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Pikeperch (*Sander lucioperca* L.) larviculture improvements using rotifers *Brachionus plicatilis*

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

Introduction

Importance of pikeperch in aquaculture

Fish are an essential source of protein in many countries (FAO, 2018) and, in the west, its consumption is promoted for its health benefits (Krauss et al., 2000; Mozaffarian and Rimm, 2006; Hibbeln et al., 2007; Ruxton, 2011; Parletta et al., 2019). The consequent growing demand for fish has led to the development and intensification of the aquaculture industry, making aquaculture the world's fastest growing industry (FAO, 2018), estimated to produce over 80.0 million tonnes in 2016 (110.2 million tonnes, including aquatic plants) worth US$ 231.6 billion (US$ 243.3 billion, including aquatic plants) (FAO, 2018). The proportion of cultured fish in Europe production increased to 18% in 2016, up from 14% in 2006 (FAO, 2018). There is a growing gap between production and the level of consumption in the European Union, given that the volume of capture fisheries has decreased to 8 million tonnes (Commission, 2013). Considering the challenges faced by capture fisheries, including, but not limited to, overfishing, pollution, and climate change, it is clear that further development of aquaculture is essential (Gjedrem et al., 2012; FAO, 2018).

Pikeperch, *Sander lucioperca* (L.) is a large predatory percid ubiquitous in lakes and rivers and in the Aral, Azov, Baltic, and Caspian Seas (Lappalainen et al., 2003; Stepien and Haponski, 2015). Pikeperch is a promising candidate for diversification of European inland aquaculture, currently dominated by rainbow trout *Oncorhynchus mykiss* and common carp *Cyprinus carpio* (Policar and Adámek, 2013), due to its excellent flesh quality and rapid growth in intensive culture (Wang et al., 2009; Policar et al., 2016a).

Recently, pikeperch prevalence in natural waters has declined due to overfishing, pollution, poor management, and other anthropogenic changes to the natural environment (Policar et al., 2019). Worldwide production of the species is estimated at 21900 tonnes, primarily originating from natural waters (FAO, 2017). Pikeperch cultured production is based on extensive/semi-intensive farming in ponds (Steenfeldt, 2015) and intensive aquaculture using recirculating aquaculture systems (RAS). Extensive aquaculture in central and eastern Europe yields 300–500 tonnes annually (Policar et al., 2016b). Intensive pikeperch aquaculture is gaining popularity among commercial farmers due to the lower water consumption, higher level of control of production conditions, and production cycle to market-sized fish of only 13 months (Overton et al., 2015; Steenfeldt, 2015). In 2017, European pikeperch production was estimated at 823 tonnes, with approximately 90% of the total from eastern and northern Europe (FAO 2020). Interest in pikeperch production has increased in countries including France, Austria, Czech Republic, Germany, Finland, Romania, Bulgaria, and Croatia (Policar et al., 2016b). Pikeperch production in the Czech Republic is stable and was at ~65 tonnes in 2017 (FAO, 2020). European Union – sponsored research projects such as Luciopercimprove and DIVERSIFY as well as government support facilitate further development of pikeperch rearing in RAS (Steenfeldt, 2015). In 2012, the European Percid Fish Culture organization was established to identify problem areas in the sector, and currently comprises members from both academia and industry with an interest in improving and developing pikeperch farming (Policar et al., 2019).

Recirculating aquaculture systems and pikeperch larviculture under intensive aquaculture

Recirculating aquaculture systems

Recirculating aquaculture systems (RAS) have proven successful for intensive production of a wide range of aquatic species (Martins et al., 2010). The technology is effective in reducing water and land use, minimizing waste, and in nutrient recycling, as well as control of biological pollution and disease (Martins et al., 2010). The system is based on a series of water treatment steps using mechanical, biological, and trickling filters (Espinal and Matulić, 2019). The function of mechanical filters is to remove undissolved waste products (uneaten feed, faeces, bacterial flocs) from the water. Without this step, solid particles would quickly accumulate and may disrupt the biological filter and damage fish gills (Chapman et al., 1987).

After the mechanical filter, water flows through a biological filter that oxidizes ammonia to nitrate via a two-step nitrification process in which ammonia-oxidizing bacteria, usually *Nitrosomonas* spp., oxidize ammonia to nitrite and, subsequently, nitrite-oxidizing bacteria oxidize nitrite to nitrate. In the second step of the process, Nitrobacter spp. are the most common bacteria used (Schreier et al., 2010). Both ammonia and nitrite can be toxic to fish (Kroupova et al., 2005; Chen et al., 2006). Biological filtration is a complex system of microbial communities interacting with the environment (Schreier et al., 2010). Its efficacy depends chiefly on water temperature and pH, as well as on dissolved oxygen concentration, organic matter, alkalinity, salinity, and water turbulence (Chen et al., 2006; Pedersen et al., 2007; Kinyage et al., 2019). The fish species introduced into system also play important role in a functioning biofilter, as they bring their own characteristic microbial flora (Schreier et al., 2010).

To remove carbon dioxide, a trickling filter or other degassing equipment can be used (Steenfeldt, 2015).

Some RAS systems use UV irradiation and ozone treatment for water disinfection (Espinal and Matulić, 2019). The UV irradiation kills microorganisms and destroys dissolved ozone (Summerfelt et al., 2009). Its efficacy depends on the concentration and size of suspended solids, UV transmittance, and the dose-response of target microorganisms (Summerfelt et al., 2009). Water treatment with ozone has been shown to improve water quality by oxidizing natural organic matter, carbon-based compounds, and nitrite, as well as eliminating colour and reducing geosmin, bacteria, and pathogens (Summerfelt et al., 2009; Spiliotopoulou et al., 2018).

Despite the above-mentioned advantages of the RAS system, obstacles to its use include high cost of installation and operation and high consumption of electricity (Badiola et al., 2012). In addition, the system requires experienced staff to conduct consistent maintenance (Badiola et al., 2012).

Culture of pikeperch in RAS has been studied for several decades, but pikeperch larviculture under controlled conditions is still a major bottleneck in its production, due to mortality related to low stress resistance, dependence on live food, small mouth gape size, swim bladder inflation failure, high growth heterogeneity, and cannibalism (Policar et al., 2019).

Intensive culture of pikeperch larvae

The survival and health of larvae are highly dependent on broodstock nutrition and management, rearing and weaning protocol, and water conditions throughout development, especially the exogenous feeding period (Tielmann et al.; 2017; Policar et al., 2019; Schaefer

et al., 2019). Current research is focused on the optimization of pikeperch larval culture under controlled conditions, specifically its relatively high mortality and frequent occurrence of skeletal deformities (Ostaszewska, 2005; Tielmann et al., 2017; Yanes-Roca et al., 2020a).

Rearing conditions are an important consideration in pikeperch larviculture (Policar et al., 2019). Pikeperch larvae are usually kept in cylindrical tanks with a conical bottom (Steenfeldt, 2015) with black or dark walls preferable (Steenfeldt, 2015; Policar et al., 2019). The optimal stocking density of pikeperch larvae in RAS has been extensively researched (Mamcarz et al. 1997; Molnar et al., 2004; Szkudlarek and Zakes, 2007, Policar et al. 2013; Steenfeldt, 2015). Stocking density for the period from 4 to 18 days post-hatching (DPH) is recommended at 100 larvae l^{-1} (Szkudlarek and Zakęś, 2007). According to Pickering and Pottinger (1989), larger fish release higher levels of the stress hormone cortisol when stocked at higher densities. Water temperature has a direct effect on larval growth and development (Policar et al., 2019), and low water temperature is recommended (Table 1). Tielmann et al. (2017) reported light at 500 and 1000 lx improves growth of pikeperch larvae, but survival is higher under lower light conditions (100 lx).

Table 1. *Environmental parameters for optimal rearing of pikeperch larvae.*

Parameter	Value	References
Water temperature	15–20 °C	(Kestemont and Henrotte, 2015; Steenfeldt, 2015)
Light regime	8–12D:12–16L	(Steenfeldt, 2015)
Light Intensity	100 lux; dim	(Tielmann et al., 2017)
Salinity	2–10 ppt	(Imentai et al., 2019; Lund et al., 2019)
Water depth	1900–2000 mm	(Steenfeldt, 2015)
Water exchange	25–50% per hour	(Steenfeldt, 2015)

Although commercial feed preparations for aquaculture have been improved considerably, the best results are still obtained using live food for the initial exogenous feeding (People Le Ruyet et al., 1993). The transfer from yolk sac absorption to exogenous feeding is a critical stage in pikeperch larviculture. Live prey is provided to pikeperch larvae after mouth opening at ~5 DPH (Steenfeldt, 2015). *Artemia* nauplii is usually used for starter feeding and larval rearing through weaned to dried feed (Ronfeldt and Nielson, 2010; Steenfeldt, 2015). Beginning at 12–15 DPH and proceeding through 19–22 DPH, live prey is gradually replaced with a dried diet formulation (Policar et al., 2019). An optimized feeding regime helps minimize the more costly period of live feeding (Policar et al., 2019). Ostaszewska et al. (2005) reported satisfactory survival (52.4%) of pikeperch larvae using high quality commercial dried feeds, but these results may also be attributed to rearing conditions and/or to the nutritional value of the tested diets (Hamza et al., 2007).

Development of the larva digestive system

In teleosts, ontogenesis is not complete at hatching (Mani-Ponset et al., 1996). Newly hatched larvae are small (5 ± 0.5 mm total length) and transparent with closed mouth and anus (Ostaszewska, 2005). The liver and pancreas are undifferentiated (Ostaszewska, 2005). Larvae are not able to actively feed and are completely dependent on yolk reserves until mouth opening (Ostaszewska, 2005). The duration of endogenous feeding is primarily dependent on water temperature and generally extends to 6 DPH (Ostaszewska, 2005). The first few days following mouth opening represent a crucial period for pikeperch larval development (Ostaszewska, 2005), as it involves the synchronization of depletion of yolk reserves with the

first exogenous feeding (Mani-Ponset et al., 1996). At the onset of exogenous feeding, the pikeperch larva digestive system is still developing, and shows low digestive enzyme activity (Hamza et al., 2007). During the transfer from endogenous to exogenous feeding, the liver increases in size and becomes functional, while the pancreas shows exocrine activity (Hamza et al., 2015). The anterior intestine becomes distinct from the posterior intestine, separated by an intestinal valve. With growth, the number and size of intestine mucosal folds and the height of the brush border are increased (Ostaszewska, 2005). The height of the mucosal folds in the anterior intestine is between 24 μm to 36 μm, and, in the posterior, ranges from 23 μm to 29 μm. The height of brush border is ~2 μm (Ostaszewska, 2005). At 15 to 20 DPH (TL 8–13 mm; 210–273°D) (Hamza et al., 2015), the primary stomach develops between the oesophagus and the anterior intestine (Ostaszewska et al., 2005; Hamza, 2015). The length of the oesophagus increases, and the secretory activity and number of mucous cells increase. Lipids are hydrolysed to fatty acids in the intestine and monoglycerides are absorbed and stored as fat droplets in enterocytes.

Critical stages in pikeperch larviculture

Recently, significant efforts have been devoted to the optimization of management protocols in pikeperch larviculture, addressing major challenges to obtaining high quality larvae, including timing of initial exogenous feeding, swim bladder inflation, and cannibalism (Policar et al., 2019).

First exogenous feeding of pikeperch larvae

During intensive larviculture, the stage at which pikeperch shift from endogenous feeding to live prey is critical, and inadequate management may result in severe losses. Larval survival is generally at its lowest during the initial exogenous feeding stage at approximately 5 DPH (~0.5 mg), with survival estimated at around 27% under controlled conditions (Klein Breteler, 1989). The mouth opening of pikeperch larvae is small compared to other freshwater fish species (Hamza et al., 2015; Yanes-Roca et al., 2018), a drawback that can be mitigated by adjusting feed particle size. Exogenous feeding should commence at the time of mouth opening, around 5 DPH, while larvae can still utilize the yolk as an energy source (Hamza et al., 2015). Given the low survival rate, a great deal of effort has been allocated to the development of better early feeding techniques, with live feed yielding the best results (Ostaszewska et al., 2005; Steenfeldt, 2015).

Live feed modifies survival and growth rates in a variety of ways. It has been suggested that the movement and metabolic secretions of live prey act as a stimulus that induces a predatory response in the pikeperch. Following a period of live feeding, pikeperch need to be gradually transitioned to formulated feeds. This is usually achieved by co-feeding *Artemia* with a starter formulated diet, beginning at 12–15 DPH and continuing through 19–22 DPH (Policar et al., 2019). The enzymes of live nauplii and their movement in the intestinal tract can enhance digestion of co-fed formulated feeds (Kestemont et al., 1996; Kolkovski et al., 1997a; Kolkovski et al., 1997b).

Swim bladder inflation

Another critical stage of larval development is the inflation of the swim bladder. The swim bladder aids in maintaining buoyancy, and its inflation is usually concomitant with the onset of exogenous feeding. Pikeperch are physoclistic fish in which the connection between the

swim bladder and the digestive tract remains open only during the few days while the swim bladder is filled (Steenfeldt, 2015; Blecha et al., 2019). Larvae begin ingesting air to inflate the swim bladder at 5–8 DPH, depending on the water temperature, and the process continues to 14 DPH (Demska-Zakęś et al., 2003; Policar et al., 2019). To ensure proper swim bladder inflation, it is important to implement a spray system to break the surface tension of the water during the first two weeks post-hatching. Bacteria in a surface film can be transferred to the swim bladder, inducing inflammation or aerocystitis (Steenfeldt, 2015). Larvae with this condition are easily recognized by a non-horizontal position in the water and atypical body movements (Steenfeldt, 2015). In addition, abiotic factors implicated in inhibition of swim bladder inflation include temperature, depth, turbidity, salinity, photoperiod, light intensity, and tank background colour (Steenfeldt, 2015; Tielmann et al., 2017; Policar et al., 2019). Pikeperch with non-inflated swim bladders must allocate more energy to swimming and less to somatic growth (Steenfeldt, 2015), resulting in reduced growth rates, higher mortality, and increased cannibalism. Fish without inflated swim bladders are also more likely to exhibit skeletal deformations such as lordosis, a result of excessive swimming to compensate for the negative buoyancy (Steenfeldt, 2015). Fish with non-inflated swim bladders are removed from culture as soon as detected, due to reduced quality, higher production costs, and low market demand (Steenfeldt, 2015; Blecha et al., 2019; Policar et al., 2019).

Cannibalism

Cannibalistic behaviour is a serious obstacle to culture of pikeperch larvae (Ljubobratovic et al., 2015). Cannibalism can be divided into two categories: Type I involves partial ingestion, mainly tail-first, and occurs from 11 to 16–18 DPH; while type II begins at an older age with prey captured head-first (Kestemont et al., 2003; Policar et al., 2019). Type I exerts a lower impact on overall survival rates, while type II can result in up to 50% of the total mortality (Policar et al., 2019). Cannibalism is the biggest factor in loss of larvae and may severely reduce batch success among predatory species such as pikeperch (Kestemont et al., 2003). Cannibalism is probably related to a high larval growth rate, which is influenced by food type and availability (Kestemont et al., 2007). According to Ljubobratovic et al. (2015) the first signs of cannibalism occur around 15 DPH (256–322 °d). Steenfeldt et al. (2010) found significant size heterogeneity among larvae at 14 DPH, contributing to cannibalistic behaviour, while non-cannibals at the same stage grew at similar rates before 35 DPH. Conspecific prey fish were found to be smaller (65.4±6.7%) than the cannibals (Steenfeldt, 2015). Therefore, Kestemont et al. (2007) suggested weaning larvae at 12 DPH, which resulted in lower growth rate but also reduced cannibalism.

Type I cannibalism can be also be mitigated by adjusting environmental factors such as stocking density, light intensity, water temperature, nutrition, and feeding schedule (Baras et al., 2003). Type II cannibalism can be prevented by size-grading of fish during early stages (Steenfeldt, 2015). Szczepkowski et al. (2007) found that grading significantly increased survival by reducing cannibalism among pikeperch larvae. Król and Zakęś (2016) found that supplementation of artificial starter with crystalline L-tryptophan at 5, 10, and 20 $g.kg^{-1}$ during 28 days intensive culture of pikeperch larvae from 15 DPH resulted in a slight decrease in both types of cannibalism.

Live prey for pikeperch larvae

Artemia

Artemia nauplii are commonly used for initiating exogenous feeding and rearing until weaning (Rønfeldt and Nielsen, 2010; Steenfeldt, 2015). Newly hatched nauplii (Instar I stage) are the most common form used in hatcheries due to their small size and nutritional value (Sorgeloos et al., 2001). The Instar I stage is free-swimming and 0.4–0.5 mm in length (Drewes, 2006). Newly hatched nauplii contain yolk material, which remains the main energy source until the development of the digestive tract (Drewes, 2006). Therefore, the nutritional content of cysts and instar stage I nauplii are not dependent on diet or environmental conditions, but are primarily affected by the parent diet (Støttrup and McEvoy, 2003). Later stages of *Artemia* are more difficult for fish larvae to capture, given their larger size and higher motility, consequently reducing larval growth (Bengtson et al., 1991; Sorgeloos et al., 2001). Also, later stages have lower nutritional value and need to be provided with feed, elevating costs.

Artemia fatty acid composition may vary according to strain and diet (Zhukova et al., 1998; Sorgeloos et al., 2001). Saturated fatty acid 16:0; the monosaturated fatty acids 16:1(n-7) and 18:1(n-9); and polyunsaturated fatty acids 18:3(n-3), 18:2(n-6), and 20:5(n-3) make up approximately 80% of the total fatty acid content (Bengston et al., 1991). However, *Artemia* is deficient in the essential fatty acids arachidonic acid (ARA, 20:4n-6), docosahexaenoic acid (DHA, 22:6n-3), and eicosapentanoic acid (EPA, 20:5n-3). *Artemia* can be nutritionally enriched (Chakraborty et al., 2007), but enrichment may decrease survival up to 82% (Harel et al., 2002; Figueiredo et al., 2009). In addition, larvae with small gape size tend to have difficulty with enriched *Artemia*, which are larger due to the enhanced nutrition (Figueiredo et al., 2009).

Rotifers

Rotifers were identified by a Japanese aquaculturist in the early 1960s as a suitable starter diet in marine fish larviculture (Ito, 1960; Lubzens et al., 1989) and are now common initial feed in fish hatcheries worldwide, with advantages including small size, slow swimming velocity, ease of culture, and potential for enrichment with fatty acids, vitamins, and therapeutics (Table 2) (Lie et al., 1997; Dhert et al., 2001; Lubzens et al., 2003; Odo et al., 2015; Yanes-Roca et al., 2020).

Since rotifers are non-selective feeders, their nutritional value is highly affected by their diet, and fatty acid enrichment is easily administered (Dhert et al., 2001; Lubzens et al., 2003). Enrichment of rotifers can be accomplished with algae, primarily *Chlorella, Pavlova* sp., *Isochrysis* spp., *Nannochloropsis salina*, *Nannocholropsis* spp., and *Phaeodactylum tricornutum*; lipid emulsions; and baker's yeast (Lie et al., 1997; Zhukova et al., 1998). The most common EPA and ARA enrichments employ *Nannocholropsis* spp. or *Chaetoceros* spp. and, for DHA, Isochrysis galbana or Pavlova spp. (Lubzens et al., 2003; Chakraborty et al., 2007; Hamre, 2016). It is also possible to enrich rotifers using oil emulsions before feeding. Without enrichment, rotifers deplete the valuable nutrients in their gut and their lipid/nutrient balance is impaired (Dhert et al., 2001). Rotifer enrichment with baker's yeast yields lower vitamin C, free fatty acids, and triacylglycerol content, but is common in industrial fish farms, due to the lower cost.

Table 2. *Characteristics of Artemia and rotifers Branchionus plicatilis and Branchionus rotundiformes Adapted from Lubzens et al., 2003.*

Characteristic	*Rotifers*	*Artemia*
Size	100–340 µm, depending on species and development stage	422–517 µm, depending on species and development stage
Body shape	Circular and flat, without spines	Segmented with thoracopodes
Salinity	Tolerance to a wide range of salinities	Requires high salinity
Reproduction	Sexual reproduction or parthenogenesis	Sexual reproduction or parthenogenesis
Maturation time	18–72 hours	3–6 weeks
Supply	Reliable, depending on culture facilities	Harvested from natural resources and farming
Nutritional quality	Flexible	Flexible
Vectors of parasites and predation on fish larvae	Minimal	Minimal
Vehicles for therapeutic agents and probiotics	Feasible	Feasible

Rotifer culture

The euryhaline rotifer species *Brachionus plicatilis* is widely used as a live food in small-scale laboratory experiments and in commercial hatcheries (Lubzens et al., 2003; Yin and Zhao, 2008; Hamre, 2016). Batch and continuous culture systems are used for its mass production. Since batch culture was developed in 1964 (Hirata, 1964), it has remained the most common method of rotifer culture with slight modifications (Dhert et al., 2001). Under the batch method, rotifers can be cultured at a constant volume by increasing rotifer density or at a constant density by increasing volume (Dhert et al., 2001). When the density or volume of the rearing tank reaches its maximum, the entire culture is harvested, and rotifers are used to feed larvae. A portion of the harvest is used to initiate another culture (Hagiwara et al., 2017). Despite the simplicity of the method, drawbacks exist, including unstable physico-chemical water parameters, low efficiency with respect to labour and utilization of infrastructure, which result in unpredictable culture conditions and low production yield with high costs (Dhert et al., 2001).

In a recirculating system, rotifer culture conditions of temperature, pH, and oxygen supply can be fully controlled, with the potential to manipulate physiological and nutritional quality for continuous culture. These culture systems use filters, protein skimmers, and ozone to maintain water quality (Hagiwara et al., 2017) and can be maintained for longer than batch cultures. Consequently, hatcheries use small scale recirculating systems to reduce production costs (Hagiwara et al., 2017).

Despite the advance of culture techniques, maintenance of the rotifer culture can present challenges, and factors such as poor water quality and bacterial infestations may cause its collapse (Hagiwara et al., 2017). To prevent colony collapse, water quality is monitored by measuring temperature, pH, ammonia, salinity, and viscosity (Hagiwara et al., 2017). The optimal rearing temperature for *B. plicatilis* is 20–25 °C with salinity from 10‰ to 25‰ (Lubzens et al., 2001).

The increase in culture temperature, pH, and salinity raises the proportion of toxic un-ionized ammonia in the total ammonia (Lubzens et al., 2003). Bacteria can cause mortality

and/or low growth in rotifer cultures, and infected rotifers may have a negative impact on fish larvae (Dhert et al., 2001). Measures to limit or eliminate the culture bacterial load include application of disinfectants and advanced oxidation processes based on their production of hydroxyl radicals (Poblete-Chávez et al., 2016), but their use is not highly effective and can create toxic by-products or compounds (Dhert et al., 2001). The application of antibiotics may have more effective results; however they can interact with the larva gut microflora, and their regular use can promote development of resistant strains and have adverse environmental impacts (Dhert et al., 2001). Bacterial populations can be controlled by introducing selected non-pathogenic species to compete with, and limit the proliferation of, pathogenic bacteria.

Water conditions are critical to rotifer culture. In particular, oxygen must be supplied to counteract the shortage of dissolved oxygen that accompanies high rotifer density. The oxygen gas supplied to the culture releases carbon dioxide from the water, consequently increasing culture pH. A low temperature is recommended for B. plicatilis culture to increase stability by reducing the bacterial proliferation rate.

Destabilization in rotifer cultures can also result from the presence of the ciliate Euplotes. According to Ushilo et al. (1998) Euplotes spp. compete with B. plicatilis for diet microbes, and active algae *Nannocholropsis oculata* is an effective food for preventing its proliferation in rotifer cultures.

Aims and objectives

Given the crucial role of live feed for the development of the digestive system and growth of pikeperch larvae, the goal of this research was to determine the impact of rotifer *Brachionus plicatilis* on the growth performance and digestive system development of pikeperch *Sander lucioperca* L. larvae during the initial exogenous feeding period.

The primary objectives of the research comprising this thesis were to

1. **Determine effects of three diets: *Artemia*, rotifers, and mixed *Artemia*/rotifers on survival and growth of pikeperch larvae during initial exogenous feeding.**
2. **Characterise the effects of feeding regimes using rotifers *Brachionus plicatilis* as the first diet on survival, growth, and digestive system development of pikeperch larvae.**
3. **Ascertain optimal salinity for rearing pikeperch larvae using euryhaline rotifers *Brachionus plicatilis*.**
4. **Determine the optimal rotifers prey density at the beginning of exogenous feeding of pikeperch larvae.**

References

Badiola, M., Mendiola, D., Bostock, J., 2012. Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. Aquacult. Eng. 51, 26–35.

Baras, E., Kestemont, P., Mélard, C., 2003. Effect of stocking density on the dynamics of cannibalism in sibling larvae of *Perca fluviatilis* under controlled conditions. Aquaculture 219, 241–255.

Bengtson, D.A., Léger, P., Sorgeloos, P., 1991. Use of *Artemia* as a food source for aquaculture. *Artemia* Biology 11, 255–285.

Blecha, M., Malinovskyi, O., Veselý, L., Křišťan, J., Policar, T., 2019. Swim bladder inflation failure in pikeperch (*Sander lucioperca*) larvae in pond culture. Aquacult. Int. 27, 983–989.

Chakraborty, R.D., Chakraborty, K., Radhakrishnan, E., 2007. Variation in fatty acid composition of *Artemia salina* nauplii enriched with microalgae and baker's yeast for use in larviculture. J. Agr. Food Chem. 55, 4043–4051.

Chapman, P.M., Popham, J.D., Griffin, J., Leslie, D., Michaelson, J., 1987. Differentiation of physical from chemical toxicity in solid waste fish bioassays. Water, Air, and Soil Pollution 33, 295–308.

Chen, S., Ling, J., Blancheton, J.-P., 2006. Nitrification kinetics of biofilm as affected by water quality factors. Aquacult. Eng. 34, 179–197.

Commission, E., 2013. Strategic Guidelines for the sustainable development of EU aquaculture: Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. European Commission.

Demska-Zakęś, K., Kowalska, A., Zakęś, Z., 2003. The development of the swim bladder of pikeperch *Sander lucioperca* (L.) reared in intensive culture. Fisheries & Aquatic Life 11, 45–55.

Dhert, P., Rombaut, G., Suantika, G., Sorgeloos, P., 2001. Advancement of rotifer culture and manipulation techniques in Europe. Aquaculture 200, 129–146.

Drewes, C., 2006. Quantitative investigations of hatching in brine shrimp cysts, Tested Studies for Laboratory Teaching, Volume. Proceedings of the 27th Workshop/Conference of the Association for Biology Laboratory Education (ABLE). pp. 299–312.

Espinal, C.A., Matulić, D., 2019. Recirculating aquaculture technologies, Aquaponics Food Production Systems, Springer, pp. 35–76.

FAO, 2020. Fishery Statistical Collections. Global Aquaculture Production. http://www.fao.org/figis/servlet/SQServlet?file=/usr/local/tomcat/8.5.16/figis/webapps/figis/temp/hqp_3625207438791431307.xml&outtype=html. Visited 1 March 2020

FAO, 2018. Aquaculture Department. The state of world fisheries and aquaculture. Rome: FAO; 2010.

Figueiredo, J., van Woesik, R., Lin, J., Narciso, L., 2009. *Artemia franciscana* enrichment model – How to keep them small, rich and alive? Aquaculture 294, 212–220.

Gjedrem, T., Robinson, N., Rye, M., 2012. The importance of selective breeding in aquaculture to meet future demands for animal protein: a review. Aquaculture 350, 117–129.

Hagiwara, A., Kim, H.-J., Marcial, H., 2017. Mass culture and preservation of *Brachionus plicatilis* sp. complex, Rotifers, Springer, pp. 35–45.

Hamre, K., 2016. Nutrient profiles of rotifers (*Brachionus* sp.) and rotifer diets from four different marine fish hatcheries. Aquaculture 450, 136–142.

Hamza, N., Mhetli, M., Kestemont, P., 2007. Effects of weaning age and diets on ontogeny of digestive activities and structures of pikeperch (*Sander lucioperca*) larvae. Fish Physiol. Biochem. 33, 121–133.

Hamza, N., Ostaszewska, T., Kestemont, P., 2015. Development and functionality of the digestive system in percid fishes early life stages, Biology and culture of percid fishes, Springer, pp. 239–264.

Harel, M., Koven, W., Lein, I., Bar, Y., Behrens, P., Stubblefield, J., Zohar, Y., Place, A.R., 2002. Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. Aquaculture 213, 347–362.

Hibbeln, J.R., Davis, J.M., Steer, C., Emmett, P., Rogers, I., Williams, C., Golding, J., 2007. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. The Lancet 369, 578–585.

Hirata, H., 1964. Cultivation of live food organisms at the Yashima Station. Saibai-Gyogyo 2, 4.

Imentai, A., Yanes-Roca, C., Steinbach, C., Policar, T., 2019. Optimized application of rotifers *Brachionus plicatilis* for rearing pikeperch *Sander lucioperca* L. larvae. Aquacult. Int. 27, 1137–1149.

Ito, T., 1960. On the culture of mixohaline rotifer *Brachionus plicatilis* OF Muller in the sea water. Report of Faculty of Fisheries, Prefectual University of Mie 3, 708–740.

Kestemont, P., Henrotte, E., 2015. Nutritional requirements and feeding of broodstock and Early life stages of Eurasian perch and pikeperch, Biology and Culture of Percid Fishes, Springer, pp. 539–564.

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

Kestemont, P., Mélard, C., Fiogbe, E., Vlavonou, R., Masson, G., 1996. Nutritional and animal husbandry aspects of rearing early life stages of Eurasian perch *Perca fluviatilis*. J. Appl. Ichthyol. 12, 157–165.

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

Kinyage, J.P.H., Pedersen, P.B., Pedersen, L.-F., 2019. Effects of abrupt salinity increase on nitrification processes in a freshwater moving bed biofilter. Aquacult. Eng. 84, 91–98.

Klein Breteler, J.G.P., 1989. Intensive culture of pikeperch fry with live food. In: DePauw, N. et al. (Eds), Aquaculture-A Biotechnology in Progress. Vol. 1. European Aquaculture Society, Bredene, pp. 203–207.

Kolkovski, S., Koven, W., Tandler, A., 1997a. The mode of action of *Artemia* in enhancing utilization of microdiet by gilthead seabream Sparus aurata larvae. Aquaculture 155, 193–205.

Kolkovski, S., Tandler, A., Izquierdo, M., 1997b. Effects of live food and dietary digestive enzymes on the efficiency of microdiets for seabass (*Dicentrarchus labrax*) larvae. Aquaculture 148, 313–322.

Krauss, R.M., Eckel, R.H., Howard, B., Appel, L.J., Daniels, S.R., Deckelbaum, R.J., Erdman Jr., J.W., Kris-Etherton, P., Goldberg, I.J., Kotchen, T.A., 2000. AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 102, 2284–2299.

Król, J., Zakęś, Z., 2016. Effect of dietary L-tryptophan on cannibalism, survival and growth in pikeperch *Sander lucioperca* (L.) post-larvae. Aquac. Int. 24, 441–451.

Kroupova, H., Machova, J., Svobodova, Z., 2005. Nitrite influence on fish: a review. Vet. Med. 50, 461–471.

Lappalainen, J., Dorner, H., Wysujack, K., 2003. Reproduction biology of pikeperch (*Sander lucioperca* (L.)) – a review. Ecol. Freshw. Fish. 12, 95–106.

Lie, O., Haaland, H., Hemre, G.-I., Maage, A., Lied, E., Rosenlund, G., Sandnes, K., Olsen, Y., 1997. Nutritional composition of rotifers following a change in diet from yeast and emulsified oil to microalgae. Aquacult. Int. 5, 427–438.

Ljubobratović, U., Péter, G., Alvestad, R., Horváth, Z., Rónyai, A., 2019. Alcalase enzyme treatment affects egg incubation and larval quality in pikeperch (*Sander lucioperca*). Aquacult. Int. 27, 917–929.

Lubzens, E., Tandler, A., Minkoff, G., 1989. Rotifers as Food in Aquaculture. Hydrobiologia 186, 387–400.

