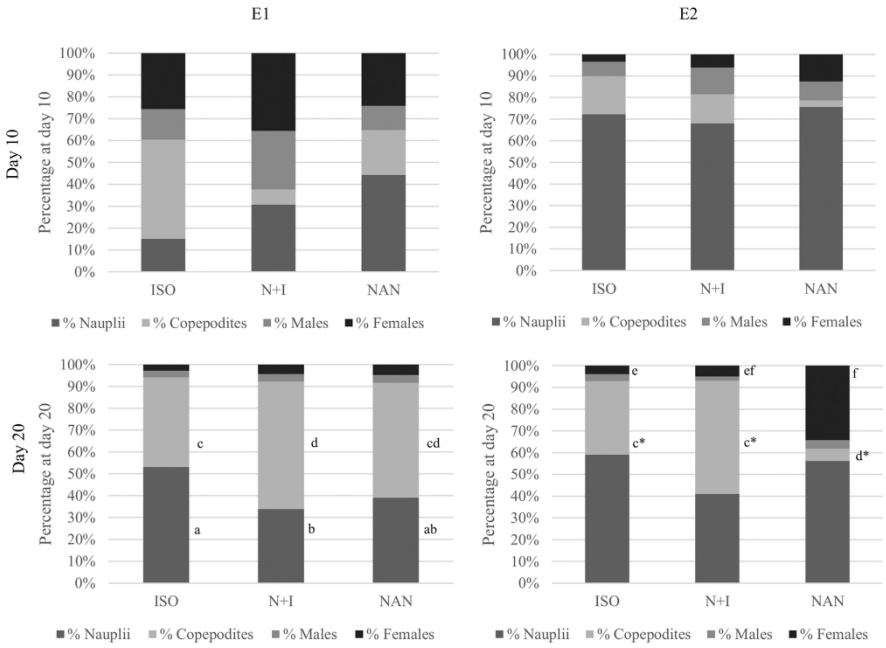

Fig. 2. Population structure of *Apocyclops panamensis* in percentage of each copepod stage on day 10 (top) and 20 (bottom) in E1 (left) and E2 (right). Different letters indicate significant for nauplii percentage (a, B), for copepodite percentage (c, d) and for female percentage (e, f). Symbol * indicates that Kruskall-Wallis test was applied.

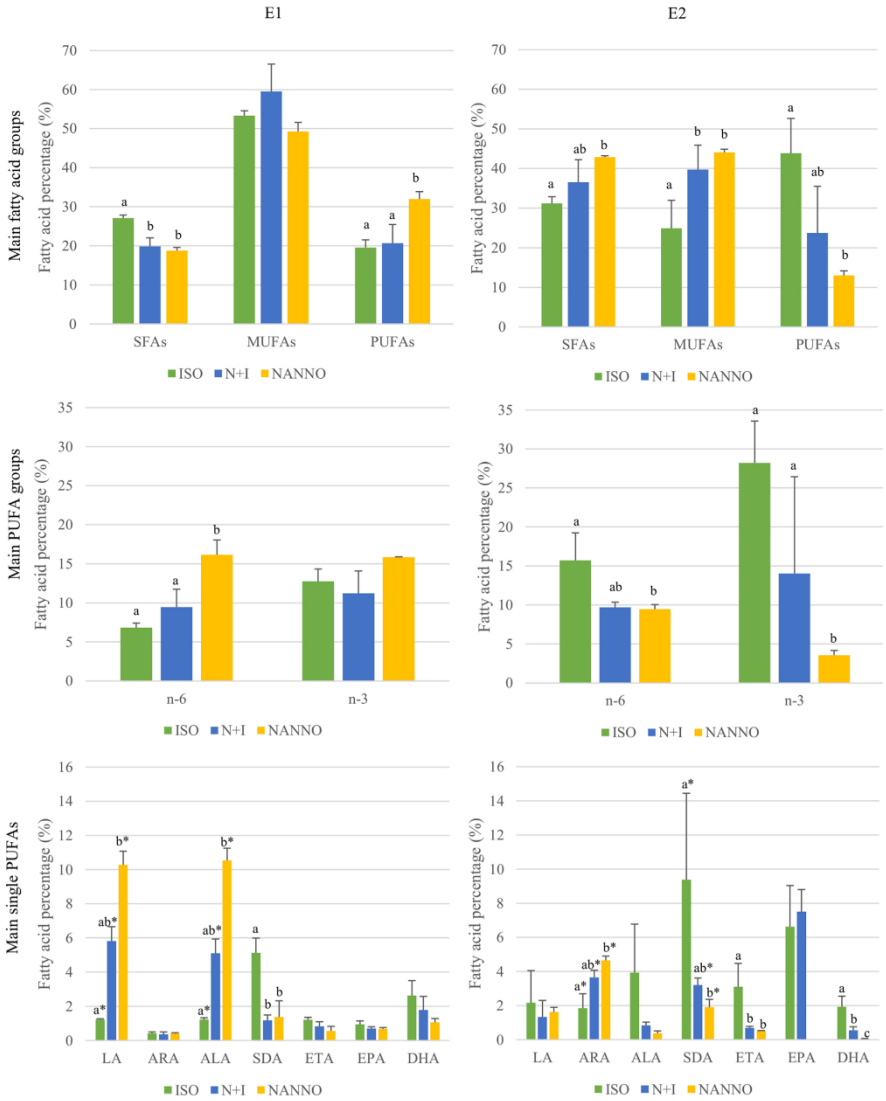

Fig. 3. Fatty acid composition of *Apocyclops panamensis*. From top to bottom: main fatty acid groups, main PUFA groups and main single PUFAs in percentage of the total fatty acid content of the copepods at the end of the experiments 1 (left) and 2 (right) after all the different diet regimes. Significant differences between diets are represented by different letters a, b and c. Symbol * indicates the application of Kruskal-Wallis test.

(MUFAs) were the most abundant FA (48.8–59.2 %), in particular oleic acid (C18:1) with the highest percentage (42.2–53.4 %) (Fig. 3). Polyunsaturated fatty acids (PUFAs) were lower in copepods fed with ISO (19.6 %). However, copepods fed with ISO had the highest n-3/n-6 ratio followed by N + I, and finally, NAN (Fig. 3). Within the omega-6 (n-6) PUFAs, linoleic acid (LA, C18:2 n-6) was the most abundant FA in copepods fed with N + I and NAN. Within the n-3 PUFAs, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) percentages were higher with diet ISO compared with N + I and NAN. Stearidonic acid (SDA, C18:4 n-3) was the most abundant PUFA in copepods fed with ISO (Fig. 3).

In E2, the fatty acid profile showed that the SFAs were lower in copepods fed with ISO compared with N + I and both lower than NAN. The same pattern was found for MUFAs (Fig. 3). PUFAs were the most abundant FA for ISO (41.7 %) and higher compared with the other two diets (Fig. 3). Moreover, copepods fed with ISO had the highest n-3/n-6 ratio followed by N + I (Fig. 3). Within the n-6 PUFAs, arachidonic acid (ARA, C20:4 n-6) was the most abundant FA in copepods fed with NAN.

Within the n-3 PUFAs, DHA was the highest in copepods fed with ISO and EPA in copepods fed with N + I. SDA was the most abundant PUFA in copepods fed with ISO (Fig. 3).

4. Discussion

4.1. Potential influence of water parameters

Apocyclops panamensis was kept at a water temperature of 23 °C, salinity of 30 ‰ and chemical parameters of ammonium, nitrite, nitrate and phosphate between 0.3–2.3, 0.16–1.7, 5.9–11.2 and 0.5–1.2 mg L^{-1}, respectively. These water conditions maintained our copepod cultures. To our knowledge, the water chemical composition for an optimal culture of *A. panamensis* has not been established so far. Cruz-Rosado et al. (2020) reported a better population growth rearing these copepods at a water temperature of 32 °C and a salinity of 28 ‰ than at 24 °C. However, the total final densities were higher at 23 °C and a salinity of 30 ‰ in the present study. In E1, ammonium (2.3 ± 1.2 mg L^{-1}), nitrate (11.2 ± 9.2 mg L^{-1}) and phosphate (1.2 ± 1.0 mg L^{-1}) were higher than in E2 even though, population performed better (see Section 4.2). In E2, highest nitrite concentrations were observed (1.70 ± 3.70 mg L^{-1}). Jepsen et al. (2015) found that *Acartia tonsa* adults performed well under around 8.4 mg L^{-1} of ammonium and 0.3 mg L^{-1} of ammonia at a pH of 8. However, *Acartia tonsa* nauplii mortality increased at an ammonium level of 1.8 mg L^{-1} and ammonia 0.81 mg L^{-1} at a pH of 8 (Jepsen et al., 2015). Our data showed ammonium values that are in the range for an adequate performance. However, ammonia depends on salinity, temperature and pH and thus, pH must be measured in copepod cultures.

Consequently, although our results demonstrate that the slightly variable water parameters and chemical concentrations between the experiments did not negatively affect the utilized *A. panamensis* culture, future studies should include the monitoring of all these parameters.

4.2. Effect of microalgae diets on population performance of A. panamensis

A high initial nauplii mortality was found in both experiments. In E1, the initial total density was 4,000 ind. (nauplii) L^{-1}, which decreased until 1,000 nauplii L^{-1} during the first 2 days. This mortality might be caused by the filtration of the original culture damaging the nauplii. For E2, the initial total density was 8,000 ind. (nauplii) L^{-1}, which decreased until 1,000 nauplii L^{-1} during the first 2 days. This higher initial mortality in E2 compared to E1 could not be explained only by the filtration because one would have expected a proportional effect to the initial densities. Additionally, nauplii are reported to be resistant against filtration (Nilsson et al., 2018) so that the higher mortality might have arisen from a negative effect of the lower feed ration per initial nauplii in E2. Since the *A. panamensis* cultures contained microalgae and were illuminated, photosynthesis should have taken place and the lack of oxygen could not have caused this mortality. However, in future experiments a monitoring of this parameter is recommended. Nevertheless, instantaneous mortality rate (Z) was not significant different between experiments showing no effect of the initial stocking density (data no included). Mortalities found in our experiments (0.146–0.280 d^{-1}) were higher than reported in Drillet et al. (2006) for *Acartia tonsa* (0.035–0.130 d^{-1}). Consequently, future studies should research specifically if there is an effect of stocking density and/or quantity of feed in the development of *A. panamensis* nauplii. Furthermore, highest mortality rates were found for NAN in E1 (0.208 d^{-1}) and E2 (0.280 d^{-1}) although only significant differences were observed in E2. Nevertheless, our data indicated a negative effect of the exclusive feeding with *Nannochloropsis* sp.

