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Comparative study on the large-scale intensive culture of pikeperch (*Sander lucioperca*) larvae: Evaluation of two different live exogenous food options

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Abstract: This study is focused on the comparison of production efficiency (growth, survival, and development) and economic evaluation of large-scale larvae culture in pikeperch (*Sander lucioperca*) using *Artemia* (Group A) compared to using rotifers and the subsequent combination of rotifers and *Artemia* (Group R) as a first exogenous feed following weaning and intensive culture of juveniles. Both experimental groups were stocked with the same initial density (100 pcs per litre) of larvae at 4 days post hatching (DPH). Each group was cultured in triplicate until 65 DPH. Assessed parameters included FBW (final body weight), FTL (final total length), SGR (specific growth rate), SR (survival rate), CR (cannibalism rate), SBI (swim bladder inflation rate), and TDR (total deformity rate). Production costs were confronted with total number of all produced and high-quality juveniles to determine the cost per one juvenile. Juveniles in Group A displayed more rapid growth (significantly higher FBW, FTL and SGR). On the other hand, parameters concerning quality of the fish (SBI, SR, Share of high quality juveniles and TDR) were significantly higher in Group R. Statistical difference in CR also benefits Group R. Production of bigger share of high-quality juveniles in Group R decreased overall production cost per one high-quality juvenile by 59.7%, from 0.72 EUR in Group A to 0.29 EUR in Group R. Use of established feeding protocols confirmed significant production and economic improvements in pikeperch larvae culture and production of high-quality juveniles. Even though the price of rotifer cultivation significantly exceeded the price of *Artemia* purchase and cultivation, the production cost of high-quality pikeperch juveniles significantly decreased.

Keywords: *Artemia*; economy; larvae; RAS; rotifers

Pikeperch (*Sander lucioperca*) has been used for the diversification of inland aquaculture due to its high-quality flesh, fast growth, and overall market profitability (Overton et al. 2015). Fully grown marketable-sized pikeperch is highly demanded by the gastronomy industry and the angling com-

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munity (Policar et al. 2019). However, most of its production comes from fishing in open waters, while the share of an RAS-cultured (Recirculating Aquaculture System) pikeperch is minor (Penka et al. 2023). During the last two decades, this species was subjected to an extensive scientific research in Central (Czech Republic, Hungary, Poland) and Western Europe (Belgium, France, Germany, Switzerland) (Policar et al. 2019). Larvae culture remains the biggest bottleneck due to low effectiveness, high mortality rates, and deformities of larvae, resulting in high production costs of pikeperch (Imentai et al. 2022). Several published studies aimed at optimizing larval culture focused on the improvement of growth, fish condition, survival rate, elimination of cannibalism, decreasing of the deformity rates and avoiding the point of no return (Kestemont et al. 2007; Imentai et al. 2019a, b; 2020; Colchen et al. 2020; Yanes-Roca et al. 2022). Today, the issue has not been adequately solved, and the highest mortality occurs during the introduction of exogenous food sources. This issue is caused by the small mouth diameter and underdeveloped digestive tract of pikeperch larvae (Yanes-Roca et al. 2018). Therefore, the presence of suitable live feed at the beginning of exogenous feeding is one of the key factors for successful larval culture. The benefits of the euryhaline rotifer *Brachionus plicatilis* for small and primitive larvae and their controlled culture and production has been well documented and proven in marine aquaculture in the last 45 years (Yuifera et al. 1993). Therefore, the same species of rotifers was recently used to tackle the issues associated with pikeperch larvae culture, and rotifers were introduced as the first exogenous live feed (Yanes-Roca et al. 2018). *B. plicatilis* are suitable for inland aquaculture for several reasons: (*i*) they are easy to culture and produce, (*ii*) they are approximately about 100 µm and are therefore acceptable for small larvae, (*iii*) they are slow swimmers easily hunted by the larvae, (*iv*) they can survive and be motile in low-salinity water (2–4‰) for a sufficient time period, and (*v*) they meet the first nutritional requirements of larvae, including complements of enzymes for the digestive system of underdeveloped larvae (Imentai et al. 2019a; 2020). A study published by Yanes-Roca et al. (2018) described the efficiency of *B. plicatilis* combined with *Artemia salina* as a primary first live feed for pikeperch larvae. Otherwise, knowledge of the application of rotifers to other freshwater species in inland aquaculture is scarce (Allen et al. 2016). It was reported that different timings of introduction of exogenous feed, their duration, proportion and enrichment significantly enhance the efficacy of the first feeding in larvae culture of pikeperch Yanes-Roca et al. (2018; 2020a, b) and Imentai et al. (2019a, b; 2022). Despite the effectiveness of these measures, these studies have little to no information about production efficacy and cost, which is crucial for large-scale commercial production. The present study is focused on the comparison of pikeperch production efficiency (growth, survival, and development) and production cost of large-scale larval culture using *Artemia* compared to first feeding with rotifers and the combination of rotifers and *Artemia* as a first exogenous feed with subsequent weaning and intensive culture of juveniles until 65 DPH.