Lubzens, E., Zmora, O., Stottrup, J., McEvoy, L., 2003. Production and nutritional value of rotifers. Live Feeds in Marine Aquaculture, 300–303.

Lund, I., Rodríguez, C., Izquierdo, M.S., El Kertaoui, N., Kestemont, P., Reis, D.B., Dominguez, D., Pérez, J.A., 2019. Influence of salinity and linoleic or α-linolenic acid based diets on ontogenetic development and metabolism of unsaturated fatty acids in pike perch larvae (*Sander lucioperca*). Aquaculture 500, 550–561.

Mamcarz, A., Kucharczyk, D., Kujawa, R., Skrzypczak, A., 1997. Influence of fish density on survival, growth and cannibalism development of pikeperch (*Stizostedion lucioperca* L.) larvae. In Island aquaculture and tropical aquaculture: Proc 2nd Int Workshop Aquacult Percid Fish. European Aquaculture Society, Oostende, Belgium, pp. 343–346.

Mani-Ponset, L., Guyot, E., Diaz, J., Connes, R., 1996. Utilization of yolk reserves during post-embryonic development in three teleostean species: the sea bream *Sparus aurata*, the sea bass *Dicentrarchus labrax*, and the pike-perch *Stizostedion lucioperca*. Marine Biology 126, 539–547.

Martins, C., Eding, E.H., Verdegem, M.C., Heinsbroek, L.T., Schneider, O., Blancheton, J.-P., d'Orbcastel, E.R., Verreth, J., 2010. New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquacult. Eng. 43, 83–93.

Molnár, T., Hancz, C., Bódis, M., Müller, T., Bercsényi, M., Horn, P., 2004. The effect of initial stocking density on growth and survival of pike-perch fingerlings reared under intensive conditions. Aquacult. Int. 12, 181–189.

Mozaffarian, D., Rimm, E.B., 2006. Fish intake, contaminants, and human health: evaluating the risks and the benefits. Jama 296, 1885–1899.

Odo, G., Agwu, J., Iyaji, F., Madu, J., Ossai, N., Allison, L., 2015. Mass production of rotifer (*Branchionus calyciflorus*) for aquaculture in south-eastern Nigeria. Int. J. Fish. Aquaculture 7, 151–159.

Ostaszewska, T., 2005. Developmental changes of digestive system structures in pike-perch (*Sander lucioperca* L.). Electronic Journal of Ichthyology 2, 65–78.

Overton, J.L., Toner, D., Policar, T., Kucharczyk, D., 2015. Commercial production: factors for success and limitations in European percid fish culture, Biology and culture of percid fishes, Springer, pp. 881–890.

Parletta, N., Zarnowiecki, D., Cho, J., Wilson, A., Bogomolova, S., Villani, A., Itsiopoulos, C., Niyonsenga, T., Blunden, S., Meyer, B., 2019. A Mediterranean-style dietary intervention supplemented with fish oil improves diet quality and mental health in people with depression: A randomized controlled trial (HELFIMED). Nutr. Neurosci. 22, 474–487.

Pedersen, L.-F., Pedersen, P.B., Sortkjær, O., 2007. Temperature-dependent and surface specific formaldehyde degradation in submerged biofilters. Aquacult. Eng. 36, 127–136.

People Le Ruyet, J., Alexandre, J.C., Thébaud, L., Mugnier, C., 1993. Marine fish larvae feeding: formulated diets or live prey? J. World Aquacult. Soc. 24, 211–224.

Pickering, A.D., Pottinger, T.G., 1989. Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish Physiol. Biochem. 7, 253–258.

Poblete-Chávez, R., Cortés-Pizarro, E., Rojas-Araya, R., 2016. Treatment of seawater for rotifer culture uses applying adsorption and advanced oxidation processes. Lat. Am. J. Aquat. Res. 44, 779–791.

Policar, T., Adámek, Z., 2013. Preface. Aquacult. Int. 21, 737–738.

Policar, T., Blecha, M., Krist'an, J., Mraz, J., Velisek, J., Stara, A., Stejskal, V., Malinovskyi, O., Svacina, P., Samarin, A.M., 2016a. Comparison of production efficiency and quality of differently cultured pikeperch (*Sander lucioperca* L.) juveniles as a valuable product for ongrowing culture. Aquacult. Int. 24, 1607–1626.

Policar, T., Křišťan, J., Blecha, M., Vaniš, J., 2016b. Adaptation and culture of pikeperch (*Sander lucioperca* L.) juveniles in recirculating aquaculture system (RAS). Edition of Methodics, FFPW USB, no. 141, 38 pp.

Policar, T., Schaefer, F.J., Panana, E., Meyer, S., Teerlinck, S., Toner, D., Żarski, D., 2019. Recent progress in European percid fish culture production technology – tackling bottlenecks. Aquacult. Int. 27, 1151–1174.

Rønfeldt, J., Nielsen, J., 2010. Filling of gas bladder, growth and the survival in pikeperch larvae (*Sander lucioperca*) in intensive aquaculture. M.Sc. thesis, University of Copenhagen 350–351, 303–323.

Ruxton, C., 2011. The benefits of fish consumption. Nutrition Bull. 36, 6–19.

Schaefer, F.J., Tielmann, M., Overton, J.L., Krüger, A., Wuertz, S., Kloas, W., Schulz, C., Meyer, S., 2019. Fate or independency: is batch-specific larval performance determined by egg traits? A case study in farmed pikeperch (*Sander lucioperca*). Aquacult. Int. 27, 957–969.

Schreier, H.J., Mirzoyan, N., Saito, K., 2010. Microbial diversity of biological filters in recirculating aquaculture systems. Curr. Opin. Biotechnol. 21, 318–325.

Sorgeloos, P., Dhert, P., Candreva, P., 2001. Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. Aquaculture 200, 147–159.

Spiliotopoulou, A., Rojas-Tirado, P., Chhetri, R.K., Kaarsholm, K.M., Martin, R., Pedersen, P.B., Pedersen, L.-F., Andersen, H.R., 2018. Ozonation control and effects of ozone on water quality in recirculating aquaculture systems. Water Res. 133, 289–298.

Steenfeldt, S., 2015. Culture methods of pikeperch early life stages. Biology and Culture of Percid Fishes, Springer, pp. 295–312.

Stepien, C.A., Haponski, A.E., 2015. Taxonomy, distribution, and evolution of the Percidae, Biology and Culture of Percid Fishes, Springer, pp. 3–60.

Støttrup, J., McEvoy, L. (Eds), 2003. Live Feeds in Marine Aquaculture, Blackwell Science, Oxford, 318 pp.

Summerfelt, S.T., Sharrer, M.J., Tsukuda, S.M., Gearheart, M., 2009. Process requirements for achieving full-flow disinfection of recirculating water using ozonation and UV irradiation. Aquacult. Eng. 40, 17–27.

Szczepkowski, M., Zakęś, Z., Szczepkowska, B., & Piotrowska, I., 2011. Effect of size sorting on the survival, growth and cannibalism in pikeperch (*Sander lucioperca* L.) larvae during intensive culture in RAS. Czech J. Anim. Sci. 56, 483–489.

Szkudlarek, M., Zakęś, Z., 2007. Effect of stocking density on survival and growth performance of pikeperch, Sander *lucioperca* (L.), larvae under controlled conditions. Aquacult. Int. 15, 67–81.

Tielmann, M., Schulz, C., Meyer, S., 2017. The effect of light intensity on performance of larval pike-perch (*Sander lucioperca*). Aquacult. Eng. 77, 61–71.

Ushilo, M.J., Hino, A., Maeda M., 1998. A growth rate and feed habit analysis of the ciliate Euplotes sp. contaminating a mass culture of the rotifer *Brachionus plicatilis*. Microb. Environ. 13, 85–92.

Wang, N., Xu, X.L., Kestemont, P., 2009. Effect of temperature and feeding frequency on growth performances, feed efficiency and body composition of pikeperch juveniles (*Sander lucioperca*). Aquaculture 289, 70–73.

Yanes-Roca, C., Mráz, J., Born-Torrijos, A., Holzer, A.S., Imentai, A., Policar, T., 2018. Introduction of rotifers (*Brachionus plicatilis*) during pikeperch first feeding. Aquaculture 497: 260–268.

Yanes-Roca, C., Holzer, A., Mraz, J., Veselý, L., Malinovskyi, O., Policar, T., 2020a. Improvements on live feed enrichments for pikeperch (*Sander lucioperca*) larval culture. Animals 10, 401.

Yanes-Roca, C., Leclercq, E., Vesely, L., Malinovskyi, O., Policar, T., 2020. Use of lactic acid bacteria during pikeperch (*Sander lucioperca*) larval rearing. Microorganisms 8, 238.

Yin, X.W., Zhao, W., 2008. Studies on life history characteristics of *Brachionus plicatilis* OF Müller (Rotifera) in relation to temperature, salinity and food algae. Aquat. Ecol. 42, 165–176.

Zhukova, N.V., Imbs, A.B., Yi, L.F., 1998. Diet-induced changes in lipid and fatty acid composition of *Artemia* salina. Comp. Biochem. Physiol. B-Biochem. Mol. Biol. 120, 499–506.

CHAPTER 2

INTRODUCTION OF ROTIFERS (*BRACHIONUS PLICATILIS*) DURING PIKEPERCH FIRST FEEDING

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Introduction of rotifers *(Brachionus plicatilis)* during pikeperch first feeding

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ABSTRACT

The influence of rotifers (*Brachionus plicatilis*) on pikeperch performance during first feeding was investigated. Significant differences between treatments were found in length, body weight, survival, feed consumption, fatty acid composition, and RNA/DNA ratio. Pikeperch larvae (*Sander lucioperca*) were reared under three different diets (artemia nauplii (A), artemia nauplii/rotifers (B), and rotifers (C)), during the first 17 days post hatch (dph). Final performance parameters in total length, myomere height (MH), body weight, eye diameter, stomach fullness, survival, fatty acid composition, and RNA/DNA ratio were measured and compared among treatments.

In terms of growth (length, body weight, and myomere height) and stomach fullness, the larvae from treatment B excelled over the other two treatments. Survival and key fatty acids such as docosahexaeonic acid (DHA) were higher in treatment C.

Results suggest that the most favorable diet during larval pikeperch first feeding is a mixed diet (rotifers/artemia). This feeding method can significantly increase efficiency of pikeperch larval culture on a commercial scale. Yet, more accurate weaning protocols of this diet during the first 12–17 dph need to be developed to further improve larval performance.

1. Introduction

Pikeperch (*Sander lucioperca*), is a fresh and brackish water fish, commonly found in Central and Eastern Europe, as well as in large areas of Northern Asia (Fao, 2013). Full-grown fish are highly demanded by the gastronomy industry and by the recreational angling community (Kestemont et al., 2015). Pikeperch is currently one of the new species targeted by several international initiatives looking for aquaculture diversification within the European Union, due to its market value and fast growth rate in recirculation systems (RAS) (Dalsgaard et al., 2013; Schäfer, 2016; Wang et al., 2009; Watson et al., 2008). Although, the bulk of pikeperch production currently comes from wild fisheries, production in RAS systems is increasing (Fao, 2013).

Recently, this species has been the subject of intense scientific study in both Central (Czech Republic, Hungary, Poland) and Western Europe (Belgium, Finland, France, Germany). Research is focused on developing methods for intense pikeperch aquaculture production, mainly in RAS (FAO, 2016).

One of the bottlenecks in this field remains the low effectiveness and high costs of rearing larval pikeperch in RAS. The nutritional requirements of juvenile pikeperch (quality and quantity of feed) have been largely identified (Kestemont et al., 2007; Nyina-wamwiza et al., 2005; Schulz et al., 2007).

The expansion of pikeperch culture depends on the development of culture in RAS; little or no expansion in the farming of pikeperch in ponds is anticipated (Steffens et al., 1996). This is supported by increasing consumer demand, concomitant with decreasing catches of this species in open waters (FAO, 2016). One of the limiting factors is the possibility of producing sufficient quantities of juveniles (FAO, 2016). Improving methods for artificial reproduction and rearing larval pikeperch in RAS are the key issue (Kestemont et al., 2015).

Recent developments in husbandry have provided improvements in growth, survival, and deformities reduction (Divanach et al., 1997; Hilge and Steffens, 1996; Kestemont et al., 2007; Policar et al., 2016; Policar et al., 2013; Szkudlarek and Zakęś, 2007; Wang et al., 2009),

Abbreviations: dph, Days post hatch; RAS, Recirculation aquaculture systems; TL, Total length; BW, Body weight; MH, Myomere height; ED, Eye diameter; SF, Stomach fullness; FA, Fatty acids; LA, Linoleic acid; ALA, Alpha linoleic acid; ARA, Arachidonic acid; EPA, Eicosapentaenoic; RAS, Recirculating aquaculture system; DHA, Docosahexaeonic acid; FFPW, Faculty of fisheries and protection of waters; USB, University of South Bohemia; LMM, Linear mixed model; GLMM, Generalized linear mixed models; EFA, Essential fatty acids

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making it a profitable business.

A key factor for larval husbandry optimization is live feed improvements. The introduction of rotifers (*Brachionus plicatilis*) marked the first successes in mass larval rearing of several marine species of economic value, such as grey mullet (*Mugil cephalus*) (Nash and Kuo, 1975), sole (*Solea solea*) (Fuchs, 1978; Howell, 1997), gilthead seabream (*Sparus aurata*) (Person-LeRuyet and Verillaud, 1980; Tandler and Helps, 1985) and sea bass (*Dicentrarchus labrax*) (Girin, 1975), which have similar small mouth gape and primitively developed digestive system as pikeperch.

Several characteristics make rotifers suitable as live food in mariculture: (1) Small size, ranging from 60 μm–1000 μm; (2) Slow swimmers; (3) Fast reproductive cycle, allowing mass production; (4) They can be enriched with the adequate nutritional supplements. Standardized mass culture techniques insure the supply of rotifers required to raise fish species. Rotifers lack of long chain highly unsaturated fatty acids (HUFA)(Cowey et al., 2017; Dendrinos and Thorpe, 1987; Gatesoupe, 1990; Owen et al., 1975; Watanabe' et al., 1983). Yet, these species are regarded as living food capsules for transferring nutrients to fish larvae. These nutrients include highly unsaturated fatty acids (mainly 20: 5 n-3 and 22: 6 n-3) essential for survival of marine fish larvae (Lubzens et al., 1989). Current data show that larvae at first feeding seem to possess the necessary complement of enzymes for digesting their prey (Govoni et al., 1986; Hamza et al., 2008). The other most commonly used starter feed, artemia nauplii, is currently used for both Eurasian perch (*Perca fluviatilis*) and pikeperch larvae (Kestemont and Henrotte, 2015). Artemia is the most widely used aquaculture live organism for marine larvae, primarily because they are readily available, very convenient to use (Narciso et al., 1999; Navarro et al., 1992) and of small size (500 μm). The biochemical composition of artemia is regarded important for optimizing larval nutrition for survival and growth of aquaculture species such as finfish and shellfish. However, Artemia nauplii are an incomplete food source for larvae of marine finfish and crustaceans, because of their paucity of essential n3 and n6 polyunsaturated fatty acids (PUFAs), such as DHA (0.1), EPA (3.18), ARA (2.3) and LA (8.5) (Chakraborty et al., 2007).

1.1. Objective

The research objective of this study was to optimize larval survival, growth and fitness of pikeperch during the first feeding by using rotifers as the first live prey instead of artemia nauplii.

2. Materials and methods

Pikeperch broodstock (TL = 515 ± 38 mm and W = 1220 ± 200 g) held under controlled conditions (Blecha et al., 2015) in recirculating aquaculture system (RAS) at the University of South Bohemia, Facility of Fisheries and Protection of Waters, Czech Republic (USB, FFPW) were used for spawning and egg production in this study. Final oocyte and sperm maturation was performed under 15 h:9 h light:darkness regime with a light intensity of 100 lx, water temperature of 15 ± 0.5 °C (Blecha et al., 2016; Blecha et al., 2015; Samarin et al., 2015) and synchronized with intramuscular hormonal injection with a dose of 500 IU kg^{-1} of Human Chorionic Gonadotropin (hCG; Chorulon, Intervet International B·V.) according to Křištan et al., 2013 and Blecha et al. (2016). After hormonal treatment, both sexes were separated until gamete stripping. Forty eight hours after the hormonal treatment, all broodstock were anesthetized with clove oil (Dr. Kulich Pharma Ltd., Czech Republic) before each manipulation at a concentration 30 mgl^{-1}(Křištan et al., 2014). Artificial egg fertilization was done respecting physiological requirements of pikeperch gametes and according to optimized protocol published by (Kristan et al., 2018). Fertilized eggs were treated with Alcalase® enzyme (*Bacillus licheniformis*, Merck EC 3.4.21.14, Darmstadt, Germany) in concentration 1.5 $ml\,l^{-1}$ of 1% NaCl solution (Křištan et al., 2016). Non-stick fertilized eggs were incubated in Zug jars (volume 10 l) at a water temperature of 16 ± 0.5 °C, during 7 days when hatching occurred at a rate of 83.5 ± 2.5%. Three days old larvae (100 per l) were stocked into each 2-l larval rearing tanks (n = 12). Water quality parameters in the RAS were monitored daily; average values were: salinity (3 ± 0.5 ppt), dissolved oxygen (8.0 ± 1 mgl^{-1}), and temperature (17.1 ± 0.2 °C). NH_3 (0.21 ± 0.05 mgl^{-1}), NO_2 (0.02 ± 0.01 mgl^{-1}), NO_3 (0.10 ± 0.02 mgl^{-1}), was measured every 3 days.

Table 1
Experiment husbandry schedule. Amount of daily feed offered, shading concentration (*Nannochloropsis occulata*) and recirculation flow changes with time are shown. Days in which sampling took places, as well as, water quality (NH_3, NO_3 and NO_2) measurements are also indicated.

DPH	Daily feed art-(art/rots)-rot/ml	Shading (cells/ml)	Flow (ml/min)
3	No feeding	0	100
4	10-(5/5)-10	300,000	100
5	10-(5/5)-10	300,000	100
6	10-(5/5)-10	300,000	100
7	10-(5/5)-10	300,000	100
8	14-(7/7)-14	400,000	160
9	14-(7/7)-14	400,000	160
10	14-(7/7)-14	400,000	160
11	14-(7/7)-14	400,000	160
12	14-(7/7)-14	500,000	200
13	14-(7/7)-14	500,000	200
14	14-(7/7)-14	500,000	200
15	16-(8/8)-16	500,000	250
16	16-(8/8)-16	750,000	250
17	16-(8/8)-16	750,000	250

Three diet treatments were tested: artemia only (A), mixed diet of rotifers/artemia (B), and rotifers only (C). Live feed (rotifers, *Brachionus plicatilis,* and artemia) was added three times per day (08:00, 11:30 and 15:30) starting at 5 dph. Treatment A was fed with artemia only at an initial density of 10 individuals per ml. Treatment B was fed an initial mixed diet (5 rotifers & 5 artemia ml^{-1}) and treatment C was fed only rotifers (10 individuals per ml). Feeding densities were steadily increased based on residual counts, performed prior to each feeding (Table 1). In addition, algal paste (Nannochloropsis 3600, Reed Mariculture, Campbell, CA) was added to the larval tanks beginning at 4 dph and maintained at a concentration of 500,000 cells ml^{-1}. By 17 dph, rotifer density reached 16 rotifers ml^{-1} in treatment C, 8 rotifers ml^{-1} and/ 8 artemia ml^{-1} in treatment B and 16 artemia ml^{-1} in treatment A. Live feed culture for the trial was done onsite. Rotifers (average size of 280 μm) were produced following a batch culture protocol fed with *Nannochloropsis occulata* (Nanno 3600, Reed Mariculture, Campbell, CA) at a rate of 1 ml of paste per liter of culture twice a day. Artemia nauplii (Micro artemia cysts, Ocean Nutrition™, Belgium) were hatched (12h) onsite and fed right away; artemia nauplii average sized was 430 μm.

Flow rates started at 100 ml min^{-1} and increased with time (Table 1). Prior to each feeding, flow was stopped and re-started 2 h after, in order to improve larval feeding efficiency.

Seven days after treatment initiation (11 dph), 40 larvae per treatment (10 per tank) were collected using a 300-μm-diameter mesh, and data total length (TL), body weight (BW), myomere height (MH), eye diameter (ED), stomach fullness (SF) and air bladder inflation was recorded using an Olympus BX41 microscope fitted with a Canon-72 digital camera and the Olympus cellSens imaging software (version 1.3).

Prior to the appearance of cannibalism and light photosensitivity, the trial was terminated seventeen days post hatching. All larvae were accounted for and samples were collected to access larvae performance. Sixty larvae per treatment were collected for fatty acids (FA) analysis, shock frozen and stored at −80 °C. The diets (prey organisms) themselves were also analyzed (3 mg) for FA composition. Thirty larvae per treatment were fixed in RNA later to determine RNA-DNA ratios. After penetration of the larvae with the RNA preservative for 3 h at room

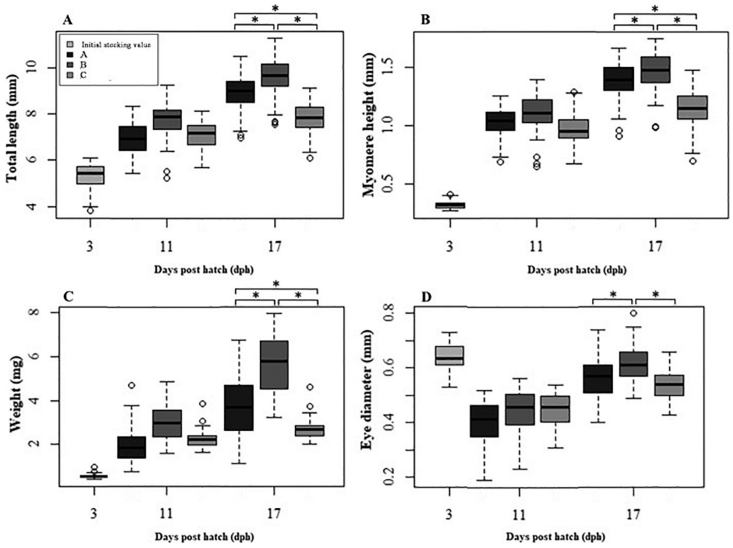

Fig. 1. Larval growth parameters (mm) from three diet treatments at days 11 (n = 40) and 17 dph (n = 75). A: Total length, B: Myomere height, C: body weight, D: Eye diameter. Dots show mean values and whiskers indicate standard error. Statistically significant differences between data from 17 dph are marked with an asterisk.

temperature, they were transferred to −80 °C freezer for storage. Another 75 larvae per treatment were collected for final morphometric analysis (TL, BW, MH, ED, SF), just like at 11 dph.

2.1. Fatty acid analysis

All frozen samples were analyzed at the USB, FFPW, Laboratory of Nutrition. Lipid extraction was carried out following the protocol of Hara and Radin (1978) with slight modifications. In brief, to approximately 0,05 g of larvae samples were added 1 ml of deionized water and mixture was homogenized in 10 ml of hexane-isopropanol (3:2) and 6 ml of Na_2SO_4 (6.67%) were added to the obtained homogenates and mixed. After centrifugation, the upper lipid phase was transferred into pre-weighted tubes and subsequently evaporated under nitrogen. Final determination of lipid content was carried out gravimetrically.

Methylation of 1 mg of lipids was induced with boron trifluoride-methanol complex solution and NaOH as described by Appelqvist (1968). Resulting fatty acid methyl esters (FAME) were checked on TLC plate and analyzed using a gas chromatograph (Trace Ultra FID; Thermo Scientific, USA) equipped with a BPX 70 column (SGE, USA). Subsequently, comparison of FAME retention times for sample and standards GLC-68D was used to identify fatty acid compositions.

Methods applied for lipid extraction and methylation of rotifers and artemia followed the same protocol as the larval analysis (Appelqvist, 1968; Hara and Radin, 1978).

2.2. DNA/RNA ratio analysis method

For DNA/RNA ratio analysis, frozen larvae were completely defrosted and picked from the eppendorfs, using sterile forceps. DNA and RNA was extracted individually from six to eight larvae (per diet), using the All Prep DNA/RNA Mini Kit (Qiagen).

2.3. Statistical analysis

Differences in body measurements, food consumption and fatty acids composition between three different diets in larvae sampled at 17dph were evaluated with linear mixed models (LMM, package *lme4*, version 1.1–7;Bates et al., 2014). The effect of the diet was tested on fish total length, body weight, MH and ED (response variables) and the tank was included as random effect. Prior to LMM, the different response variables were transformed with the Box-Cox transformation, which gives the best power estimate for each one (package *car*, version 2.1.2; Fox and Weisberg, 2011). Thereafter, multiple pairwise comparisons between diets were obtained using Tukey's all-pair comparisons, applying the Bonferroni correction to adjust the *p*-values (package *multcomp*, version 1.3–3; (Hothorn et al., 2008)). The same analyses were run to test for differences in the fatty acid composition between diets and between artemia and rotifers used as preys (LA, ALA, ARA, EPA, DHA as different response variables).

Differences in stomach fullness (1 to 4, 1 being empty gut and 4 full gut) were evaluated with generalized linear mixed models (GLMM, package *lme4*), fitted with a binomial error structure and using stomach fullness as response variable and tank as random factor. These analyses were followed by multiple pairwise comparisons with Tukey's all-pair comparisons.

Survival of pikeperch fish was compared between diet groups using a Generalized Linear Mixed Model (GLMM), with survival i.e. proportion of alive fish at 17dph as response variable, fitted with a binomial error structure, and with diet as fixed effect and the tank as random effect. After GLMM, pairwise comparisons were obtained with Tukey's all-pair comparison test. Bonferroni correction was applied to adjust the *p*-values of multiple comparisons.

Concentrations, quality and purity (260/280 and 260/230 ratios) of DNA and RNA were determined by nanodrop. RNA/DNA ratios, transformed with Box-Cox transformation, was compared between diets

by a Linear Mixed Model (LMM), with ratio as response variable and diets as random effect, followed by Tukey's all-pair comparison test to obtain pairwise comparisons between diets. Bonferroni correction was applied to adjust the *p*-values of multiple comparisons.

All analyses were conducted in R (R Core Team, 2014) and statistical significance was set at $\alpha = 0.050$.

3. Results

3.1. Larval growth, stomach fullness and air bladder inflation

Initial pikeperch larval total length and body weigh at 3 dph was 5.32 ± 0.5 mm and 0.55 ± 0.1 mg. After 11 days, treatment B (Fig. 1A), had the larvae with the largest average total length (7.72 ± 0.79 mm) and wet weigh (2.97 ± 0.81 mg) (Fig. 1C). By the end of the trial (17dph), average total length and wet weight was greater in B (9.60 ± 0.79 mm and 5.66 ± 1.37 mg) than A and C (Fig. 1A, C).

Significant differences in total length and body weight at 17dph were found between all diet groups, with larvae fed diet B (mixed rotifers-artemia) being 1.08 and 1.22 times longer (LMM, *p*-value < 0.001) and 1.61 and 2.05 times heavier (LMM, p-value < 0.001) than larvae fed with diet A and C, respectively. Furthermore, fish fed with diet A were 1.13 times longer and 1.27 times heavier than the ones fed with diet C (LMM, *p*-value < 0.001).

Significant differences in myomere height and eye diameter at 17dph were found between all diet groups, with larvae fed diet B (mixed rotifers-artemia) having 1.07 and 1.26 times higher myomeres height (LMM, *p*-value < 0.001) and 1.09 and 1.14 times bigger eye diameters than larvae fed with diet A and C, respectively (LMM, p-value < 0.01 and p-value < 0.001, respectively). Larvae fed with diet A had 1.19 times higher myomere height than the ones fed with diet C (LMM, *p*-value < 0.001), but these showed no significant differences in their eye diameter (LMM, *p*-value > 0.05).

Significant differences were found between the stomach fullness of larvae fed with diet B with respect to diets A and C, with larvae from diet B being 1.04 more full than those fed with A and C (GLMM, diet A p-value = 0.03; diet C p-value = 0.02), with no significant differences between the latter two (GMM, p-value > 0.05). However, these differences were not supported by the multiple pairwise test. Otherwise, pairwise comparisons were generally concordant with LMM and GLMM results. No differences in air bladder inflation were found among treatments.

3.2. Survival

Survival rates were also significantly different between all diets (GLMM and pairwise comparisons $p < 0.001$), showing that the survival of larvae fed exclusively with rotifers (diet C) was 2.14 times higher than larvae fed with artemia (diet A) and 1.16 times higher than larvae receiving a mixed diet (diet B), while the survival of larvae from diet B was 1.85 times higher than of larvae receiving diet A (Fig. 2A).

3.3. RNA/DNA ratio

The RNA/DNA ratio analysis showed significant differences between diets, showing that diet B (mixed rotifers-artemia) has a 2.03 and 2.53 times higher RNA/DNA ratio than diet A (LMM, *p*-value = 0.002) and C (LMM, p-value < 0.001), respectively (Fig. 2). Diet A showed the highest variability of RNA/DNA ratios. However, no difference was found between diets A and C (LMM, *p*-value > 0.05). Pairwise comparisons were concordant with LMM results.

3.4. Fatty acid composition

The fatty acid composition of artemia and rotifers used for the treatments in this study are shown in Fig. 3. Rotifers as prey had 2.7 times higher LA levels, 5.32 times higher Alpha-LA and over 20.1 times higher DHA values than artemia. On the contrary, artemia as prey had 1.38 times higher ARA values, 1.46 times higher EPA values than rotifers (LMM analyses, all with *p*-value < 0.001).

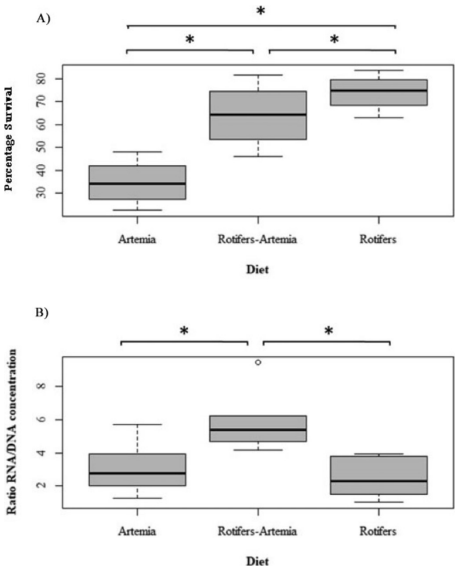

Fig. 2. A: Larval survival percentage, B: RNA/DNA concentration ($n = 40$) and 17 dph ($n = 75$). Dots show mean values and whiskers indicate standard error. Statistically significant differences between data from 17 dph are marked with an asterisk.

The fatty acid composition of larvae receiving the different diets, are shown in Fig. 3. LA levels were highest in diet C, which was 1.78 and 1.68 times higher than in larvae from diets A and B, respectively (LMM, *p*-value < 0.001). Larvae from diet B showed 1.06 times significantly higher LA values than larvae from diet A (LMM, *p*-value < 0.001). ALA levels was highest in diet C, which were 1.93 and 1.82 times higher than in larvae from diets A and B, respectively (LMM, *p*-value < 0.001) and with no significant differences between these two groups (LMM, p-value > 0.05). No significant differences were found between larvae from different diets in their levels of ARA (LMM, *p*-value > 0.05). EPA levels were higher in larvae fed diet B, with a difference of 1.03 and 1.36 times higher compared to larvae fed diets A and C, respectively (LMM, *p*-value < 0.001). Furthermore, larvae from diet A showed 1.33 times higher EPA values than larvae fed with diet C (LMM, p-value < 0.001). DHA levels were higher in larvae fed with diet C, with a difference of 2.05 and 2.47 times compared to larvae fed with diets A and B, respectively (LMM, p-value < 0.001). Furthermore, larvae from diet A showed 1.21 times higher DHA values than larvae fed with diet B (LMM, p-value < 0.001). Pairwise comparisons were generally concordant with LMM results. Fig. 4.