After the first decline, the populations developed adequately in both experiments. In E1 a peak in total density was found on day 12, corresponding to the nauplii density increase and thus, indicating the start of the reproduction. At the end of the experiment, the treatments with ISO and N + I showed the highest total density between 11,000 and 14,000 ind. L^{-1} while the lowest total density was recorded for NAN (8,000 ind. L^{-1}). In E2 the peak in total density appeared on day 6, corresponding to the nauplii density increase. At the end of the experiment, the treatments ISO and N + I showed also the highest total density between 6,000 and 8,000 ind. L^{-1} while the final total density for NAN was < 1,000 ind. L^{-1}. Except for NAN in E2, our results exceed the density numbers reported in other studies like Cruz-Rosado et al. (2020) who used *Tetraselmis chuii* (2×10^4 cells mL^{-1}) and obtained a maximum of 1, 200 ind. L^{-1} at a temperature of 32 °C and a salinity of 28 ‰. Our densities were also higher than recorded in Phelps et al. (2005) who obtained a maximum copepod density of 5000 ind. L^{-1} when using *I. galbana* at 5×10^5 cells mL^{-1}. Moreover, other studies such as Guenther et al. (2015), Lee et al. (2006) and Ruiz-Guzmán et al. (2012) have already shown high densities when using *I. galbana* for other cyclopoid species. Consequently, our data indicate an adequate population development. Nevertheless, regarding initial conditions, total final densities were higher when starting with 4,000 nauplii L^{-1} that seems to be more adequate.

In our study, the two microalgae species influenced the development of the *A. panamensis* population. In both experiments total densities where higher when the copepods fed on *I. galbana* instead of exclusive *Nannochloropsis* sp. This is in accordance with Farhadian et al. (2008), who reported higher densities of *Apocyclops dengizicus* when feeding them with *I. galbana* compared with *Nannochloropsis* sp. In E1, the copepodites percentage on day 10 was higher with the diet ISO and the proportion of nauplii higher in NAN. This result indicates a better development of the nauplii into copepodites when feeding ISO. Higher nauplii densities indicate a delay or failure in naupliar development (Pan et al., 2018). As Rasdi and Qin (2018) reviewed, feed quality (microalgae species) influences the naupliar development. Consequently, our data show that the fed microalgae have influenced the duration of the nauplii and copepodite stages as observed in Amarasinghe et al. (1997).

Furthermore, on day 20 in both of our experiments, significantly more copepodites were found in N + I followed by ISO. Therefore, diets including *I. galbana* showed a better development with a percentage of nauplii and copepodites exceeding 85 % being an adequate population composition to be useful as live feed for larviculture and likewise to continue the copepod culture (Pan et al., 2018).

Our *A. panamensis* population reached a mean size of 143.4–158.7 μm for the nauplii, 367.7–372.4 μm for the copepodites and 637.2–707.4 μm for the adults. No comparable literature was found for *A. panamensis*. Nevertheless, our size data are within the range of a closely related species, *Apocyclops royi* (Lindberg, 1940) (Jepsen et al., 2021). The authors reported a size range of 78–250 μm for nauplii, and of 260–997 μm for copepodites and adults. Consequently, nauplii of *A. panamensis* might be used as earlier suggested to feed small-mouthed larvae species as a first live feed by Planas and Cunha (1999) and copepodites for later larval stages by Jepsen et al. (2021).

Feed quality influenced also the reproduction parameters. The first ovigerous female appeared under the diet ISO in both experiments, indicating better copepod development and thus, related with the high final density. The number of eggs per female found was always higher when the diets contained *I. galbana*. This result indicates that the feed quality can influence the production of eggs by the females as observed in Kleppel et al. (2015). Although a ratio M:F of 1:1 is known to be adequate (Drillet et al., 2011), our results in E1 (M:F 0.5:1) are comparable to those found in Farhadian et al. (2014). Consequently, our data showed better reproduction parameters when *A. panamensis* was fed with *I. galbana*.

4.3. Effect of microalgae diets on fatty acid composition of A. panamensis

The fatty acid composition of the two studied microalgae resembles the pattern reported in the same species and also closely related species

by Roncarati et al. (2004) and Wacker et al. (2002). The authors reported a higher amount of oleic acid, linoleic acid, DHA and a higher DHA:EPA ratio in *I. galbana*, compared with a higher amount of palmitoleic acid and EPA in *Nannochloropsis* sp. (Appendix). Consequently, based on the given algae as feed, different suits of fatty acids were transferred to the *A. panamensis*.

In general, the fatty acid composition of *A. panamensis* showed no clear pattern for SFAs and MUFAs with regard to the different microalgae diets. This suggests that they were not directly affected by the different dietary fatty acid group composition. In E1, total MUFAs were higher than the SFAs while in E2, the MUFAs were lower than the SFAs. The best population performance coincided with higher content of MUFAs compared with SFAs. We could not find any literature that studied the fatty acid composition of *A. panamensis* after feeding with microalgae. Nevertheless, Sumiarsa and Phelps (2007) showed higher SFAs than MUFAs in *A. panamensis* when feeding them with fertilizers in ponds. Pan et al. (2018) and Nielsen et al. (2019) reported SFAs and MUFAs data for *A. royi* under different microalgae diets. However, the authors found no pattern in relation with different microalgae diets.

On the other hand, our study showed that PUFAs in the microalgae diet determined the PUFAs in *A. panamensis*. Within PUFAs, higher n-3/n-6 ratio in the microalgae lead to an improved n-3/n-6 ratio in the copepods. In E1, PUFAs were lower in copepods fed with ISO and N + I. Nevertheless, copepods fed with ISO had the highest n-3/n-6 ratio (2.20). In E2, copepods with highest n-3/n-6 ratio were fed with ISO (1.98) and N + I (1.91), which coincided with the highest final densities. In both experiments, the highest n-3/n-6 ratio in copepods was observed with the diet ISO, which contained a higher n-3/n-6 ratio (4) than *Nannochloropsis* sp. (3.6). For *A. royi*, the same pattern was observed when feeding the copepods with *I. galbana* vs. *Nannochloropsis oculata* (Pan et al., 2018). Consequently, our data demonstrate that the PUFAs in the microalga diet determined the PUFAs in *A. panamensis*.

In E1, the high PUFAs content in the copepods fed with NAN corresponded to the higher content of LA and ALA than in copepods fed with ISO and N + I, although *I. galbana* had a higher percentage of LA and ALA than *Nannochloropsis* sp. (Appendix). Our copepod samples for the fatty acid analysis contained different proportion of the different life stages. As mentioned previously on day 20 of E1, copepodites percentage was significantly higher in N + I. Amparyup et al. (2022) reported for *A. royi* higher LA and ALA in copepodites and adults than in nauplii. Consequently, mix of stages in our samples cannot explain our fatty acid profile for NAN in E1. In E2, PUFAs were higher in copepods fed ISO followed by N + I and NAN. In E2, copepods fed with NAN had the highest ARA content coinciding with the worst performance. Considering the fatty acid profile of *Nannochloropsis* sp. with a higher content of ARA, we can conclude that during E1 the copepod culture did not exclusively feed on *Nannochloropsis* sp.

The single PUFA content in *A. panamensis* also depended on the single PUFAs composition of the microalgae diet. *I. galbana* had a higher content in SDA and eicosatetraenoic acid (ETA, C20:4 n-3) than *Nannochloropsis* sp. Consequently, copepods fed ISO showed the highest SDA and ETA, followed by N + I and NAN in both experiments. The same pattern applied for DHA. Its content was higher in copepods fed with ISO being 2.6 % in E1 and 1.9 % in E2. Moreover, in E2, DHA content in copepods fed with diet ISO was similar to N + I (1.8 %) in E1, which had the same amount of *I. galbana* per copepod at the beginning of the experiment. DHA content in *A. royi* was the highest for the adults followed by the copepodites and finally, the nauplii (Amparyup et al., 2022). However, ISO in E1 reached the highest DHA content, which had the highest proportion of nauplii and thus, evidencing that the fatty acid composition did not show the stages composition of the sample. Nevertheless, although *A. royi* is a close-related species, fatty acids for each stages of *A. panamensis* should be studied.

Our data is lower than DHA levels found for *A. royi* in Jepsen et al. (2021), Nielsen et al. (2019) and Pan et al. (2018). Nevertheless, as seen previously, population dynamics and reproduction parameters indicated an appropriate development. Since DHA is associated with the fecundity of copepods (Kleppel et al., 2015), DHA levels in our copepods were adequate. Moreover, Tocher (2010) suggested that live feed for fish larvae should contain a minimum level of 1–2.6 % DHA. Consequently, our data demonstrate that *A. panamensis* has the potential to fulfill the DHA requirements of fish larvae.

In E1, the DHA/EPA ratio (1.6–2.9) was similar to those found in Phelps et al. (2005) and Sumiarsa and Phelps (2007) (1.6–2.4) under cultivation of *A. panamensis* in fertilized ponds, and reached densities of 1,000–5,000 nauplii L^{-1}, also similar to the final nauplii densities in E1 (3,300–5,250 nauplii L^{-1}). Moreover, the higher DHA/EPA ratio was found when feeding *A. panamensis* with *I. galbana* (2.5–2.9). This result was higher than the DHA/EPA ratio reported in species like *Acartia tonsa* (1.35) and *Tisbe holothuridae* (1.63) (Drillet et al., 2006). Nevertheless, in E2, this ratio was not reached since EPA was higher than DHA for all treatments. A DHA/EPA ratio of 0.3–2.0 is essential for fish larvae (Tocher, 2010). Therefore, *A. panamensis* might fill the nutritional requirements of fish larvae.

Moreover, in E1, copepods fed with ISO showed a content of EPA+DHA of 3.5 %. El Kertaoui et al. (2019) reported that 3.5 % of EPA+DHA was the best dietary composition, together with the Ca/P ratio, for pikeperch larvae. Consequently, the fatty acid profile of *A. panamensis* also seems to fulfill the nutritional requirements of pikeperch larvae.

In summary, our study showed that *A. panamensis* populations might be optimally maintained when starting with 4,000 nauplii L^{-1} and feeding 20 days on *I. galbana* (0.5–1 × 10^5 cells mL^{-1} day^{-1}), resulting in densities of 11,000–14,000 ind. L^{-1}, an adequate percentage between 33.8 % and 59.1 % of nauplii and 33.7–58.5% of copepodites and a suitable fatty acid profile with DHA/EPA ratios of 2.5–2.9, and DHA levels of 1.8–2.6 % and EPA+DHA levels of 3.5 %. Therefore, the copepod *A. panamensis* is a suitable life feed organism to feed fish larvae in larviculture.

5. Conclusion

Our study illustrates the population dynamics of *A. panamensis* under intensive culture conditions and different microalgae feed. The results provide important information for an efficient copepod culture and adequate feeding management for fish larvae. Our data shows that *I. galbana* rather than *Nannochloropsis* sp. is nutritionally suitable as diet for *A. panamensis*. Additionally, it has the potential to provide fish larvae with a favorable DHA/EPA ratio and DHA content, matching the nutritional requirements of marine and freshwater fish larvae. Therefore, *A. panamensis* can be suggested as a good candidate for finfish larviculture in future.