MATERIAL AND METHODS

Origin of the experimental larvae

Pikeperch broodstock were produced in pond culture of a commercial fishery company Rybářství Nové Hrady Ltd. (Czech Republic). In autumn, broodstock were collected from pond harvesting and transported to the facilities of the Laboratory of Intensive Aquaculture (LIA) of the Faculty of Fisheries and Protection of the Waters of the University of South Bohemia (FFPW USB, Vodňany, Czech Republic), where fish were wintered and successfully stimulated for gonadal development under conditions of earthen ponds with live prey fish according to Malinovskyi et al. (2020). During the natural pikeperch reproduction season, females at the 3–4 maturation oocyte stage and males spontaneously releasing sperm were identified as ready for spawning according to Zarski et al. (2012). In total, six pairs of broodstock were moved to the controlled environment of the RAS (Recirculating Aquaculture System) of LIA FFPW USB, with a controlled and constant water temperature of 14.5 ± 0.5 °C and a natural light regime.

After one day of acclimatization in RAS, both sexes of broodstock were hormonally treated with intramuscular injection of human chorionic gonadotropin (hCG, Chorulon, Intervet International B.V.) at a dose of 500 IU/kg according to Kristan et al. (2013). After hormonal treatment of brood-

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stock, semiartificial nest spawning and egg incubation were performed. For this study, larvae at the age of 4 days post hatching (DPH) were collected and mixed from all used nests in one hatchery cage and finally stocked into the experimental tanks.

Used aquaculture technology and abiotic conditions

The experimental RAS consisted of 6 black plastic cylindrical tanks with a diameter of 885 mm, height of 620 mm, and usable volume of 333 l. Water outlets were connected to overflow with a water surface skimmer described by Fazekas et al. (2021), ensuring a clean water surface enabling larvae to successfully inflate the swim bladder. The overflow was wrapped in a fine net (300 µm) to prevent larvae from escaping from the tank to other parts of RAS. From the skimmer, wastewater went to a BaseDrum15 mechanical filter (Ratz Aqua & Polymer Technik GmbH, Ramscheid, Germany). After mechanical filtration, water went through biological filtration into a bed moving filter with a volume of 890 L and into the commercially produced filter unit Nexus 310 (Evolution Aqua Ltd. Wigan, UK) (100 l). The air blower Secoh EL-S 250 W(Secoh Shanghai Mec. Ltd., Shanghai, China) was used for the aeration of both mentioned filters. After biological filtration, water was treated with UV light (UV lamp EVO 110, Evolution Aqua, Ltd. Wigan, UK). After UV treatment, water was pumped back into rearing tanks by bottom inflow, esuring the movement of feeding organisms in the water column.

During the experiment, the following water quality was maintained: water temperature of 20.0 ± 1.5 °C, oxygen saturation 85.0 ± 10.0%, pH 6.8 ± 0.3, total ammonia concentration 0.3 ± 0.1 mg/l, and nitrite concentration 0.2 ± 0.1 mg/l. Water temperature and oxygen levels were measured using a YSI ProODO oximeter (YSI Inc., Yellow Springs, OH, USA) twice a day (7:00 and 15:00). The pH value was measured by a WTW 3 310 pH meter (WTW, Prague, Czech Republic) once a day at 7 a.m. Total ammonia and nitrite were measured every day at 7:30 a.m. using simple titration and colorimetry reference kits according to (Penka et al. 2021).