4. Discussion

4.1. Larval growth

One of the key factors for success in fish larval culture, is the

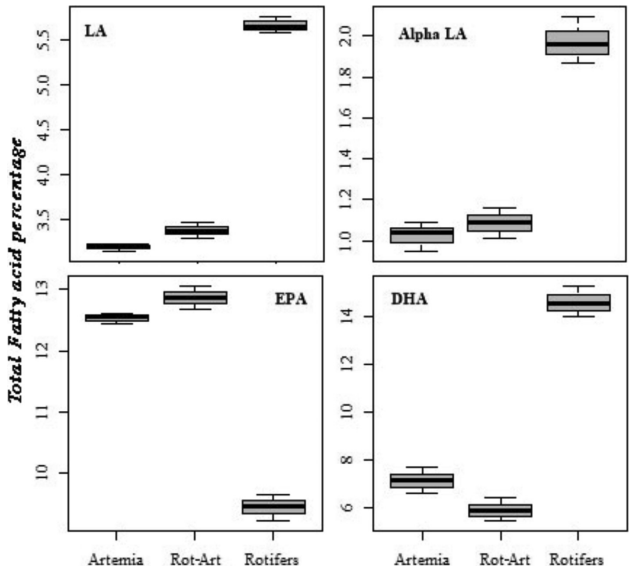

Fig. 3. Larval Essential Fatty acids composition after 17 days post hatch. Statistically significant differences between data from 17 dph are marked with an asterisk.

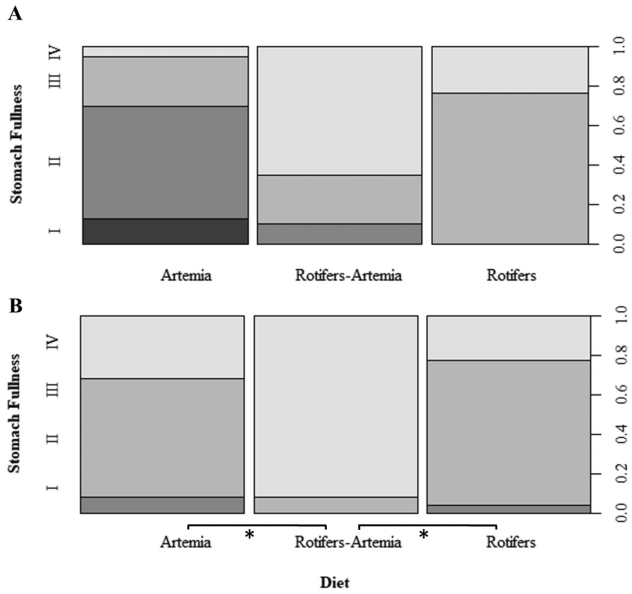

Fig. 4. Differences in stomach fullness (1 to 4, being 4 the maximum fullness, from darkest to lightest grey) at 11dph (A) and 17 dph (B). Statistically significant differences between data from 17 dph are marked with an asterisk.

capacity to develop faster, which allows them to overcome size-related problems (small mouth gape), such as prey size or enzyme development (appearance of enzymes that can break down dry diet) (Dabrowski and Bardega, 1984; Ghan and Sprules, 1993). Therefore, one of the objectives of this trial was to improve pikeperch larval growth by introducing rotifers during first feeding. Although rotifers are mainly used for marine fish larval culture, the use of such live feed in pikeperch larval culture could optimize results due to the following main characteristics: size, distribution, total amount available, digestibility, absorption, and nutritional quality.

As Rønnestad et al. (2013) stated, "feeding success depends on the progressive development of anatomical characteristics and physiological functions and on the availability of suitable food items throughout larval development". Prey dimensions, as well as other factors, are crucial and should be accounted for, so larval development can be optimized, but there are not always taken into consideration, as in pikeperch larval culture. Although rotifers have been tested and used in Eurasian perch (*Perca fluviatilis*) and yellow perch (*Perca flavescens*) (Kestemont and Henrotte, 2015). Hilge and Steffens (1996) stated that the size of mouth gape in pikeperch allows for early ingestion of large organisms (500–1100 μm total length). If such conclusions are taken into consideration, the success of pikeperch is limited to the cohort size variability, which can be affected by broodstock quality and environmental conditions (Schaerlinger and Żarski, 2015). The introduction of saltwater rotifers (*Brachionus plicatilis*) has helped the progressive development of pikeperch during this trial mainly due to its size and nutritional value. The availability of smaller size prey has allowed smaller larvae to start exogenous feeding and avoid the point of no return, and this had a direct effect on larval growth and survival. Steenfeldt (2015), argued against the use of marine rotifers due to their inability to stay alive in a freshwater environment, estimating rotifers to live 2 to 3 h. In order to extend the life and motility of these marine rotifers, pikeperch larvae in our experiment were raised in brackish conditions (3 ppt), which allowed rotifers to survive for up to 10 h. As a result, larvae in treatments B and C were able to ingest rotifers. A significant effect was observed between the larvae fed only on artemia and the larvae fed with the mix (artemia nauplii/rotifers) diet (B), especially in terms of body weight (p-value < 0.001), total length, myomere height, RNA/DNA ratio and eye diameter. Such benefits were also found in a long list of marine fish larvae species (John and Tucker Jr, 1998) such as: Gulf menhaden *(Brevoortia patronus)* (Hettler, 1981), Northern anchovy *(Engraulis mordax)* (Hunter and Kimbrell, 1980), milkfish (*Chanos chanos*) (Villegas, 1990), and barramundi (*Lates calcarifer*) (Tookwinas, 1989).

Size of prey fed to larvae must increase for larvae to optimize growth (Hunter, 1980; Hunter and Kimbrell, 1980; Lasker et al., 1970). Prey size is likely one of the factors that determined the difference between treatments during this trial. Beyer and Laurence (1981) stated that as larvae reach certain sizes, the energetic cost of each attack on prey exceeds the gain from ingesting smaller food particles, which explained treatment C's lower growth results.

Another factor to take into consideration is size heterogeneity within the cohort, which is known to induce cannibalism (Geffen, 2002; Steenfeldt et al., 2011). The larvae from treatment A showed a significant size variability compared to treatment B and C (Fig. 1). Therefore, larvae feed only on artemia nauplii will be more prone to cannibalism in a later stage. The excessive feeding (prey) of larger larvae vs smaller ones is reflected not only in the bigger size variability but also in strong differences in physiological activity (reflected in RNA/DNA ratios).

4.2. Survival

Larval growth and survival are directly correlated. Optimal growth conditions tend to result in good survival, (Finn et al., 2002; Rønnestad et al., 1999) although, as in the case of this trial, the treatment with the best survival does not always have the best growth. Treatment C had the highest survival (Fig. 2), despite having the lowest growth out of the three treatments. There are several factors that could have been responsible for such results. When a series of treatments are compared, prey competition could affect growth (Beyers et al., 1994; Connolly and Connolly, 2011; Lundberg and Persson, 1993). Density wise, feeding across treatments is equal, in order to give the initial number of larvae the same opportunity to capture preys. This assumption is based on an equal stocking density, which with time will vary depending on the treatment effects over the cohort. The first 4 to 5 days of this trial, in terms of size, treatment C (rotifers only) was offered a higher density of adequate prey than the other two treatments, since treatment B had half the amount rotifers and A had none at all. As a result, a more uniform cohort was observed, in which larvae had a higher chance for first feeding and avoiding the point of no return, yet their growth is limited to the amount of prey available, which is less since there are more larvae feeding.

As larvae from treatment C grow in size (10-11dph), the rotifer's size and nutritional value becomes insufficient to provide optimal growth (Pedersen, 1997; Rønnestad et al., 2013). At this point in time, bigger prey (such as artemia) shall be introduced. Such an introduction is essential to maintain an exponential growth adequate to the larval fast metabolism (Zambonino Infante and Cahu, 2007). The effectiveness of such a weaning process can be observed in treatment B, where, although the mixed diet was offered from the beginning, survival was lower, yet, growth was significantly higher than the other two single prey treatments. The opposite prey size effect was observed in treatment A, where prey size during the first 4–5 days was only adequate for only those larvae with bigger mouth gape and although the overall percentage of larvae that can ingest artemia is higher, not all the larvae can capture and ingest the artemia. This limiting factor is key to improve survival.

The highest survival (74%) from this study is in the range of previous studies (Szkudlarek and Zakęś, 2007; Tielmann et al., 2017). It can be argued why to use rotifers when overall survival remains similar, but we attribute such results to the rearing tanks volume. In this study, tank volume was 2 l tanks compared to other studies (Szkudlarek and Zakęś, 2007 and Tielmann et al., 2017), where 200 l were used; such difference might of have an effect on airbladder inflation across all the treatment lowering the overall survival.

4.3. Stomach fullness

The assessment of individual larval consumption carried out during this experiment showed a significant difference between treatments. In accordance with the prior discussed results, treatment B larvae had the best results (highest number of larvae with full stomachs), with no differences between exclusively artemia or rotifer diet. Treatment C had the highest amount of larvae with "stage III" of stomach fullness. Stomach fullness during this experiment was influenced by the early introduction of rotifers, which allowed the larvae to develop their digestive system faster by ingesting prey from the moment larval mouth gape was adequate for capturing preys, due to the availability of suitable food items throughout larval development (Rønnestad et al., 2013). The results from treatment C could be argued that they were influence by prey size and density, which at 17 days post hatch are not sufficient to meet the larval ingestion and evacuation rate (Houde, 1989).

4.4. Fatty acids

Feeding of pikeperch larvae is, in most cases, limited to the administration of one or two species of live prey (Kestemont and Henrotte, 2015). This limitation in the range of food used for the cultured larvae can lead to nutritional imbalances or deficiencies. The most used prey, artemia, has deficiencies in essential fatty acids (EFA) and, unless

Table 2
Total Fatty acid percentage composition and standard deviation (±) from the live feed used and 17 dph larvae from the three different treatments.

FA [%]	Rotifers	Artemia	A	B	C
C14:0	2.93 ± 0.62	2.62 ± 0.14	1.03 ± 0.07	0.98 ± 0.06	1.11 ± 0.06
C14:1	1.24 ± 0.12	1.09 ± 0.04	0.49 ± 0.04	0.46 ± 0.03	0.21 ± 0.01
C16:0	20.23 ± 0.09	16.03 ± 0.44	18.3 ± 0.25	18.25 ± 0.15	21.72 ± 0.21
C16:1	10.41 ± 0.23	16.02 ± 0.46	9.01 ± 0.53	9.01 ± 0.67	4.25 ± 0.13
C18:0	7.08 ± 0.14	5.22 ± 0.19	8.18 ± 0.14	8.43 ± 0.27	11.68 ± 0.30
C18:1n-9	4.89 ± 1.27	18.17 ± 0.28	16.47 ± 0.37	16.52 ± 0.49	9.47 ± 0.32
C18:1n-7	2.81 ± 0.11	13.05 ± 0.81	11.25 ± 0.13	11.25 ± 0.19	4.33 ± 0.27
C18:2n-6	13.97 ± 0.46	5.24 ± 2.65	3.19 ± 0.05	3.37 ± 0.09	5.67 ± 0.09
C18:3n-3	11.17 ± 0.42	2.12 ± 0.04	1.03 ± 0.07	1.09 ± 0.08	1.97 ± 0.11
C20:0	0 ± 0.00	0.08 ± 0.01	0.14 ± 0.06	0.12 ± 0.01	0.21 ± 0.03
C20:1n-9	1.12 ± 0.16	0.38 ± 0.05	0.41 ± 0.01	0.42 ± 0.02	0.62 ± 0.02
C20:2n-6	1.16 ± 0.40	0.06 ± 0.03	0.16 ± 0.01	0.18 ± 0.04	0.73 ± 0.14
C20:4n-6	2.9 ± 0.20	4.01 ± 0.29	5.51 ± 0.16	5.59 ± 0.26	5.47 ± 0.06
C20:3n-3	0.96 ± 0.08	0.03 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.47 ± 0.02
C20:5n-3	10.73 ± 0.38	15.66 ± 1.12	12.54 ± 0.08	12.87 ± 0.18	9.45 ± 0.21
C22:0	1.08 ± 0.08	0.08 ± 0.02	0.16 ± 0.02	0.16 ± 0.01	0.06 ± 0.05
C22:1	0.22 ± 0.20	0 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0 ± 0.00
C22:5n-3	4.93 ± 0.16	0 ± 0.00	4.83 ± 0.12	5.18 ± 0.28	7.83 ± 0.07
C22:6n-3	2.09 ± 0.08	0.1 ± 0.01	7.14 ± 0.55	5.93 ± 0.48	14.59 ± 0.65
C24:0	0 ± 0.00	0.07 ± 0.01	0.08 ± 0.00	0.07 ± 0.00	0.08 ± 0.02
C24:1	0.09 ± 0.15	0 ± 0.00	0 ± 0.00	0.01 ± 0.01	0.11 ± 0.02
SFA	31.32 ± 0.37	24.08 ± 0.15	27.88 ± 0.39	28 ± 0.37	34.9 ± 0.07
MUFA	20.77 ± 1.38	48.71 ± 0.51	37.69 ± 1.03	37.73 ± 1.38	18.98 ± 0.56
PUFA	47.9 ± 1.05	27.21 ± 0.66	34.43 ± 0.77	34.28 ± 1.05	46.17 ± 0.63
n-3	29.88 ± 0.88	17.9 ± 0.67	25.58 ± 0.65	25.14 ± 0.88	34.3 ± 0.59
n-6	18.03 ± 0.20	9.31 ± 0.19	8.85 ± 0.14	9.14 ± 0.20	11.86 ± 0.19
n-3/n-6	1.66 ± 0.06	2.02 ± 0.10	2.89 ± 0.04	2.75 ± 0.06	2.89 ± 0.07

enriched, pikeperch larvae are not provided with an optimal nutritional diet in terms of EFA (Abi-Ayad et al., 2004), thus producing negative effects such as stress sensitivity and long term impaired neural development (Lund et al., 2012; Lund and Steenfeldt, 2011). As a result, it can be observed (Table 2) that treatment A and B have a lower amount of PUFA and SFA compared to treatment C. Larvae fed on rotifers only, had close to double the amount of DHA and LA. Such differences mainly are due the rotifer diet, since nutritional values in terms of EFA between rotifers and artemia are different (Table 2).

Rotifers for this experiment were raised on *Nannocholropsis occulata* and artemia were just simply hatched and fed without any enrichment. Both prey had different fatty acid composition (Table 2) and as a result, the larval fatty acid profiles from the three treatments are different. Looking at the FA profile obtained from the three treatments (Table 2), a clear prey selection can be observed by the larvae in treatment B, whose FA profile is very similar to the artemia only treatment. At day 17 post hatching, pikeperch larvae are clearly selecting artemia over rotifers (also observed in the stomach contents), most likely due to the difference in size (Jackson and Lenz, 2016). Such selection was expected, due to the larval size at this age, therefore switching to artemia shall take place before day 17 post hatched. Rotifer weaning shall take place around day 10 post hatching when the majority of pikeperch larvae have a mouth gape capable of capturing and ingesting artemia. Observing larval FA profile, LA, ALA and DHA had the highest values in diet C, suggesting that larvae fed with this diet had a higher concentration of such essential fatty acids. The direct effect was observed in the overall treatment survival, which was 2.14 and 1.16 higher in diet C when compared to diets A and B. Given the fact that treatment C larvae were not offered size-adequate prey after 10 days, having a higher survival can be attributed to the difference in EFA, which are known to be of key importance during larval development stages (Izquierdo et al., 2000; Watanabe, 1982). When looking closely at the specific fatty acids composition from the live feeds (Table 2), DHA values differ between feeds, although it would not explain the significant difference in DHA found between larvae from treatment C and the other two. On the other hand, LA and ALA composition is significantly different, which could be responsible for the high level of DHA in treatment C larvae. Buzzi et al. (1997) and Fonseca-Madrigal et al. (2014) reported the biosynthesis of LA to DHA in northern pike (*Esox lucius*) and silverside pike (*Chirostoma estor)*. In pikeperch, Kowalska et al. (2012) reported benefits from high concentration of LA in the diet since the biochemical transformation of ALA to EPA and DHA in pikeperch occurs to a greater degree than that of LA to arachidonic acid (C20:4 n-6, ARA) (Jankowska et al., 2003). Work done with pikeperch juveniles (Schulz et al., 2005), indicates the capability for pikeperch to produce EPA and DHA from shorter chain precursor, such capability will explained the results obtained in this trial.

4.5. RNA/DNA ratio

RNA/DNA ratio concentrations were significantly higher (p-value < 0.002) in larvae from the mixed diet when compared to the other two diets (Fig. 2). RNA/DNA ratios give a representation of the physiological activity in an organism (Foley et al., 2016; Richard et al., 2018), which was found to be the highest in diet B fed larvae. Based on the largely different FA content of the two prey organisms used, a mixed diet would require a higher diversity of gene transcripts to digest and convert the mixed diet. Although the rotifers only treatment has a higher RNA/DNA than the artemia treatment, no significant difference was found between larvae fed diet A and diet C, due to the large variation detected in diet A. Similar as in diet B, high RNA/DNA ratios in diet A are likely based on fish consuming not just artemia but also congeners.

5. Conclusions

The use of rotifers as live feed starter for pikeperch larvae has shown to benefit larval development during the first 17 days post hatching. The key factors for such improvements are due to the rotifer size and its added nutritional value. The smaller size increases the changes of smaller larvae to be able to start feeding as sooner, avoiding the point of no return, therefore cohort mortality is reduced during such a critical

period. On the other hand, rotifers fed with *Nannochloropsis* are supplying the larvae with a much more complete diet in terms of EFA, when compared to artemia, allowing the larvae to develop faster and stronger. However, once larvae reach a critical size, rotifers become too small. We hence recommend the introduction of rotifers during the first 12 days post hatch, followed by a period of co-feeding with artemia and gradually wean larvae off rotifers as they grow is size. Future work is needed to establish optimal weaning periods and adequate prey enrichments.

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References

Abi-Ayad, S.-M.E.-A., Boutiba, Z., Mélard, C., Kestemont, P., 2004. Dynamics of Total body fatty acids during early ontogeny of pikeperch (*Sander lucioperca*) larvae. Fish Physiol. Biochem. 30, 129–136. https://doi.org/10.1007/s10695-005-3417-9.

Appelqvist, L.-A., 1968. Lipids in cruciferae. III. Fatty acid composition of diploid and tetraploid seeds of brassica campestris and sinapis alba grown under two climatic extremes. Physiol. Plant. 21, 615–625. https://doi.org/10.1111/j.1399-3054.1968.tb07286.x.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting Linear Mixed-Effects Models using lme4 67. https://doi.org/10.18637/jss.v067.i01.

Beyer, J.E., Laurence, G.C., 1981. Aspects of stochasticity in modelling growth and survival of clupeoid fish larvae. Rapp. P. -v. Reun. Cons. Int. Explor. Mer. 178, 17–23.

Beyers, D.W., Muth, R.T., Fhgrg, M.S., 1994. LFL-072-Beyers_et_al-1994-Rpt.pdf. Colorado State University, Fort Collins.

Blecha, M., Kristan, J., Samarin, A.M., Rodina, M., Policar, T., 2015. Quality and quantity of pikeperch (*Sander lucioperca*) spermatozoa after varying cold water treatments. J. Appl. Ichthyol. 31, 75–78. https://doi.org/10.1111/jai.12853.

Blecha, M., Samarin, A.M., Křištan, J., Policar, T., 2016. Benefits of hormone treatment of both sexes in semi-artificial reproduction of pikeperch (Sander lucioperca L.). Czech J. Anim. Sci. 61, 2016–2203. https://doi.org/10.17221/60/2015-CJAS.

Buzzi, M., Henderson, R.J., Sargent, J.R., 1997. Biosynthesis of docosahexaenoic acid in trout hepatocytes proceeds via 24-carbon intermediates. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 116, 263–267. https://doi.org/10.1016/S0305-0491(96)00210-6.

Chakraborty, R.D., Chakraborty, K., Radhakrishnan, E.V., 2007. Variation in fatty acid composition of *Artemia salina* nauplii enriched with microalgae and baker's yeast for use in larviculture. J. Agric. Food Chem. https://doi.org/10.1021/jf063654l.

Connolly, A.J., Connolly, B.Y.J., 2011. On difficulties with replacement-series methodology in mixture experiments on difficulties with replacement-series methodology in mixture experiments. Society 23, 125–137.

Cowey, C.B., Owen, J.M., Adron, J.W., Middleton, C., 2017. Studies on the nutrition of marine flatfish. In: The Effect of Different Dietary Fatty Acids on the Growth and Fatty Acid Composition of Turbot (Scophthalmus maximus). https://doi.org/10.1079/BJN19760102.

Dabrowski, K., Bardega, R., 1984. Mouth size and predicted food size preferences of larvae of three cyprinid fish species. Aquaculture 40, 41–46. https://doi.org/10.1016/0044-8486(84)90214-X.

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., 2013. Farming different species in RAS in Nordic countries: current status and future perspectives. Aquac. Eng. 53, 2–13. https://doi.org/10.1016/J.AQUAENG.2012.11.008.

Dendrinos, P., Thorpe, J.P., 1987. Experiments on the artificial regulation of the amino acid and fatty acid contents of food organisms to meet the assessed nutritional requirements of larval, post-larval and juvenile Dover sole [Solea solea (L.)]. Aquaculture 61, 121–154. https://doi.org/10.1016/0044-8486(87)90364-4.

Divanach, P., Papandroulakis, N., Anastasiadis, P., Koumoundouros, G., Kentouri, M., 1997. Effect of water currents on the development of skeletal deformities in sea bass (Dicentrarchus labrax L.) with functional swimbladder during postlarval and nursery phase. Aquaculture 156, 145–155. https://doi.org/10.1016/S0044-8486(97)00072-0.

Fao, 2013. The State of Food and Agriculture. (Rome).

FAO, 2016. FAO. Fisheries and Aquaculture statistics, Rome.

Finn, R., Rønnestad, I., Van Der Meeren, T., Fyhn, H., 2002. Fuel and metabolic scaling during the early life stages of Atlantic cod Gadus morhua. Mar. Ecol. Prog. Ser. 243, 217–234. https://doi.org/10.3354/meps243217.

Foley, C.J., Bradley, D.L., Höök, T.O., 2016. A review and assessment of the potential use of RNA:DNA ratios to assess the condition of entrained fish larvae. Ecol. Indic. 60, 346–357. https://doi.org/10.1016/J.ECOLIND.2015.07.005.

Fonseca-Madrigal, J., Navarro, J.C., Hontoria, F., Tocher, D.R., Martínez-Palacios, C.A., Monroig, Ó., 2014. Diversification of substrate specificities in teleostei Fads2: characterization of Δ4 and Δ6Δ5 desaturases of Chirostoma estor. J. Lipid Res. 55, 1408–1419. https://doi.org/10.1194/jlr.M049791.

Fox, J., Weisberg, S., 2011. An R Companion to Applied Regression, 2nd. ed. Sage, Thousand Oaks, CA.

Fuchs, J., 1978. Influence de la photoperiode sur la croissance et la survie de la larve et du juvenile de sole (Solea solea) en elevage. Aquaculture 15, 63–74. https://doi.org/10.1016/0044-8486(78)90072-8.

Gatesoupe, F.-J., 1990. The continuous feeding of turbot larvae, Scophthalmus maximus, and control of the bacterial environment of rotifers. Aquaculture 89, 139–148. https://doi.org/10.1016/0044-8486(90)90306-8.

Geffen, A.J., 2002. Length of herring larvae in relation to age and time of hatching. J. Fish Biol. 60, 479–485. https://doi.org/10.1006/jfbi.2001.1859.

Ghan, D., Sprules, W.G., 1993. Diet, prey selection, and growth of larval and juvenile burbot Lota lota (L.). J. Fish Biol. 42, 47–64. https://doi.org/10.1111/j.1095-8649.1993.tb00305.x.

Girin, M., 1975. Marine fish culture in France: recent developments. Aquaculture 5, 113. https://doi.org/10.1016/0044-8486(75)90036-8.

Govoni, J.J., Boehlert, G.W., Watanabe, Y., 1986. The physiology of digestion in fish larvae. Environ. Biol. Fish 16, 59–77.

Hamza, N., Mhetli, M., Khemis, I. Ben, Cahu, C., Kestemont, P., 2008. Effect of dietary phospholipid levels on performance, enzyme activities and fatty acid composition of pikeperch (Sander lucioperca) larvae. Aquaculture. https://doi.org/10.1016/j.aquaculture.2008.01.014.

Hara, A., Radin, N.S., 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem. 90, 420–426.

Hettler, W.F., 1981. Spawning and rearing Atlantic Menhaden. Progress. Fish-Culturist 43, 80–84. https://doi.org/10.1577/1548-8659(1981)43[80:SARAM]2.0.CO;2.

Hilge, V., Steffens, W., 1996. Aquaculture of fry and fingerling of pikeperch (Stizostedion lucioperca L.) a short review. J. Appl. Ichthyol. 12, 167–170. https://doi.org/10.1111/j.1439-0426.1996.tb00083.x.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50, 346–363. https://doi.org/10.1002/bimj.200810425.

Houde, E.D., 1989. Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fish. Bull. 87.

Howell, B.R., 1997. A re-appraisal of the potential of the sole, Solea solea (L.), for commercial cultivation. Aquaculture 155, 355–365. https://doi.org/10.1016/S0044-8486(97)00103-8.

Hunter, J.R., M, R.J., 1980. The feeding behaviour and ecology of marine fish larvae. In: Bardach, J.E., Magnuson, J.J., M, R.C. (Eds.), Ehaviour and Its Use in the Capture and Culture of Fishes. ICLARM Conf Proc 5. Manila, Philippines, pp. 287–330.

Hunter, R., Kimbrell, C., 1980. Early life history of Pacific mackerel, Scomber japonicus. Fish. Bull. 78, 89–101.

Izquierdo, M.S., Socorro, J., Arantzamendi, L., Hernández-Cruz, C.M., 2000. Recent advances in lipid nutrition in fish larvae. Fish Physiol. Biochem. 22, 97–107.

Jackson, J.M., Lenz, P.H., 2016. Predator-prey interactions in the plankton: larval fish feeding on evasive copepods. Sci. Rep. 6, 33585. https://doi.org/10.1038/srep33585.

Jankowska, B., Zakes, Z., Zmijewski, T., Szczepkowski, M., 2003. A comparison of selected quality features of the tissue and slaughter yield of wild and cultivated pikeperch Sander lucioperca (L.). Eur. Food Res. Technol. 217, 401–405. https://doi.org/10.1007/s00217-003-0757-5.

John, W., Tucker Jr., , 1998. Marine fish culture. In: John, W., Tucker Jr. (Eds.), Google Books, 1st. ed. Springer-Science.

Kestemont, P., Henrotte, E., 2015. Nutritional requirements and feeding of broodstock and early life stages of Eurasian Perch and Pikeperch. In: Biology and Culture of Percid Fishes. Springer Netherlands, Dordrecht, pp. 539–564. https://doi.org/10.1007/978-94-017-7227-3_20.

Kestemont, P., Xueliang, X., Hamza, N., Maboudou, J., Imorou Toko, I., 2007. Effect of weaning age and diet on pikeperch larviculture. Aquaculture 264, 197–204. https://doi.org/10.1016/J.AQUACULTURE.2006.12.034.

Kestemont, P., Dabrowski, K., Summerfelt, R.C., 2015. Biology and Culture of Percid Fishes: Principles and Practices. Principles and Practices, Biology and Culture of Percid Fishes. https://doi.org/10.1007/978-94-017-7227-3.

Kowalska, A., Zakęś, Z., Siwicki, A.K., Jankowska, B., Jarmołowicz, S., Demska-Zakęś, K., 2012. Impact of diets with different proportions of linseed and sunflower oils on the growth, liver histology, immunological and chemical blood parameters, and proximate composition of pikeperch Sander lucioperca (L.). Fish Physiol. Biochem. 38, 375–388. https://doi.org/10.1007/s10695-011-9514-z.

Křištan, J., Alavi, S.M.H., Stejskal, V., Policar, T., 2013. Hormonal induction of ovulation in pikeperch (Sander lucioperca L.) using human chorionic gonadotropin (hCG) and mammalian GnRH analogue. Aquac. Int. 21, 811–818.

Křištan, J., Stara, A., Polgesek, M., Drasovean, A., Kolarova, J., Priborsky, J., Blecha, M., Svacina, P., Policar, T., Velisek, J., 2014. Efficacy of different anaesthetics for pikeperch (Sander lucioperca L.) in relation to water temperature. Neuroendocrinol. Lett. 35, 81–85.

Křištan, J., Blecha, M., Policar, T., 2016. Alcalase treatment for elimination of stickiness in pikeperch (Sander lucioperca L.) eggs under controlled conditions. Aquac. Res. 47, 3998–4003. https://doi.org/10.1111/are.12850.

Kristan, J., Zarski, D., Blecha, M., Policar, T., Malinovskyi, O., Samarin, A.M., Palinska-Zarska, K., Nowosad, J., Krejszeff, S., Kucharczyk, D., 2018. Fertilizing ability of gametes at different post-activation times and the sperm-oocyte-ratio in the artificial reproduction of pikeperch Sander lucioperca. Aquacult. Res. 49, 1383–1388. https://doi.org/10.1111/are.13570.