CRediT authorship contribution statement

Conceptualization: Laura Ballesteros-Redondo, Adrian A. Bischoff. Methodology: Laura Ballesteros-Redondo, Adrian A. Bischoff, Alexander Wacker. Formal analysis and investigation: Laura Ballesteros-Redondo, Alexander Wacker. Writing – original draft: Laura Ballesteros-Redondo. Writing – review & editing: Harry W. Palm, Hanno Bährs, Alexander Wacker, Adrian A. Bischoff. Funding acquisition: Harry W. Palm, Hanno Bährs, Adrian A. Bischoff. Resources: Harry W. Palm, Hanno Bährs, Alexander Wacker. Supervision: Adrian A. Bischoff, Alexander Wacker. All authors read and approved the final manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Consent for publication

All authors approved the manuscript and its submission to "Aquaculture Reports".

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2023.101535.

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6. Dynamics of fatty acids in pikeperch (*Sander lucioperca*) larvae and juveniles during early rearing and weaning in a commercial RAS – Implications for dietary refinement.

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Dynamics of Fatty Acids in Pikeperch (*Sander lucioperca*) Larvae and Juveniles during Early Rearing and Weaning in a Commercial RAS—Implications for Dietary Refinement

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Abstract: The aquaculture production of pikeperch has reached commercial scale in a number of European countries, but the high mortality of early life cycle stages and minor understanding of nutritional requirements are still major bottlenecks. To investigate the fate of fatty acids during early development, weaning and rearing, pikeperch larvae and juveniles from a commercial recirculating aquaculture system (RAS) were sampled over 2 months for morphometric data, as well as fatty acid composition, with a total of 6 sampling days, with four to five replicates per sampling day and between 1 and 25 pikeperch larvae per individual sample, depending on larval biomass. The biomass of sampled pikeperch larvae varied from 0.1 to 420 mg (dry mass DM), depending on the age of the larvae, and the initial length of the pikeperch larvae was about 4.5 mm. Our data confirm that, accompanied by an exponential increase in dry mass, total fatty acids (TFAs) in larval tissues increased with the beginning of exogenous feed uptake and were depleted between days 13 and 25 post hatch, most likely associated with the weaning and metamorphosis of the larvae. We conclude that all fatty acid classes may serve as metabolic fuel during metamorphosis, but the ultimate fatty acid composition is strongly impacted by the available feed. The chosen diet probably caused a lack of alpha-linolenic (18:3n-3; ALA) and docosahexaenoic acid (22:6n-3; DHA) during larval development and a shortage of vaccenic (18:1n-7), alpha-linolenic (18:3n-3; ALA) and arachidonic acid (20:4n-6; ARA) in juvenile pikeperch. This led to low DHA/EPA ratios 13 days post hatch, a high EPA/ARA ratio at days 41 and 56 post hatch and a fluctuating ratio of alpha-linolenic acid to linoleic acid (18:2n-6; LA). A temporary lack of essential fatty acids can cause dysfunctions and eventually mortalities in pikeperch larvae and juveniles. Despite high larval growth rates, the biochemical composition of the first fed *Artemia* and microdiets was most likely not sufficient and in need of improvement. We suggest that deficiencies must be compensated, e.g., through the substitution of the offered *Artemia* with more suitable live feed organisms, such as freshwater rotifers, and the enrichment of current microdiets in order to prevent high mortalities during pikeperch rearing and weaning.

Keywords: *Sander lucioperca*; commercial aquaculture; feeding regime; fatty acid composition; larviculture management

Key Contribution: The focus of this study was on the fatty acid composition of pikeperch larvae and their dynamics during development. By investigating the fatty acids of the pikeperch larvae over a

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period of 56 days post hatch, the importance of the nutrient supply during the metamorphosis of the pikeperch larvae, as well as the species-specific nutrient composition, could be demonstrated.

1. Introduction

Pikeperch (*Sander lucioperca* L., (1758)) is a fast-growing and valuable fish species of European freshwater systems [1–3] and a promising candidate for aquaculture in Northern Europe, including Germany. In a number of countries, pikeperch rearing has already reached commercial scale, but sufficient production of fry is still a bottleneck [4]. Survival of pikeperch in early life stages under hatchery conditions depends strongly on the amount and quality of the feed. Summerfelt (1996) [5] identified the end of the post-larval and the beginning of the juvenile stages as most critical during early development. At temperatures below 20 °C, walleye (*Sander vitreus*) larvae performed metamorphosis between days 16 and 19 post hatch [5]. Pikeperch was successfully weaned with artificial diets [6,7], and, despite the fragility of the larvae, Ostaszewska et al. (2005) [8] suggested that pikeperch larvae can be adapted to high-quality microdiets already at day 5 post hatch, without significant disorders in the digestion and absorption of dry feeds. Kestemont et al. (2007) [7] demonstrated that the addition of microdiets to larval diets from day 12 post hatch onward caused significantly lower deformities than exclusive feeding with *Artemia* and that early weaning reduced cannibalism.

Brine shrimp (*Artemia* spp.) are still the most commonly used first feed for pikeperch to date. Numerous studies have shown that pikeperch larvae can be reared with *Artemia* [7–14] under aquaculture conditions but still with unsatisfactory mortality rates. It should thus be investigated whether *Artemia* can completely satisfy the nutritional requirements of pikeperch larvae.

Already in 1988, Heming and Buddington [15] suggested that the composition of larval yolk reserves could be a helpful tool in the identification of larval requirements and the development of suitable diets for first-feeding larvae. Palm and co-workers [16] assumed that a high sum of monounsaturated fatty acid (ΣMUFA) contents and high levels of eicosapentaenoic acid 20:5n-3 (EPA) in combination with the absence of 22:6n-3 (docosahexaenoic acid; DHA) in brine shrimp are unfavorable for the early development of pikeperch. This leads to a high EPA/ARA ratio and especially a low DHA/EPA ratio, which might be possible reasons for high mortality rates in the conventional rearing process of the fry. Inside their natural habitats, freshwater fish species are generally characterized by higher levels of C18 polyunsaturated fatty acids (PUFAs) and the highly unsaturated fatty acid (HUFA) 20:4n-6 arachidonic acid (ARA) compared to marine species [17–19]. Nevertheless, freshwater species also contain substantial amounts of n-3 (omega-3) HUFAs, especially 20:5n-3 (EPA) and 22:6n-3 (DHA) [19], and several studies confirmed the importance of HUFAs for pikeperch larvae [7,10,12].

The aim of the present study was to analyze the fatty acid composition and its dynamics in pikeperch larvae and juveniles under commercial aquaculture conditions until day 56 post hatch. We hypothesized that the most important fatty acids for the early life cycle development reach their maximum in newly hatched yolk-sac larvae and that the fatty acid ratios can serve as a reference point also in the later larval and juvenile development. We further hypothesized that the fatty acid patterns in pikeperch larvae are highly influenced by the diet and may not necessarily meet the actual larval requirements.

2. Materials and Methods

For a comprehensive representative and reproducible analysis of the effects of live feed on the development of pikeperch larvae and juveniles, further parameters such as mortality, deformations or the effects of stress would be helpful and necessary in addition to the fatty acid and growth data (specific growth rate (SGR)), which were used here. However, due to limited financial and personnel capacities, not all these parameters could

be determined in the course of this experiment. This is also due to the fact that the thematic priorities of the Institute for Fisheries of the State Research Centre for Agriculture and Fishery Mecklenburg-Western Pomerania (LFA MV) in Hohen Wangelin at that time were the investigation of breeding and hatching rates, as well as growth parameters, but not yet the above-mentioned parameters.

These parameters have already been collected for subsequent experiments and will now also be collected for future experiments, as long as this is appropriate for the thematic priority of the research.

2.1. Hatching and Rearing of Pikeperch in a Commercial Recirculating Aquaculture System (RAS)

The pikeperch nests were obtained from a pond production in Saxony, Germany. Fish used as broodstock originated from wild catches and had a minimum total wet mass of 4 kg, and they were therefore assumed to spawn repeatedly. After spawning, the nests, including the fertilized eggs, were transferred to the facilities of the Institute for Fisheries of the State Research Centre for Agriculture and Fishery Mecklenburg-Western Pomerania (LFA MV) in Hohen Wangelin, which is operated as a commercial hatchery with research tasks. There, the nests were inserted in conical 500 L breeding tanks. Each breeding tank was part of a freshwater recirculating system. Pikeperch larvae hatched at a temperature of 19 °C and were immediately transferred to separate conical 500 L larval rearing tanks of another recirculating system, to avoid a decline in water quality due to the hatching process. Fish larvae were reared in freshwater at an average temperature of 21.01 ± 0.99 °C under dim light with a light intensity between 20 and 40 Lux. Within the first thirty days post hatch, the tanks were permanently illuminated (24 h); thereafter, a light cycle of 11L:13D (light:dark) was chosen. The larval rearing tanks were gently aerated. The nitrogen compounds were analyzed regularly and were 0.11 ± 0.05 mg·L^{-1} for ammonium (NH_4^+), 0.58 ± 0.84 mg·L^{-1} for nitrite (NO_2^-) and 57.72 ± 9.69 mg·L^{-1} for nitrate (NO_3^-). The recorded average pH was 8.21 ± 0.09.

2.2. Feed and Feeding Regime

Pikeperch larvae had an initial length of 4.5 ± 0.1 mm and an initial biomass of 0.1 ± 0.0 mg (DM). External feeding started four days post hatch (dph). Following a set feeding regime; Micro *Artemia* (Ocean Nutrition Micro *Artemia* Cysts AF430, Rijkmakerlaan 15, 2910 Essen, Belgium) were first given to the larvae over a period of four days (dph 4–7). Micro *Artemia* are small *Artemia* selected by mechanical separation, which can hatch faster. Due to the earlier hatching, Micro *Artemia* are smaller than conventional *Artemia* and can therefore be taken up by small and sensitive fish larvae. The feeding regime was continued with a mixture of Micro *Artemia* and *Artemia* sp. (Coppens, Premium *Artemia* Cysts (GSL), Dwarsdijk 4, 5705 DM Helmond, The Netherlands) at day 8 post hatch and a gradual adaption to dry feeds (weaning period) by feeding a combination of different-sized *Artemia* and microdiets (O.range Start, INVE, Dendermonde, Belgium) between days 9 and 20 post hatch. Larvae were fed ad libitum by adding *Artemia* four times a day to the fish-rearing tanks. After the weaning period, pikeperch juveniles were exclusively fed microdiets (dph 21–56) of increasing particle size (100–200 µm, 200–300 µm and 300–500 µm) until the end of the sampling period (Table 1).

Cysts of *Artemia* were incubated in 25 L cylindrical-conical polyethylene tanks (PE), at a salinity of 30–35 psu and a temperature of 27 °C. Hatching of *Artemia* eggs was performed under intense continuous aeration. *Artemia* nauplii were given to the fish larvae without additional food or enrichments to the culture flasks. The cysts of larger-sized *Artemia* were decapsulated before adding them to the rearing tanks. Fatty acid compositions of all applied feeds were analyzed to study their effects on the fatty acid composition of pikeperch larvae over the period of dph 4–56 (Table 2).

Table 1. The applied feeding regime in a pikeperch hatchery in a commercial recirculating aquaculture system (RAS) over the course of the first 56 days post hatch.