To prolong the lifespan and motility of rotifers and *Artemia* inside the rearing tanks, a water salinity of 3–4 g/l was set and maintained by the addition of sea salt (Instant Ocean, Blacksburg, VA, USA) to the whole RAS according to Imentai et al. (2019a). The light regime was set as 12 h of light and 12 h of dark. Light intensity was 80 ± 15 lux measured by a UNITEST 93514 luxmeter (Beha-Amprobe GmbH, Glottertal, Germany) once per day.

Experimental groups and their feeding protocol

In total, 199 800 larvae were stocked in 6 tanks (3 tanks per group) at 4 DPH. In each tank, 33 300 larvae were stocked with an initial larval density of 100 larvae per litre. The number of larvae was measured using the volumetric method. Larvae are concentrated in a small portable tank (10 l). After homogenization (gentle hand mixing) 3 samples are taken, using 10 ml cup. After calculation of exact number of the larvae in each sample mean number of the larvae is calculated and multiplied by 1 000 to determine the amount of larvae in a portable tank. In total, 99 900 larvae were stocked per each group. The whole experiment was divided into 4 periods: the 1st period was set up to 5–12 DPH, the 2nd to 13–17 DPH, the 3rd to 18–26 DPH and the 4th to 27–65 DPH. Two feeding protocols were tested in two experimental groups as follows.

During the 1st period, larvae in Group R (rotifers) were fed by rotifers, maintaining the density of rotifers at 10 ind/ml in the rearing tanks. During the 2nd period, this group was fed rotifers (5 ind/ ml), and *Artemia nauplii* (Micro *Artemia* cysts, Ocean Nutrition, Belgium) were added at a density of 5 ind/ml. During the 3rd period, larvae of this group were adapted to dry feed. This adaptation was performed by the co-feeding (mix of *Artemia* and dry feed) with the share of dry starter Otohime A, with grain size 75–250 µm, nutritional content: 8.0% moisture, 60.5% crude protein, 11.1% crude fat, 2.3% crude fibre, 15.8% crude ash, 3.1% calcium and 2.4% phosphorus (Marubeni Nisshin Feed Co., Ltd, Tokyo, Japan). The relative share of dry feed started at 11% of DFR (Daily Feeding Ratio) on the first day of weaning. The share of dry feed was gradually increased every day. As the share of dry feed in DFR was increasing amount of *Artemia* decreased accordingly and resulted in complete transition between *Artemia* and dry feed in 9 days. Dry feeding of larvae was carried out during the 4th period at *ad libitum*. The particle size of dry feed

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was gradually increased to keep up with the needs of growing larvae and early juveniles up to particle size 1 410 µm. The same commercial brand of feed was maintained during the whole trial. First, Otohime A than Otohime B1 (250–360 µm) and B2 (360–350 µm) had slightly modified nutrition compositions compared to Otohime A: 6.3% moisture, 55.8% crude protein, 14.9% crude fat, 2.8% crude fibre, 15% crude ash, 2.5% calcium, and 2.2% phosphorus.

Later, towards the end of the trial, Otohime C1/C2 (580–1 410 µm) was used with 6.3% moisture, 55.1% crude protein, 14.3% crude fat, 2.9% crude fibre, 16.4% crude ash, 2.9% calcium and 2.4% phosphorus. The feeding protocol is summarized in Table 1.

During the 1st period of larval culture in Group A (*Artemia*), larvae were fed with *Artemia nauplii* at a feeding density of 5 ind/ml in the rearing tanks, and at the 2nd period density of *Artemia* was increased up to 10 ind/ml. At the 3rd period included juvenile weaning to dry food and following juvenile culture (4th period) with dry feed, Otohime A was applied identically as described in Group R. As stated by Ronnestad et al. (2013) and later Yanes-Roca et al. (2018) rotifers size and nutritional value becomes insufficient in 10–11 DPH. At this point bigger size of prey organisms such as *Artemia* must be introduced to cope with exponential growth of the larvae.