Lasker, R., Feder, H.M., Theilacker, G.H., May, R.C., 1970. Feeding, growth, and survival of Engraulis mordax larvae reared in the laboratory. Mar. Biol. 5, 345–353. https://

doi.org/10.1007/bf00346901.
Lubzens, E., Tandler, A., Minkoff, G., 1989. Rotifers as food in aquaculture. Hydrobiologia 186–187, 387–400. https://doi.org/10.1007/BF00048937.
Lund, I., Steenfeldt, S.J., 2011. The effects of dietary long-chain essential fatty acids on growth and stress tolerance in pikeperch larvae (Sander lucioperca L.). Aquac. Nutr. 17, 191–199. https://doi.org/10.1111/j.1365-2095.2009.00724.x.
Lund, I., Vilhelm, P., Winding, B., 2012. Comparative Biochemistry and Physiology, Part A Dietary supplementation of essential fatty acids in larval pikeperch (*Sander lucioperca*); short and long term effects on stress tolerance and metabolic physiology. Comp. Biochem. Physiol. Part A 162, 340–348. https://doi.org/10.1016/j.cbpa.2012.04.004.
Lundberg, S., Persson, L., 1993. Optimal body size and resource density. J. Theor. Biol. 164, 163–180. https://doi.org/10.1006/JTBI.1993.1146.
Narciso, L., Pousão-Ferreira, P., Passos, A., Luís, O., 1999. HUFA content and DHA/EPA improvements of Artemia sp. with commercial oils during different enrichment periods. Aquac. Res. 30, 21–24. https://doi.org/10.1046/j.1365-2109.1999.00293.x.
Nash, C.E., Kuo, C.-M., 1975. Hypotheses for problems impeding the mass propagation of grey mullet and other finfish. Aquaculture 5, 119–133. https://doi.org/10.1016/0044-8486(75)90093-9.
Navarro, J.C., Amat, F., Sargent, J.R., 1992. Fatty acid composition of coastal and inland Artemia sp. populations from Spain. Aquaculture 102, 219–230. https://doi.org/10.1016/0044-8486(92)90150-J.
Nyina-wamwiza, L., Xu, X.L., Blanchard, G., Kestemont, P., 2005. Effect of Dietary Protein, LIPID and carbohydrate Ratio on Growth, Feed Efficiency and Body Composition of pikeperch *Sander lucioperca* Fingerlings. 36. pp. 486–492. https://doi.org/10.1111/j.1365-2109.2005.01233.x.
Owen, J.M., Adron, J.W., Middleton, C., Cowey, C.B., 1975. Elongation and desaturation of dietary fatty acids in turbot *Scophthalmus maximus* L., and rainbow trout, Salmo gairdnerii rich. Lipids 10, 528–531. https://doi.org/10.1007/BF02532354.
Pedersen, B.H., 1997. The cost of growth in young fish larvae, a review of new hypotheses. Aquaculture 155, 259–269. https://doi.org/10.1016/S0044-8486(97)00127-0.
Person-LeRuyet, J., Verillaud, P., 1980. Techniques d'elevage intensif de la daurade doree (sparus aurata (L.)) de la naissance a l'age de deux mois. Aquaculture 20, 351–370. https://doi.org/10.1016/0044-8486(80)90096-4.
Policar, T., Stejskal, V., Kristan, J., Podhorec, P., Svinger, V., Blaha, M., 2013. The effect of fish size and stocking density on the weaning success of pond-cultured pikeperch Sander lucioperca L. juveniles. Aquac. Int. 21, 869–882. https://doi.org/10.1007/s10499-012-9563-z.
Policar, T., Blecha, M., Křišťan, J., Mráz, J., Velíšek, J., Stará, A., Stejskal, V., Malinovskyi, O., Svačina, P., Samarin, A.M., 2016. Comparison of production efficiency and quality of differently cultured pikeperch (Sander lucioperca L.) juveniles as a valuable product for ongrowing culture. Aquac. Int. 24, 1607–1626. https://doi.org/10.1007/s10499-016-0050-9.
R Core Team, 2014. A Language and environment for Statistical Computing. Vienna, Austria. http://www.R-project.org.
Richard, P., Bergeron, J.P., Boulhic, M., Galois, R., Person-Leruyet, J., 2018. Effect of starvation on RNA, DNA and protein content of laboratory-reared larvae and juveniles of *Solea solea*. Mar. Ecol. Prog. Ser. 72, 69–77.
Rønnestad, I., Thorsen, A., Finn, R.N., 1999. Fish larval nutrition: a review of recent advances in the roles of amino acids. Aquaculture 177, 201–216. https://doi.org/10.1016/S0044-8486(99)00082-4.
Rønnestad, I., Yú Fera, M., Ueberschä, R.B., Ribeiro, L., Saele, Ø., Boglione, C., 2013. Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. Rev. Aquac. 5, S59–S98. https://doi.org/10.1111/raq.12010.
Samarin, M.A., Miroslav, Blecha, Bytyutskyy, D., Policar, T., 2015. Post-ovulatory oocyte ageing in pikeperch (Sander lucioperca L.) and its effect on egg viability rates and the occurrence of larval malformations and ploidy anomalies. Turk. J. Fish. Aquat. Sci. 15, 429–435. https://doi.org/10.4194/1303-2712-v15_2_29.
Schaerlinger, B., Żarski, D., 2015. Evaluation and Improvements of Egg and Larval Quality in Percid Fishes, in: Biology and Culture of Percid Fishes. Springer Netherlands, Dordrecht, pp. 193–223. https://doi.org/10.1007/978-94-017-7227-3_6.
Schäfer, F.J., 2016. Reproductive Management and Gamete Quality in Pikeperch (*Sander lucioperca*). Humboldt-Universität zu Berlin.
Schulz, C., Knaus, U., Wirth, M., Rennert, B., 2005. Effects of varying dietary fatty acid profile on growth performance, fatty acid, body and tissue composition of juvenile pike perch (Sander lucioperca). Aquac. Nutr. 11, 403–413. https://doi.org/10.1111/j.1365-2095.2005.00369.x.
Schulz, C., Böhm, M., Wirth, M., Rennert, B., 2007. Effect of dietary protein on growth, feed conversion, body composition and survival of pike perch fingerlings (*Sander lucioperca*). Aquac. Nutr. 13, 373–380.
Steenfeldt, S., 2015. Culture methods of Pikeperch early life stages. In: Biology and Culture of Percid Fishes. Springer Netherlands, Dordrecht, pp. 295–312. https://doi.org/10.1007/978-94-017-7227-3_10.
Steenfeldt, S., Lund, I., Höglund, E., 2011. Is batch variability in hatching time related to size heterogeneity and cannibalism in pikeperch (Sander lucioperca)? Aquac. Res. 42, 727–732. https://doi.org/10.1111/j.1365-2109.2010.02681.x.
Steffens, W., Geldhauser, F., Gerstner, P., Hilge, V., 1996. German experiences in the propagation and rearing of fingerling pikeperch (Stizostedion lucioperca). Ann. Zool. Fenn. 33, 627–634.
Szkudlarek, M., Zakęś, Z., 2007. Effect of stocking density on survival and growth performance of pikeperch, Sander lucioperca (L.), larvae under controlled conditions. Aquac. Int. 15, 67–81. https://doi.org/10.1007/s10499-006-9069-7.
Tandler, A., Helps, S., 1985. The effects of photoperiod and water exchange rate on growth and survival of gilthead sea bream (Sparus aurata, Linnaeus; Sparidae) from hatching to metamorphosis in mass rearing systems. Aquaculture 48, 71–82. https://doi.org/10.1016/0044-8486(85)90053-5.
Tielmann, M., Schulz, C., Meyer, S., 2017. The effect of light intensity on performance of larval pikeperch (Sander lucioperca). Aquac. Eng. https://doi.org/10.1016/j.aquaeng.2017.03.001.
Tookwinas, S., 1989. Larviculture of seabass (Lates calcarifer) and grouper (Epinephelus malabaricus) in Thailand. Adv. Trop. Aquac. Work. Tahiti, French Polyn. 9, 645–659 20 Feb - 4 Mar 1989 (Actes de colloque).
Villegas, C.T., 1990. The effects on growth and survival of feeding water fleas (Moina macrocopa Straus) and rotifers (Brachionus plicatilis) to milkfish (Chanos chanos Forsskal) fry. Isr. J. Aquac. 42, 10–17.
Wang, N., Mandiki, S.N.M., Henrotte, E., Bouyahia, A.-G., Mairesse, G., Rougeot, C., Melard, C., Kestemont, P., 2009. Effect of partial or total replacement of forage fish by a dry diet on the quality of reproduction in pikeperch, *Sander lucioperca*. Aquac. Res. 40, 376–383. https://doi.org/10.1111/j.1365-2109.2008.02108.x.
Watanabe, T., 1982. Lipid nutrition in fish. Comp. Biochem. Physiol. Part B Comp. Biochem. 73, 3–15. https://doi.org/10.1016/0305-0491(82)90196-1.
Watanabe', T., Kitajima, C., Fujita, S., 1983. Nutritional Values of Live Organisms Used in Japan For Mass Propagation of Fish: A Review. vol. 34. Aquac Elsevier Sci Publ B, pp. 115–143.
Watson, L., Fontaine, P., Kestemont, P., Teletchea, F., Wang, N., 2008. Percid fish culture. In: From Research to Production. Research to Production, Percid Fish Culture From.
Zambonino Infante, J.L., Cahu, C.L., 2007. Dietary modulation of some digestive enzymes and metabolic processes in developing marine fish: applications to diet formulation. Aquaculture 268, 98–105. https://doi.org/10.1016/J.AQUACULTURE.2007.04.032.

CHAPTER 3

EFFECTS OF FIRST FEEDING REGIME ON GROWTH PERFORMANCE, SURVIVAL RATE AND DEVELOPMENT OF DIGESTIVE SYSTEM IN PIKEPERCH (*SANDER LUCIOPERCA*) LARVAE

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Effects of first feeding regime on growth performance, survival rate and development of digestive system in pikeperch (*Sander lucioperca)* larvae

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ABSTRACT

This study evaluated the effects of first feeding regime on growth performance, survival rate, and development of digestive organs (intestine, liver and pancreas) in pikeperch (*Sander lucioperca*) larvae. The fish larvae at 5 days post-hatch (DPH), were initially fed with rotifers (*Brachionus plicatilis*) for 3 days and from 8 to 17 DPH were fed with rotifers/*Artemia* for different time periods as follows: (A) only rotifers; (B) 8–13 DPH rotifers/14–17 DPH *Artemia*; (C) 8–10 DPH rotifers/ 11–17 DPH *Artemia*; (D) only Artemia; (E) a combination of rotifers and *Artemia*. Growth performance, survival rate and histological features of intestine, liver and pancreas were assessed at 11, 14 and 17 DPH to examine the effects of feeding regime. The groups fed rotifers for initial 3 days followed by feeding on *Artemia* (group D) (53 ± 5.43%) and combination of rotifers and *Artemia* (group E) (68 ± 5.51%), respectively, for the following 9 days showed significantly ($P < .05$) higher survival rates than the other groups (36–50%). The group fed merely on rotifers (groups A) exhibited significantly lower specific growth rate (SGR) than the other groups, and the highest SGR was found in the group fed with combination of rotifers and *Artemia* after 3 day rotifer feeding. Moreover, the highest total length (8.57 ± 0.57 mm), myotome height (0.75 ± 0.09 mm) and eye diameter (0.58 ± 0.05 mm) were obtained by combined feeding of rotifers and *Artemia* after 3 day of initial rotifer feeding. Significant differences among groups were found in morphometric parameters in the anterior intestine and liver. The results of histological examination of the liver, intestine and pancreas did not show any obvious pathological changes in all groups. In conclusion, feeding with rotifers from 5 to 8 DPH and afterwards with *Artemia* could be suggested as an economical feeding regime for first feeding of pikeperch larvae as comparable survival and growth to co-feeding with rotifers and *Artemia* were achieved.

1. Introduction

Pikeperch (*Sander lucioperca* L.) is a large predatory fish inhabiting fresh and brackish waters of central and eastern Europe and large areas of Northern Asia (FAO, 2020). It has been recognized as one of the most promising candidates for diversification of European inland aquaculture (Hilge and Steffens, 1996; Policar et al., 2019) owing to its high growth rate and flesh quality, and attractiveness to anglers (Blecha et al., 2016; Krist'an et al., 2013; Schulz et al., 2007).

Nowadays the considerable decline in pikeperch stock in the natural environment mainly as a result of overfishing and poor fishery management has urged the development of rearing methods for pikeperch production particularly in recirculating aquaculture systems (RAS) (Overton et al., 2015; Policar et al., 2019; Steenfeldt, 2015). To date, most studies have mainly focused on efficient rearing methods for pikeperch juveniles, ongrowing and broodstock (Hermelink et al., 2017; Jarmołowicz et al., 2018; Khendek et al., 2018; Ljubobratovic et al., 2016; Ljubobratović et al., 2017; Malinovskyi et al., 2018; Policar et al., 2016; Steinberg et al., 2018; Steinberg et al., 2017). Little attention has been paid to larval rearing of pikeperch while high larval mortality remains one of the main challenges for fish farmers (Colchen et al., 2020; El Kertaoui et al., 2019; Ljubobratovic et al., 2020; Szkudlarek and Zakes, 2007; Yanes-Roca et al., 2018).

The transition from endogenous to exogenous feeding is a critical period during the early life stages of fishes (Abi-Ayad et al., 2004). Suitability of live feeds, as essential food sources, for fish larvae in terms of size, movement and nutritional content is crucial for the onset of exogenous feeding in fish larvae particularly in percid fishes. Size of

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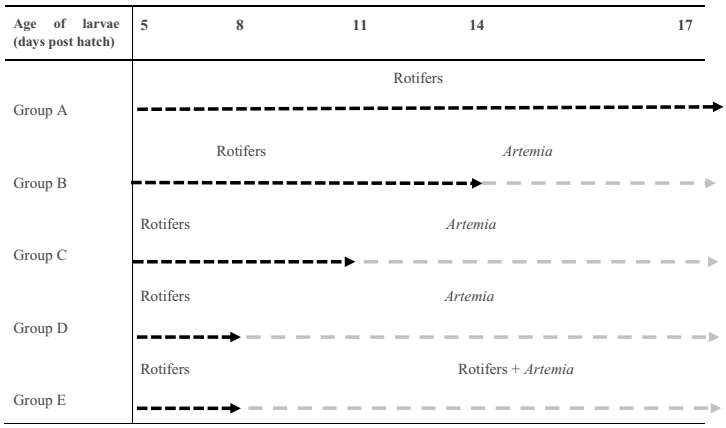

Fig. 1. The graph of applied feeding regimes for pikeperch larvae throughout the experiment.

the mouth opening in pikeperch larvae is rather small compared to other freshwater fish species (Hamza et al., 2015; Kestemont and Henrotte, 2015). Thus, the size of selected live feed should match the size of mouth opening and diameter of the esophagus of larvae (Busch, 1996). The wide variations in the body size of rotifers which ranges from small (50–100 μm) to large (100–200 μm) sizes make them a suitable candidate for first feeding of marine and freshwater fish larvae (Awaiss et al., 1992; Dhert and Sorgeloos, 1995; Steenfeldt, 2015). Moreover, abundant occurrence of rotifers in ponds is an additional benefit making them a superior feed choice for the first feeding of pikeperch larvae by providing normal development accompanied by a rapid and similar growth of all individuals in the population and minimizing cannibalism (Verreth and Kleyn, 1987; Peterka et al., 2003). The results of a recent research showed that using rotifers for first feeding significantly improves survival and growth performance of pikeperch larvae (Yanes-Roca et al., 2018). These improvements by the introduction of the euryhaline rotifer (*Brachionus plicatilis*) could be attributed to its smaller size and higher content of important poly-unsaturated fatty acids such as docosahexaenoic (DHA; 22:6n-3) and linoleic (LA; 18:2n-6) compared to *Artemia* (Yanes-Roca et al., 2018).

One of the main issues in fish larvae culture is the evaluation of their digestive system and functional anatomy of liver, intestine and exocrine pancreas. The method of choice is usually histology of these organs, as improper choice of feed/ingredients could induce various pathological and/or morphological alterations leading to adverse impacts on fish development, growth and survival (Gisbert et al., 2008; Segner et al., 1993). If no distinct changes in the histological organization are observed in the liver, intestine and exocrine pancreas, then planar morphometry of cells or their nuclei is suggested to assess metabolic state of organs via their cellular activity. In this context, the height of absorptive epithelium in intestine, surface area of hepatocytes or pancreocytes (and their nuclei) can be determined and compared for evaluation of nutritional status of fish larvae (Fontagne et al., 1998; Ostaszewska et al., 2018). This approach has been implemented in pikeperch larvae for evaluation of commercial starter feed and pointing to malnutrition of larvae fed unbalanced commercial diets or wrong protein source in diets compared to *Artemia* as a control (Hamza et al., 2007; Ostaszewska et al., 2005). There are several reports on using *B. plicatilis* for pikeperch larval culture (Imentai et al., 2019a; Imentai et al., 2019b; Yanes-Roca et al., 2018), however, there are no studies on evaluation of suitability of *B. plicatilis* on digestive histology of pikeperch larvae.

The aim of the present study was optimization of the first feeding regime for pikeperch larvae using rotifers and *Artemia* under controlled conditions. The assessment of the nutritional status of larvae was done through histological methods to determine cellular/histological microanatomy development of liver, intestine and exocrine pancreas.

2. Materials and methods

2.1. Fish and experimental design

Rearing of pikeperch larvae and *B. plicatilis* was performed at the Experimental Fish Facility of the Faculty of Fisheries and Protection of Waters, University of South Bohemia, Czech Republic. Rotifers were cultured in 50-l flat-bottomed polyethylene tanks ($n = 3$) using a batch culture protocol and fed with commercial microalgal paste of *Nannochloropsis* sp. (Nanno 3600, ReedMariculture Inc., USA) at a rate of 1 ml l^{-1} of culture twice a day. *Artemia* nauplii (Micro artemia cysts, Ocean Nutrition^{tm}, Belgium) were hatched (20-24 h) onsite following provided manual from the producer and upon hatching, *Artemia* were fed right away.

Hatched pikeperch larvae originating from nest spawning of pond-cultured broodstock (Malinovskyi et al., 2018) were acclimated to experimental RAS with water temperature of 15 ± 0.5 °C at 3 days post-hatch (DPH). Then, the larvae at 4 DPH (total length = 5.62 ± 0.03 mm, body weight = 0.66 ± 0.16 mg) were divided into five experimental groups with four replicates (2-l tanks) at initial density of 100 larvae per liter. All larvae at 5 DPH were initially fed with rotifers for 3 days and thereafter from 8 to 17 DPH were divided to 5 (A-E) different feeding regimes (Fig.1) and fed with rotifers and *Artemia* as follows: (A) larvae fed only with rotifers till 17 DPH; (B) larvae fed with rotifers till 14 DPH followed by feeding with *Artemia* till 17 DPH; (C) larvae fed with rotifers till 11 DPH followed by feeding with *Artemia* till 17 DPH; (D) larvae fed only with *Artemia* till 17 DPH; (E) larvae fed a combination of rotifers and *Artemia* till 17 DPH. Nanno 3600 was added to the larval tanks twice daily (2 × 300, 000 cells/ml) beginning at 5 DPH and continuing throughout the experiment according to the mentioned protocol. Rotifers and *Artemia* were provided as live feed to larvae three times daily with residual counts prior to each feeding. Feeding densities were steadily increased based on residual counts, performed prior to each feeding (Table 1). In order to improve larval

Table 1
Experiment husbandry schedule. Amount of daily feed offered, shading concentration (*Nannochloropsis sp.*) and recirculation flow changes with time are shown.

DPH	Daily feed rot-(rot-art)-art/ml	Shading (cells/ml)	Flow (ml/min)
4	No feeding	0	100
5	Rot 5/ml	300,000	100
6	Rot 5/ml	300,000	100
7	Rot 5/ml	300,000	100
8	Rot 10/ml; art 5/ml	400,000	150
9	Rot 10/ml; art 5/ml	400,000	150
10	Rot 10/ml; art 5/ml	400,000	150
11	Rot 14/ml; art 7/ml	600,000	200
12	Rot 14/ml; art 7/ml	600,000	200
13	Rot 14/ml; art 7/ml	600,000	200
14	Rot 16/ml; art 8/ml	700,000	250
15	Rot 16/ml; art 8/ml	700,000	250
16	Rot 16/ml; art 8/ml	700,000	250
17	Rot 16/ml; art 8/ml	700,000	250

feeding efficiency, prior to each feeding water flow was stopped and re-started in each tank after 2 h. Flow rates started at 100 ml/min and increased with time (Table 1).

Water temperature, pH, and dissolved oxygen (DO) were measured before each feeding with a pH/temperature tester (HI98129, Hanna Combo) and oximeter (OxyGuard International A/S, Farum, Denmark) and the values were 17.8 ± 0.17 °C, 7.3 ± 0.04 and 88.5 ± 2.53%, respectively. Total ammonia and nitrite concentrations were determined twice a week and maintained below 0.5 and 0.1 mg l^{-1}, respectively. Light intensity on the water surface was 90–100 lx and photoperiod was set at 13 L: 11D (07:00 to 20:00 h). Salinity was 2 ± 0.2 g l^{-1} which was kept by the adding of salt (Instant Ocean® Sea Salt) to water of the whole culture system. The tanks were cleaned twice per day by siphoning out the faeces and dead larvae.

2.2. Sampling, survival and growth assessment of larvae

Total length (TL ± 0.1 mm), eye diameter (ED ± 0.1 mm) and myotome height (MH ± 0.1 mm) were measured using a stereo microscope SMZ75T (Nikon, Japan) with Quick PHOTO MICRO 3 in total 60 larvae on stocking day (4 DPH) and 60 larvae from each group (15 per replicate) at the 11, 14 and 17 DPH. Body weight of the same larvae (BW ± 0.01 mg) was measured using a Kern ABT analytical balance (Kern & Sohn GmbH, Germany). All larvae were randomly sampled and anesthetized with MS-222 (tricaine methanesulphonate, Sigma; 100 mg l^{-1}) prior to handling. Also, the number of larvae in each tank was counted at the end of experiment for calculation of survival rate. Growth and survival rates were calculated using the following formula:

Specific growth rate (SGR,%day^{-1}) = 100 ($\ln_{TL_f} - \ln_{TL_i}$)/t

where TL_i and TL_f are initial and final total lengths, respectively, and t is the period in days.

Survival rate (%) = Final fish number/Initial fish number × 100

2.3. Histological analyses

Twelve larvae (3 per replicate) were sampled at 11, 14 and 17 DPH for histological analyses. Whole larvae were sacrificed humanely by immersion in overdose of MS-222 anaesthetic, immediately transferred to Davidson's fixative (preserved overnight) and subsequently transferred into ethanol (70%). The samples were dehydrated in ascending ethanol concentrations (70%, 95% and 100%), cleared in xylene, embedded in paraffin, and cut into a series of 5 μm longitudinal sections using a rotary microtome (Galileo, Italy). Sections were subsequently stained with hematoxylin and eosin (H&E) using a staining robot (Tissue-Tek DRS 2000, Sakura). The slides were assessed for general histopathological alterations and tissue structure, and later assessment of selected cells in each tissue was conducted. Anterior portion of intestine was analyzed for: (a) surface areas of enterocyte nuclei and (b) height of enterocytes; liver was analyzed for: (c) surface areas of hepatocyte nuclei (d), vacuolation of hepatocytes and (e) frequency of small (possibly pyknotic) nuclei; pancreas was analyzed for: (f) frequency of zymogen granules. To avoid sampling bias, all measurements were done at 1–3 serial sections of the same fish. Estimation of nuclei surface area was performed using the point counting method (Weibel et al., 1966). In brief, we superimposed a grid of 3927 test points (77 × 51) on a micrograph of tissue section, with surface of rectangle between 4 points amount 8.28 μm^2. Each test point falling in the frame of one nucleus (accounting also nuclear envelope) was counted and the number of points hitting the same nucleus was multiplied by 8.28 μm^2 in order to estimate surface area (Fig. 2a,b). Only nuclei with visible nucleoli were assessed, as nucleoli served as pivotal point in order to reduce bias as recommended by Rašković et al. (2019). Due to the technical reasons, the cell membrane was not always visible in each cell, so we opted not to estimate surface areas of cells but only of their nuclei. Height of enterocytes was measured by drawing a straight line between the basal membrane and apex of the enterocyte, while taking into account that line is passing through the nucleus (Fig. 2a). Vacuolation of hepatocytes was assessed semi-quantitatively, as a number between 0 and 3 was given to each cell depending on the frequency of vacuolation in relation to complete cell: 0 - no vacuolation; 1 - vacuolation present in frequency below 1/3 of the cellular surface area; 2 - vacuolation present in frequency between 1/3 and 2/3 of the cellular surface area; 3 - vacuolation present in frequency above 2/3 of the cellular surface area (Figueiredo-Silva et al., 2005). In the exocrine

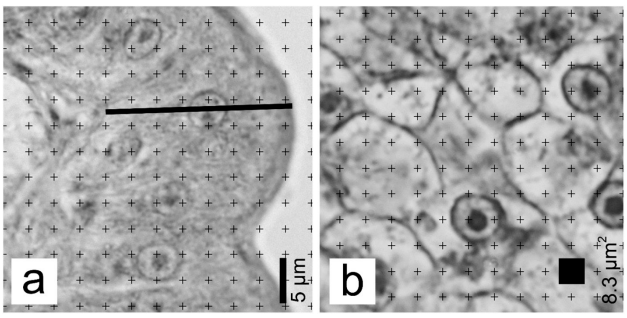

Fig. 2. Illustration of methods of planar morphometry used in the study: a) anterior intestine of pikeperch larvae; test grid is superimposed to each slide for estimating profile area of enterocyte nuclei, while line represent morphometric method for measuring enterocyte height; b) micrograph of liver section; same test grid is used for estimation of profile area of hepatocyte nuclei (H&E, ×1000).

pancreas, the frequency of zymogen granules was evaluated as an indicator for the secretion of proenzymes, using the same semi-quantitative scoring system. Histological slides were analyzed and photographed using an Olympus EX51 light microscope fitted with Canon E600 digital camera, while planar morphometry was conducted using ImageJ v. 1.50i (National Institutes of Health, USA) software package.

2.4. Statistical analyses

All data are presented as mean ± the standard error of the mean (SEM). Data were analyzed with the program RStudio (R Core Team, 2014). Normal distribution of data was confirmed by Shapiro-Wilk's test. The frequency of the zymogen granules among the groups was analyzed using the non-parametric Kruskal-Wallis test. Comparisons for the rest of the morphometrical parameters, survival and SGR were made using one-way ANOVA followed by Tukey *post-hoc* test when significant differences were found at $P < 0.05$.

3. Results

The results of survival rate and SGR of pikeperch larvae are presented in Fig. 3. Significantly higher survival rates were achieved in group D (68%) and E (53%) which received rotifers for initial 3 days followed by feeding on *Artemia* and combination of rotifers and *Artemia*, respectively, for the following 9 days. Extension of feeding period on rotifers resulted in a significantly reduced survival rate (33–50%). A similar trend was observed for SGR where the group offered exclusively rotifers exhibited the lowest SGR value. Furthermore, the highest values of TL, MH and ED were found in the group fed with combination of rotifers and *Artemia* after 8 DPH (group E) (Table 2). Moreover, replacement by *Artemia* from 8 DPH (group D) produced comparable MH and ED values to the group fed with combination of rotifers and *Artemia* at 17 DPH.

The results of histological examination of the anterior intestine, liver and pancreas did not show any changes in the histological organization of target tissues associated with the changes in feeding strategies. The intestine was characterized by well-differentiated enterocytes, lined up by eosinophilic brush border membrane, and intestinal microvilli height of ~1.5 μm (Fig.4). The highest values of enterocyte height in the anterior intestine were observed in groups C, D and E at 14 DPH which were significantly different from those of A and B groups. However, there were no significant differences among groups at 11 and 17 DPH. The highest profile area of enterocytes nuclei was found in group D at 11 DPH. The highest profile area of hepatocyte

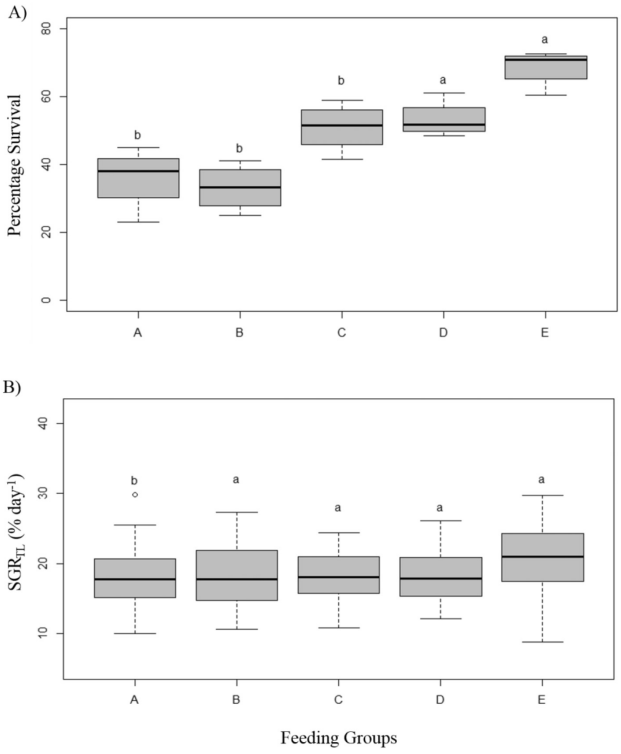

Fig. 3. Survival and specific growth rate (SGR) ($n = 60$) of pikeperch larvae at the end of experimental period. Different letters denote significant ($p < .05$) differences between feeding groups. Box limits correspond to upper and lower quartiles, horizontal bar to the median, and points show outliers outside the 1.5 times interquartile range.

Table 2
Growth and morphometric parameters of *S. lucioperca* larvae at 11 (n = 40), 14 (n = 40) and 17 (n = 60) days post hatch (DPH).

Parameter	DPH	Groups				
		A	B	C	D	E
Total length (mm)	11	7.18 ± 0.05^{b}	7.18 ± 0.05^{b}	7.18 ± 0.05^{b}	7.90 ± 0.07^{a}	7.77 ± 0.08^{a}
	14	7.88 ± 0.06^{b}	7.88 ± 0.06^{b}	8.08 ± 0.09^{ab}	8.42 ± 0.10^{a}	8.36 ± 0.07^{a}
	17	8.21 ± 0.09^{b}	8.21 ± 0.08^{b}	8.22 ± 0.07^{b}	8.22 ± 0.08^{b}	8.57 ± 0.09^{a}
Myotome height (mm)	11	0.52 ± 0.01^{b}	0.52 ± 0.01^{b}	0.52 ± 0.01^{b}	0.65 ± 0.01^{a}	0.63 ± 0.01^{a}
	14	0.67 ± 0.01^{b}	0.67 ± 0.01^{b}	0.69 ± 0.01^{ab}	0.75 ± 0.01^{a}	0.73 ± 0.01^{a}
	17	0.70 ± 0.01^{ab}	0.69 ± 0.01^{b}	0.71 ± 0.02^{ab}	0.73 ± 0.00^{ab}	0.75 ± 0.01^{a}
Eye diameter (mm)	11	0.46 ± 0.00^{b}	0.46 ± 0.00^{b}	0.46 ± 0.00^{b}	0.50 ± 0.01^{a}	0.52 ± 0.01^{a}
	14	0.53 ± 0.01^{b}	0.53 ± 0.01^{b}	0.56 ± 0.01^{b}	0.60 ± 0.01^{a}	0.54 ± 0.01^{b}
	17	0.57 ± 0.01^{ab}	0.55 ± 0.01^{b}	0.55 ± 0.01^{b}	0.56 ± 0.01^{ab}	0.58 ± 0.01^{a}

Values are presented as mean ± SEM. Values in the same row with different superscript letters are significantly different (P < .05).

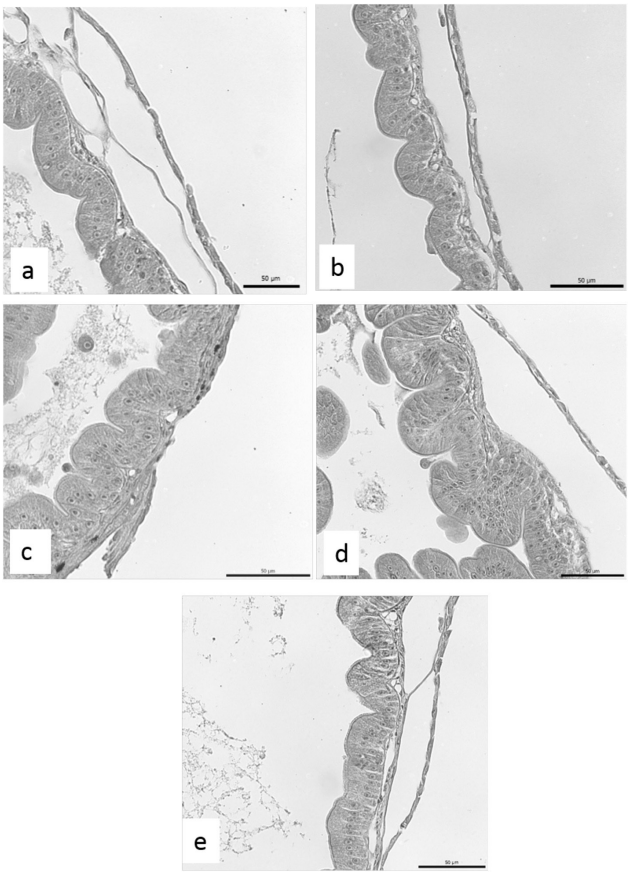

Fig. 4. Longitudinal section of the anterior intestine of pikeperch larvae fed: a) only with rotifers till 17 DPH; b) with rotifers till 14 DPH followed by feeding with *Artemia* till 17 DPH; c) with rotifers till 11 DPH followed by feeding with *Artemia* till 17 DPH; d) only with *Artemia* till 17 DPH; e) a combination of rotifers and *Artemia* till 17 DPH. (H&E). Scale bar = 50 μm.

Table 3
Morphometric parameters (n = 12) of anterior intestine, liver and pancreas of *S. lucioperca* larvae at 11, 14 and 17 day post hatch (DPH).