Days Post Hatch	Fish Larval Feeds
0–3	no exogenous feed
4–7	Micro *Artemia* (AF430)
8	Micro *Artemia* and *Artemia* (GSL)
9–10	Micro *Artemia*, *Artemia* and microdiets (O.range Start, INVE)
11–20	*Artemia* and microdiets
21–56	microdiets

Table 2. Fatty acid contents (means ± SD in µg·mg^{-1} DM of n = 4 replicates for Micro *Artemia* and *Artemia* spp. and n = 3 for the microdiet) and fatty acid ratios of live feeds and microdiet utilized in a commercial recirculating aquaculture system (RAS); ΣSFA = sum of saturated fatty acids, ΣMUFA = sum of monounsaturated fatty acids, ΣPUFA = sum of polyunsaturated fatty acids, HUFA = highly unsaturated fatty acids (i.e., PUFA ≥ 20 carbon chain length), TFAs = total fatty acids.

Fatty Acids	4–10 dph	8–20 dph	9–56 dph
	Micro *Artemia*	*Artemia* spp.	microdiet
14:0	2.3 ± 0.4	0.6 ± 0.4	1.3 ± 0.4
16:0	18.8 ± 1.3	13.7 ± 0.7	11.6 ± 1.5
18:0	6.1 ± 0.4	6.2 ± 0.4	5.2 ± 0.5
∑SFA	28.0 ± 2.2	20.4 ± 0.8	18.0 ± 2.3
16:1n-7	17.9 ± 1.4	3.0 ± 0.1	2.1 ± 0.3
18:1n-9	25.0 ± 1.5	23.7 ± 1.5	8.3 ± 1.0
18:1n-7	17.2 ± 1.0	7.8 ± 0.5	1.8 ± 0.2
20:1n-9	0.7 ± 0.0	0.6 ± 0.0	3.1 ± 0.4
22:1n-11	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.3
∑MUFA	62.4 ± 4.0	35.9 ± 2.1	18.6 ± 2.2
16:3n-4	11.3 ± 0.8	1.2 ± 0.1	0.3 ± 0.0
18:2n-6	4.9 ± 0.4	7.5 ± 0.6	8.2 ± 1.1
18:2n-4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:3n-3	1.8 ± 0.2	34.4 ± 3.5	1.1 ± 0.2
18:4n-3	0.9 ± 0.1	5.5 ± 0.6	0.8 ± 0.1
20:4n-6	4.4 ± 0.5	0.8 ± 0.1	0.5 ± 0.1
20:5n-3	15.4 ± 1.8	1.9 ± 0.3	5.2 ± 0.9
22:6n-3	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 1.8
∑PUFA	41.9 ± 4.0	54.1 ± 5.3	27.2 ± 4.4
∑TFAs	132.2 ± 10.1	110.4 ± 8.0	63.8 ± 8.3
∑C18 PUFA	8.5 ± 0.7	48.0 ± 4.7	10.1 ± 1.3
∑C20 + 22 HUFA	19.8 ± 2.3	4.5 ± 0.6	16.8 ± 3.1
∑n-3	18.1 ± 2.1	43.6 ± 4.57	17.55 ± 3.24
∑n-6	10.2 ± 0.9	9.0 ± 0.7	8.6 ± 1.1
n-3/n-6	1.8:1.0	4.9:1.0	2.0:1.0
DHA/EPA	0.0:15.4	0.0:1.9	1.7:1.0
EPA/ARA	3.5:1.0	2.3:1.0	11.5:1.0
18:3n-3/18:2n-6	1.0:2.8	4.6:1.0	1.0:7.4
SFA/MUFA	1.0:2.2	1.0:1.8	1.0:1.0
SFA/PUFA	1.0:1.5	1.0:2.7	1.0:1.5
MUFA/PUFA	1.5:1.0	1.0:1.5	1.0:1.5

2.3. Determination of Fish Larvae Mass

Samples of fish larvae were collected according to the protocol below in the course of the fatty acid analysis (see Section 2.4 Sampling and fatty acid analyses). After lyophilization for 48 h, dry mass (DM) of the fish larvae was determined using a Sartorius micro balance (MC21 S, ± 2 µg).

Specific growth rate (SGR) [%·day^{-1}] of the pikeperch larvae was calculated on dry matter basis modified after Jorgensen (1990), applying the formula

$$SGR = \left(\ln\left(\frac{M_{tE}}{M_{tI}}\right)\cdot t^{-1}\right)\cdot 100 \quad (1)$$

where M_{tE} and M_{tI} are the average dry mass of the larvae at time tE (end of the growth interval) and time tI (beginning of the interval).

Additionally, for the dry mass, which was recorded for each sampling day, total length was only recorded at days 1, 7 and 13 post hatch. Each sample encompassed 4 to 16 individuals. Total length of the fish larvae was measured using a stereo light microscope (SZX10 Olympus, Hamburg, Germany) connected to a UC30 digital camera (Olympus, Hamburg, Germany) and the software package cellSens Dimension 1.6 (Olympus Soft Imaging Solutions, Hamburg, Germany). Therefore, fish larvae were individually placed under the stereo light microscope, and the longest distance between the tip of the head and the tip of the tail was recorded.

The data on dry mass and total length were used to determine Fulton's condition index (k_c) according to the formula

$$k_c = 100\cdot\left(M_i\cdot L_i^{-3}\right) \quad (2)$$

where M_i is the average dry mass, and L_i is the total length of the corresponding sampling days.

2.4. Sampling and Fatty Acid Analyses

For the fatty acid analyses of the feeds, all *Artemia* samples consisted of approximately 500 individuals per replicate sample. Respective to culture density, a volume of 10 to 20 mL was filtered through gauze (mesh net size 23 µm) and rinsed three times with saltwater (3 psu) to remove small particles. Pooled samples were subsequently collected in pre-combusted (12 h at 400 °C) and pre-cooled glass vials and stored at −80 °C until analysis.

For the fatty acid analyses of the pikeperch larvae and juveniles, 4 to 5 replicate samples were taken in regular intervals from the rearing tanks at days 1, 7, 13, 25, 41 and 56 post hatch. One sample encompassed 5 to 25 individual pikeperch larvae, with a reduced number of individuals per sample with increasing body size and mass. At days 25, 41 and 56 post hatch, only two individuals and one individual, respectively, were taken per replicate sample. Immediately after sampling, the larvae were sorted in pre-combusted (12 h at 400 °C) and pre-cooled glass vials. Thereafter, the samples were stored at −80 °C until analysis. Dry mass (DM) was determined using a Sartorius micro balance (MC21 S, ±2 µg).

Samples were analyzed according to the method described by Fink [20] and Kattner and Fricke [21]. Lipid extraction was conducted by immersing homogenized tissue in a dichloromethane and methanol solution (2:1/*v*:*v*) for at least 12 h. Thereafter, fish and zooplankton samples were sonicated and centrifuged for 5 min (4500× *g*). After taking up the supernatant quantitatively, the solvent was evaporated to dryness under a N_2 atmosphere at 40 °C. In the following, total fatty acids were transesterified with 5 mL of 3 N methanolic HCl (SUPELCO) at 70 °C for 20 min to their respective fatty acid methyl esters (FAMEs) [20]. FAMEs were extracted with 2 × 2 mL iso-hexane, and after evaporation, the samples were finally dissolved in 100 µL iso-hexane. For gas chromatographic analyses, 1 µL of each sample was injected splitless into an Agilent 6890N GC System (Agilent Technologies. Waldbronn, Germany) equipped with a DB-225 capillary column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) and an FID detector. Helium was used as the carrier gas at a flow rate of 1.5 mL min^{-1} and an oven temperature gradient as described elsewhere [20]. FAMEs were identified by comparing the retention times with those of reference compounds and quantified using two internal standards (nonadecanoic

(C19:0 ME) and tricosanoic acid (C23:0 ME) methyl esters) and previously established calibration functions for each individual FAME [20].

2.5. Statistical Analyses

All statistical analyses were performed by applying the software IBM SPSS Statistics, Version 22 (IBM Corp, Armonk, NY, USA). For the test of normal distribution, the Shapiro–Wilk test was applied. To test the homogeneity of variance, the Levene test was used. To analyze differences between means, an analysis of variance (ANOVA) was performed. In case significant results were obtained, post hoc tests followed, either Tukey–Kramer (with variance homogeneity) or Dunnett T3 post hoc tests (without variance homogeneity). As non-parametric tests either a Mann–Whitney U-Test or a Kruskal–Wallis analysis of variance (ANOVA) was chosen. All significance levels α were set to 0.05.

3. Results

3.1. Growth

The early development of the pikeperch larvae was characterized by exponential growth between days 1 and 56 post hatch (Figure 1 and Table 3). Pikeperch larvae were fed according to the feeding protocol in Table 1.

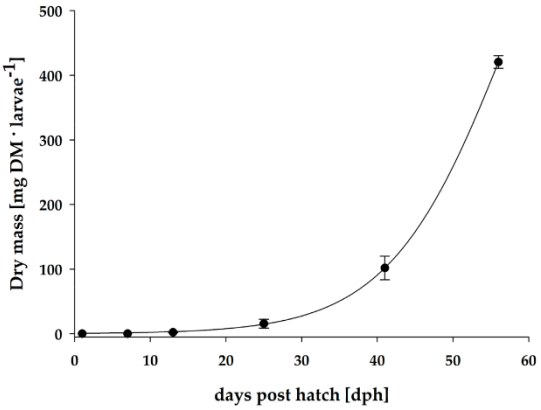

Figure 1. Individual dry mass of pikeperch larvae from the RAS over a course of 56 days.

Table 3. Individual pikeperch dry mass, specific growth rate (SGR) and mass-specific and individual fatty acids on day 1 post hatch (dph 1, n = 5; dph 7, n = 5; dph 13, n = 5; dph 25, n = 4; dph 41, n = 4; dph 56, n = 5) and Fulton's condition index on day 1 post hatch (dph 1, n = 5; dph 7, n = 5; dph 13).

	Pikeperch of a Commercial Recirculating Aquaculture System (RAS)				
Days post hatch	Dry mass ($mg \cdot Ind^{-1}$)	Fulton's condition index	SGR ($\%\cdot day^{-1}$)	Total fatty acids ($\mu g \cdot mg^{-1}$ DM)	Total fatty acids ($\mu g \cdot Ind^{-1}$)
1	0.1 ± 0.0	0.138	-	97.8 ± 7.8	13.0 ± 0.7
7	0.3 ± 0.0	0.085	10.9	90.9 ± 4.3	23.0 ± 4.3 *
13	2.2 ± 0.3	0.118	36.3	95.0 ± 2.9	212.7 ± 46.4 *
25	15.6 ± 6.8	# n.d.	16.3	39.8 ± 7.0	59.1 ± 14.6 *
41	101.8 ± 18.4	# n.d.	11.7	85.5 ± 22.0	425.8 ± 239.0
56	420.5 ± 9.7	# n.d.	9.5	81.4 ± 20.6	473.2 ± 144.6

* Significantly different to the previous sampling day (*t*-test). # n.d. stands for not detectable.