Given the fact that larvae must enter the phase of weaning in overall good condition inclusion of group fed exclusively by rotifers was not feasible to achieve production goals (Yanes-Roca et al. 2018). Larvae in both groups were fed live feed three times a day, providing continuous movement of feeding organisms in the water column of the rearing tanks and supporting continuous larval feeding (Imentai et al. 2019b). The density of feeding organisms in the rearing tanks was checked prior to feeding using an Olympus BX41 microscope (Olympus Corp, Tokyo, Japan), and the cells were counted. Live feed culture was performed on-site. Rotifers were produced in 50-l flat-bottomed buckets following a batch culture protocol with feeding by commercially sold *Nannochloropsis occulata* suspension (Nanno 3 600, Reed Mariculture, Campbell, CA, USA) according to Yanes-Roca et al. (2018). *Artemia nauplii* were also hatched on-site (hatching after 20–24 h under controlled conditions with temperature 28 °C; pH 8.5; continuous light and aeration). After the introduction of live feed into the cultured tanks, water inflow was stopped and restarted after 2 h to improve larval feeding efficiency and decrease the waste of feeding organisms otherwise escaping from the tanks according to Yanes-Roca et al. (2020b).

At the beginning of the experiment, 100 larvae were fixated for a biometrical measurement. The mean initial TL (total length) was 5.7 ± 0.2 mm, and the mean IBW (initial body weight) was 0.7 ± 0.15 mg. TL was measured with an accuracy of 0.01 mmusing an Olympus BX41 microscope fitted with a Canon-72 digital camera (Canon Inc. Tokyo, Japan) connected to the imaging software cellSens (Olympus corp., Tokyo, Japan). BW was measured in the laboratory using analytical scales KERN ABT 220-5DM (Kern

Table 1. Feeding protocol of both experimental groups under intensive larvae culture of pikeperch. Group R used rotifers as the first exogenous feeding, and Group A used the conventional method of intensive culture of larvae using only *Artemia nauplii*

Rearing period (DPH)	Group R fed with rotifers and *Artemia* as first exogenous feeding	Group A fed exclusively with *Artemia* as first exogenous feeding
5th–12th	rotifers 10 ind/ml	*Artemia* 5 ind/ml
13th–17th	rotifers and *Artemia* 5 + 5 ind/ml	*Artemia* 10 ind/ml
18th–26th	process of weaning using co-feeding with *Artemia* and dry starter Otohime A (75–250 µm) gradually increasing by 11% per day of DFR starting at 11% of DFR	
27th–65th	Otohime A (75–250 µm) 27–40 day Otohime B1/B2 (250–650 µm) 35–55 day Otohime C1/C2 (580–1 410 µm) 45–65 day	

DFR = daily feeding ratio

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& Sohn GmbH, Balingen, Germany) with an accuracy of 0.01 mg. After 60 days of culture, the experiment was terminated. The whole biomass of fish in tanks was determined using KERN KB 2400-2 N scales (Kern & Sohn, GmbH, Balingen, Germany) with an accuracy of 0.1 g. All cultured surviving fish from each tank were counted by hand. In total, 100 fish from each tank (300 fish per group) were anaesthetized using MS-222 (tricaine methanesulfonate, Sigma-Aldrich, St. Louis, MO, USA, 100 mg/l), and TL (total length) and BW (body weight) were determined using the same method as at the beginning of the experiment.

After the measurement, fish were put back into the tanks to recover from anaesthesia. During the biometrical measurement, the total percentage of fish deformities (total deformity rate – TDR) in both groups was assessed as well as the number of fish with successfully inflated swim bladders to determine the SBI parameter. Fish without deformities with normal development and shape with inflated swim bladder were graded as high-quality juveniles. In malformed fish, the proportion of each deformity – lordosis, scoliosis and zig-zag shape (%) was evident by one expert person according to Policar et al. (2016a) as well as the proportion of fish with uninflated swim bladders. All these fish were excluded from further culture, and all these parameters were then used for calculation of production cost and comparison of economic profitability.

Evaluated production parameters

At the end of the experiment, the following production parameters were calculated according to the following formulas.

Specific growth rate (%/d) was calculated as follows:

$$SGR = \frac{\ln FBW - \ln IBW}{t} \times 100 \quad (1)$$

Swim bladder inflation rate (%) as follows:

$$SBI = \frac{Ninf}{N} \times 100 \quad (2)$$

Survival rate (%) was calculated as follows:

$$SR = \frac{NF}{NI} \times 100 \quad (3)$$

Cannibalism rate (%) as follows:

$$CR = \frac{NI - (NF + RM)}{NI} \times 100 \quad (4)$$

The total deformity rate (%) was calculated as follows:

$$TDR = \frac{NDF}{NF} \times 100 \quad (5)$$

where:

FBW – final body weight;
IBW – initial body weight;
FTL – final total length;
t – number of days of the experiment;
Ninf – number of fish with an inflated swim bladder;
N – number of fish in the sample;
NF – final number of fish;
NI – initial number of fish;
RM – recorded mortality;
NDF – number of deformed fish.