Parameter	DPH	Groups				
		A	B	C	D	E
Anterior intestine						
Height of enterocytes (μm)	11	23.2 ± 0.70	23.2 ± 0.70	23.2 ± 0.70	26.4 ± 0.47	26.0 ± 2.04
	14	20.1 ± 0.45^{b}	20.1 ± 0.45^{b}	23.6 ± 0.88^{a}	24.9 ± 0.42^{a}	24.0 ± 0.83^{a}
	17	20.4 ± 1.78	20.3 ± 0.53	24.6 ± 3.26	22.3 ± 1.94	24.4 ± 2.30
Profile area of enterocyte nuclei (μm^2)	11	11.5 ± 0.60^{b}	11.5 ± 0.60^{b}	11.5 ± 0.60^{b}	14.4 ± 0.54^{a}	13.5 ± 0.46^{b}
	14	12.3 ± 0.65	12.3 ± 0.65	14.4 ± 0.53	14.6 ± 0.59	13.8 ± 0.96
	17	13.0 ± 0.34^{ab}	10.3 ± 0.76^{b}	11.8 ± 0.29^{b}	14.2 ± 0.51^{a}	15.9 ± 1.55^{a}
Liver						
Profile areas of hepatocyte nuclei (μm^2)	11	25.2 ± 0.86^{b}	25.2 ± 0.86^{b}	25.2 ± 0.86^{b}	26.3 ± 0.62^{ab}	28.6 ± 0.62^{a}
	14	28.1 ± 0.78^{b}	28.1 ± 0.78^{b}	26.8 ± 0.67^{b}	32.6 ± 0.97^{a}	28.4 ± 1.24^{b}
	17	23.2 ± 1.01	23.8 ± 1.23	22.0 ± 1.21	22.4 ± 0.84	21.0 ± 0.70
Vacuolation of hepatocytes	11	0.87 ± 0.29^{b}	0.87 ± 0.29^{b}	0.87 ± 0.29^{b}	2.30 ± 0.30^{a}	0.83 ± 0.30^{b}
	14	0.20 ± 0.20	0.20 ± 0.20	1.43 ± 0.43	1.50 ± 0.37	1.40 ± 0.40
	17	0.40 ± 0.24	0.30 ± 0.15	0.60 ± 0.24	0.57 ± 0.20	0.30 ± 0.15
Pancreas						
Frequency of zymogen granules	11	1.75 ± 0.47	1.75 ± 0.47	1.75 ± 0.47	2.00 ± 0.25	1.50 ± 0.20
	14	1.78 ± 0.67	1.78 ± 0.67	1.38 ± 0.92	1.75 ± 0.48	1.80 ± 0.37
	17	1.38 ± 0.26	2.00 ± 0.32	1.43 ± 0.42	1.50 ± 0.22	1.60 ± 0.40

Values are presented as mean ± SEM. Values with different superscript letters in the same row are significantly different (P < .05). The lack of superscript letter indicates no significant differences among treatments.

nuclei was detected in groups E and D at 11 and 14 DPH, respectively (Table 3).

The appearance of hepatocytes was typical for the liver of fish. Most of the hepatocytes showed granular eosinophilic cytoplasm with large euchromatic nuclei (Fig. 5b). However, variable size/number of vacuoles were observed in the cytoplasm of hepatocytes in most analyzed fish (Fig. 5a). Frequency of cytoplasm vacuolation corresponded the ingested feed at the first sample point (11 DPH) where group A had higher scores compared to D and E groups (P < .05). A vast majority of cells in group A had cytoplasm almost entirely occupied with vacuoles at 11 DPH, but that statistical trend did not continue at other sampling points where the degree of cellular vacuolation decreased in group A at 14 DPH while this trend continued in all groups at 17 DPH. In addition, in some fish, clearly visible lipid droplets were present in the cytoplasm of hepatocytes (Fig. 5c), but number of fish with this characteristic was low. Droplets were present in fish from all experimental groups and there was no significant difference among the groups. Mean profile area of nuclei showed a significant difference among groups at first two sampling points. At 11 DPH mean profile area of nuclei was significantly higher in E group compared to the other groups (P < .05), while at 14 DPH profile area of nuclei was the highest in D group (P < .05). Detailed breakdown of profile area of hepatocyte nuclei to classes (Fig. 4 abc) showed domination of larger nuclei during 11 DPH and 14 DPH sampling points, while small, possibly pyknotic nuclei (profile area less than 8 μm^2) averaged only 1.2% and 0.9% of total nuclei, respectively. At 17 DPH, smallest class of nuclei accounted for 6.8% of total nuclei, while largest classes reduced frequency in total number of nuclei.

All fish presented well-developed exocrine pancreas characterized by pyramidal cells with a basophilic cytoplasm containing eosinophilic zymogen granules. No significant differences among the feeding regimes were observed for the frequency of the zymogen granules (Kruskal-Wallis-Test; P > .05).

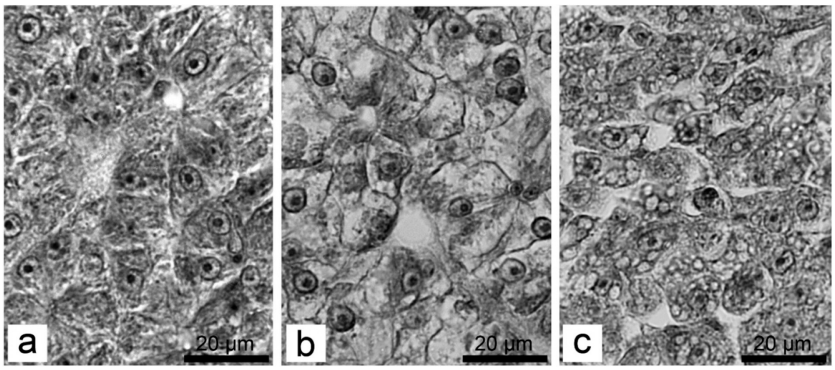

Fig. 5. Three different aspects of hepatocyte morphology obtained from liver sections of *S. lucioperca*: a) hepatocytes containing granular eosinophilic cytoplasm; b) large vacuoles are occupying hepatocellular space, giving look of "clear cells", as cytoplasm is present in low frequency comparing to vacuolation of cells; c) large number of relatively small droplets present in hepatocytes (H&E, ×1000).

4. Discussion

This study provides the first optimized rotifers feeding protocol recommended for pikeperch larvae culture. As a result, statistically same survival rates were achieved for group D (53%) and E (68%) which received rotifers for initial 3 days followed by feeding on *Artemia*, and combination of rotifers and *Artemia*, respectively, for the following 9 days. This could be attributed to the prey size preference during larval growth.

Starting from the mouth opening, larvae preference of prey is dependent to mouth width and prey size (Hamza et al., 2015; Kestemont and Henrotte, 2015; Lubzens et al., 1989). The predator-prey size plays a vital role in the introduction of rotifers during the early larval stages (Faleiro and Narciso, 2009). The amount of energy on capture and ingestion of bigger prey can be much higher to larvae affecting feeding efficiency (Faleiro and Narciso, 2009). Yanes-Roca et al. (2018) suggested that rotifers are suitable for pikeperch larvae from the beginning of exogenous feeding till 10–11 DPH. Size of prey fed to larvae must increase to optimize the growth performance (Hunter, 1980; Hunter and Kimbrell, 1980; Lasker et al., 1970). In the present study, the highest growth indices including TL, ED and MH were found in the group fed with combination of rotifers and *Artemia* from 8 DPH (group E). Similarly, Yanes-Roca et al. (2018) reported that the highest growth of pikeperch larvae occurs by feeding combination of rotifers and *Artemia*.

Although data suggest that the prey size is the main factor influencing the feeding efficiency of pikeperch larvae, there are several other factors that may affect the larval performance. Besides the size differences, *Brachionus* and *Artemia* differ in motion, behavior, form, colour and nutritional composition. Furthermore, not only amino acids but also fatty acids, which could not be synthesized efficiently, are essential for normal growth and survival during early life stages of larvae (Hamza et al., 2008). Therefore, their supply from an exogenous source is crucial for normal development of larvae. The dietary n-3 long-chain polyunsaturated fatty acids (PUFA) such as eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) play important roles in pikeperch larval development and stress tolerance (Lund et al., 2018). Yanes-Roca et al. (2020a) reported higher survival and growth rates for larval pikeperch fed rotifers enriched with *Chlorella vulgaris* rather than *Nannochloropsis* sp. They ascribed such improvements to the higher essential fatty acids content of *Chlorella vulgaris* compared to *Nannochloropsis* sp. In addition, in this study a diluted paste of *Nannochloropsis* sp. was added to all larval tanks. It has been earlier reported that "green water" technique using freeze-dried *Nannochloropsis oculata* during rearing larval gilthead seabream (*Sparus aurata*) has a positive effect on survival, growth and good development of the digestive tract (Navarro and Sarasquete, 1998). Fatty acid composition of *Artemia* is also determined by diet and could vary by the changes of lipid composition in different developmental stages (Zhukova et al., 1998). It is deficient in essential fatty acids such as DHA and LA (Yanes-Roca et al., 2018). In this study, rotifers were fed on *Nannochloropsis* sp. and *Artemia* were used immediately after hatching without any enrichment. As larvae from group E were fed rotifers and *Artemia* continuously the survival of this group was the highest (68%). The obtained survival rates for pikeperch larvae in this study was in the range of reported values in previous studies (Yanes-Roca et al., 2020a; Yanes-Roca et al., 2018; Yanes-Roca et al., 2020b).

The improper feeding can be distinguished through the histological alterations in the structure of the digestive tract of fish (Gisbert and Doroshov, 2003; Kamaszewski and Ostaszewska, 2014; Ostaszewska et al., 2006; Rašković et al., 2011). There are several studies on the effects of diet on histology of digestive tract in pikeperch larvae (Hamza et al., 2007; Hamza et al., 2015; Kamaszewski and Ostaszewska, 2014; Kamaszewski et al., 2010; Kowalska et al., 2006; Mani-Ponset et al., 1994; Ostaszewska, 2005; Ostaszewska et al., 2005), however, this is the first study to evaluate the effects of *B. plicatilis* on histology of digestive tract in pikeperch larvae. The results of histological examination of the anterior intestine did not show any obvious pathological changes associated with the implemented feeding strategies. Morphological changes in the intestine such as the height of enterocytes and brush border can reflect the nutritional condition of fish (Dumitrescu et al., 2014; Gisbert and Doroshov, 2003; Ostaszewska et al., 2006). A low height of the intestinal folds, decrease in the profile areas of the enterocytes and the lack of absorptive vacuoles can indicate possible pathology, from improper nutritional status to starvation (Gisbert and Doroshov, 2003; Kamaszewski and Ostaszewska, 2014; Ostaszewska et al., 2006). In the current study, significantly lower enterocyte height in anterior intestine was observed at 14 DPH in groups A and B. This indicates that feeding larvae with rotifers for over 12 DPH can adversely affect growth, development of intestine and economical profit of this culture in pikeperch larvae. Yanes-Roca et al. (2018) suggested that optimal feeding duration with rotifers for pikeperch larvae is between 3 and 7 days from 8 to 11 DPH which is in agreement with the results of the present study.

Apart from intestine, liver structure has also been used as a means to assay the effects of dietary treatment (Hur et al., 2006; Kestemont et al., 1996; Storch and Juario, 1983; Strussmann and Takashima, 1990). Appearance of hepatocytes is regarded as a vital criteria for assessment of liver nutritional status. In the present study hepatocyte vacuolation was found in all fish livers, but the degree of vacuolation was variable. Higher degree of vacuolation generally corresponds with the higher lipid accumulation in the liver as reported in yellow perch (*Perca flavescens*) (Jiang et al., 2019). Jiang et al. (2019) evaluated the effects of starch with various origins on liver histology in yellow perch, and hepatocytes appearance resembled those in the present study. Even more similar appearance of hepatocellular vacuolation was found in wild European perch (*Perca fluviatilis*) (Nikolić et al., 2020). Presence of lipid droplets in vacuolated hepatocytes was confirmed in ultrastructure/frozen section histology in European sea bass (*Dicentrarchus labrax*), rainbow trout (*Oncorhynchus mykiss*) (Caballero et al., 2004; Figueiredo-Silva et al., 2005), Senegalese sole (*Solea senegalensis*) (Mandrioli et al., 2012) and common carp (*Cyprinus carpio*) (Rašković et al., 2016). We assume that *Artemia* contained higher concentration of lipids than *B. plicatilis* as larvae fed *Artemia* had higher frequency of vacuolated hepatocytes at first sampling point. Moreover, it has been shown that type of lipids (neutral/polar) has also an effect on the degree of lipid accumulation considering the role of phospholipids in lipid transport (Gisbert et al., 2005). Higher storage of lipids in liver as demonstrated by hepatocytes appearance could potentially explain the higher SGR and survival in groups D and E. This is in line with previous studies where pikeperch larvae were fed formulated starter diets, and *Artemia* was used as a control (Ostaszewska et al., 2005; Kamaszewski et al., 2010). Hepatocytes from control group had higher percentage of vacuoles, but not glycogen, comparing to larvae fed compound diets, which reflected on highest weight and length during the course of the experiment (Ostaszewska et al., 2005).

In the current study, the changes in profile areas of hepatocytes nuclei was also assessed which is used as an indirect measure of hepatocytes metabolism where nuclei with higher profile area will inherently have higher DNA transcription level (Rašković et al., 2019). It has been demonstrated that nuclei size correlates with the nutritional status of larvae of different fish species at first-feeding (Strussmann and Takashima, 1990; Wold et al., 2009) which was also the case in the present study. Larvae fed *Artemia* and combination of *Artemia* and *B. plicatilis* (groups D and E) at 11 and 14 DPH showed the highest profile area of the nuclei which further confirmed the beneficial nutritional status in the these two groups. On the other hand, the reduction in profile areas of nuclei in all tested groups at 17 DPH is probably due to decreasing metabolic activity of developing liver usually depicted in the lower RNA/DNA ratio (another metabolic marker in nutritional status). Correlation of RNA/DNA ratio with nuclear diameter is documented in various fish species which all show similar decrease of nuclear size

(Segner et al., 1993).

If nutritional status of larvae goes to the point-of-no-return, then a number of hepatocytes will undergo either to apoptosis or necrosis (Di Pane et al., 2020). Prior to this, condensation of chromatin in nucleus called pyknosis occurs while size of nuclei drops below physiologically active point. Therefore, pyknosis is used as one of the important markers of malnutrition in fish (Di Pane et al., 2020; Gisbert et al., 2008). In the present study, nuclei with the profile areas below 8 μm^2 could possibly be considered as an early sign of pyknosis but as there were no differences among the groups and the fact that number of small nuclei increased during time in all groups, we believe this phenomenon is part of the process of decreasing metabolic activity in liver as described earlier.

In the exocrine pancreas, zymogen granules contain proenzymes that participate in digestion of proteins, carbohydrates, fats and nucleotides (Genten, 2009). In this organ, apart from a smaller size and condensed nucleus, atrophy is characterized by a reduced number of zymogen granules (Takashima and Hibiya, 1995). Therefore, this depletion can indicate inadequate feeding or starvation (Kamaszewski et al., 2010). For example, in previous studies pikeperch larvae fed with artificial compound diets presented a lower abundance of zymogen granules compared to those fed with *Artemia* (Kamaszewski et al., 2010; Ostaszewska, 2005). In the present study, the frequency of zymogen granules was not impacted by feeding regimes, while additional pathological alterations in the exocrine pancreas were not present. Therefore, detrimental effects of feeding regimes on the developing larvae can be excluded.

In conclusion, the findings in this study showed that feeding pikeperch larvae with *B. plicatilis* from 5 to 8 DPH and afterwards exclusively with *Artemia* or combination of rotifers and *Artemia* till 17 DPH can ensure high survival and growth rates, and better development of digestive organs. Feeding pikeperch larvae with rotifers from 5 to 8 DPH and afterwards replacing with *Artemia* till 17 DPH is recommended as an optimum feeding regime because larval survival and growth were satisfying and it reduces the costs of production.

Ethic statement

The experimental procedures were performed according to the ethical rules of the EU-harmonized Animal Welfare Act of the Czech Republic. The experimental unit is licensed (No. 2293/2015-MZE-17214 in project NAZV QK1820354) according to the Czech National Directive (Law against Animal Cruelty, No. 246/1992).

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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References

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

Awaiss, A., Kestemont, P., Micha, J.C., 1992. Nutritional suitability of the rotifer, *Brachionus calyciflorus* pallas for rearing fresh-water fish larvae. J. Appl. Ichthyol. 8, 263–270.

Blecha, M., Kristan, J., Policar, T., 2016. Adaptation of intensively reared pikeperch (*Sander lucioperca*) juveniles to pond culture and subsequent se-adaptation to a re-circulation aquaculture system. Turk. J. Fish. Aquat. Sc. 16, 15–18.

Busch, A., 1996. Transition from endogenous to exogenous nutrition: larval size parameters determining the start of external feeding and size of prey ingested by Ruegen spring herring *Clupea harengus*. Mar. Ecol. Prog. Ser. 130, 39–46.

Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Fernandez, A.J., Rosenlund, G., 2004. Histological alterations in the liver of sea bream, *Sparus aurata* L., caused by short-or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. J. Fish Dis. 27 (9), 531–541.

Colchen, T., Gisbert, E., Krauss, D., Ledore, Y., Pasquet, A., Fontaine, P., 2020. Improving pikeperch larviculture by combining environmental, feeding and populational factors. Aqua. Rep. 17, 100337. https://doi.org/10.1016/j.aqrep.2020.100337.

Dhert, P., Sorgeloos, P., 1995. Live feeds in aquaculture. Infofish Int. 2, 31–39.

Di Pane, J., Gendrot, F., Giraldo, C., Marchal, P., Koubbi, P., Loots, C., 2020. Evaluating the histological-based condition of wild collected larval fish: a synthetic approach applied to common sole (*Solea solea*). J. Mar. Syst. 103309.

Dumitrescu, G., Grozea, A., Szilagyi, P., Ciochina, L.P., Grozea, I., Gal, D., 2014. Natural vs. controlled rearing conditions in pikeperch (*Sander lucioperca*) fingerlings. A histological approach at the liver and intestine level. Rom. Biotech. Lett. 19, 9531–9542.

El Kertaoui, N., Lund, I., Assogba, H., Domínguez, D., Izquierdo, M.S., Baekelandt, S., Cornet, V., Mandiki, S.N., Montero, D., Kestemont, P., 2019. Key nutritional factors and interactions during larval development of pikeperch (*Sander lucioperca*). Sci. Rep.-Uk. 9, 1–15.

Faleiro, F., Narciso, L., 2009. Brachionus vs Artemia duel: optimizing first feeding of *Upogebia pusilla* (Decapoda: Thalassinidea) larvae. Aquaculture. 295, 205–208.

FAO, 2020. Cultured Species Information Programme. *Sander lucioperca*. In: Cultured Aquatic Species Infromation Programme. Text by Zakes, Z. *FAO Fisheries and Aquaculture Department* (online), Rome Updated January 2012. http://www.fao.org/fishery/culturedspecies/Sander_lucioperca/en. Visited 1 February 2020.

Figueiredo-Silva, A., Rocha, E., Dias, J., Silva, P., Rema, P., Gomes, E., Valente, L.M.P., 2005. Partial replacement of fish oil by soybean oil on lipid distribution and liver histology in European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) juveniles. Aquac. Nutr. 11, 147–155.

Fontagne, S., Geurden, I., Escaffre, A.M., Bergot, P., 1998. Histological changes induced by dietary phospholipids in intestine and liver of common carp (*Cyprinus carpio* L.) larvae. Aquaculture. 161, 213–223.

Genten, F., 2009. Atlas of Fish Histology. CRC Press.

Gisbert, E., Doroshov, S.I., 2003. Histology of the developing digestive system and the effect of food deprivation in larval green sturgeon (*Acipenser medirostris*). Aquat. Living Resour. 16, 77–89.

Gisbert, E., Villeneuve, L., Zambonino-Infante, J.L., Quazuguel, P., Cahu, C.L., 2005. Dietary phospholipids are more efficient than neutral lipids for long-chain polyunsaturated fatty acid supply in European sea bass *Dicentrarchus labrax* larval development. Lipids 40 (6), 609–618.

Gisbert, E., Ortiz-Delgado, J.B., Sarasquete, C., 2008. Nutritional cellular biomarkers in early life stages of fish. Histol. Histopathol. 23, 1525–1539.

Hamza, N., Mhetli, M., Kestemont, P., 2007. Effects of weaning age and diets on ontogeny of digestive activities and structures of pikeperch (*Sander lucioperca*) larvae. Fish Physiol. Biochem. 33, 121–133.

Hamza, N., Mhetli, M., Khemis, I.B., Cahu, C., Kestemont, P., 2008. Effect of dietary phospholipid levels on performance, enzyme activities and fatty acid composition of pikeperch (*Sander lucioperca*) larvae. Aquaculture 275 (1-4), 274–282.

Hamza, N., Ostaszewska, T., Kestemont, P., 2015. Development and functionality of the digestive system in percid fishes early life stages. In: Biology and culture of Percid Fishes. Springer, pp. 239–264.

Hermelink, B., Kleiner, W., Schulz, C., Kloas, W., Wuertz, S., 2017. Photo-thermal manipulation for the reproductive management of pikeperch *Sander lucioperca*. Aquac. Int. 25, 1–20.

Hilge, V., Steffens, W., 1996. Aquaculture of fry and fingerling of pike-perch (*Stizostedion lucioperca* L) - a short review. J. Appl. Ichthyol. 12, 167–170.

Hunter, R., Kimbrell, C., 1980. Early life history of Pacific mackerel, Scomber japonicus. Fish. Bull 78, 89–101.

Hunter, J.R., 1980. The feeding behavior and ecology of marine fish larvae. In: Fish Behavior and its Use in the Capture and Culture of Fishes. ICLARM Conference Proceedings, pp. 287–330.

Hur, J.W., Jo, J.H., Park, I.S., 2006. Effects of long-term starvation on hepatocyte ultrastructure of olive flounder *Paralichthys olivaceus*. Ichthyol. Res. 53, 306–310.

Imentai, A., Yanes-Roca, C., Malinovskyi, O., Policar, T., 2019a. Effect of *Brachionus plicatilis* density on pikeperch (*Sander lucioperca* L.) larva performance at first feeding. J. Appl. Ichthyol. 35, 1292–1294.

Imentai, A., Yanes-Roca, C., Steinbach, C., Policar, T., 2019b. Optimized application of rotifers *Brachionus plicatilis* for rearing pikeperch *Sander lucioperca* L. larvae. Aquac. Int. 27, 1137–1149.

Jarmołowicz, S., Rożyński, M., Kowalska, A., Zakęś, Z., 2018. Growth in juvenile pikeperch (*Sander lucioperca* L.) stimulated with yeast, *Saccharomyces cerevisiae*, extract. Aquac. Res. 49, 614–620.

Jiang, M., Zhao, H., Zhai, S.W., Newton, R.J., Shepherd, B., Tian, J., Lofald, A.G., Teh, S., Binkowski, F.P., Deng, D.F., 2019. Nutritional quality of different starches in feed fed to juvenile yellow perch, *Perca flavescens*. Aquac. Nutr. 26, 671–682 2020.

Kamaszewski, M., Ostaszewska, T., 2014. The effect of feeding on morphological changes in intestine of pike-perch (*Sander lucioperca* L.). Aquac. Int. 22, 245–258.

Kamaszewski, M., Napora-Rutkowski, L., Ostaszewska, T., 2010. Effect of feeding on digestive enzyme activity and morphological changes in the liver and pancreas of pike-Perch (*Sander lucioperca*). Isr. J. Aquacult. 62, 225–236.

Kestemont, P., Mélard, C., Fiogbe, E., Vlavonou, R., Masson, G., 1996. Nutritional and animal husbandry aspects of rearing early life stages of Eurasian perch Perca fluviatilis. Journal of Applied Ichthyology 12 (3-4), 157–165.

Kestemont, P., Henrotte, E., 2015. Nutritional requirements and feeding of broodstock

and early life stages of Eurasian perch and pikeperch. In: Biology and Culture of Percid Fishes. Springer, pp. 539–564.
Khendek, A., Chakraborty, A., Roche, J., Ledoré, Y., Personne, A., Policar, T., Żarski, D., Mandiki, R., Kestemont, P., Milla, S., 2018. Rearing conditions and life history influence the progress of gametogenesis and reproduction performances in pikeperch males and females. Animal. 12, 2335–2346.
Kowalska, A., Zakęś, Z., Demska-Zakęś, K., 2006. The impact of feeding on the results of rearing larval pikeperch, *Sander lucioperca* (L.), with regard to the development of the digestive tract. Elec. J. Polish Agric. Uni., Fish. 9.
Krist'an, J., Alavi, S.M.H., Stejskal, V., Policar, T., 2013. Hormonal induction of ovulation in pikeperch (*Sander lucioperca* L.) using human chorionic gonadotropin (hCG) and mammalian GnRH analogue. Aquac. Int. 21, 811–818.
Lasker, R., Feder, H., Theilacker, G., May, R., 1970. Feeding, growth, and survival of *Engraulis mordax* larvae reared in the laboratory. Mar. Biol. 5 (4), 345–353.
Ljubobratovic, U., Kucska, B., Sandor, Z., Peteri, A., Rónyai, A., 2016. Effects of stocking density, feeding technique and vitamin C supplementation on the habituation on dry feed of pikeperch (*Sander lucioperca*) pond reared juveniles. Iran. J. Fish. Sci. 15, 1337–1347.
Ljubobratović, U., Péter, G., Horváth, Z., Żarski, D., Ristović, T., Percze, V., Sándor, Z., Lengyel, S., Rónyai, A., 2017. Reproductive performance of indoor-reared pikeperch (*Sander lucioperca*) females after wintering in outdoor earthen ponds. Aquac. Res. 48, 4851–4863.
Ljubobratovic, U., Kosanovic, D., Demeny, F.Z., Krajcsovics, A., Vukotic, G., Stanisavljevic, N., Golic, N., Jeney, G., Lukic, J., 2020. The effect of live and inert feed treatment with lactobacilli on weaning success in intensively reared pike-perch larvae. Aquaculture 516, 734608. https://doi.org/10.1016/j.aquaculture.2019.734608.
Lubzens, E., Tandler, A., Minkoff, G., 1989. Rotifers as food in aquaculture. Hydrobiologia. 186, 387–400.
Lund, I., El Kertaoui, N., Izquierdo, M.S., Dominguez, D., Hansen, B.W., Kestemont, P., 2018. The importance of phospholipids combined with long-chain PUFA in formulated diets for pikeperch (*Sander lucioperca)* larvae. Br. J. Nutr. 120, 628–644.
Malinovskyi, O., Vesely, L., Blecha, M., Kristan, J., Policar, T., 2018. The substrate selection and spawning behaviour of pikeperch *Sander lucioperca* L. broodstock under pond conditions. Aquac. Res. 49, 3541–3547.
Mandrioli, L., Sirri, R., Gatta, P.P., Morandi, F., Sarli, G., Parma, L., Fontanillas, R., Bonaldo, A., 2012. Histomorphologic hepatic features and growth performances of juvenile Senegalese sole (*Solea senegalensis*) fed isogenertic practical diets with variable protein/lipid levels. J. Appl. Ichthyol. 28 (4), 628–632.
Mani-Ponset, L., Diaz, J.-P., Schlumberger, O., Connes, R., 1994. Development of yolk complex, liver and anterior intestine in pike-perch larvae, *Stizostedion lucioperca* (Percidae), according to the first diet during rearing. Aquat. Living Resour. 7, 191–202.
Navarro, N., Sarasquete, C., 1998. Use of freeze-dried microalgae for rearing gilthead seabream, *Sparus aurata*, larvae: I. growth, histology and water quality. Aquaculture. 167, 179–193.
Nikolić, D., Skorić, S., Rašković, B., Lenhardt, M., Krpo-Ćetković, J., 2020. Impact of reservoir properties on elemental accumulation and histopathology of European perch (*Perca fluviatilis*). Chemosphere. 244, 125503.
Ostaszewska, T., 2005. Developmental changes of digestive system structures in pikeperch (*Sander lucioperca* L.). Elec. J. Ichthyol. 2, 65–78.
Ostaszewska, T., Dabrowski, K., Czuminska, K., Olech, W., Olejniczak, M., 2005. Rearing of pikeperch larvae using formulated diets - first success with starter feeds. Aquac. Res. 36, 1167–1176.
Ostaszewska, T., Korwin-Kossakowski, M., Wolnicki, J., 2006. Morphological changes of digestive structures in starved tench *Tinca tinca* (L.) juveniles. Aquac. Int. 14, 113–126.
Ostaszewska, T., Krajnik, K., Adamek-Urbańska, D., Kasprzak, R., Rzepkowska, M., Luczynski, M., Karczewska, T.A., Dabrowski, K., 2018. Effect of feeding strategy on digestive tract morphology and physiology of lake whitefish (Coregonus lavaretus). Aquaculture 497, 32–41.
Overton, J.L., Toner, D., Policar, T., Kucharczyk, D., 2015. Commercial production: factors for success and limitations in European percid fish culture. In: Biology and Culture of Percid Fishes. Springer, pp. 881–890.
Peterka, J., Matína, J., Lipka, J., 2003. The diet and growth of larval and juvenile pikeperch (*Stizostedion lucioperca* (L.)): a comparative study of fishponds and a reservoir. Aquac. Int. 11 (4), 337–348.
Policar, T., Blecha, M., Krist'an, J., Mraz, J., Velisek, J., Stara, A., Stejskal, V., Malinovskyi, O., Svacina, P., Samarin, A.M., 2016. Comparison of production efficiency and quality of differently cultured pikeperch (*Sander lucioperca* L.) juveniles as a valuable product for ongrowing culture. Aquac. Int. 24, 1607–1626.
Policar, T., Schaefer, F.J., Panana, E., Meyer, S., Teerlinck, S., Toner, D., Żarski, D., 2019. Recent progress in European percid fish culture production technology—tackling bottlenecks. Aquac. Int. 27, 1151–1174.
R Core Team, 2014. A language and environment for statistical computing. Retrieved from. R Foundation for statistical computing, Vienna, Austria. http://www.Rproject.org**, Accessed date: 22 November 2017.**
Rašković, B.S., Stanković, M.B., Marković, Z.Z., Polekšić, V.D., 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. J. Agric. Sci. 56, 87–100.
Rašković, B., Ćirić, M., Koko, V., Stanković, M., Marković, Z., Poleksić, V., 2016. Effect of supplemental feeds on liver and intestine of common carp (*Cyprinus carpio*) in semi-intensive rearing system: histological implications. Biologia 71 (2), 212–219.
Rašković, B., Cruzeiro, C., Poleksić, V., Rocha, E., 2019. Estimating volumes from common carp hepatocytes using design-based stereology and examining correlations with profile areas: revisiting a nutritional assay and unveiling guidelines to microscopists. Microsc. Res. Tech. 82 (6), 861–871.
Schulz, C., Bohm, M., Wirth, M., Rennert, B., 2007. Effect of dietary protein on growth, feed conversion, body composition and survival of pike perch fingerlings (*Sander lucioperca*). Aquac. Nutr. 13, 373–380.
Segner, H., Rösch, R., Verreth, J., Witt, U., 1993. Larval nutritional physiology: studies with *Clarias gariepinus, Coregonus lavaretus* and *Scophthalmus maximus*. J. World Aquacult. Soc. 24, 121–134.
Steenfeldt, S., 2015. Culture methods of pikeperch early life stages. In: Biology and Culture of Percid Fishes. Springer, pp. 295–312.
Steinberg, K., Zimmermann, J., Stiller, K.T., Meyer, S., Schulz, C., 2017. The effect of carbon dioxide on growth and energy metabolism in pikeperch (*Sander lucioperca*). Aquaculture. 481, 162–168.
Steinberg, K., Zimmermann, J., Meyer, S., Schulz, C., 2018. Start-up of recirculating aquaculture systems: how do water exchange rates influence pikeperch (*Sander lucioperca*) and water composition? Aquac. Eng. 83, 151–159.
Storch, V., Juario, J.V., 1983. The effect of starvation and subsequent feeding on the hepatocytes of *Chanos chanos* (Forsskal) fingerlings and fry. J. Fish Biol. 23, 95–103.
Strussmann, C.A., Takashima, F., 1990. Hepatocyte nuclear size and nutritional condition of larval pejerrey, *Odontesthes bonariensis* (Cuvier Et Valenciennes). J. Fish Biol. 36, 59–65.
Szkudlarek, M., Zakes, Z., 2007. Effect of stocking density on survival and growth performance of pikeperch, *Sander lucioperca* (L.), larvae under controlled conditions. Aquac. Int. 15, 67–81.
Takashima, F., Hibiya, T., 1995. An Atlas of Fish Histology: Normal and Pathological Features.
Verreth, J., Kleyn, K., 1987. The effect of biomanipulation of the zooplankton on the growth, feeding and survival of pikeperch (*Stizostedion lucioperca*) in nursing ponds. J. Appl. Ichthyol. 3 (1), 13–23.
Weibel, E.R., Kistler, G.S., Scherle, W.F., 1966. Practical stereological methods for morphometric cytology. J. Cell Biol. 30, 23–38.
Wold, P.A., Hoehne-Reitan, K., Cahu, C., Infante, J.Z., Rainuzzo, J., Kjørsvik, E., 2009. Comparison of dietary phospholipids and neutral lipids: effects on gut, liver and pancreas histology in Atlantic cod (*Gadus morha* L.) larvae. Aquac. Nutr. 15, 73–84.
Yanes-Roca, C., Mraz, J., Born-Torrijos, A., Holzer, A.S., Imentai, A., Policar, T., 2018. Introduction of rotifers (*Brachionus plicatilis*) during pikeperch first feeding. Aquaculture. 497, 260–268.
Yanes-Roca, C., Leclercq, E., Vesely, L., Malinovskyi, O., Policar, T., 2020a. Use of lactic acid bacteria during pikeperch (*Sander lucioperca*) larval rearing. Microorganisms. 8, 238.
Yanes-Roca, C., Holzer, A., Mraz, J., Veselý, L., Malinovskyi, O., Policar, T., 2020b. Improvements on live feed enrichments for pikeperch (*Sander lucioperca*) larval culture. Animals. 10, 401.
Zhukova, N.V., Imbs, A.B., Yi, L.F., 1998. Diet-induced changes in lipid and fatty acid composition of *Artemia salina*. Comp. Biochem. Physiol. B 120, 499–506.