3.2. Fatty Acid Composition of Pikeperch Larvae

The total fatty acid (TFA) content per individual pikeperch larvae increased significantly (ANOVA, $p = 0.005$) within the first 13 days post hatch compared to the initial content (Table 3). By contrast, the TFA content in relation to dry mass did not change significantly (ANOVA, $p = 0.714$) at the same time (Table 3). Between day 13 and day 25 post hatch, the TFA content per individual larva, as well as in relation to dry mass, decreased significantly (ANOVA, $p = 0.009$ and $p = 0.001$) by 72% and 58%, respectively. After day 25 post hatch, again, a distinct increase in the TFA level per individual fish larva was observed, with significant (ANOVA, $p = 0.023$) differences between days 25 and 56 post hatch. The TFA content per dry mass also increased, but the differences were not significant (ANOVA, $p = 0.134$), despite an increase in the TFA content per individual larva by a factor of 36 over the first 56 days post hatch. The final TFA content per dry mass 56 days post hatch was not significantly (ANOVA, $p = 0.751$) different compared to the initial content of newly hatched larvae.

In terms of fatty acid classes, PUFAs were the dominant fatty acids in pikeperch larvae one day post hatch, with a content twice as high as the initial MUFA content and five times higher compared to the initial SFA level (Table 4). Each fatty acid class, SFAs, MUFAs and PUFAs, increased per individual after the beginning of exogenous feeding within the first seven days of development. Due to only a slight absolute increase in PUFAs compared to SFAs and MUFAs, the PUFA content in relation to dry mass decreased significantly (ANOVA, $p < 0.001$) from day 1 to day 7 post hatch. In terms of fatty acid composition, 18:1n-9, 22:6n-3 (DHA), 18:2n-6 (LA), 16:0 and 18:3n-3 (ALA) (>7 $\mu g \cdot mg^{-1}$ DM and ≥ 1 $\mu g \cdot Ind^{-1}$) were the dominant fatty acids in larvae one day post hatch. Despite an absolute increase, the content of each fatty acid class in relation to dry mass did not change significantly (ANOVA, $p = 0.860$).

Similarly, the level of each fatty acid per individual increased in larval tissues, with the highest increase in 18:1n-9, 18:3n-3 (ALA), 16:0 and 18:1n-7 (in that order). Despite the fact that the content of 18:1n-9 was similar in both types of *Artemia*, 18:1n-9 was accumulated to the highest extent between days 7 and 13 post hatch.

After 13 days, the content per individual larva increased by a factor of about 10, and also, the content per dry mass was higher than the initial content. In relation to dry mass, decreasing levels of 16:1n-7, 18:1n-7, 16:3n-4, 20:4n-6 (ARA), 20:5n-3 (EPA) and 22:6n-3 (DHA) were seen. On the other hand, the content of 18:3n-3 (ALA) per individual increased by a factor of 57.

Between day 13 and day 25 post hatch, the absolute amount of each fatty acid class and the amount of each principal fatty acid per individual larva also decreased, with the exception of 22:6n-3 (DHA). The 22:6n-3 (DHA) level per individual increased by a factor of about 6 between days 13 and 25 post hatch. While the content of MUFAs and PUFAs in relation to dry mass also decreased significantly (Kruskal–Wallis, $p = 0.007$) between days 13 and 25 post hatch, the relative amount of SFAs per dry mass did not change significantly (Kruskal–Wallis, $p = 0.415$). Compared to the initial contents, the SFA level per dry mass was higher, while the PUFA content was significantly (ANOVA, $p < 0.001$) lower compared to the initial content. The most pronounced decrease was detected for the levels of 18:1n-9, 18:3n-3 (ALA), 16:0, 18:1n-7 and 16:1n-7. While the contents of 16:0 and 18:0 in relation to dry mass were higher compared to the initial proportions 25 days post hatch, the relative content of any other principal fatty acid was reduced compared to the starting values.

Table 4. Fatty acid contents (means ± SD in µg·mg^{-1} DM of n = 5 replicates at dph 1, 7, 13 and 56 as well as n = 4 at dph 25 and 41) during early development of pikeperch in a commercial recirculating aquaculture system under conventional rearing conditions over 56 days post hatch with a feeding period from day 4 to day 56 post hatch. One replicate sample for dry masses and fatty acids encompassed 5 to 25 individual larvae with a reduced number of individuals per sample with increasing body mass. At days 25, 41 and 56 post hatch, only two individuals and one individual, respectively, were taken per replicate sample. ΣSFA = sum of saturated fatty acids, ΣMUFA = sum of monounsaturated fatty acids, ΣPUFA = sum of polyunsaturated fatty acids, HUFA = highly unsaturated fatty acids (i.e., PUFA ≥ 20 carbon chain length), TFAs = total fatty acids. Data in a row with different characters indicate statistical differences over the course of the experiment.

Fatty Acids	dph 1	dph 7	dph 13	dph 25	dph 41	dph 56
16:0	7.9 ± 0.7 ^{a}	13.0 ± 0.6 ^{b}	13.1 ± 0.6 ^{b}	8.4 ± 1.2 ^{a}	16.9 ± 4.3 ^{b}	14.6 ± 3.5 ^{b}
18:0	2.8 ± 0.3 ^{a}	5.5 ± 0.3 ^{b}	6.6 ± 0.4 ^{c}	4.1 ± 0.7 ^{b}	5.3 ± 0.8 ^{b}	3.6 ± 0.6 ^{a}
∑SFA	11.3 ± 1.0 ^{a}	20.0 ± 0.9 ^{b}	21.0 ± 1.0 ^{b}	12.8 ± 1.9 ^{a}	24.1 ± 5.8 ^{b}	20.1 ± 4.8 ^{b}
16:1n-9	2.2 ± 0.2 ^{a}	1.2 ± 0.1 ^{b}	1.3 ± 0.1 ^{b}	0.4 ± 0.1 ^{c}	0.4 ± 0.1 ^{c}	0.4 ± 0.2 ^{c}
16:1n-7	4.4 ± 0.3 ^{a}	9.0 ± 0.4 ^{b}	5.4 ± 0.2 ^{c}	1.0 ± 0.2 ^{d}	3.2 ± 1.2 ^{a}	3.9 ± 1.0 ^{ac}
18:1n-9	18.6 ± 1.4 ^{ab}	16.7 ± 0.7 ^{a}	20.3 ± 0.7 ^{b}	6.5 ± 1.1 ^{c}	16.0 ± 4.6 ^{a}	19.8 ± 5.1 ^{ab}
18:1n-7	4.7 ± 0.4 ^{a}	8.9 ± 0.3 ^{b}	8.4 ± 0.5 ^{b}	1.9 ± 0.3 ^{c}	2.8 ± 0.8 ^{c}	2.7 ± 0.7 ^{c}
∑MUFA	30.4 ± 2.3 ^{a}	36.6 ± 1.7 ^{b}	35.9 ± 1.2 ^{b}	10.3 ± 1.7 ^{c}	26.2 ± 7.7 ^{a}	31.3 ± 8.2 ^{a}
16:3n-4	1.7 ± 0.2 ^{a}	4.9 ± 0.2 ^{b}	3.6 ± 0.1 ^{c}	0.5 ± 0.1 ^{d}	0.0 ± 0.0 ^{d}	0.2 ± 0.1 ^{d}
18:2n-6	10.9 ± 0.9 ^{a}	4.4 ± 0.2 ^{b}	5.1 ± 0.2 ^{c}	3.3 ± 0.5 ^{d}	11.9 ± 3.7 ^{a}	12.1 ± 3.2 ^{a}
18:3n-3	7.7 ± 0.6 ^{a}	2.0 ± 0.1 ^{b}	12.8 ± 0.8 ^{c}	1.4 ± 0.3 ^{d}	1.7 ± 0.6 ^{d}	1.8 ± 0.5 ^{d}
20:4n-6	4.5 ± 0.4 ^{a}	4.0 ± 0.2 ^{a}	2.4 ± 0.1 ^{b}	1.0 ± 0.2 ^{c}	0.7 ± 0.1 ^{d}	0.6 ± 0.1 ^{d}
20:5n-3	4.2 ± 0.3 ^{a}	8.5 ± 0.5 ^{b}	5.6 ± 0.4 ^{c}	2.2 ± 0.5 ^{d}	4.7 ± 1.0 ^{a}	3.7 ± 1.0 ^{a}
22:6n-3	16.3 ± 1.3 ^{a}	5.8 ± 0.5 ^{b}	1.2 ± 0.1 ^{c}	6.5 ± 1.5 ^{b}	13.4 ± 2.6 ^{a}	8.5 ± 2.3 ^{b}
∑PUFA	56.1 ± 4.6 ^{a}	34.2 ± 1.7 ^{b}	38.0 ± 1.5 ^{b}	16.7 ± 3.4 ^{c}	35.2 ± 8.6 ^{b}	30.0 ± 7.8 ^{b}
∑TFAs	97.8 ± 7.8 ^{a}	90.9 ± 4.3 ^{a}	95.0 ± 2.9 ^{a}	39.8 ± 7.0 ^{b}	85.5 ± 22.0 ^{a}	81.4 ± 20.6 ^{a}
∑C18 PUFA	22.7 ± 2.0 ^{a}	7.9 ± 0.4 ^{b}	21.3 ± 1.1 ^{a}	5.1 ± 0.8 ^{b}	14.5 ± 4.6 ^{c}	15.0 ± 4.0 ^{c}
∑C20 + 22 HUFA	31.3 ± 2.6 ^{a}	20.5 ± 1.1 ^{b}	13.1 ± 0.7 ^{c}	11.1 ± 2.5 ^{c}	20.7 ± 4.0 ^{b}	14.8 ± 3.9 ^{c}
∑n-3	33.0 ± 2.5 ^{a}	18.5 ± 1.1 ^{b}	25.9 ± 1.4 ^{c}	11.6 ± 2.6 ^{d}	22.5 ± 4.8 ^{bc}	16.5 ± 4.5 ^{b}
∑n-6	19.4 ± 1.8 ^{a}	9.6 ± 0.4 ^{b}	8.5 ± 0.2 ^{c}	4.4 ± 0.7 ^{d}	12.6 ± 3.8 ^{e}	13.3 ± 3.4 ^{e}
n-3/n-6	1.7:1.0	1.9:1.0	3.0:1.0	2.6:1.0	1.8:1.0	1.2:1.0
DHA/EPA	3.9:1.0	1.0:1.5	1.0:4.7	3.0:1.0	2.9:1.0	2.3:1.0
EPA/ARA	1.0:1.1	2.1:1.0	2.3:1.0	2.2:1.0	6.7:1.0	6.2:1.0
18:3n-3/18:2n-6	1.0:1.4	1.0:2.2	2.5:1.0	1.0:2.4	1.0:7.0	1.0:6.7
SFA/MUFA	1.0:2.7	1.0:1.8	1.0:1.7	1.2:1.0	1.0:1.1	1.0:1.6
SFA/PUFA	1.0:5.0	1.0:1.7	1.0:1.8	1.0:1.3	1.0:1.5	1.0:1.5
MUFA/PUFA	1.0:1.8	1.1:1.0	1.0:1.1	1.0:1.6	1.0:1.3	1.0:1.0

Between days 25 and 41 post hatch, the level of fatty acid classes, as well as the content of each dominant fatty acid, increased per individual as well as per dry mass. While the PUFA level per dry mass increased significantly (ANOVA, $p = 0.001$) at this time, the accumulation of SFAs and MUFAs was statistically not significant (Kruskal–Wallis, $p = 0.168$ and $p = 1.000$) in relation to dry mass. The highest increase per individual was observed for 16:0, 18:1n-9, 18:2n-6 (LA) and 22:6n-3 (DHA). On the other hand, a low increase per individual was observed for 18:1n-7, 20:1n-9, 22:1n-11, 18:3n-3 (ALA), 18:4n-3 and 20:4n-6 (ARA), and the level of 16:3n-4 per individual even decreased. In relation to dry mass, 20:4n-6 (ARA) was the only fatty acid with a decreasing amount relative to dry mass from days 25 to 41 post hatch.