The recorded mortality of the cultured fish was checked three times a day, and all the deceased individuals were counted by hand and recorded. These records were later used to calculate CR.

Calculation of production cost and comparison of economic profitability

To analyse the production cost and assess the economic profitability of the two tested feeding protocols, the 12 main production costs were evaluated as follows: Personal costs including insurance and taxes; *Artemia* (including the purchase of the eggs, their cultivation, used chemicals and tap water); rotifers (including their production cost for incubation, such as labour, chemicals, electricity and tap water), dry feed, price of larvae, preparation of RAS, electricity, water, chemicals applied in RAS, other consumables, and depreciation.

To enhance the understanding of the economic profitability of both tested feeding protocols and address the challenges posed by escalating prices across various aspects of production. Each cost was also transformed into a relative percentage distribution. This approach was employed to ensure the long-term applicability and significance of this study for future application in practice.

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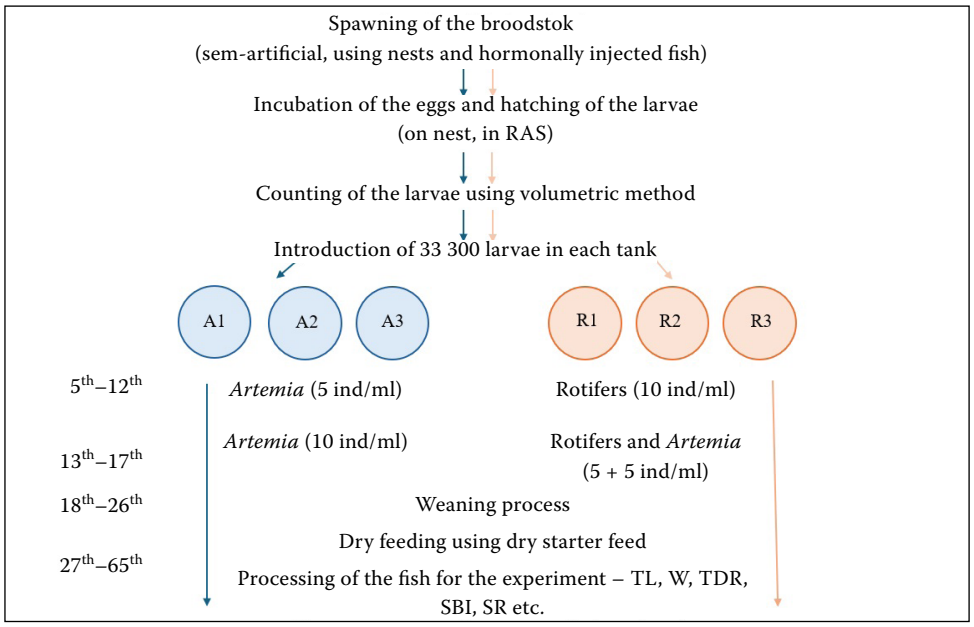

Figure 1. Scheme of an experiment providing an overview of two experimental rearing strategies of pikeperch larvae from 5 up to 65 DPH

Ethical statement

The experimental procedures conformed to the European Communities Directive (No. 2010/63/EU) and were authorized by the Czech Ministry of Health (No. MSMT-6744/2018–2) regarding the protection of animals used for scientific purposes with valid legislative regulations in the Czech Republic (Act No. 166/1996 and No. 246/1992).

Statistical analysis

Data were analysed with the program R Studio (R Core Team, 2014). The normal distribution of data was confirmed by the Shapiro–Wilk test. A comparison of production parameters was made by *t*-test with a margin of significance set at $P < 0.05$. The homogeneity of the data was assessed by the Tukey HSD method. All the data are presented as the mean ± standard deviation (SD).