CHAPTER 4

OPTIMIZED APPLICATION OF ROTIFERS *BRACHIONUS PLICATILIS* FOR REARING PIKEPERCH *SANDER LUCIOPERCA* L. LARVAE

Imentai, A., Yanes-Roca, C., Steinbach, Ch., Policar, T., 2019. Optimized application of rotifers *Brachionus plicatilis* for rearing pikeperch *Sander lucioperca* L. larvae. Aquaculture International 27, 1137–1149.

My contribution to this work was about 60%.

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EUROPEAN PERCID FISH CULTURE

Optimized application of rotifers *Brachionus plicatilis* for rearing pikeperch *Sander lucioperca* L. larvae

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Abstract

This study aimed to optimize the use of euryhaline rotifer *Brachionus plicatilis* for rearing larval pikeperch *Sander lucioperca* L. We assessed motility of rotifers under salinities of 0‰, 2‰, 4‰, 8‰, and 16‰ over a 6-h period. Rotifers stocked into freshwater were completely immotile within 2 h. The motility of rotifers after 6 h was 10 ± 2.27% at 2‰, 35 ± 5.58% at 4‰, 79 ± 30.35% at 8‰, and 92 ± 1.98% at 16‰. A second trial quantified the effect of the target salinity levels on pikeperch gut fullness over the course of 11 h. The gut fullness of larvae fed with rotifers was significantly lower at 8‰ salinity than in other tested groups. Salinity of 16‰ resulted in 100% mortality. Survival and growth of pikeperch larvae from 4 to 11 days post-hatching (DPH) was analyzed at low (2‰ and 4‰) and medium (8‰) salinity. Differences in larval performance under tested salinities were found in survival (S), total length, SGR, eye diameter, myotome height, and gut fullness. The highest mean survival (S) and specific growth rate (SGR) were obtained at 2‰ (S = 64%; SGR = 10 ± 5.4% day^{-1}) and 4‰ (S = 65%; SGR = 8.23 ± 3.4% day^{-1}) salinity and significantly differed from 8‰ (S = 36%; SGR = 5 ± 2.3% day^{-1}) and control (S = 34%; SGR = 7 ± 3% day^{-1}) at 11 DPH. At the conclusion of the trial (11 DPH), larvae of all exposures and control presented normal histology with no signs of pathology. Morphometric analyses revealed significant differences in brush border and fold height of anterior intestine among treatment groups. Larvae at 2‰ salinity showed significantly higher intestinal fold height (31.2 ± 5.40 μm) compared to those at 4‰ (26.5 ± 6.11 μm), 8‰ (26.3 ± 4.52 μm), and control (22.8 ± 5.13 μm) ($p < 0.05$). Larvae at 4‰ salinity had significantly higher brush border height (2.50 ± 0.73 μm) compared to those at 2‰ (2.27 ± 0.63 μm) and 8‰ (1.87 ± 0.44 μm) salinity and control (1.66 ± 0.56 μm) ($p < 0.05$). The results showed 2‰ and 4‰ salinity supported higher numbers of motile *B. plicatilis* and higher larval survival and growth rate to 11 DPH.

Keywords Exogenous feeding · Growth · Gut fullness · Intestine · Motility · *Sander lucioperca* · Survival

Abbreviations

RAS Recirculation aquaculture system

Highlights

- Improving survival and fitness of pikeperch larvae
- The use of rotifers for first feeding of pikeperch larvae

DPH Days post-hatching
BW Body weight
TL Total length
SGR Specific growth rate

Introduction

Pikeperch *Sander lucioperca* L. inhabit both fresh and brackish waters (FAO 2012). Its delicate flesh, rapid growth, and attractiveness to anglers (Kestemont et al. 2007; Policar et al. 2016; Wang et al. 2009) make it a commercially valuable fish for European freshwater aquaculture. Pikeperch has been traditionally farmed in ponds and lakes (Hilge and Steffens 1996), making production dependent on natural conditions. Recently developed technologies using recirculating aquaculture systems (RAS) (Molnar et al. 2004) or a combination of pond and RAS (Blecha et al. 2016; Policar et al. 2013) have been successfully applied for pikeperch production; however, high larva mortality is a serious problem (Ostaszewska et al. 2005).

Yanes-Roca et al. (2018) suggest the most effective first-feeding diet of pikeperch larvae to be euryhaline rotifers *Brachionus plicatilis* in combination with *Artemia nauplii*. The benefits of euryhaline rotifers for marine larvae are well documented (Girin 1975; Howell 1997; Nash and Kuo 1975; Yufera et al. 1993), but information of its application in freshwater species is scarce (Allen et al. 2016; Awaiss et al. 1992; Yanes-Roca et al. 2018).

The motility of prey is a key factor in feeding fish larvae (Battaglene 1995; Fielder et al. 2000). *Brachionus plicatilis* tolerate a wide range of salinities, but transfer to freshwater reduces rotifer availability to larvae (Epp and Winston 1978; Epp and Lewis 1984; Fielder et al. 2000; Oie and Olsen 1993). Pikeperch swim horizontally from the beginning of exogenous feeding (Xu et al. 2017), thus requiring more time to capture prey, resulting in an energy deficit due to low numbers of rotifers in the water column (Dowd and Houde 1980; Munk and Kiorboe 1985; Tandler and Sherman 1981). Insufficient prey negatively impacts larva growth and survival rate (Dowd and Houde 1980; Parra and Yufera 2000; Wang and Eckmann 1994), but overfeeding on rotifers can also have a negative effect on survival and growth (Lubzens et al. 1989; Wang and Eckmann 1994) as well as decrease profits and hygiene (Fielder et al. 2000).

The goal of the present study was to evaluate the effect of salinity level on (a) motility of rotifers *B. plicatilis* in a water column over a "6-h period"; (b) gut fullness of pikeperch larvae fed on *B. plicatilis* over a 11 h; and (c) survival, growth, and intestine morphology of pikeperch larvae fed on *B. plicatilis* to 11 DPH.

Materials and methods

Rearing of pikeperch larvae *S. lucioperca* L. and rotifers *B. plicatilis* was performed at the Experimental Fish Facility of the Faculty of Fisheries and Protection of Waters, University of South Bohemia, Czech Republic. Two adult pikeperch females ($W = 1544 \pm 419$ g, TL = 544 ± 38 mm) and two males ($W = 1414 \pm 290$ g, TL = 534 ± 35 mm) were used in this study. For each experiment, one pikeperch pair was used. Pikeperch larvae were stocked in RAS at an optimal temperature of 15.15 ± 0.43 °C until the onset of exogenous feeding at 4–6 DPH. Experiments were conducted separately. Rotifers (mean length 280 μm) were cultured in flat-

bottomed 50-l polyethylene tanks using a batch culture protocol fed with microalgae paste of *Nannochloropsis* sp. (Nanno 3600, Reed Mariculture Inc., USA) at a rate of 1 ml of paste per liter of culture twice daily. Aerated seawater (35 ± 1%) at 24 ± 1 °C and pH 7.95 ± 0.36 were used. The mean density of rotifers was 127 ind ml^{-1}.

Three independent experiments were designed and carried out to study the effect of salinity on motility of rotifers, pikeperch gut fullness, and pikeperch survival and growth performance.

Experiment 1: the effect of salinity on rotifer motility

Rotifers were harvested with a 42-μm sieve and equally distributed among clear plastic 500-ml beakers containing 200 ml water of salinity 0‰, 2‰, 4‰, 8‰, or 16‰. All tested solutions were prepared by mixing freshwater and salt (Instant Ocean® Sea Salt) and measured by a temperature-compensated salinity refractometer (Salinity Refractometer SR5-AQ, Pentair Aquatic Eco-Systems, USA). Each salinity level trial was conducted in triplicate. The temperature in experimental beakers was measured hourly and maintained at 24 ± 0.7 °C. Microalgae and aeration were not provided to the beakers. Before sampling, each beaker was stirred to avoid rotifer sedimentation on the bottom. Representative samples (1 ml per replicate) were randomly taken with a pipette and transferred to a Sedgwick Rafter Chamber Cell to count motile rotifers under a light microscope. Samples were not returned to the beakers. Sampling was repeated at 1-h intervals during the 6-h experiment. The mean number of motile rotifers of the three replicates per treatment was calculated and converted to percentage of motile rotifers.

Experiment 2: effect of salinity on gut fullness of pikeperch larvae after feeding with *B. plicatilis*

Pikeperch larvae (TL = 5.53 ± 0.16 mm; BW = 0.42 ± 0.08 mg) at 6 DPH were randomly stocked at initial density 40 l into 2-l rearing tanks containing 1 l water of the following salinity: 2‰ (group A), 4‰ (group B), 8‰ (group C), and 16‰ (group D). Water preparation and measurement of salinity were conducted as in experiment 1. A control group was placed in freshwater. Each group was conducted in triplicate. Larvae were fed three times daily at 0800, 1200, and 1600 h with the rotifer *B. plicatilis* at 10 rotifers ml^{-1}. Water temperature, pH, and concentration of dissolved oxygen (DO) were measured before each feeding with an oximeter (OxyGuard International A/S, Farum, Denmark) and pH tester (HI98129, Hanna Combo). Mean values were temperature 16 ± 0.1 °C, pH 8.4 ± 0.2, and DO 9.1 ± 0.36 mg l^{-1}. Ammonium concentration was < 0.1 mg l^{-1} and nitrate concentration was < 0.01 mg l^{-1}. Light regime was set at 13 L:11D (0700 to 2000-h light). Light intensity on the water surface ranged from 90 to 100 lx. Aeration was provided to ensure uniform distribution of prey. Forty larvae from one replicate of each group were collected using a 300-μm diameter mesh net 3 h after each feeding (1100, 1500, 1900 h) and anesthetized with 100 mg l^{-1} tricaine methanesulfonate (MS-222; Sigma). Gut fullness was recorded using an Olympus BX41 microscope fitted with Canon-72 digital camera and the Olympus cellSens v.1.3 imaging software and evaluated based on a scale of I (empty) to IV (maximum) (Tielmann et al. 2017). The gut fullness of larvae was evaluated over the course of 11 h and the percentage of each scale (I–IV) was calculated.

Experiment 3: the effect of salinity on survival, growth, and intestine morphology of pikeperch larvae from 4 to 11 DPH

Pikeperch larvae (TL = 5.19 ± 0.08 mm, BW = 0.56 ± 0.1 mg) at 4 DPH were stocked randomly into 2-l rearing tanks at initial density 100 larvae l^{-1} in four replicates of salinity 2‰ (group A), 4‰ (group B), and 8‰ (group C). Preparation and measurements of tested salinities were conducted as in experiment 1. A control group was reared in freshwater. Water temperature, pH, and concentration of dissolved oxygen were measured twice daily at 0700 and 1500 h as in experiment 2. Mean values were temperature 16.9 ± 0.5 °C, pH 8.18 ± 0.32, and DO 8.3 ± 0.36 mg l^{-1}. Nitrate and ammonia levels were measured on the first, middle, and final days of the trial. Ammonium concentration was < 0.1 mg l^{-1} and nitrate concentration was < 0.01 mg l^{-1}. Photoperiod, light intensity, aeration, and larval feeding were as in experiment 2. The tanks were cleaned twice daily by siphoning to remove excrement and uneaten food. Dead larvae were removed and counted daily before each feeding. Water was exchanged daily (50%) before the first feeding.

On the middle (8 DPH) and final days (11 DPH) of the experiment, 10 randomly selected larvae were removed from each rearing tank and anesthetized with 100 mg l^{-1} tricaine methanesulfonate (MS-222; Sigma). Total length, myotome height at anus level, and eye diameter to the nearest (± 0.01 μm) were measured using a stereo microscope SMZ75T (Nikon, Tokyo, Japan) with Quick PHOTO MICRO 3. The survival rate was estimated as

$$\text{Survival}\,(\text{S},\%) = \text{NSF}/\text{NFB} \times 100$$

where NSF is the number of surviving fish and NBF is the initial number of stocked fish (Policar et al. 2011).

Specific growth rate (SGR) (% day^{-1}) was calculated as

$$\text{SGR} = 100\,(\text{In}\,L_2 - \text{In}\,L_1)/t$$

where L_1 and L_2 are the initial and final total lengths, and t is the period of time between L_2 and L_1 in days (Policar et al. 2011). At the conclusion of the trial at 11 DPH, differences in gut fullness (n = 40) prior to feeding were evaluated as in experiment 2.

Histology

Seven fish from each group were collected for histology at 11 DPH. Fish were anesthetized with MS-222, preserved in Davidson's fixative overnight, dehydrated in an ascending ethanol concentration (70, 95, 100%), cleared in xylene, embedded in paraffin, and cut into 5-μm longitudinal sections using a rotary microtome (Galileo, Italy). Sections were stained with hematoxylin and eosin and used to evaluate tissue morphology and to determine height of the fold (± 0.01 μm) and height of brush border (± 0.01 μm) of the anterior intestine. All the histomorphometry measurements were conducted on the anterior intestine, since folds in posterior intestine were not well differentiated. Seven specimens from each treatment group were analyzed. Twenty measurements of the height of intestinal folds and brush border were taken from each fish. Histological preparations were analyzed using an Olympus EX51 light microscope fitted with Canon E600 digital camera and the software ImageJ v.1.50i (National Institutes of Health, USA).

Statistical analyses

Data were presented as mean ± SD and analyzed with the program RStudio (R Core Team 2014). For experiment 1, the significance of differences in motility among groups was evaluated using two-way ANOVA, with *time* and *salinity* as categorical factors and *beaker* nested within time and salinity. For experiments 2 and 3, the differences in survival, SGR, TL, myotome height, eye diameter, gut fullness, intestinal fold height, and brush border height among groups were evaluated using one-way ANOVA analyses with Tukey post hoc test. The level of significance was set at $p < 0.05$.

Results

Effect of salinity on rotifer motility

In all saline groups, rotifers were found on the bottom of the beakers within an hour of stocking (Fig. 1). Rotifers stocked into freshwater showed 100% mortality within 2 h (Fig. 1), and the group was omitted from further analysis. Salinity significantly affected the rotifer motility rate relative to time ($F = 4.88$, $p < 0.01$), with the highest percentage motile rotifers (93 ± 1.9%) observed at the highest level of salinity (16‰) and the lowest (10 ± 2.2%) at the lowest salinity (2‰). In general, the mean percentage of motile rotifers was > 50% at 8‰ and 16‰ salinity over the 6-h trial period. At salinity of 2 and 4‰, the mean percentage of motile rotifers was > 50% 2 h post-stocking. After 4 h, the mean percentage of motile rotifers was significantly higher at salinity of 4‰ (37 ± 4%) than at 2‰ (15 ± 5%) ($p < 0.05$). At the end of the trial (6 h post-stocking) rotifers at 16‰ salinity showed the highest motility rate (93 ± 1.9%), while, at 2 and 4‰, the percentage of motile rotifers was 10 ± 2.2% and 35 ± 5.5%, respectively (Fig. 1).

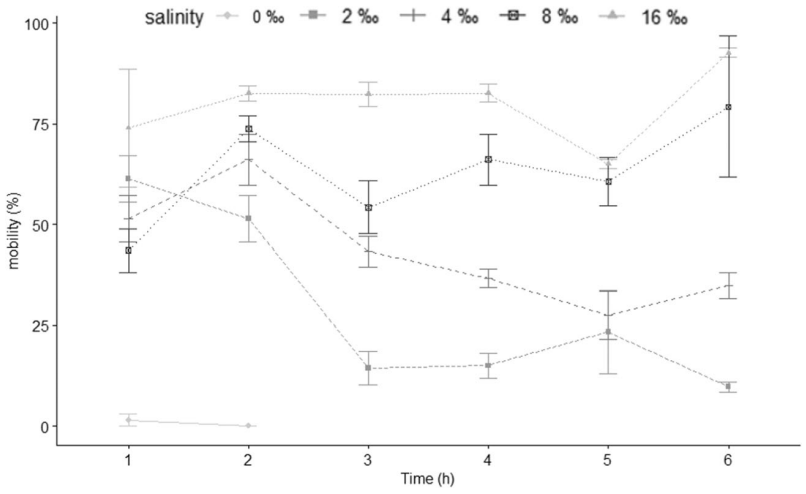

Fig. 1 Mean percentage of motile *Brachionus plicatilis* in the water column 1 to 6 h after transfer from culture medium (salinity 25‰) to salinity 0‰, 2‰, 4‰, 8‰, and 16‰ (three replicates per treatment)

Effect of salinity on gut fullness of pikeperch larvae after feeding with *B. plicatilis* for 11 h

Larvae reared at 16‰ salinity showed 100% mortality, and this group was omitted from the analysis. After first feeding (11:00 h), larvae at 8‰ salinity exhibited 1.14, 1.11, and 1.08 times lower gut fullness than those at 4‰, control, and 2‰, respectively ($p < 0.05$) (Fig. 2). At 15:00 h, gut fullness of larvae at 8‰ was 2.22, 2.22, and 2.16 times lower than at 4‰, 2‰, and control, respectively. At 19:00 h, larvae at 2 at 4‰ salinity and control exhibited gut fullness between II and IV, higher than the group at 8‰ salinity. In general, gut fullness increased after each feeding in 2 and 4‰ salinity groups and in control.

Effect of salinity on survival, growth, and intestine morphology of pikeperch larvae fed with *B. plicatilis*

The highest survival rate at 11 DPH was observed at 4‰ (65.31 ± 15.69%) and 2‰ salinity (63.9 ± 23%) and significantly differed from 8‰ (36.25 ± 25.81%) and control (34.53 ± 17.96%) (Fig. 3).

No significant differences were found in SGR among groups at 8 DPH ($p > 0.05$, Fig. 4A). The highest growth rates at 11 DPH were found at 4‰ (10 ± 5.4% day^{-1}) and 2‰ (8.23 ± 3.4% day^{-1}) salinity, compared to control (7 ± 3% day^{-1}) and 8‰ (5 ± 2.3% day^{-1}) ($p < 0.05$) (Fig. 4B).

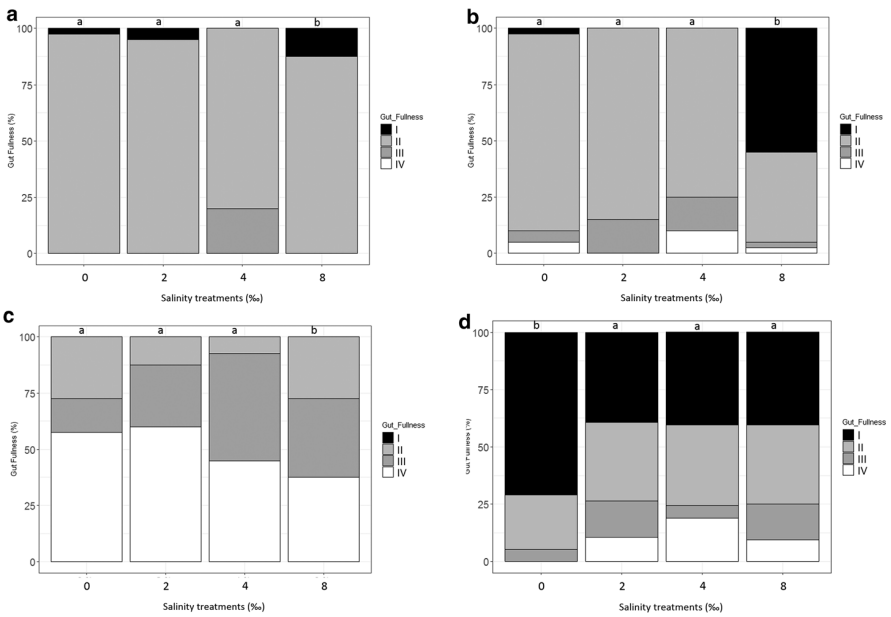

Fig. 2 Larva gut fullness percentage (I–IV; I, empty—IV, maximum fullness) relative to salinity and feeding time. (A) First feeding at 11:00 (6 DPH). (B) Second feeding at 15:00 (6 DPH). (C) Third feeding at 19:00 (6 DPH). (D) Before feeding (11 DPH). Different letters indicate significant difference among groups ($p < 0.05$)

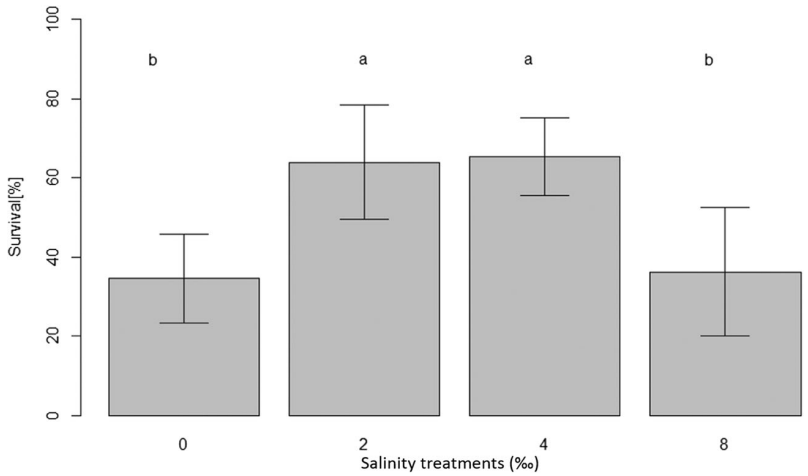

Fig. 3 Larva survival (%) at 11 DPH relative to salinity. Different letters indicate significant difference between groups ($p < 0.05$)

No significant differences were found in TL among groups at 8 DPH (Fig. 5A). The greatest TL at 11 DPH was observed at 4‰ salinity (5.89 ± 0.38 mm) compared to control (5.69 ± 0.21 mm) and salinity of 8‰ (5.55 ± 0.16 mm) ($p < 0.05$, Fig. 5A). No significant differences were found between 4‰ (5.89 ± 0.38 mm) and 2‰ salinity (5.77 ± 0.23 mm) at 11 DPH ($p > 0.05$, Fig. 5A).

Significantly greater myotome height at 8 DPH was found at 8‰ salinity (0.35 ± 0.01 mm) compared to 4‰ salinity (0.34 ± 0.02 mm) and control (0.33 ± 0.01 mm) ($p < 0.05$, Fig. 5B). No significant differences were found between 2‰ salinity and other groups at 8 DPH ($p > 0.05$, Fig. 5B). At 11 DPH, with larvae at 4‰ salinity (0.37 ± 0.04 mm), it showed significantly greater myotome height than those at to 2‰ (0.35 ± 0.03 mm), control (0.35 ± 0.03 mm), and 8‰ (0.33 ± 0.03 mm) ($p < 0.05$) (Fig. 5B).

No significant differences were found in eye diameter at 8 DPH among groups ($p > 0.05$, Fig. 5C). At 11 DPH, larvae at salinity of 2 (0.37 ± 0.02 mm) and 4‰ (0.36 ± 0.02 mm) displayed significantly greater eye diameter compared to controls (0.35 ± 0.01 mm) ($p < 0.05$, Fig. 5C). No significant differences were found between larvae at 8‰ salinity and other groups ($p > 0.05$, Fig. 5C).

At 11 DPH, control larvae showed significantly lower gut fullness than other tested groups, with 0% categorized as level IV compared to 19% at 4‰ salinity, 10.5% at 2‰ salinity, and 9.3% at 8‰ salinity ($p < 0.05$, Fig. 2D).

Histology

At the end of the experiment, all examined fish presented normal histology with no signs of pathology. The enterocytes were well differentiated and adjacent to the eosinophilic brush border membrane. Larvae at 2‰ salinity showed significantly higher intestinal fold height (31.2 ± 5.40 μm) compared to those at 4‰ (26.5 ± 6.11 μm), 8‰ (26.3 ± 4.52 μm), and control (22.8 ± 5.13 μm) ($p < 0.05$, Figs. 6 and 7). Larvae at 4‰ salinity had significantly

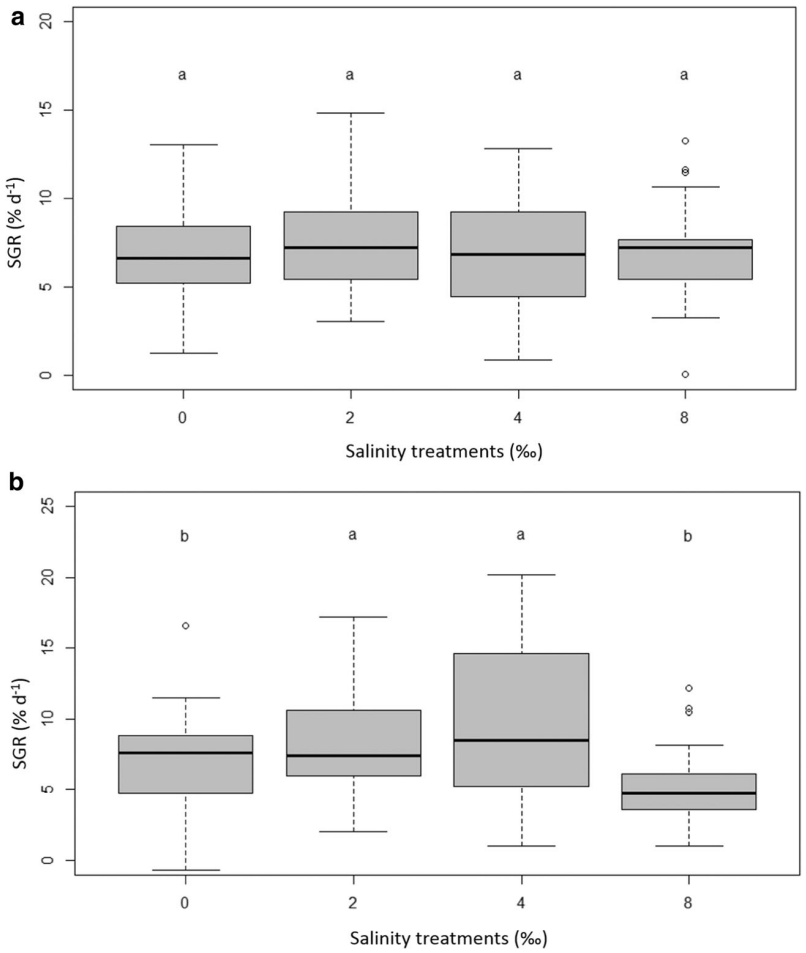

Fig. 4 Larva SGR (% day^{-1}) at 8 DPH (A) and 11 DPH (B). Different letters indicate significant difference among groups ($p < 0.05$)

higher brush border height (2.50 ± 0.73 μm) compared to those at 2‰ (2.27 ± 0.63 μm) and 8‰ (1.87 ± 0.44 μm) salinity and control (1.66 ± 0.56 μm) ($p < 0.05$, Figs. 6 and 7B).

Discussion

Rotifers are commonly used in marine larva culture and were recently introduced for pikeperch larviculture (Yanes-Roca et al. 2018). Rotifer motility was evaluated at salinities above 10‰ by Fielder et al. (2000). However, this salinity could be damaging to pikeperch larvae. In freshwater, *B. plicatilis* were completely immotile within 2 h, while those stocked at 2‰ and 4‰ salinity retained motility over a 6-h period. Motility of *B. plicatilis* stocked at 8‰ and 16‰ salinity exhibited increased duration of motility.

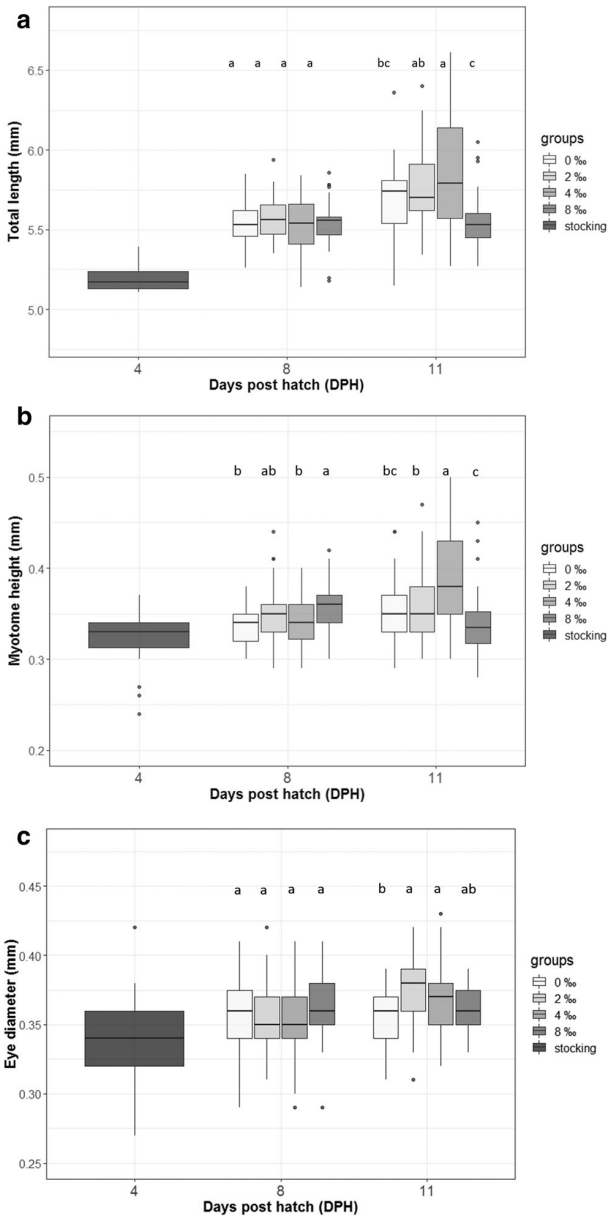

Fig. 5 Larval growth parameters (mm) relative to salinity at 4, 8, and 11 DPH. (A) Total length. (B) Myotome height. (C) Eye diameter. Dots indicate outliers. Different letters indicate significant differences among groups ($p < 0.05$)

We measured eye diameter, which might reflect visual acuity (Trabelsi et al. 2013) and influence detection of prey. Pikeperch larvae stocked at 2 and 4‰ salinity

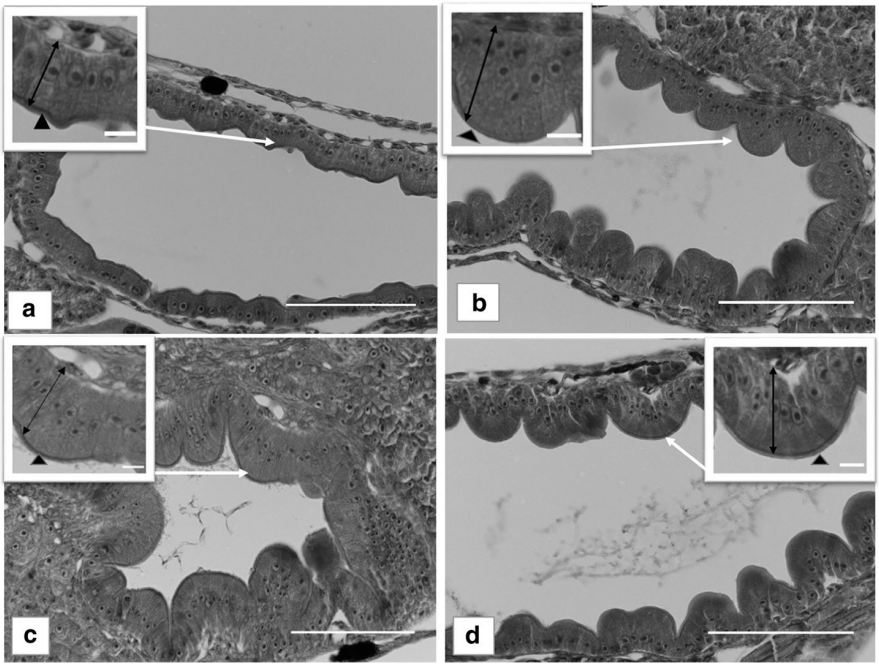

Fig. 6 Longitudinal section of the anterior intestine of pikeperch larvae at 11 DPH. **a** Control—low fold (double arrow) and brush border (arrow) height. **b** Reared at 2‰ salinity—greater fold (double arrow) and brush border (arrow) height. **c** Reared at 4‰ salinity—higher folds (double arrow) and brush border (arrow). **d** Reared at 8‰ salinity—greater of fold height (double arrow) and lower brush border height (arrow). Hematoxylin and eosin staining. Scale bar, 50 μm

showed significantly higher survival (64% and 65%, respectively) during first exogenous feeding compared to the freshwater control (34%). The main source of the high mortality of controls was likely the lack of motile rotifers in the water column over time. Yanes-Roca et al. (2018) reported rotifer survival up to 10 h between feedings at 3‰ salinity. Positive effects of low to medium salinity in inhibiting microbial pathogens such as bacteria have been documented for percid fishes (Guo et al. 1993; Lozys 2004).