Between days 41 and 56 post hatch, the level of fatty acid classes per dry mass did not change significantly (Kruskal–Wallis, $p = 1.000$) at this time. Nevertheless, compared to the initial content, total SFAs, as well as the level of both dominant SFAs, 16:0 and 18:0, in relation to dry mass, increased significantly (Mann–Whitney U-Test, $p = 0.016$) at 56 days post hatch. On the other hand, total MUFAs, as well as the levels of 16:1n-7, 18:1n-9, 18:2n-6 (LA) and 20:5n-3 (EPA) per dry mass, were not significantly (Mann–Whitney U-Test, $p = 0.421$ and $p = 0.151$) different compared to the starting values, while total PUFAs and the level of 18:1n-7, 18:3n-3 (ALA), 20:4n-6 (ARA) and 22:6n-3 (DHA) decreased significantly (Mann–Whitney U-Test, $p < 0.001$) within the first 56 days of pikeperch development.

In accordance with the highest n-3/n-6 ratio in *Artemia* fed between days 4 and 20, the highest n-3/n-6 ratio in pikeperch larvae was observed on days 13 and 25 post hatch. Concerning the DHA/EPA ratio, the proportion of DHA was about four times higher in larvae one day post hatch. Due to the absence of DHA in Micro *Artemia*, as well as in *Artemia*, the proportion of EPA in relation to DHA increased during early development to the lowest DHA/EPA ratio 13 days post hatch. Associated with the presence of DHA in microdiets, the proportion of DHA was higher compared to EPA from day 25 post hatch onward. Whereas the EPA/ARA ratio was balanced already one day post hatch, the proportion of EPA increased during early development and was twice as high as the ARA level until 25 days post hatch. Associated with the highest EPA/ARA ratio in microdiets, the EPA proportion in pikeperch juveniles was six times higher in relation to ARA at 41 and 56 days post hatch. Caused by the highest ALA level in *Artemia*, the highest ALA/LA ratio was detected in larvae on day 13 post hatch. On the other hand, accompanied by comparable levels of LA but distinctly lower levels of ALA in microdiets compared to *Artemia*, the lowest ALA/LA ratio was detected in pikeperch 41 and 56 days post hatch.

The proportion of MUFAs was generally higher than SFAs. Exceptions were days 25 and 41 post hatch, where either the proportion of SFAs was higher or a balanced SFA/MUFA ratio was observed. Further, the PUFA level was higher compared with SFAs at all sampling times, with the highest proportion of PUFAs in relation to SFAs in newly hatched larvae. The MUFA/PUFA ratio was the lowest in newly hatched larvae and 25 days post hatch, while the MUFA proportion compared to PUFA was higher 7, 13 and 56 days post hatch.

4. Discussion

4.1. Effect of Feeding on the Growth of Pikeperch Larvae and Juveniles

The present study analyzed the growth and fatty acid composition of conventionally reared pikeperch larvae during their first 56 days post hatch. The observed growth was comparable to growth rates described in the literature, such as Colchen et al. (2020) [22], who described SGRs of $16.9 \pm 1.7\%{\cdot}d^{-1}$, or Péter et al. (2023) [23], who described $17.8 \pm 0.7\%{\cdot}d^{-1}$, but exceeding other studies where SGRs were ranging between 0.8 and $3.0\%{\cdot}d^{-1}$ (Ballesteros et al., 2023) [24] and 0.9 and $4.1\%{\cdot}d^{-1}$ (Bischoff and Kubitz et al., 2022) [25]. Consequently, the sampled pikeperch larvae performed according to other studies, also mainly based on *Artemia* as first life feed. However, this data comparison should be considered carefully and together with the survival rates as well as other parameters, such as the condition index, which shows already a strong variation for the first 13 days of larval development, which indicates a possible lack of nutrients very early post hatch. The survival rates of $7.9 \pm 3.5\%$ per tank described by Colchen et al. (2020) [22] and 11.3% by Péter et al. (2023) [23] were rather low survival rates compared to the survival rates ranging from 64.0 to 93.7% by Ballesteros et al. (2023) [24] and up to 94.0% by Bischoff and Kubitz et al. (2022) [25]. Therefore, we hypothesize that a low survival rate might lead to growth rates that do not represent the whole population but rather the small group of survivors, which are strong and fast-growing. In terms of ecologically and economically sustainable aquaculture, the best possible performance should be a good compromise between high survival rates and good growth rates, and the focus should not be on only one of the two factors. Consequently, a lower growth rate can be compensated by a significantly higher number of individuals.

4.2. Effect of Feed on Pikeperch Fatty Acid Composition

4.2.1. Period 1–7 dph

Larvae were fed exclusively Micro *Artemia* from dph 4 to dph 7.

For the cultivated pikeperch larvae, the FAs are described as FA classes and single FAs in the order of PUFAs, MUFAs and SFAs.

While PUFAs and MUFAs were, in that order, the dominant fatty acids in larvae one day post hatch, SFAs increased at most during the first seven days post hatch.

Within the PUFAs, the absolute contents of LA, ALA and DHA per individual decreased within the first seven days. This could be due to a reduced concentration or the absence within the microdiets or an increased demand during the early development. The level of C18 PUFAs also seemed to be highly related to the diet. These observations could be confirmed by previous studies, where the level of C18 PUFAs of pikeperch larvae and juveniles was highly influenced by the concentration in the corresponding diet [15,24]. Low contents of LA and especially ALA in Micro *Artemia* might cause the detected depletion of C18 PUFAs within the first seven days of larval development. In a previous study, we supposed that the availability of both C18 PUFA LA and ALA in the diet is important during the early development of pikeperch [16]. The highest survival rates could be observed in pikeperch larvae with a final content of LA, as well as ALA, of about 3 µg mg^{-1} DM.

The larval EPA content increased during the first feeding period. This reflected that the larvae fed on Micro *Artemia*. However, LA and DHA decreased to low levels. DHA contents on dph 7 were lower than those reported by Bischoff et al. (2018) [26] on dph 10 under starvation. Ballesteros-Redondo et al. (2023) [24] showed that the provision of dietary LA is particularly important during this period. Consequently, our data indicate that Micro *Artemia* is not adequate for this period and that EPA should not be considered an essential fatty acid for pikeperch larvae during this early life stage.

As Micro *Artemia* are rich in each principal MUFA, they had the highest net increase in pikeperch larvae. These results can be confirmed by observations made in a previous feeding study, where the principal MUFAs, 16:1n-7, 18:1n-7 and 18:1n-9, were highly accumulated in pikeperch larvae within the first ten days post hatch, if high amounts were available in the diet [16].

Due to depletions without a minimal threshold when being less abundant in the diet, we already concluded 16:1n-7 and 18:1n-7 as non-essential fatty acids for pikeperch larvae. On the other hand, the structural importance of 18:1n-9 could be confirmed by a minimal threshold of this MUFA in pikeperch larval tissues. We hypothesize that if non-essential MUFAs, such as 16:1n-7 and 18:1n-7, are highly available in the diet during early development, they will be stored as energy for the upcoming metamorphosis. If non-essential fatty acids are not available, probably any other alternative energy source, like proteins, has to be utilized during metamorphosis.

Concerning the ratios of the FAs, the initial SFA:MUFA:PUFA ratio in yolk-sac larvae was 1:3:5 and changed to a ratio of 1:2:2 after 7 days. In a previous feeding study, we hypothesized that an increasing level of SFAs during early larval development might be an indicator for a good feeding constitution of pikeperch larvae [16]. In that study, a similar SFA:MUFA:PUFA ratio of 1:3:4 was observed in newly hatched larvae, and after a ten-day feeding period, the level of fatty acid classes (SFA:MUFA:PUFA ratios) differed between the feeding groups. While the proportion of SFAs was higher in relation to MUFAs in pikeperch larvae fed *Brachionus calyciflorus* and *Eurytemora affinis*, the proportion of MUFAs exceeded total SFAs in larvae fed conventional *Artemia*.

A balanced ALA/LA ratio ten days post hatch was described by [16]. A similar ALA/LA ratio of 1.0:1.4 could be observed in newly hatched larvae of the present study and therefore could be a kind of recommended value. Sargent et al. [27] already revealed that freshwater fish species, unlike most marine fish species, have high requirements for both n-3 and n-6 PUFAs. In perch breeders, Henrotte et al. [28] observed that an n-3/n-6 ratio between 0.8:1.0 and 2.5:1.0 was optimal for the reproduction success of the perch breeder, while a higher proportion of n-3 PUFAs decreased the fertilization and the spawning rate, and too high levels of n-6 PUFAs, on the other hand, can cause inflammations and diseases in fish [28].

4.2.2. Period 7–13 dph

During this period, the pikeperch larvae were still fed Micro *Artemia* until dph 10, which was followed by a co-feeding of regular *Artemia* and microdiets.

Accompanied by the exponential growth of the pikeperch dry mass during the experiment, a sigmoidal increase in TFAs per individual was observed, and the TFAs showed the highest increase between days 7 and 13 post hatch.

From dph 7 onward, the C18 PUFA level also increased in pikeperch larvae, and the content of ALA in relation to dry mass after thirteen days post hatch was between six and nine times higher than during the rest of the sampling period.

Due to a lack of DHA in both Micro *Artemia* and *Artemia*, combined with the highest growth of pikeperch larvae between days 7 and 13 post hatch, we conclude a shortage of DHA in pikeperch larvae at this stage. Moreover, although regular *Artemia* are rich in ALA, a depletion of DHA within this period indicated that a lack of synthesis from C18 PUFAs to their higher homologous n-3 and n-6 HUFAs occurred, which was also observed in other freshwater species [28–30]. Lund et al. [12] made similar observations in pikeperch larvae, revealing that, in spite of DHA deprivation in the diet, ALA was not converted to their higher homologous HUFAs. Instead, they obtained elevated stress sensitivity in pikeperch larvae fed on a diet lacking DHA. The authors consequently hypothesized that pikeperch, like most marine fish species and some strict piscivorous freshwater species such as pike (*Esox lucius*), are unable to synthesize essential HUFAs from their C18 omega-3 precursors. Nevertheless, Mourente [31] found the DHA levels detected in neural tissues of marine fish to be mainly of dietary origin and independent of the capability of in vivo DHA synthesis. These findings emphasize the need for sufficient supplies of dietary DHA. Bischoff and Kubitz et al. (2022) [25] and Ballesteros-Redondo et al. (2023) [24] have shown recently that rotifers are adequate feed for pikeperch larvae since they apparently help the larvae to maintain sufficient DHA levels. Nonetheless, DHA has to be provided for the later life stages. Our data demonstrate that the microdiets were either eaten or digested by the larvae, and thus, DHA should be offered via alternative live feeds such as copepods (Ballesteros-Redondo et al. submitted) for this life stage.