RESULTS

Production parameters

Significant differences were found in final body weight (FBW) and total length (FTL) between the two tested feeding protocols (groups). Better growth results were observed in Group A. The FBW in Group A reached 0.67 ± 0.38 g compared to 0.39 ± 0.11 g in Group R. The FTL in Group A was 41.33 ± 8.18 mm compared to 36.03 ± 3.95 mm in Group R. Fish from Group A managed to outgrow Group R by 42% of body weight and by 13% of total length. A significantly higher SGR was found in Group A (7.37 ± 0.04%/day) than in Group R (6.83 ± 0.04%/day) at the end of this trial. Group A reached a rather low survival rate of 16 ± 2.29% compared to Group R, which reached a significantly higher survival rate of 37 ± 3.92%, 2.31 times higher than that in Group A.

Comparison of the cannibalism rate in both groups indicated a decreased cannibalism rate in Group R. Group A displayed a 2.86 times higher CR than Group R. In Group R, the cannibalism rate reached only 6.97 ± 2.35% compared to Group A (20.03 ± 4.32%). SBI was significantly higher in Group R (85.00 ± 6.76%) than in Group A (66.50 ± 7.37%).

The TDR in Group A was 33.0 ± 7.21% compared to the TDR in Group R (15.0 ± 5.29%). Lordosis contributed by 40.0 ± 8.19% and 62 ± 10.54% in Group R and A, respectively. Scoliosis contributed to TDR by 35.0 ± 10.15% in Group R compared to 24.0 ± 5.57% in Group A. Zig-zag deformity shape was observed at 25.0 ± 2.00% of all malformed fish

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Table 2. Summary of production parameters during the intensive culture of larvae and early juveniles of pikeperch (*Sander lucioperca*) under controlled RAS conditions until 65 DPH, including relative shares of each type of deformity as a subcategory of TDR

Production parameters			
Groups	Group R	Group A	*P*-value
FTL (mm)	36.0 ± 3.95[b]	41.3 ± 8.18[a]	$P < 0.001$
FBW (g)	0.39 ± 0.11[b]	0.67 ± 0.38[a]	$P < 0.001$
SGR (%/day)	6.83 ± 0.04[b]	7.37 ± 0.04[a]	$P < 0.001$
SR (%)	37.0 ± 3.92[a]	16.0 ± 2.29[b]	$P = 0.001$
CR (%)	6.97 ± 2.35[b]	20.0 ± 4.32[a]	$P = 0.010$
SBI (%)	85.0 ± 6.76[a]	66.5 ± 7.37[b]	$P = 0.033$
TDR (%)	15.0 ± 5.29[b]	33.0 ± 7.21[a]	$P = 0.025$
TDR – lordosis (%)	40.0 ± 8.19[b]	62.0 ± 10.5[a]	$P = 0.046$
TDR – zig-zag (%)	25.0 ± 2.00[a]	14.0 ± 5.00[b]	$P = 0.024$
TDR – scoliosis (%)	35.0 ± 10.2[a]	24.0 ± 5.57[b]	$P = 0.018$
All produced juveniles (pcs.)/ HQ juveniles (pcs.)	37 000 / 31 450	16 000 / 10 720	–
Proportion of HQ juveniles (%)	85	67.2	–

[a,b]Different letters in the same row indicate significant differences ($P < 0.05$)

CR = cannibalism rate; FBW = final body weight; FTL = final total length; HQ = high-quality; SBI = swim bladder inflation rate; SGR = specific growth rate; SR = survival rate; TDR = total deformity rate

in Group R compared to 14.0 ± 5.00% in Group A. After 60 days of this trial, in total 37 000 juveniles were produced with a share of 31 450 pcs. (85%) of high-quality RAS juveniles without any morphological deformities and with normal development in Group R. However, only 16 000 juveniles were produced with a share of 10 720 pcs. of high-quality juveniles (67.2%) in Group A. For a convenient use a complete summary of all the production parameters is shown in Table 2.

Economical evaluation

The overall production cost of one juvenile (mean FBW= 0.67 g) raised in Group A was 0.48 EUR, while that of the juvenile raised in Group R (mean FBW= 0.39 g) was 0.25 EUR, which suggests a 48% reduction in production costs. Lower production cost in Group R was achieved with a higher survival rate and lower TDR. Differences between both groups were confirmed in high-quality juvenile production.