Morphology of the intestine such as the height of folds and brush border can reflect the feeding status of fish (Dumitrescu et al. 2014; Gisbert and Doroshov 2003; Ostaszewska et al. 2006). A low intestine fold height can indicate inadequate feeding, including starvation (Gisbert and Doroshov 2003; Kamaszewski and Ostaszewska 2014; Ostaszewska et al. 2006). We observed pikeperch reared in freshwater to have lower height of anterior intestine folds than those in saline water. Larvae reared in freshwater and at 8‰ salinity displayed the lowest brush border height of the anterior intestine. These observations suggest that the pikeperch reared in freshwater had inadequate rotifer intake. This is further supported by our findings with respect to motility of rotifers in different salinities and the survival and growth of pikeperch larvae. Larvae at salinity of 8‰ did not feed for the first 48 h of the trial, which could have affected the height of the anterior intestine as well as survival and growth.

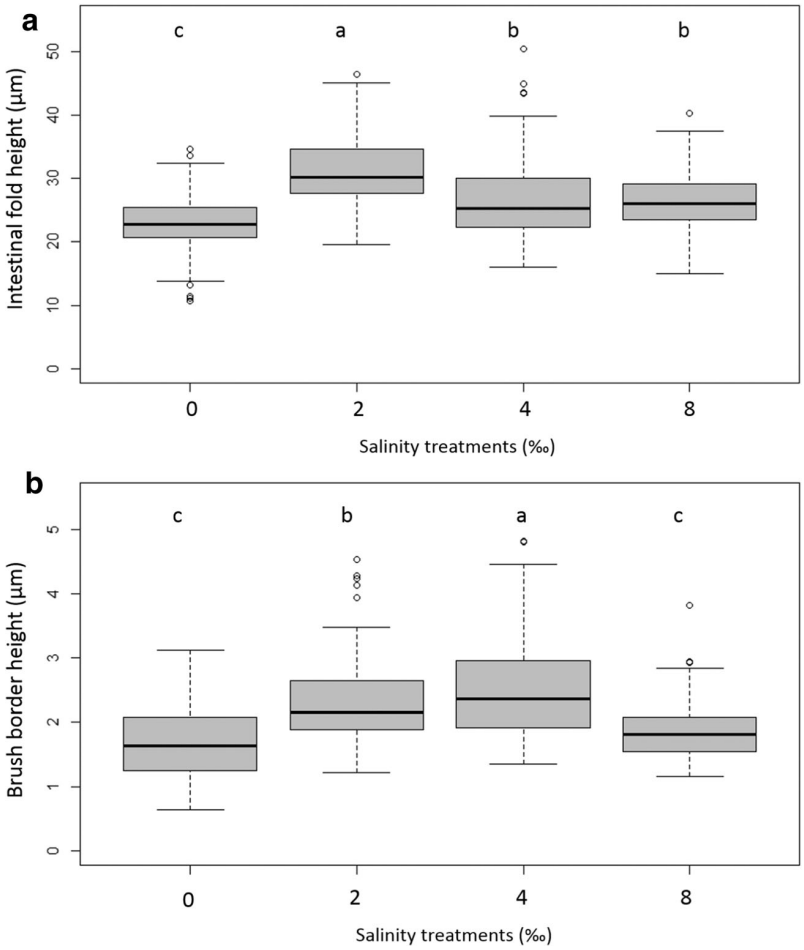

Fig. 7 Larval anterior intestinal morphology (μm) relative to salinity at 11 DPH. (A) Intestinal fold height. (B) Brush border height. Dots indicate outliers. Different letters indicate significant difference among groups ($p < 0.05$)

Low saline water (2 and 4‰) in pikeperch larviculture resulted in higher rates of motile rotifers to supply continual larva feeding, simpler management, and likely lower costs compared to freshwater culture.

Conclusions

The rearing of pikeperch larvae in water of low salinity was shown to benefit survival and growth during initial exogenous feeding with *B. plicatilis*. The key factor is high availability of prey of accepted size for pikeperch larvae due to greater numbers of motile rotifers compared to freshwater. Future study is needed to investigate the effect of salinity on pikeperch larvae culture over a longer time and to optimize rotifer

feeding in terms of rotifer density, combination with *Artemia*, and weaning to *Artemia* or artificial starter.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics statement The experimental procedures were performed according to the ethical rules of the EU-harmonized Animal Welfare Act of the Czech Republic. The unit is licensed (No. 53100/2013-MZE-17214) according to the Czech National Directive (the Law against Animal Cruelty, No. 246/1992).

References

Allen RL, Wallace RL, Sisson BE (2016) A rotifer-based technique to rear zebrafish larvae in small academic settings. Zebrafish 13:281–286

Awaiss A, Kestemont P, Micha JC (1992) Nutritional suitability of the rotifer, Brachionus-calyciflorus Pallas for rearing fresh-water fish larvae. J Appl Ichthyol 8:263–270

Battaglene SC (1995) Induced ovulation and larval rearing of four species of Australian marine fish. PhD thesis, University of Tasmania, Australia. 215 pp.

Blecha M, Kristan J, Policar T (2016) Adaptation of intensively reared pikeperch (Sander Lucioperca) juveniles to pond culture and subsequent re-adaptation to a recirculation aquaculture system. Turk J Fish Aquat Sci 16:15–18

Dowd C, Houde E (1980) Bream, Archosargus rhomboidalis (Sparidae). Mar Ecol Prog Ser 3:181–185

Dumitrescu G, Grozea A, Szilagyi P et al (2014) Natural vs. controlled rearing conditions in pikeperch (Sander lucioperca) fingerlings. A histological approach at the liver and intestine level. Rom Biotechnol Lett 19:9531–9542

Epp RW, Lewis WM (1984) Cost and speed of locomotion for rotifers. Oecologia 61:289–292

Epp RW, Winston PW (1978) Effects of salinity and Ph on activity and oxygen-consumption of Brachionus-Plicatilis (Rotatoria). Comp Biochem Physiol A Physiol 59:9–12

FAO (2012) Cultured aquatic species information programme. Food and Agriculture Organization of the United Nations. Available at: http://faostat.fao.org (Last accessed 12 November 2018).

Fielder DS, Purser GJ, Battaglene SC (2000) Effect of rapid changes in temperature and salinity on availability of the rotifers Brachionus rotundiformis and Brachionus plicatilis. Aquaculture 189:85–99

Girin M (1975) Marine fish culture in France - recent developments. Aquaculture 5:113–113

Gisbert E, Doroshov SI (2003) Histology of the developing digestive system and the effect of food deprivation in larval green sturgeon (Acipenser medirostris). Aquat Living Resour 16:77–89

Guo R, Mather P, Capra MF (1993) Effect of salinity on the development of silver perch (Bidyanus-Bidyanus) eggs and larvae. Comp Biochem Physiol A Physiol 104:531–535

Hilge V, Steffens W (1996) Aquaculture of fry and fingerling of pike-perch (Stizostedion lucioperca L) - a short review. J Appl Ichthyol 12:167–170

Howell BR (1997) A re-appraisal of the potential of the sole, Solea solea (L.), for commercial cultivation. Aquaculture 155:355–365

Kamaszewski M, Ostaszewska T (2014) The effect of feeding on morphological changes in intestine of pike-perch (Sander lucioperca L.). Aquac Int 22:245–258

Kestemont P, Xu XL, Hamza N et al (2007) Effect of weaning age and diet on pikeperch larviculture. Aquaculture 264:197–204

Lozys L (2004) The growth of pikeperch (Sander lucioperca L.) and perch (Perca fluviatilis L.) under different water temperature and salinity conditions in the Curonian Lagoon and Lithuanian coastal waters of the Baltic Sea. Hydrobiologia 514:105–113

Lubzens E, Tandler A, Minkoff G (1989) Rotifers as food in aquaculture. Hydrobiologia 186:387–400

Molnar T, Hancz C, Bodis M et al (2004) The effect of initial stocking density on growth and survival of pike-perch fingerlings reared under intensive conditions. Aquac Int 12:181–189

Munk P, Kiorboe T (1985) Feeding-behavior and swimming activity of larval herring (Clupea-Harengus) in relation to density of copepod nauplii. Mar Ecol Prog Ser 24:15–21

Nash CE, Kuo CM (1975) Hypotheses for Problems impeding mass propagation of grey mullet and other finfish. Aquaculture 5:119–133

Oie G, Olsen Y (1993) Influence of rapid changes in salinity and temperature on the mobility of the rotifer Brachionus-Plicatilis. Hydrobiologia 255:81–86

Ostaszewska T, Dabrowski K, Czuminska K, Olech W, Olejniczak M (2005) Rearing of pike-perch larvae using formulated diets - first success with starter feeds. Aquac Res 36:1167–1176

Ostaszewska T, Korwin-Kossakowski M, Wolnicki J (2006) Morphological changes of digestive structures in starved tench Tinca tinca (L.) juveniles. Aquac Int 14:113–126

Parra G, Yufera M (2000) Feeding, physiology and growth responses in first-feeding gilthead seabream (Sparus aurata L.) larvae in relation to prey density. J Exp Mar Biol Ecol 243:1–15

Policar T, Blaha M, Kristan J, Stejskal V (2011) High-quality and stable production of pondcultured pikeperch (*Sander lucioperca*) juveniles under pond conditions. Edition of handbooks. USB FFPW 110 33p. (in Czech, only)

Policar T, Stejskal V, Kristan J, Podhorec P, Svinger V, Blaha M (2013) The effect of fish size and stocking density on the weaning success of pond-cultured pikeperch Sander lucioperca L. juveniles. Aquac Int 21: 869–882

Policar T, Blecha M, Krist'an J et al (2016) Comparison of production efficiency and quality of differently cultured pikeperch (Sander lucioperca L.) juveniles as a valuable product for ongrowing culture. Aquac Int 24:1607–1626

R Core Team (2014) A language and environment for Statistical Computing. Vienna, Austria. http://www.R-project.org. Accessed 22 Nov 2017

Tandler A, Sherman R (1981) Food organism concentration, environmental temperature and survival of the gilthead bream (Sparus aurata) larvae. Spec Publ Europ Maricult Soc 6:237–248

Tielmann M, Schulz C, Meyer S (2017) The effect of light intensity on performance of larval pike-perch (Sander lucioperca). Aquacultural Engineering 77:61–71

Trabelsi A, Gardeur JN, Teletchea F, Brun-Bellut J, Fontaine P (2013) Hatching time effect on the intra-spawning larval morphology and growth in Northern pike (Esox luciusL.). Aquac Res 44:657–666

Wang N, Eckmann R (1994) Effects of temperature and food density on egg development, larval survival and growth of perch (Perca-Fluviatilis L). Aquaculture 122:323–333

Wang N, Xu XL, Kestemont P (2009) Effect of temperature and feeding frequency on growth performances, feed efficiency and body composition of pikeperch juveniles (Sander lucioperca). Aquaculture 289:70–73

Xu ZC, Li CJ, Ling QF, Gaughan S, Wang G, Han X (2017) Early development and the point of no return in pikeperch (Sander lucioperca L.) larvae. Chin J Oceanol Limnol 35:1493–1500

Yanes-Roca C, Mraz J, Born-Torrijos A et al (2018) Introduction of rotifers (Brachionus plicatilis) during pikeperch first feeding. Aquaculture 497:260–268

Yufera M, Pascual E, Polo A, Sarasquete MC (1993) Effect of starvation on the feeding ability of gilthead seabream (Sparus-Aurata L) Larvae at 1st Feeding. J Exp Mar Biol Ecol 169:259–272

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CHAPTER 5

EFFECT OF *BRACHIONUS PLICATILIS* DENSITY ON PIKEPERCH (*SANDER LUCIOPERCA* L.) LARVA PERFORMANCE AT FIRST FEEDING

Imentai, A., Yanes-Roca, C., Malinovskyi, O., Policar, T., 2019. Effect of *Brachionus plicatilis* density on pikeperch (*Sander lucioperca* L.) larva performance at first feeding. Journal of Applied Ichthyology https://doi.org/10.1111/jai.13963

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Effect of *Brachionus plicatilis* density on pikeperch (*Sander lucioperca* L.) larva performance at first feeding

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1 | INTRODUCTION

The transition from endogenous yolk reserves to exogenous food sources is a crucial period for fish larvae (Hjort, 1914). The rotifer *Brachionus plicatilis* has been recognized as a suitable first food for pikeperch *Sander lucioperca* L. larvae (Yanes-Roca et al., 2018). There are many studies of the effects of rotifer density on fish larvae that suggest that optimal prey concentration is species-specific (Parra & Yufera, 2000; Rosalescasian, 1994; Wang & Eckmann, 1994). Overfeeding can result in lower survival and growth (Dabrowski, 1984; Laurel, Brown, & Anderson, 2001; Lubzens, Tandler, & Minkoff, 1989), as does low prey concentration when the energy requirements of larvae are not met (Dowd & Houde, 1980; Tandler & Sherman, 1981). The aim of this study was to determine the minimum *B. plicatilis* density effective for acceptable survival and growth of pikeperch larvae during first exogenous feeding.

2 | MATERIALS AND METHODS

Pikeperch larvae and *B. plicatilis* were cultured at the Experimental Fish Facility of the Faculty of Fisheries and Protection of Waters, University of South Bohemia, Czech Republic. Larvae were obtained from controlled reproduction (Krist'an, Alavi, Stejskal, & Policar, 2013; Kristan et al., 2018) and acclimated in a recirculating aquaculture system until the onset of exogenous feeding five days post-hatching (DPH). Rotifers were cultured in 50-L flat-bottomed polyethylene tanks using a batch culture protocol and fed with a microalga paste of *Nannochloropsis* sp. (Nanno 3600, ReedMariculture Inc., USA) at a rate of 1 ml/L of culture twice a day.

Pikeperch larvae (TL = 5.98 ± 0.08 mm, BW = 0.55 ± 0.06 mg, 4 DPH) were stocked randomly into sixteen 2-L rearing tanks at initial density of 100 larvae/L. Water temperature, pH, and the concentration of dissolved oxygen (DO) were measured before each feeding with an oximeter (OxyGuard International A/S, Farum, Denmark) and pH tester (HI98129, Hanna Combo). Mean values were temperature 16 ± 0.1°C, pH 7.5 ± 0.2, and DO 6.7 ± 0.63 mg/L. Ammonium and nitrite were measured on stocking days, in the middle, and at the conclusion of the trial with a multiparameter photometer (HI83300, Hanna). Ammonium concentration was <0.1 mg/L and nitrite concentration was <0.01 mg/L. Light regime was set at 13L:11D (0700 to 2000 hr light). Light intensity on the water surface was 90–100 lux. Salinity was 5 ± 0.5 ppt. The tanks were cleaned twice daily by siphoning to remove excrement, dead larvae, and uneaten feed.

Nanno 3600 was added to the larva tanks twice daily (2 × 300,000 cells/ml) beginning at 4 DPH and continuing throughout the experiment. Beginning at mouth opening (5 DPH), four densities of *B. plicatilis* were provided: 2 ml^{-1} (Group A), 6 ml^{-1} (Group B), 10 ml^{-1} (Group C), and 20 ml^{-1} (Group D). Each density trial was conducted in four replicates. Rotifers were provided as live feed to the fish larvae three times daily with residual counts prior to each feeding. In the morning before feeding and 2 hr after each feeding during the light phase, representative samples (1 ml per replicate) were randomly taken by pipette and transferred to a Sedgwick Rafter Chamber Cell under a light microscope to count rotifers, and density in the larva tanks was adjusted to the designated level.

On the first day (4 DPH), 40 larvae and at 9 DPH, 10 randomly selected larvae (40 per treatment group) were removed from each rearing tank and anesthetized with MS-222 (tricaine methanesulphonate, Sigma; 100 mg/L). The anesthetized larvae were measured for total length (TL) to the nearest ± 0.01 mm using a stereo microscope SMZ75T (Nikon, Tokyo, Japan) with Quick PHOTO MICRO 3. Wet weight (±0.1 mg) was measured using a Kern ABT analytical balance (Kern & Sohn GmbH, Germany) after removal of water by placing the larvae on a dry paper tissue. Specific growth rate (SGR), survival and Fulton's condition factor (K) were calculated at 9 DPH (Policar et al., 2011).

2.1 | Statistical analyses

Data were analyzed with the program RStudio (R Core Team, 2014) and presented as mean ± *SD* Normal distribution of data was confirmed by Shapiro-Wilk's test. One-way ANOVA analyses with Tukey post-hoc test were used for the comparison of the survival, SGR, K, and wet weight among experimental groups. The level of significance was set at $p < .05$.

3 | RESULTS

A high survival rate was found among all tested groups (78.9%–89.4%) at 9 DPH, with no significant differences ($p > .05$, Table 1). The highest growth rates were observed in Group D (15.16 ± 3.10% d^{-1}), which differed significantly from Group A (10.24 ± 3.95% d^{-1}; $p < .05$). No significant differences were found in SGR among groups D, B (12.52 ± 3.55% d^{-1}), and C (12.08 ± 3.28% d^{-1}). Larvae from Group D showed higher final wet weight (1.34 ± 0.2 mg) compared to group A (1.1 ± 0.16 mg; $p < .05$, Figure 1). No significant differences were found in wet weight among groups B, C, and D at 9 DPH ($p > .05$, Figure 1). All tested larvae showed similar K (0.37–0.42).

4 | DISCUSSION

Brachionus plicatilis concentration of 6 ml^{-1} was sufficient to support larval survival (81%) and growth (SGR = 12.52% d^{-1}) from 5 DPH to 9 DPH. Similar results have been obtained for perch *Perca fluviatilis* larvae (Wang & Eckmann, 1994). Density of 7 rotifers/ml was suggested for grunion *Leuresthes tenuis* larvae (Rosalescasian, 1994). A critical factor to consider is that the trial began at mouth opening, when yolk reserves were not yet exhausted. In previous studies, the mixed-feeding transition period in pikeperch from endogenous to exogenous feeding occurred from 6 or 7 DPH to 15 DPH at water temperature 20°C (Ostaszewska, 2005) and from 8 to 14 DPH at 15°C (Xu et al., 2017). The absorption rate of the yolk sac can vary depending on environmental factors, especially water temperature, and yolk quantity at fertilization, (Yufera & Darias, 2007). In the present study, water temperature was maintained at 16.5°C and mouth

TABLE 1 Survival, specific growth rate (SGR), and Fulton's condition factor (K) assessed at 9 DPH fed *B. plicatilis* at four concentrations

	Experimental groups			
	A	B	C	D
Parameter	2 rotifers/ml	6 rotifers/ml	10 rotifers/ml	20 rotifers/ml
Survival (%)	89.37 ± 14.43	81.87 ± 8.02	87.87 ± 9.60	78.87 ± 5.13
SGR (% d^{-1})	10.24 ± 3.95^{a}	12.52 ± 3.55^{ab}	12.08 ± 3.28^{ab}	15.16 ± 3.10^{b}
K	0.37 ± 0.08	0.42 ± 0.12	0.37 ± 0.05	0.37 ± 0.07

Different superscripts indicate significant difference ($p < .05$).

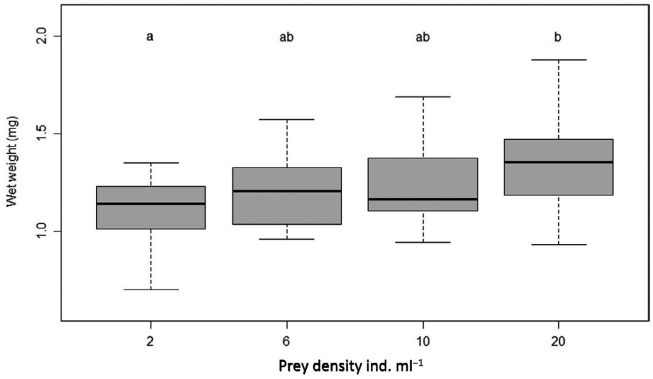

FIGURE 1 Boxplot of wet weight (mg) ($n = 40$) of pikeperch larvae at 9 DPH from different rotifer densities showing median, quartiles and whiskers. Different letters indicate significant difference among groups ($p < .05$)

opening was at 5 DPH. A low saline environment (5 ppt; Imentai, Yanes-Roca, Steinbach, & Policar, 2019) and Nanno 3600 was used to maintain rotifer availability in the tank.

At 9 DPH yolk-sac absorption was complete and the oil globule was exhausted. Therefore, feeding of *B. plicatilis* provided sufficient support for growth of pikeperch larvae during a combination of exogenous and endogenous feeding.

5 | CONCLUSION

Feeding pikeperch larvae on *B. plicatilis* at 20 ml^{-1} from 5 to 9 DPH at 16.5°C provided the highest growth rates. However, 6 rotifers/ml produces adequate survival and growth and reduces costs of production (Kestemont, Dabrowski, & Summerfelt, 2015).

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ETHICS STATEMENT

The experimental procedures were performed according to the criteria of the EU-harmonized Animal Welfare Act of the Czech Republic. The unit is licensed (No. 53100/2013-MZE-17214) according to the Czech National Directive (Law against Animal Cruelty, No. 246/1992).

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REFERENCES

Dabrowski, K. (1984). The feeding of fish larvae - Present state of the art and perspectives. *Reproduction Nutrition Development*, *24*, 807–833. https://doi.org/10.1051/Rnd:19840701

Dowd, C., & Houde, E. (1980). Bream, *Archosargus rhomboidalis* (Sparidae). *Marine Ecology Progress Series*, *3*, 181–185.

Hjort, J. (1914). Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. *Rapports Et Procès-Verbaux Des Réunions Conseil Permanent International Pour L'exploration De La Mer*, *20*, 1–228.

Imentai, A., Yanes-Roca, C., Steinbach, C., & Policar, T. (2019). Optimized application of rotifers *Brachionus plicatilis* for rearing pikeperch *Sander lucioperca* L. larvae. *Aquaculture International*, *27*, 1137–1149. https://doi.org/10.1007/s10499-019-00420-3

Kestemont, P., Dabrowski, K., & Summerfelt, R. C. (Eds.) (2015). *Biology and culture of percid fishes: Principles and practices*. Dordrecht, Netherlands: Springer. https://doi.org/10.1007/978-94-017-7227-3

Krist'an, J., Alavi, S. M. H., Stejskal, V., & Policar, T. (2013). Hormonal induction of ovulation in pikeperch (*Sander lucioperca* L.) using human chorionic gonadotropin (hCG) and mammalian GnRH analogue. *Aquaculture International*, *21*, 811–818. https://doi.org/10.1007/s10499-012-9572-y

Kristan, J., Zarski, D., Blecha, M., Policar, T., Malinovskyi, O., Samarin, A. M., & Kucharczyk, D. (2018). Fertilizing ability of gametes at different post-activation times and the sperm-oocyte ratio in the artificial reproduction of pikeperch *Sander lucioperca*. *Aquaculture Research*, *49*, 1383–1388. https://doi.org/10.1111/are.13570

Laurel, B. J., Brown, J. A., & Anderson, R. (2001). Behaviour, growth and survival of redfish larvae in relation to prey availability. *Journal of Fish Biology*, *59*, 884–901. https://doi.org/10.1006/jfbi.2001.1704

Lubzens, E., Tandler, A., & Minkoff, G. (1989). Rotifers as food in aquaculture. *Hydrobiologia*, *186*, 387–400. https://doi.org/10.1007/BF00048937

Ostaszewska, T. (2005). Developmental changes of digestive system structures in pike-perch (*Sander lucioperca* L.). *Electronic Journal of Ichthyology*, *2*, 65–78.

Parra, G., & Yufera, M. (2000). Feeding, physiology and growth responses in first-feeding gilthead seabream (*Sparus aurata* L.) larvae in relation to prey density. *Journal of Experimental Marine Biology and Ecology*, *243*, 1–15. https://doi.org/10.1016/S0022-0981(99)00106-9

Policar, T., Podhorec, P., Stejskal, V., Kozak, P., Svinger, V., & Alavi, S. M. H. (2011). Growth and survival rates, puberty and fecundity in captive common barbel (*Barbus barbus* L.) under controlled conditions. *Czech Journal of Animal Science*, *56*, 433–442. https://doi.org/10.17221/3236-CJAS

R Core Team. (2014). *A language and environment for statistical computing*. Vienna, Austria: R Foundation for statistical computing. Retrieved from http://www.Rproject.org (accessed 22 November 2017).

Rosalescasian, J. A. (1994). The effect of rotifer (*Brachionus plicatilis*) density on the survival and growth of California Grunion (Leuresthes-Tenuis Ayres) larvae. *Marine Environmental Research*, *38*, 71–75. https://doi.org/10.1016/0141-1136(94)90001-9

Tandler, A., & Sherman, R. (1981). Food organism concentration, environmental temperature and survival of the gilthead bream (*Sparus aurata*) larvae. *Special Publication. European Mariculture Society*, *6*, 237–248.

Wang, N., & Eckmann, R. (1994). Effects of temperature and food density on egg development, larval survival and growth of perch (*Perca fluviatilis* L). *Aquaculture*, *122*, 323–333. https://doi.org/10.1016/0044-8486(94)90341-7

Xu, Z. C., Li, C. J., Ling, Q. F., Gaughan, S., Wang, G. C., & Han, X. F. (2017). Early development and the point of no return in pikeperch (*Sander lucioperca* L.) larvae. *Chinese Journal of Oceanology and Limnology*, *35*, 1493–1500. https://doi.org/10.1007/s00343-017-6042-4

Yanes-Roca, C., Mraz, J., Born-Torrijos, A., Holzer, A. S., Imentai, A., & Policar, T. (2018). Introduction of rotifers (*Brachionus plicatilis*) during pikeperch first feeding. *Aquaculture*, *497*, 260–268. https://doi.org/10.1016/j.aquaculture.2018.08.004

Yufera, M., & Darias, M. J. (2007). The onset of exogenous feeding in marine fish larvae. *Aquaculture*, *268*, 53–63. https://doi.org/10.1016/j.aquaculture.2007.04.050

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CHAPTER 6

General discussion

Rotifers have proven to be an appropriate starter diet for larval fish and crustaceans (Awaiss et al., 1992; Das et al., 2012; Hamre, 2016; Theilacker and McMaster, 1971), and it is common practice in commercial marine and some freshwater hatcheries to use rotifers along with *Artemia* nauplii for initial feeding. Although there are some reports of using rotifers in pond culture of larval pikeperch (Verreth and Kleyn, 1987; Peterka et al., 2003), many questions regarding the introduction of rotifers into pikeperch first exogenous feeding remain largely unanswered.

Introduction of rotifers

The transfer from endogenous to exogenous feeding is a critical period in intensive pikeperch larviculture that can lead to high mortality (Steenfeldt, 2015; Policar et al., 2019). The major obstacle to successful early pikeperch larval feeding is their small mouth opening (Hamza et al., 2015; Yanes-Roca, et al., 2018). Consequently, prey size plays an important role in the initial exogenous feeding (Faleiro and Narciso, 2009). Usually, small strains of brine shrimp (*Artemia*) nauplii (420–480 μm) are used as starter feed in pikeperch hatcheries (Kestemont and Henrotte, 2015). However, the even smaller (123–292 μm) rotifers *Brachionus plicatilis* (Snell and Carrillo, 1984) might help to reduce the time when first feeding can be initiated (Steenfeldt, 2015). Chapter 2 (Yanes-Roca et al., 2018) reports comparison of the impact on pikeperch larval performance of two common live prey, *Artemia* and *B. plicatilis*, and their combination, as starter feed. This was the first study assessing use of *B. plicatilis* as initial feed for pikeperch initial exogenous feeding. The results confirmed the benefit of rotifers to pikeperch larval survival, development, and growth at the beginning of exogenous feeding. Both rotifers and the rotifers/*Artemia* combination showed advantages in terms of growth and survival of pikeperch larvae to 17 DPH. Larvae fed on rotifers only obtained the highest survival (74%), similar to that reported in earlier studies by Szkudlarek and Zakęś (2007) and Tielmann et al. (2017), who reared fish in 200l, compared to our 2l tanks, which may have affected swim bladder inflation and impacted our results. Although larvae fed only rotifers showed the highest survival rate, the growth of larvae fed rotifers in combination with the larger *Artemia* was significantly higher. During larviculture, the size of prey must be increased as larval size increases in order to optimize growth (Lasker et al., 1970; Hunter and Kimbrell, 1980).

Better growth performance of larvae fed the *Artemia*/rotifers combination could also be attributed to nutritional content. Highly unsaturated fatty acids are crucial to the survival and development of fish larvae (Whyte and Nagata, 1990). We found that larvae fed only *B. plicatilis* exhibited almost twice the DHA and LA of those fed on *Artemia*. This could be related to enrichment of live feed. The nutritional composition of rotifers and *Artemia* is primarily determined by their food source (Chakraborty et al., 2007; Das et al., 2012). In our study, rotifers were enriched with *Nannochloropsis* spp., and *Artemia* were fed to larvae immediately after hatching. A principal finding of the study was that the combination of rotifers and *Artemia* is the most favourable diet for larval pikeperch first exogenous feeding. Obtained data could be useful to pikeperch hatcheries and may help to increase efficiency of pikeperch larval culture on a commercial scale.

Feeding regime

In Chapter 2 (Yanes-Roca et al., 2018), we concluded that a diet combining *Artemia* and rotifers for larval pikeperch first exogenous feeding provided highest growth and gut fullness. We observed a clear larva preference for *Artemia* as opposed to *B. plicatilis* from 10–11 DPH, based on morphometric parameters and gut fullness. The main drawback of feeding larva on rotifers is the high cost and labour required for their production (Steenfeldt, 2015; Policar et al., 2019). Therefore, we aimed to establish the optimal feeding regime with rotifers during pikeperch larval first exogenous feeding.

We found significantly higher survival in larvae fed rotifers for the initial 3 days followed by feeding the rotifer/*Artemia* combination (68 ± 5.51%) or *Artemia* only (53 ± 5.43%) for the following 9 days (Imentai et al., 2020). The highest SGR was observed in the group fed the rotifer/*Artemia* combination after the 3-day rotifer feeding. High survival and growth rates could be attributed to prey size preference and nutritional value coordinated with growth of larvae. The beginning of exogenous feeding is associated with mouth opening, and larvae are likely to select prey smaller than their mouth width. The mouth gape of pikeperch, along with that of other percids, is small (Steenfeldt, 2015). The difference between size of predator and prey in plays a key role in success of introduction of rotifers at early larval stages (Faleiro and Narciso, 2009). With the growth of larvae, the size of prey must be increased to optimize growth performance (Hunter, 1980; Hunter and Kimbrell, 1980; Lasker et al., 1970). The quantity of energy expended on capture and ingestion of disproportionately large prey can be high and affect feeding efficiency (Faleiro and Narciso, 2009). In addition to the size discrepancy, *B. plicatilis* and *Artemia* differ in motion, behaviour, form, colour, and nutritional composition.