As a result of the high importance of DHA for the development of neural tissues, such as the retina of the eye and the brain [10,12] and, as already discussed, the general importance of HUFAs in larvae of other carnivorous fish [22], we expect that the absence of DHA in different-sized *Artemia* probably caused dysfunctions, a high stress sensitivity and/or probably mortalities in our experiment.

Due to significant increases in ALA and 18:4n-3 and concurrent decreases in 16:1n-7, ARA and EPA in the larvae between days 7 and 13 post hatch, we assume a higher influence of *Artemia* compared to Micro *Artemia* at this time. Moreover, due to an ongoing reduction in the DHA content relative to dry mass, we conclude that the impact of microdiets is minor at this life stage.

Furthermore, the absence of DHA in the first feeds in combination with the highest EPA contents in Micro *Artemia* probably led to the lowest DHA/EPA ratio in pikeperch larvae 13 days post hatch. Henrotte et al. [32] already revealed the importance of an adequate DHA/EPA ratio in Eurasian perch. In this study, the best hatching rates, as well as the highest stress resistance in larvae, were observed for perch eggs with the highest DHA/EPA ratio. In a previous study, we assumed a DHA/EPA ratio of 5:1 in larval tissues as optimal during early development [16], which is comparable to the DHA/EPA ratio observed in newly hatched larvae [16] as well as in juvenile pikeperch [33] as well as perch [32].

4.2.3. Period 13–25 dph

During the period between dph 13 and 20, the pikeperch larvae were fed with a combination of regular *Artemia* and microdiets, which was followed by exclusively microdiets from dph 21 onward.

The constant increase in TFAs in the larvae was intermitted between days 13 and 25 post hatch, where a significant decrease in TFAs per individual larvae, as well as per dry mass, was observed. During this period, the TFA content in relation to dry mass was about three times lower at day 25 post hatch than in larvae one day post hatch. Such a similar

decrease in the TFA content within the first 21 days of pikeperch development was also detected by Lund and Steenfeldt [10], where the TFA content per dry mass was three to six times lower after a 21-day feeding period with different-enriched *Artemia* compared to newly hatched larvae.

During the feeding period dph 13–25, the level of LA in pikeperch larvae fed with microdiets was comparable to larvae fed *Artemia*, but the ALA content was markedly lower, leading to an ALA/LA ratio of approx. 1:7. The differences in the diet probably caused high variations in the ALA/LA ratio in pikeperch fry, with the highest ALA/LA ratio at day 13 post hatch. DHA was the only fatty acid with increasing amounts between days 13 and 25 post hatch.

As explained before, probably influenced by the fatty acid composition of the diet, 16:0, 18:0, 16:1n-7, 18:1n-7, 18:1n-9 and ALA were the dominant fatty acids before metamorphosis and also the most depleted during metamorphosis. These results provide evidence that the influence of bigger-sized *Artemia* was the highest before metamorphosis and that each abundant fatty acid in the diet can principally be stored and utilized during metamorphosis. Nevertheless, the lowest final level per dry mass after 25 days post hatch could be observed for 16:1n-7, 18:1n-7, ALA and ARA, while 16:0, DHA, 18:1n-9 and 18:0 were the most abundant fatty acids. One reason could be that these fatty acids were low-abundant in microdiets, but another reason could be the higher structural importance of SFAs and 18:1n-9 compared to 16:1n-7, 18:1n-7 and ALA.

As mentioned for the previous period, despite an increase in SFAs, the highest net increase was observed concerning MUFAs and PUFAs, and the levels per larvae were almost twice as high as the level of SFAs before metamorphosis. Due to the fact that MUFAs and PUFAs were depleted to the same extent between days 13 and 25 post hatch, we conclude that these fatty acids were consumed for growth and during metamorphosis. These results provide evidence that not only MUFAs but also PUFAs are generally utilized for metabolic purposes, as already observed in early pikeperch larvae [16].

As no DHA could be detected in both types of *Artemia*, this possibly caused critical DHA levels in larval tissues with a subsequent need for the accumulation (instead of utilization) of dietary DHA during metamorphosis as earlier mentioned by Imentai et al. (2020 and 2022) [34,35]. This resulted in DHA being the only fatty acid that increased in larval tissues during metamorphosis.

4.2.4. Period from dph 25 Onward

On the basis of the morphological analyses of the sampling material, we concluded that pikeperch larvae had completed their morphogenesis to juveniles at 25 days post hatch. In percid larvae, the metamorphosis usually occurs 29 days post hatch and at a total length of about 25 mm, and the completed metamorphosis comes along with the complete functionality of the stomach and pepsin activity. This early development of the digestive system is comparable to other carnivorous species, like the European sea bass (*Dicentrarchus labrax*) [36]. In Asian seabass (*Lates calcarifer*), Dhert et al. [37] already demonstrated the high importance of HUFAs in the diet with the onset of and especially during metamorphosis for the stress resistance and survival of seabass fry. Due to a high TFA reduction during metamorphosis in the present study, we conclude a significant consumption of fatty acids as, e.g., metabolic energy at that time, and it indicates that the feed either did not provide the right fatty acid composition or was not well assimilated (digestion and absorption) by the larvae. Consequently, especially during the early life stages in preparation for metamorphosis, fatty acids must be taken up and accumulated from an appropriate diet.

Due to the lowest net depletion during metamorphosis compared to other fatty acid classes, our results provide evidence that SFAs played a minor role as an energy source not only post hatch but also during metamorphosis. We conclude a substantial role as structural fatty acids and potential storage fatty acids during early development, due to an early accumulation in larval tissues and the fact that the relative SFA content per dry mass

at each point in time was higher compared to the initial content, even after metamorphosis. Due to a continuous supply of SFAs from the diet, it is difficult to conclude whether SFAs were accumulated from exogenous food or synthesized de novo. Following metamorphosis, each fatty acid class highly increased again until day 41 post hatch. Due to an ongoing accumulation of MUFAs between days 41 and 56, MUFAs were the dominant fatty acids at the end of this study.

An ongoing deficiency of DHA in the applied diet after metamorphosis might be associated with increased mortalities in the present study, but no survival data are available, and therefore this can only be speculated. Due to increasing amounts of DHA during metamorphosis, we can conclude that the surviving pikeperch were already successfully weaned on microdiets.

The observed general trend of fatty acids also provides evidence that principally each fatty acid is able to serve as an energy source during metamorphosis, except an essential fatty acid, like DHA, that was missing in the first feeds. On the basis of our results, we conclude that the extent of fatty acid utilization was highly dependent on the fatty acid composition of the diet. If important fatty acids are absent in the diet during early life cycle stages and especially metamorphosis, the respective fatty acids will be depleted inside the fish tissues, as observed for ALA and ARA.

After metamorphosis, pikeperch were exclusively fed with fatty acid rich microdiets. Consequently, high amounts of fatty acids accumulated in fish tissues in this phase; thus, the fatty acid composition of the larvae reflects the composition of the feed. Similar fatty acid compositions between fish and their diets were found for 16:1n-7, 18:1n-9, LA and EPA, sometimes even significantly higher within the larvae, like for 16:0 and 18:0. On the other hand, accompanied by low amounts in the diet, only low levels of 18:3n-3 (ALA) and 20:4n-6 (ARA) could be found in pikeperch. We hypothesize that, if higher amounts of these fatty acids were available in the diet, they would be accumulated in pikeperch and that the initial level could be a kind of reference. In a previous study, a physiological ARA threshold of 1.7 µg mg^{-1} DM was identified for pikeperch larvae [16]. The low ARA content in the microdiet in our study suppressed the ARA content in pikeperch tissues below this threshold and thus possibly reached a critical point for their ongoing development. The lowest level in relation to dry mass was not observed after metamorphosis (dph 25) but at day 56 post hatch, leading to the lowest EPA/ARA ratios at days 41 and 56 post hatch. Henrotte et al. [32] found a high EPA/ARA ratio in the diet of perch to negatively influence reproductivity as well as larval quality. In their study, the highest fertilization and hatching rates, as well as the highest survival of larvae exposed to osmotic stress, could be achieved with a dietary DHA:EPA:ARA ratio of 3:2:2, while, on the other hand, egg and larval quality was degraded if the EPA content was higher compared to DHA and ARA. Therefore, the dietary ARA supply in our study may not have been sufficient to increase its relative content in fish tissues. Furthermore, a conversion of dietary LA to ARA seems unlikely, as already discussed for the n-3 HUFAs. These results provide evidence that ARA supplementation to larval diets is of high importance and that microdiets probably caused a shortage in pikeperch.

In summary, the final (dry-mass-specific) contents of 16:0, 18:0, 16:1n-7, 18:1n-9, LA and EPA were not significantly different from the initial levels or even higher after 56 days post hatch. Therefore, we conclude that these fatty acids were present in sufficient amounts in the respective diets and that these levels could be representative for well-supplied pikeperch. On the other hand, the relative final contents of 18:1n-7, ALA, ARA and DHA were lower than the initial values, suggesting a shortage of these fatty acids in the offered diets relative to the larval demands.

5. Conclusions

All offered feeds were ingested by larval and juvenile pikeperch, and dietary fatty acids were incorporated into larval tissues. Increasing levels of certain fatty acids in the larvae, which were highly abundant in the diet at this time, indicate that the fatty acid

pattern of pikeperch is highly influenced by the diet. However, the supply of some specific PUFAs through the applied first feeds was apparently insufficient, which might have caused dysfunctions and mortalities in pikeperch larvae and juveniles. In the future, more efforts should be made to incorporate parameters such as mortality, deformities and the effects of stress into data collection. On the other hand, the observed relationship between feeds and larval deficiencies should be easy to compensate by different enrichments that may ultimately prevent mortalities during pikeperch rearing and weaning.

In general, the contents of fatty acids in pikeperch seemed to be highly dependent on their respective availability in the diet. Conversion of HUFAs from precursors appears to be impossible, at least at sufficient rates. As a consequence, an adequate exogenous supply of essential fatty acids is necessary. *Artemia* provided critically low DHA levels and hence unfavorable DHA/EPA ratios for pikeperch larvae. Our results further suggest that the ARA content in *Artemia* and microdiets is insufficient or unbalanced to fulfill the requirements of developing pikeperch.

Overall, our study emphasizes the importance of suitable dietary fatty acid compositions in aquaculture feeds that are well-tailored to the specific requirements of fishes and their larval stages.

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7. Statement of contribution and list of publications

- Bischoff AA, Kubitz M, Wranik CM, **Ballesteros-Redondo L**, Fink P, Palm HW (2022) The Effect of *Brachionus calyciflorus* (Rotifera) on larviculture and fatty acid composition of pikeperch (*Sander lucioperca* (L.)) cultured under pseudo-greenwater conditions. Sustainability, 14, 6607. https://doi.org/10.3390/su14116607.