The Group R production cost was 0.29 EUR for one pc. of high-quality juveniles compared to the total production cost of 0.72 EUR for one high-quality juvenile in Group A. A significant cost difference of 59.7% between one high-quality raised juvenile from each group was observed. Since the larvae were raised in the same experimental RAS and were fed with the same frequency using the same technology the personal cost (3 500 EUR), depreciation (1 500 EUR), preparation of RAS (350 EUR), tap water consumption (250 EUR), oxygen consumption (300 EUR), electricity consumption (210 EUR), other materials (105 EUR), cost of larvae (280 EUR) chemicals (125 EUR) and dry feed (650 EUR) were the same for both experimental groups. Major difference in production cost was caused by different live feed – rotifers and *Artemia*.

Overall price of rotifers cultivation including added labour and chemicals, electricity, tap water consumption and amortization of the used infrastructure resulted in 1 710 EUR (19%) of overall cost of juveniles. Together with subsequent *Artemia* cultivation (250 EUR, 3%) overall cost of live feed for Group R was 1960 EUR (22%). In comparison production in Group A solely depending on *Artemia* cultivation resulted in 450 EUR (6% of all production cost in this group) which is 4.35 times less than in Group R.

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DISCUSSION

The survival rate of currently produced pikeperch juveniles after weaning with an FBW of approximately 0.5 g on commercial-scale farms is approximately 10% (2023 conversation with Lukas Shneeberger; see "References") The issue of increasing the intensive production of juveniles and market-sized pikeperch needs to be addressed. One of the possible solutions to this problem was the recent introduction of euryhaline rotifers to pikeperch larval culture (Yanes-Roca et al. 2018). Pikeperch has one of the smallest fresh hatched larvae of all inland freshwater species around the world with a small mouth diameter, so to increase the survival rate, small feeding organisms such as rotifers were introduced. In recent years, research has pointed towards the optimization of the feeding regime (Imentai et al. 2020), application of rotifers (Imentai et al. 2019b) and enrichment (Yanes-Roca et al. 2020a, b), with results further increasing the efficacy of utilizing rotifers as a primary exogenous live feed for pikeperch larvae. The production of live feed is expensive. Therefore, much effort must be put into the production of a stable quantity and quality of live feed for larvae (Policar et al. 2019). A cost-effectiveness evaluation is then in place to determine the influence of the euryhaline rotifers on larval culture not only from the production point of view but also from the economic point of view to help further increase the efficacy and rentability of larvae culture of pikeperch.

The results in this study confirmed that the use of rotifers as a first live feed provides lower growth of pikeperch larvae and early juveniles. On the other hand, they significantly increase the survival rate and stimulate correct development. At this stage of pikeperch production survival and development with less frequent malformation is more important than rapid growth and may increase the culture efficiency. In this study, the final body weight after 60 days of the feeding protocol increased from 0.07 mg to 0.67 g (Group A) and 0.39 g (Group R). This means that fish in Group A were 1.7 times heavier than those in Group R. As published by Yanes-Roca et al. (2018), larvae fed a mixture of rotifers and *Artemia* were 2.05 times heavier than those fed rotifers after 17 DPH. Yanes-Roca et al. (2018) achieved higher disproportion among the groups, which is probably caused by the absence of subsequent weaning using dry starter feed, which may help decrease size variability after initial growth deprivation and a shorter trial period. In a study published by Kestemont et al. (2007), larvae were weaned from 19 DPH (this study 18 DPH) and achieved a survival rate of 15.3% resp. 13.3% at 26 DPH compared to 16% and 37% at 65 DPH in the present study. However, larvae produced by Kestemont et al. (2007) were fed exclusively by *Artemia nauplii* (such as Group A) and were subsequently weaned to a commercial diet, Kyowa FFK B-400 (BioKyowa, Inc., Chesterfield, MO, USA). In the present study, the Japanese starter Otohime A was used for the initial part of the weaning process as well as early grow-out.

Pikeperch larvae display a significant mortality rate when switching to exogenous feed. This period is the most critical part of larval culture and at the same time has the highest potential for production improvement. The smaller size of the rotifers and enzymes provided by rotifers to the underdeveloped small larvae significantly increases the survival rate, while feeding with *Artemia nauplii* favours the larger and stronger larvae and supports cannibalism directly linked to a decreased survival rate.