Histological analysis of liver, pancreas, and anterior intestine allows analysis of larva nutritional status (Gisbert and Doroshov, 2003; Kamaszewski and Ostaszewska, 2014; Ostaszewska, et al., 2006; Rašković, et al., 2011). Our histomorphometric results revealed that choice of prey and duration of feeding affect the anterior intestine and liver of larvae. Significantly lower enterocyte height in the anterior intestine was observed in groups with a longer rotifer-feeding period. Low height of intestinal folds, decrease in the profile area of enterocytes, and the lack of absorptive vacuoles can indicate possible diet-related pathology ranging from inadequate nutritional status to starvation (Gisbert and Doroshov, 2003; Ostaszewska et al., 2006; Kamaszewski and Ostaszewska, 2014).

The results of this research present the first optimized feeding protocol using live rotifers as starter diet for pikeperch larvae during the initial exogenous feeding period.

Optimized application of rotifers

The rotifers *B. plicatilis* can tolerate a wide range of salinities (Øie and Olsen, 1993; Fielder et al., 2000), although transfer from a high-salinity culture to freshwater larva tanks reduces rotifers availability to the predator (Epp and Winston, 1978; Epp and Lewis, 1984; Øie and Olsen, 1993; Fielder et al., 2000). The motility of prey is a major factor affecting fish larvae feeding success (Battaglene, 1995; Fielder et al., 2000). In rapid transfer, rotifers sink and stick to the bottom and sides of tanks and are not accessible to fish larvae (Lubzens et al., 1989; Øie and Olsen, 1993). In this situation, fish farmers cannot effectively utilize the potential of rotifers, resulting in lower survival with higher production costs.

In Chapter 3 (Imentai et al., 2019a), we report the effect of different salinity levels on *B. plicatilis* motility and, consequently, on pikeperch larva performance. Motility of rotifers was assessed at 0‰, 2‰, 4‰, 8‰, and 16‰ salinity. *Brachionus plicatilis* were immotile

in freshwater, but were motile in 2‰ and 4‰ salinity. It has been reported that *B. plicatilis* oxygen consumption and activity decrease after transfer to low salinity, increasing after a period of time (Fielder et al., 2000). Generally, our study showed that rapid changes in salinity altered motility of rotifers. Nevertheless, rotifers remained motile at low salinity levels over a 6-hour period.

In addition, larvae were exposed to the same salinity levels as the rotifers in the first days of exogenous feeding. Under natural conditions, pikeperch occurs in both fresh and brackish waters (Policar et al., 2019). In Yanes-Roca et al. (2018) pikeperch larvae were reared at 3‰ salinity for preventative measures without analysing effects on survival or rotifer swimming behaviour. The larvae exposed to low salinity survived and grew with no negative impact. This has been reported previously for pikeperch larvae by Lund et al. (2019). Some authors have suggested that rearing selected percids in slightly saline water might have a beneficial effect with regard to parasite and bacterial infection (Guo et al., 1993; Ložys, 2004). However, we found larvae exposed to salinity above 8‰ grow slowly. For feeding to pikeperch larvae, it is advantageous to culture rotifers at low salinity to reduce osmotic stress upon transfer to pikeperch cultures, and limit impairment of swimming and survival. Results of this research demonstrated the efficacy of rearing pikeperch larvae in water of 2‰ or 4‰ salinity during first exogenous feeding period when feeding *B. plicatilis*.

Effect of rotifer prey density on pikeperch larva performance

Prey density plays an essential role in determining time and energy expended in prey capture (Shan and Lin, 2014). Both high (Dabrowski, 1984; Laurel et al., 2001) and low (Dowd and Houde, 1980; Tandler and Sherman, 1981) prey density can be associated with reduced larva survival and growth.

Chapter 4 (Imentai et al., 2019b) reports results of investigation into the effect of *B. plicatilis* density (2, 6, 10, and 20 rotifers mL^{-1}) on survival and growth of pikeperch larvae from 5–9 DPH. The primary objective of the study was to determine the minimum *B. plicatilis* density effective for acceptable survival and growth of pikeperch larvae during the period of initial exogenous feeding. We found that rotifers density of 6 mL^{-1} is optimal for larval growth and survival during the period from 5–9 DPH. At density fewer than 6 mL^{-1}, pikeperch larvae may consume excessive energy searching for food, delaying development and growth. Densities greater than 20 mL^{-1} can constitute overfeeding, negatively affecting growth and survival. The SGR of pikeperch larvae provided rotifers at 20 mL^{-1} was higher than observed in other experimental groups. Larvae showed lowest SGR values at rotifers density of 2 mL^{-1}. Similar results were obtained in studies of perch *Perca fluviatilis* (Wang and Eckmann, 1994), who reported optimal survival and growth of *P. fluviatilis* larvae at prey densities of 6 mL^{-1} at water temperature 20 °C. Rosales-Casián (1994) reported highest survival and growth of California grunion *Leuresthes tenuis* larvae fed rotifers at 7 mL^{-1}. Prey density was also reported to have a profound impact on the survival of first-feeding miiuy croaker *Miichthys miiuy* larvae (Shan and Lin, 2014). We observed no significant differences in survival among prey-density groups. It is likely that yolk nutrition was still available. The transition of fish larvae from endogenous to exogenous feeding is dependent on time and environmental factors such as water temperature (Yufera and Darias, 2007). Pikeperch larvae begin the shift from endogenous to exogenous feeding from 5 to 7 DPH at water temperature of 20 °C (Ostaszewska, 2005) and from 8 to 14 DPH at 15 °C (Xu et al. 2017). In Yanes-Roca et al. (2018) larvae were fed rotifers from 4 DPH at water temperature 17 °C. In Imentai et al. (2019) we observed mouth opening at 5 DPH in water temperature maintained at 16 °C. Higher rotifer density might affect the feeding behaviour of fish larvae and impact water quality (Shan and Lin, 2014), with

consequences for fish survival and growth (Dabrowski, 1984; Lubzens et al., 1989). Culture of rotifers is costly and labour intensive, thus lower prey density per larva reduces the costs of production. Data obtained from these experiments expand our knowledge of prey availability to pikeperch larvae and provide results applicable to fisheries management.

Conclusions

The research presented in this thesis investigated rotifers Brachionus plicatilis as an alternative to *Artemia* nauplii during the first exogenous feeding of pikeperch larvae. Our study confirms the effectiveness of rotifers for initial pikeperch exogenous feeding and warrant the following conclusions:

- The feeding of *Brachionus plicatilis* to pikeperch larvae significantly improves larval development during the period of initial exogenous feeding compared to feeding *Artemia*. Feeding of rotifers enhances the ability of smaller larvae to begin feeding. *Brachionus plicatilis* provides larvae with important essential fatty acids not contained in *Artemia*.

- Pikeperch survival and growth rate are significantly affected by type of live feed and the duration of feeding. Providing larvae with rotifers from 5 to 8 DPH followed by a diet of *Artemia* alone or in combination with rotifers through 17 DPH significantly increases survival, growth rate, and development of the digestive tract compared to feeding rotifers alone. Feeding larvae with rotifers from 5 to 8 DPH followed by *Artemia* alone through 17 DPH is recommended for effective and profitable commercial production.

- The rearing of pikeperch larvae in slightly saline water (2‰ and 4‰) during initial exogenous feeding is an efficient technique allowing longer duration of marine rotifer availability in larva tanks, and is associated with rapid larval growth. It can also contribute to decreasing incidence of pathogens, ease of management, and reduce costs compared to freshwater culture. Pikeperch survival and growth is significantly affected by rotifers density at the onset of exogenous feeding. *Brachionus plicatilis* density of 6 mL^{-1} at 16.5 °C satisfies the nutritional requirements of pikeperch larvae from 5 to 9 DPH and minimizes production costs.

References

Awaiss, A., Kestemont, P., Micha, J.C., 1992. Nutritional suitability of the rotifer, *Brachionus calyciflorus* Pallas for rearing freshwater fish larvae. J. Appl. Ichthyol. 8, 263–270.

Battaglene, S.C., 1995. Induced ovulation and larval rearing of four species of Australian marine fish. Ph.D. thesis, University of Tasmania, 215 pp.

Chakraborty, R.D., Chakraborty, K., Radhakrishnan, E., 2007. Variation in fatty acid composition of *Artemia salina* nauplii enriched with microalgae and baker's yeast for use in larviculture. J. Agr. Food Chem. 55, 4043–4051.

Dabrowski, K., 1984. The feeding of fish larvae – present state of the art and perspectives. Reprod. Nutr. Dev. 24, 807–833.

Das, P., Mandal, S.C., Bhagabati, S., Akhtar, M., Singh, S., 2012. Important live food organisms and their role in aquaculture. Frontiers in Aquaculture 5, 69–86.

Dowd, C., Houde, E., 1980. Bream, *Archosargus rhomboidalis* (Sparidae). Mar. Ecol. Prog. Ser 3, 181–185.

Epp, R., Winston, P., 1978. The effects of salinity and pH on the activity and oxygen consumption of *Brachionus plicatilis* (Rotatoria). Comp. Biochem. Physiol. A-Physiol. 59, 9–12.

Epp, R.W., Lewis, W.M., 1984. Cost and speed of locomotion for rotifers. Oecologia 61, 289–292.

Faleiro, F., Narciso, L., 2009. Brachionus vs *Artemia* duel: Optimizing first feeding of *Upogebia pusilla* (Decapoda: Thalassinidea) larvae. Aquaculture 295, 205–208.

Fielder, D.S., Purser, G.J., Battaglene, S.C., 2000. Effect of rapid changes in temperature and salinity on availability of the rotifers *Brachionus rotundiformis* and *Brachionus plicatilis*. Aquaculture 189, 85–99.

Gisbert, E., Doroshov, S.I., 2003. Histology of the developing digestive system and the effect of food deprivation in larval green sturgeon (*Acipenser medirostris*). Aquat Living Resour 16, 77–89.

Guo, R., Mather, P., Capra, M.F., 1993. Effect of salinity on the development of dilver perch (*Bidyanus bidyanus*) eggs and larvae. Comp. Biochem. Physiol. A-Physiol. 104, 531–535.

Hamre, K., 2016. Nutrient profiles of rotifers (*Brachionus* sp.) and rotifer diets from four different marine fish hatcheries. Aquaculture 450, 136–142.

Hamza, N., Ostaszewska, T., Kestemont, P., 2015. Development and functionality of the digestive system in percid fishes early life stages. Biology and culture of percid fishes, Springer, pp. 239–264.

Hunter, J.R., Kimbrell, C.A., 1980. Early life history of Pacific mackerel, *Scomber japonicus*. Fish. Bull. US 78, 89–101.

Imentai, A., Yanes-Roca, C., Steinbach, Ch., Policar, T., 2019a. Optimized application of rotifers Brachionus plicatilis for rearing pikeperch Sander lucioperca L. larvae. Aquacult. Internat. 27: 1137–1149

Imentai, A., Yanes-Roca, C., Malinovskyi, O., Policar, T., 2019b. Effect of *Brachionus plicatilis* density on pikeperch (*Sander lucioperca* L.) larva performance at first feeding. J. Appl. Ichthyol. 35, 1292–1294.

Imentai, A., Rašković, B., Steinbach, Ch., Rahimnejad, S., Yanes-Roca, C., Policar, T., 2020. Effects of first feeding regime on growth performance, survival rate and development of digestive system in pikeperch (*Sander lucioperca*) larvae. Aquaculture 529, 735636.

Kamaszewski, M., Ostaszewska, T., 2014. The effect of feeding on morphological changes in intestine of pike-perch (*Sander lucioperca* L.). Aquacult. Int. 22, 245–258.

Kestemont, P., Henrotte, E., 2015. Nutritional requirements and feeding of broodstock and Early life stages of Eurasian perch and pikeperch, Biology and Culture of Percid Fishes, Springer, pp. 539–564.

Lasker, R., Feder, H., Theilacker, G., May, R., 1970. Feeding, growth, and survival of *Engraulis mordax* larvae reared in the laboratory. Mar. Biol. 5, 345–353.

Laurel, B.J., Brown, J.A., Anderson, R., 2001. Behaviour, growth and survival of redfish larvae in relation to prey availability. J. Fish Biol. 59, 884–901.

Ložys, L., 2004. The growth of pikeperch (*Sander lucioperca* L.) and perch (*Perca fluviatilis* L.) under different water temperature and salinity conditions in the Curonian Lagoon and Lithuanian coastal waters of the Baltic Sea. Hydrobiologia 514, 105–113.

Lubzens, E., Tandler, A., Minkoff, G., 1989. Rotifers as Food in Aquaculture. Hydrobiologia 186, 387–400.

Lund, I., Rodríguez, C., Izquierdo, M.S., El Kertaoui, N., Kestemont, P., Reis, D.B., Dominguez, D., Pérez, J.A., 2019. Influence of salinity and linoleic or α-linolenic acid based diets on ontogenetic development and metabolism of unsaturated fatty acids in pike perch larvae (*Sander lucioperca*). Aquaculture 500, 550–561.

Øie, G., Olsen, Y., 1993. Influence of rapid changes in salinity and temperature on the mobility of the rotifer *Brachionus plicatilis*. Rotifer Symposium VI, Springer, pp. 81–86.

Ostaszewska, T., Korwin-Kossakowski, M., Wolnicki, J., 2006. Morphological changes of digestive structures in starved tench *Tinca tinca* (L.) juveniles. Aquacult. Int. 14, 113–126.

Peterka, J., Matína, J., Lipka, J., 2003. The diet and growth of larval and juvenile pikeperch (*Stizostedion lucioperca* (L.)): a comparative study of fishponds and a reservoir. Aquacult. Int. 11, 337–348.

Policar, T., Schaefer, F.J., Panana, E., Meyer, S., Teerlinck, S., Toner, D., Żarski, D., 2019. Recent progress in European percid fish culture production technology – tackling bottlenecks. Aquacult. Int. 27, 1151–1174.

Rašković, B.S., Stanković, M.B., Marković, Z.Z., Poleksić, V.D., 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. J. Agric. Sci. (Belgrade). 56, 87–100.

Rosales-Casián, J. A., 1994. The effect of rotifer (*Brachionus plicatilis*) density on the survival and growth of california grunion (*Leuresthes tenuis ayres*) larvae. Mar. Environ. Res. 38, 71–75

Shan, X., Lin, M., 2014. Effects of algae and live food density on the feeding ability, growth and survival of miiuy croaker during early development. Aquaculture 428, 284–289.

Snell, T.W., Carrillo, K., 1984. Body size variation among strains of the rotifer *Brachionus plicatilis*. Aquaculture 37, 359–367.

Steenfeldt, S., 2015. Culture methods of pikeperch early life stages. Biology and Culture of Percid Fishes, Springer, pp. 295–312.

Szkudlarek, M., Zakęś, Z., 2007. Effect of stocking density on survival and growth performance of pikeperch, *Sander lucioperca* (L.), larvae under controlled conditions. Aquacult. Int. 15, 67–81.

Tandler, A., Sherman, R., 1981. Food organism concentration, environmental temperature and survival of the gilthead bream (*Sparus aurata*) larvae. Spec. Publ. Europ. Maricult. Soc. 6, 237–248.

Theilacker, G.H., McMaster, M.F., 1971. Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as a food for larval anchovies. Mar. Biol. 10, 183–188.

Tielmann, M., Schulz, C., Meyer, S., 2017. The effect of light intensity on performance of larval pike-perch (*Sander lucioperca*). Aquacult. Eng. 77, 61–71.

Verreth, J., Kleyn, K., 1987. The effect of biomanipulation of the zooplankton on the growth, feeding and survival of pikeperch (*Stizostedion lucioperca*) in nursing ponds. J. Appl. Ichthyol. 3, 13–23.

Wang, N., Eckmann, R., 1994. Effects of temperature and food density on egg development, larval survival and growth of perch (*Perca fluviatilis* L.). Aquaculture 122, 323–333.

Whyte, J.N., Nagata, W.D., 1990. Carbohydrate and fatty acid composition of the rotifer, *Brachionus plicatilis*, fed monospecific diets of yeast or phytoplankton. Aquaculture 89, 263–272.

Xu, Z., Li, C., Ling, Q., Gaughan, S., Wang, G., Han, X., 2017. Early development and the point of no return in pikeperch (*Sander lucioperca* L.) larvae. Chin. J. Oceanol. Limnol. 35, 1493–1500.

Yanes-Roca, C., Mraz, J., Born-Torrijos, A., Holzer, A.S., Imentai, A., Policar, T., 2018. Introduction of rotifers (*Brachionus plicatilis*) during pikeperch first feeding. Aquaculture 497, 260–268.

Yufera, M., Darias, M.J., 2007. The onset of exogenous feeding in marine fish larvae. Aquaculture 268, 53–63.

English summary

Pikeperch (*Sander lucioperca* L.) larviculture improvements using rotifers *Brachionus plicatilis*

Aiman Imentai

Pikeperch *Sander lucioperca* L. is a promising candidate for intensive aquaculture. Currently, a critical bottleneck in pikeperch larviculture is the period of initial exogenous feeding when high mortalities occur. Since recognition of the rotifer as potential feed for larva culture, its use has grown tremendously in fish hatcheries. Although use of rotifers and *Artemia* as a starter feed has become a common practice in hatcheries, rotifers have not been used for pikeperch larval culture. Questions regarding introduction of rotifers as pikeperch larva first exogenous feeding remain unanswered. The aim of the research contained in this thesis was to maximize survival, growth, and proper development of pikeperch larvae during first exogenous feeding using rotifers *Brachionus plicatilis*.

The effects of *B. plicatilis* on survival rate, growth performance, stomach fullness, fatty acid composition of pikeperch larvae during first feeding were evaluated in Chapter 2 (Yanes-Roca et al., 2018). Larvae were reared under three diets (*Artemia*; *Artemia*/rotifer; rotifer) from 3 to 17 days post-hatching (DPH). Using rotifers as first diet for pikeperch larvae was shown to benefit survival and growth rates. Larvae fed on rotifers only or combined with *Artemia* obtained higher survival and growth compared to feeding on *Artemia* alone. The essential fatty acid content and smaller size of rotifers had a considerable effect on larval survival and growth, confirming that the mixed *Artemia*/rotifer diet can be recommended for initial exogenous feeding in pikeperch larvae.

The objective in Chapter 3 (Imentai et al., 2020) was to optimize the first exogenous feeding regime for pikeperch larvae using rotifers and *Artemia*. Larvae were provided with B. plicatilis for 3 days followed by one of five diet regimes from 8 to 17 DPH: rotifers only; 8–13 DPH rotifers/14–17 DPH *Artemia*; 8–10 DPH rotifers/11–17 DPH *Artemia*; *Artemia* only; and a rotifer/*Artemia* combination. Feeding on rotifers from 5 to 8 DPH followed by *Artemia* or rotifer/*Artemia* to 17 DPH can ensure high survival and growth rates, and optimal development of digestive organs. Feeding larvae with rotifers from 5 to 8 DPH followed by *Artemia* alone to 17 DPH is recommended as an optimum feeding regime, larval survival and growth are satisfactory, and production costs are lower.

The main aim in Chapter 4 (Imentai et al., 2019a) was to determine the optimal salinity for rearing of pikeperch larvae while feeding B. plicatilis. Rotifers were stocked at 0‰, 2‰, 4‰, 8‰, 16‰ salinity, and motility was investigated over a 6-h period. The same salinities were used in second trial to quantify the effect on pikeperch gut fullness over the course of 11 h. In a third trial, the survival and growth rate of larvae from 4 to 11 DPH at low and medium salinity were analysed. Results of this study showed that rotifers stocked at all tested salinities, except for freshwater, retained motility for over a 6-h period. Pikeperch larvae reared at 2‰ and 4 ‰ showed higher survival and growth rate compared to freshwater. The results of this study showed advantages of rearing larvae in low salinity water during initial exogenous feeding with *B. plicatilis*.

The aim in Chapter 5 (Imentai et al., 2019b) was to determine the optimal rotifer density as pikeperch larvae prey at the beginning of exogenous feeding. Larvae were divided into four groups provided rotifers at 2, 6, 10, and 20 ind/mL from 5 to 9 DPH. The optimal growth performance was achieved at 20 ind/mL, however survival rate did not significantly differ among groups. Results suggested that *B. plicatilis* at density of 6 ind/mL can be recommended for larval feeding from 5 to 9 DPH when balanced with production costs.

Czech summary

Inovace chovu larev candáta obecného (*Sander lucioperca* L.) při použití vířníků druhu *Brachionus plicatilis*

Aiman Imentai

Candát obecný (*Sander lucioperca*) je jedním z perspektivních druhů ryb pro světovou akvakulturu. Technologie průmyslového a stabilního chovu ryb candáta obecného je v současné době limitována celou řadou chovatelských problémů. Jedním z hlavních problémů odchovu candáta obecného je jeho optimální počáteční exogenní výživa. Vířníci, kteří jsou dnes v kombinaci se žábronožkou solnou *Artemia* hojně využíváni pro výživu mořských a sladkovodních ryb, nebyli zatím u candáta obecného testováni. Cílem této dizertační práce bylo ověřit a optimalizovat využití vířníků *Brachionus plicatilis* při počáteční exogenní výživě candáta obecného.

Vliv využití vířníků zmíněného druhu na přežití, růst a kondiční stav byl sledován v prvním vědeckém článku. Larvy byly krmeny na začátku exogenní výživy třemi různými krmnými variantami: *Artemia*; Art/vířníci; vířníci. Výsledky ukázaly, že použití vířníků jako počáteční exogenní výživy larev candáta podporuje jejich přežití a rychlost růstu. Larvy krmené pouze vířníky či v kombinaci vířníků se žábronožkou dosáhly vyššího přežití a růstu ve srovnání se skupinou, která byla krmena pouze žábronožkou. Bylo zjištěno, že esenciální mastné kyseliny, které jsou obsaženy v těle vířníků, a jejich menší velikost měly rozhodující vliv na přežití, růst a kondici odchovávaných larev. Výsledky této první studie ukázaly, že kombinovaná potrava, která je složena z vířníků a žábronožek, by mohla být doporučena jako inovativní počáteční exogenní výživa larev candáta obecného.

Cílem druhého vědeckého článku bylo optimalizovat režim počáteční exogenní výživy larev candáta obecného pomocí vířníků a žábronožek. V této studii byly larvy nejprve krmeny vířníky po dobu prvních tří dnů a poté byly od 8. do 17. dne po vylíhnutí – DPH rozděleny do 5 různých skupin. V této studii bylo zjištěno, že počáteční krmení larev candáta obecného od 5 do 8 DPH vířníky a poté výhradně žábronožkou či kombinací žábronožky a vířníků do 17 DPH může zajistit vysokou míru přežití a růstu odchovávaných ryb včetně rychlejšího vývoje trávicího traktu. Počáteční exogenní výživa larev candáta obecného vířníky od 5 do 8 DPH a poté jejich nahrazení žábronožkou do 17 DPH je považovaná a doporučovaná za optimální režim, jelikož podporuje vysoké přežití a růst larev při nízkých produkčních nákladech.

Hlavním cílem třetího vědeckého článku bylo zjistit optimální salinitu vody pro chov larev candáta obecného při jejich výživě vířníky druhu *B. plicatilis*. Vířníci byli nasazováni do různých salinit vody a byla sledována pohyblivost vířníků po dobu 6 hodin. Stejná salinita vody byla použita v dalším experimentu této studie s cílem sledovat vliv salinity vody na zaplněnost střeva larev v průběhu 11 hodin. Ve třetím experimentu byla sledována míra přežití a růstu larev od 4 do 11 DPH při nízkých a středních hodnotách salinity vody. Výsledky této studie ukázaly, že vířníci, kteří byli vysazováni do všech testovaných salinit vody s výjimkou sladké vody, si udrželi pohyblivost po dobu 6 hodin. Ovšem larvy v salinitách o hodnotě 2‰ a 4‰ se vyznačovaly vyšším přežitím a rychlostí růstu než larvy, které byly chovány ve sladké vodě. Výsledky této studie ukázaly, že larvy candáta obecného při počátečním exogenním krmení vířníky druhu *B. plicatilis* ve vodě s nízkou salinitou lépe přežívají a rychleji rostou než larvy ze sladké vody.

Cílem čtvrtého vědeckého článku této dizertační práce bylo zjistit optimální hustotu aplikovaných vířníků pro počáteční exogenního výživu larev candáta obecného. Larvy v této studii byly rozděleny do 4 skupin při různých hustotách vířníků od 5 do 9 DPH. Nejlepšího růstu

bylo dosaženo při nejvyšší hustotě vířníků, ale míra přežití larev se mezi skupinami významně nelišila. Výsledky této studie ukázaly, že pro dobrý růst larev candáta obecného od 5 do 9 DPH je ideální využívat vířníky při hustotě 6 jedinců.ml^{-1}. Tato hustota je optimálním kompromisem mezi produkčními výsledky a náklady ve srovnání s vyšší použitou hustotou vířníků.

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List of publications

Peer-reviewed journals with IF

Imentai, A., Rašković, B., Steinbach, Ch., Rahimnejad, S., Yanes-Roca, C. Policar, T., 2020. Effects of first feeding regime on growth performance, survival rate and development of digestive system in pikeperch (*Sander lucioperca*) larvae. Aquaculture 529, 735636. (IF 2019 = 3.224)

Imentai, A., Yanes-Roca, C., Steinbach, Ch., Policar, T., 2019. Optimized application of rotifers *Brachionus plicatilis* for rearing pikeperch *Sander lucioperca* L. larvae. Aquaculture International 27, 1137–1149. (IF 2018 = 1.455)

Imentai, A., Yanes-Roca, C., Malinovskyi, O., Policar, T., 2019. Effect of *Brachionus plicatilis* density on pikeperch (*Sander lucioperca* L.) larva performance at first feeding. Journal of Applied Ichthyology 35, 1292–1294. (IF 2018=0.877)

Yanes-Roca, C., Mráz, J., Born-Torrijos, A., Holzer, A. S., **Imentai, A.,** Policar, T., 2018. Introduction of rotifers (*Brachionus plicatilis*) during pikeperch first feeding. Aquaculture 497, 260–268. (IF 2018 = 3.022)

Policar, T., Bondarenko, V., Bezusyj, O., Stejskal, V., Kristan, J., Malinovkyi, O., **Imentai, A.**, Blecha M., Pylypenko, Y., 2018. Crayfish in central and southern Ukraine with special focus on populations of indigenous crayfish *Astacus pachypus* (Rathke, 1837) and their conservation needs. Aquatic Conservation: Marine and Freshwater Ecosystems 28, 6–16 (IF 2018 = 2.935)

Abstracts and conference proceedings

Imentai, A., Yanes-Roca, C., Policar, T., 2018. Optimization of pikeperch (*Sander lucioperca*) first feeding with rotifers (*Brachionus plicatilis*). In: EAS (Eds), Aquaculture Europe 2018 Abstracts, Montpellier, France, August 25–29, 2018, p. 360. (oral presentation)

Imentai, A., Steinbach, C., Yanes-Roca, C., Policar, T., 2019. Effect of feeding strategy with rotifers (*Brachionus plicatilis*) on pikeperch (*Sander lucioperca*) larval performance. In: EAS (Eds), Aquaculture Europe 2019 Abstracts, Berlin, Germany, October 7–10, 2019, pp. 613–614. (oral presentation)

Training and supervision plan during study

Name	Aiman Imentai
Research department	2016–2020 – Laboratory of Intensive Aquaculture of FFPW
Supervisor	Assoc. Prof. Tomáš Policar
Period	17th October 2016 until 17th September 2020

Ph.D. courses	**Year**
English language	2016
Basic of scientific communication	2017
Pond aquaculture	2017
Ichthyology and fish taxonomy	2017
Biostatistics	2018

Scientific seminars	**Year**
Seminar days of RIFCH and FFPW	2017 2018 2019 2020

International conferences	**Year**
Imentai, A., Yanes-Roca, C., Policar, T., 2018. Optimization of pikeperch (*Sander lucioperca*) first feeding with rotifers (*Brachionus plicatilis*). In: EAS (Eds), Aquaculture Europe 2018 Abstracts, Montpellier, France, August 25–29, 2018, p. 360. (oral presentation)	2018
Imentai, A., Steinbach, C., Yanes-Roca, C., Policar, T., 2019. Effect of feeding strategy with rotifers (*Brachionus plicatilis*) on pikeperch (*Sander lucioperca*) larval performance. In: EAS (Eds), Aquaculture Europe 2019 Abstracts, Berlin, Germany, October 7–10, 2019, 613–614. (oral presentation)	2019

Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
Prof. Pascal Fontaine, University of Lorraine, UR AFPA, Vandoeuvre-les-Nancy, France. Rearing pikeperch broodstock under controlled conditions (1 month)	2017
Prof. Teresa Ostaszewska, Department of Ichthyology, Fisheries and Aquaculture Biotechnology, Warsaw University of Life Sciences, Poland. Analysis of histological slides prepared from the series of experiments on pikeperch larvae (1 month)	2018

Pedagogical activities	**Year**
Announcing the project entitled *The effects of feeding strategy with rotifers (Brachionus plicatilis) on digestive tract of pikeperch (Sander lucioperca) larvae* at Summer school	2020
Leading project entitled *The optimized application of rotifers (Brachionus plicatilis) for the first feeding of pikeperch (Sander lucioperca L.) larvae* at Summer school	2019
Tutoring of students of bachelor and master studies, discipline Fishery at USB FFPW and Ph.D. student Meirambek Pazylbekov during his internship at USB FFPW, in the range of 80 teaching hours	2019

Curriculum vitae

PERSONAL INFORMATION

Name: Aiman
Surname: Imentai
Title: M.Sc.
Born: 29th January, 1989, Almaty, Kazakhstan
Nationality: Kazakh
Languages: English (IELTS Certificate: 7.0 score), Kazakh, Russian (native), Czech
Contact: aimentai@frov.jcu.cz

EDUCATION

2016–present	Ph.D. student in Fishery, Faculty of Fisheries and Protection of Waters, University of South Bohemia, Ceske Budejovice, Czech Republic
2011–2013	M.Sc. in Biology, Faculty of Biology and Biotechnology, al-Farabi Kazakh National University, Almaty, Kazakhstan
09/2012–03/2013	M.Sc. in Landscape Ecology and Nature Conservation, Greifswald University, Germany
2007–2011	B.Sc. in Fishery, Faculty of Biology and Biotechnology, al-Farabi Kazakh National University, Almaty, Kazakhstan
1996–2007	Private School Senim, Almaty, Kazakhstan

PROFESSIONAL EXPERIENCE

06/2013–10/2016	Junior research scientist, Laboratory of Applied Hydrobiology, Institute of Zoology, Almaty, Kazakhstan
03–07/2014	Fellow of the Marion Donhoff Fellowship in the Michael Succow Foundation, Greifswald, Germany
01/2010–09/2013	Laboratory assistant, Research Institute "Problem of Ecology", Almaty, Kazakhstan

TRAINING

26/02–27/02/2015	Workshop: Ecosystem conservation and sustainable land use in the Ili-Delta, Balkhash Lake, Kazakhstan, under decreasing water resources. Almaty, Kazakhstan
19/05–23/05/2014	Training School: Indicators of desertification: early warning signs. University of Lisbon, Portugal
12/2012–01/2013	Course: Integrated Approaches to Sustainable Development. Earth Institute, Columbia University, NY, USA
1/04–2/04/2010	Workshop: The methodological basis and optimization procedures for environmental studies. KAAE LLC, Almaty, Kazakhstan

RESEARCH AND COLLABORATIONS

2017	Prof. Pascal Fontaine, University of Lorraine, UR AFPA, Vandoeuvre-les-Nancy, France
2018	Prof. Teresa Ostaszewska, Department of Ichthyology, Fisheries and Aquaculture Biotechnology, Warsaw University of Life Sciences, Poland