Author contribution: Conceptualization, A.A.B., M.K., C.M.W. and H.W.P.; methodology, A.A.B., M.K., C.M.W. and P.F.; validation, A.A.B., M.K., P.F. and H.W.P.; formal analysis, A.A.B. and M.K.; investigation, A.A.B., M.K. and C.M.W.; resources, H.W.P.; data curation, M.K. and **L.B.-R.;** writing—original draft preparation, M.K. and A.A.B.; writing—review and editing, A.A.B., M.K., C.M.W., **L.B.- R**., P.F. and H.W.P.; visualization, A.A.B. and M.K.; supervision, P.F. and H.W.P.; project administration, A.A.B. and C.M.W.; funding acquisition, H.W.P. All authors have read and agreed to the published version of the manuscript.

- **Ballesteros-Redondo L**., Palm H. W., Bährs H., Wacker A. and Bischoff A. A. (2023a). Pikeperch larviculture (*Sander lucioperca* (L., 1758)) with *Brachionus plicatilis* (Mueller, 1786) (Rotifera) and *Apocyclops panamensis* (Marsh, 1913) (Copepoda). Journal of the World Aquaculture Society. https://doi.org/10.1111/jwas.12940

Author contribution: Conceptualization: Harry W. Palm, Hanno Bährs, Adrian A. Bischoff. Methodology: Laura Ballesteros-Redondo, Harry W. Palm, Hanno Bährs, Adrian A. Bischoff. Formal analysis and investigation: Laura Ballesteros-Redondo, Alexander Wacker. Writing - original draft preparation: Laura Ballesteros-Redondo. Writing - review and editing: Harry W. Palm, Hanno Bährs, Alexander Wacker, Adrian A. Bischoff. Funding acquisition: Harry W. Palm, Adrian A. Bischoff. Resources: Harry W. Palm, Hanno Bährs, Alexander Wacker. Supervision: Harry W. Palm, Adrian A. Bischoff

- **Ballesteros-Redondo L**., Palm H.W., Bährs, H., Rapp T., Stueken M., Wacker A. and Bischoff A.A. (2023c) Growth and fatty acid composition of pikeperch (*Sander lucioperca* [L., 1758]) larvae under altered feeding protocol including the copepod *Apocyclops panamensis* (Marsh, 1913). Sci Rep 13, 19574 (2023). https://doi.org/10.1038/s41598-023-46988-y

Author contribution: L.B.R. performed the experiments and prepared the first draft of the manuscript. L.B.R., H.W.P., H.B. and A.A.B. designed the study. H.W.P., H.B. and A.A.B. obtained the funding. L.B.R. and A.W. performed the extraction and identification of the fatty acid data. L.B.R., M.S., T.R. and A.W. analysed the data. All authors further developed and reviewed the manuscript.

- **Ballesteros-Redondo L.,** Palm H.W., Bährs H., Wacker A., and Bischoff A.A. (2023b). Effect of microalgae diets on population performance and fatty acid composition of *Apocyclops panamensis* (Marsh, 1913) (Cyclopoida, Copepoda). Aquaculture Reports, Volume 29, 101535, ISSN 2352-5134, https://doi.org/10.1016/j.aqrep.2023.101535.

Author contribution: Conceptualization: Laura Ballesteros-Redondo, Adrian A. Bischoff. Methodology: Laura Ballesteros-Redondo, Adrian A. Bischoff, Alexander Wacker. Formal analysis and investigation: Laura Ballesteros-Redondo, Alexander Wacker. Writing - original draft preparation: Laura Ballesteros-Redondo. Writing - review and editing: Harry W. Palm, Hanno Bährs, Alexander Wacker, Adrian A. Bischoff. Funding acquisition: Harry W. Palm, Hanno Bährs, Adrian A. Bischoff. Resources: Harry W. Palm, Hanno Bährs, Alexander Wacker. Supervision: Adrian A. Bischoff, Alexander Wacker. All authors read and approved the final manuscript

- Bischoff, A.A., Kubitz, M., **Ballesteros-Redondo, L**., Stüeken, M., Rapp, T., Fink, P., Hagen, W., Palm, H.W. (2023). Dynamics of Fatty Acids in Pikeperch (*Sander lucioperca*) Larvae and Juveniles during Early Rearing and Weaning in a Commercial RAS—Implications for Dietary Refinement. Fishes 2023, 8, 444. https://doi.org/10.3390/fishes8090444

Author Contribution: Conceptualization, A.B., M.K. and H.P.; methodology, A.B. and H.P.; software, A.B. and M.K.; validation, A.B., M.K, H.P., W.H., T.R. and M.S.; formal analysis, A.B. and M.K.; investigation, A.B. and M.K..; resources, H.P., W.H. and P.F.; data curation, A.B. and M.K.; writing—original draft preparation, A.B, M.K., L.B.-R. and H.P.; writing—review and editing, A.B., M.K., H.P., L.B.-R., W.H., P.F., M.S. and T.R.; visualization, A.B. and L.B.-R.; supervision, H.P., W.H. and P.F.; project administration, A.B. and H.P.; funding acquisition, H.P. All authors have read and agreed to the published version of the manuscript.

8. Independence Declaration for the Dissertation

Hiermit erkläre ich durch eigenhändige Unterschrift, die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben. Die aus den Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Dissertation ist in dieser Form noch keiner anderen Prüfungsbehörde vorgelegt worden.

Rostock, 14.07.2023 Ort, Datum Unterschrift der Doktorandin/des Doktoranden

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10. Curriculum vitae

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AUSBILDUNG

Aktuell	**Promotion** an der **Universität Rostock**	Rostock
Sep 14 - Jun 15	Universität Oviedo **Master in Fernerkundung und Geographischen Informationssystemen (GIS)** ● Masterarbeit: Analyse ozeanographischer Variablen mittels Satellitenbildern	Mieres, Spanien
Sep 12 - Jul 14	Vrije Universiteit Brüssel, Universität Gent und Universität Antwerpen **Master in Marine and Lacustrine Science and Management** ● Spezialisierung: Biodiversität und Ökologie	Gent, Belgien
Sep 08 - Jul 13	Universität Oviedo **Bachelor in Biologie** ● Fachrichtung: Umweltbiologie	Oviedo, Spain
Sep 11 - Jul 12	Universität Santiago de Compostela - **Séneca Stipendium**	Galicia, Spanien
Sep 12 - Jul 13	Vrije Universiteit Brüssel - **Erasmus Programm**	Brussels, Belgien

BERUFSERFAHRUNG

Jul 19 -Mar 23	**Wissenschaftliche Mitarbeiterin** der **Universität Rostock** ● Fischlarvenaufzucht, Kultivierung von Algen und Hälterung von Zooplanktonorganismen	Rostock
Sep 15-Okt 16	**Wissenschaftliche Mitarbeiterin** der **Universität Oviedo** ● Kultivierung von Algen und Hälterung von Zooplanktonorganismen (Appendicularia)	Oviedo, Spanien
Feb 15 - Mar 15	**Praktikum** bei "**Instituto Oceanográfico Español (IEO)"** ● Analyse und Verwaltung von ozeanographischen Variablen aus Satellitenbildern	Gijón, Spanien
Juli 14	**Praktikum** bei **"The Institute for Agricultural and Fisheries Research (ILVO)"** ● Taxonomische Analyse von Nordsee-Makrobenthos	Oostende, Belgien
August 13	**Studentenjob** bei **"Vlaams Instituut vor de zee (VLIZ)"** ● Datenverarbeitung über die handwerkliche Fischerei in der Nordsee im Rahmen der Datenpolitik	Oostende, Belgien
Juli 13	**Praktikum** bei "**Instituto Oceanográfico Español (IEO)"** ● Verarbeitung von Proben für die taxonomische Analyse von antarktischen Schwämmen	Gijón, Spanien

TEILNAHME

Okt 21- May 22	Lecture and Attendance in **Master FishFarmer Class (MFFC)**	Online
Jun-Jul 21	Presentation and Attendance in **Innovative Aquaculture Summer School**	Klaipeda, Litaneien
Apr 21- Mai 22	**Mentoring-Program** Uni Rostock	Rostock
September 21	Teilnahme des Kongresses: ● **"Aquaculture Europe 2021"** - **Best Student Poster Award:** *Apocyclops panamensis* as live feed for *Sander lucioperca* larviculture	Madeira, Portugal
April 21	Teilnahme des Kongresses: ● **"Aquaculture Europe 2020"** - **Oral presentation:** Effect of different microalgae diets on *A. panamensis* as a live food for fish larval rearing - **Oral presentation:** Effect of different diets containing rotifers and copepods as first feeding on *S. lucioperca* larvae	Online
Oktober 19	Besuch des Kongresses: ● **"Aquaculture Europe 2019"**	Berlin
Feb 13 + Feb 14	Besuch des Kongresses: ● **"VLIZ YOUNG MARINE SCIENTISTS' DAY"**	Brugge, Belgien
November 13	Poster: ● **"Porifera collected in the Weddell Sea (Antarctica) on board R/V Polarstern by the Ecoquim Project"** 9th World Sponge Conference	Perth, Australia

SPRACHEN & FÄHIGKEITEN

Sprachen	Spanisch (Muttersprache) Englisch B2 Deutsch B2/C1 (TestDaF 4/5 von Dez 2016 bis Dez 2018)
Informatik	Fortgeschrittener Benutzer: Microsoft Office (Word, Excel, PowerPoint, Outlook, Access) ArcGIS, QGIS, Geomedia, ERDAS, ERMapper, R, Primer
Berechtigungen	Führerschein Klasse B PADI Open Water Diver
Mitgliedschaften	European Aquaculture Society. National Coordinator for Germany in the EAS Student Group

VERÖFFENTLICHUNGEN

November 15	"Community dynamics of nematodes after Larsen ice-shelf collapse in the Eastern Antarctic Peninsula"
Juni 22	The Effect of *Brachionus calyciflorus* (Rotifera) on Larviculture and Fatty Acid Composition of Pikeperch (*Sander lucioperca* (L.)) Cultured under Pseudo-Green Water Conditions
Januar 23	Pikeperch larviculture (*Sander lucioperca* (L., 1758)) with *Brachionus plicatilis* (Mueller, 1786) (Rotifera) and *Apocyclops panamensis* (Marsh, 1913) (Copepoda)"
April 23	Effect of microalgae diets on population performance and fatty acids composition of *Apocyclops panamensis* (Marsh, 1913) (CYCLOPOIDA, COPEPODA)
August 23	Dynamics of Fatty Acids in Pikeperch (*Sander lucioperca*) Larvae and Juveniles during Early Rearing and Weaning in a Commercial RAS—Implications for Dietary Refinement.
Nov. 23	Growth and fatty acid composition of pikeperch (*Sander lucioperca* [L., 1758]) larvae under altered feeding protocol including the copepod *Apocyclops panamensis* (Marsh, 1913).

Rostock, 14.07.2023