Yanes-Roca et al. (2018) reached a survival rate of 35% in the experimental group fed exclusively by *Artemia nauplii* at 18 DPH. The survival rate in the group fed by both *Artemia* and rotifers (such as Group R in the present study) was almost 65% at 18 DPH, suggesting almost doubled survival while using rotifers. However, these authors did not organize weaning of larvae which is critical point of pikeperch culture. Imentai et al. (2020) found that the highest survival rate was linked to a group fed by rotifers until 8 DPH and then by a mixture of *Artemia* and rotifers and reached a survival rate of almost 70% at 17 DPH, again without weaning. Other studies display similar survival rates (Imentai et al. 2019a, b; Yanes-Roca et al. 2020a). Even though these results are much higher than the survival rate displayed in the present study, a possible explanation of this disproportion lies in subsequent mortality during weaning and following 60 days intensive rearing until 65 DPH. Mortality during these upcoming stages of rearing was determined by Kestemont et al. (2007) as 6–14% mortality during weaning and 4–6% mortality during the after-weaning period (26+ DPH). Further small disproportion could be caused by the different origins

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of broodstock, as previously stated by Schaerlinger and Zarski (2015). After taking that into account, all the studies display comparable survival rates. As suggested by Yanes-Roca et al. (2018), rotifers are suitable for larvae from 5 DPH to 10–12 DPH.

During the first larval stages, growth is exponential, contrary to juveniles or subadults, where SGR drops to 0.5–2% per day (Penka et al. 2023; Policar et al. 2013; Ronyai and Csengeri 2008). A higher SGR was observed in Group A (fed by *Artemia* only) at 7.37%/day. The group fed by rotifers and then *Artemia* accounted for 6.83%/day. Other authors stated 7.96 resp. 7.15%/day at 25 DPH (Kestemont et al. 2007). Imentai et al. (2019b) reached SGR 15–18%/day, and 7–10%/day at 17 DPH. Higher SGR stated by all mentioned authors including this study is probably determined by the size of the feed organism. As the larvae reaches certain size the energetic cost of attacking small prey exceeds the gain from digesting smaller food particles (rotifers).

For intensive aquaculture in RAS, there are significant challenges. In RAS facilities, fish are reared at high density; therefore, cannibalism occurrence can lead to significant economic losses. Cannibalism is one the biggest issues of pikeperch larvae culture (Policar et al. 2019). One of the most effective strategies for eliminating this issue is regular sorting of the fish. However, in early life stages, sorting is not applicable because of the small size of the fish. Therefore, providing high accessibility of life feed for pikeperch larvae is the only option to reduce the variation in size (Yanes-Roca et al. 2018). In this study cannibalism rate was almost three times higher in Group A than in Group R (Table 2). This result could be explained as follows. Initial feeding with rotifers decreased the share of slowly growing nonfeeding larvae, which would be eventually predated by larger individuals within the cohort. This meant decreased growth heterogeneity and cannibalism. Larger and stronger larvae in Group A started to feed on large *Artemia nauplii* early and overgrew their siblings, which were unable to capture, swallow and digest *Artemia nauplii*. In a short time, the size difference of larvae was so significant that members of the large cohort started to attack small and underdeveloped larvae.

This phenomenon was previously described by Yanes-Roca et al. (2018) and Steenfeldt et al. (2011) in both larvae and early juvenile cultures fed with *Artemia*. Cannibalism is rooted into a behavioural quality and learning of predatory abilities. In a study conducted by Colchen et al. (2023) was proven that cannibals are more efficient hunters than noncannibals. Similar tendencies were observed in study conducted by Molnar et al. (2018).

Pikeperch larvae feature a pneumatic duct, an organ that enables the inflation of the swim bladder (Bagowski et al. 2011), larvae need to expend a significant effort to breach the water surface to gulp the air bubble. If the surface is covered with slime, lipid residues or other impurities, this issue may become a serious bottleneck due to the irreversibility of the process. Aberrant energy-consuming swimming behaviour causes a higher mortality or deformity rate (Clayton and Summerfelt 2010; Blecha et al. 2019). Fish able to breach the water surface are at risk of transferring bacteria from the water surface to the swim bladder. Fazekas et al. (2021) reached an SBI of 70% with the help of water jets spraying the surface. Blecha et al. (2019) observed 83.4% to 99% SBI in ponds during traditional pond larvae rearing. The higher SBI in pond culture may be explained by optimal nutrition and thus better viability and ability to breach the clean water surface.

Another explanation of this difference lies in the natural selection of individuals with uninflated swim bladders by predators and natural conditions. In the present study, the SBI of Group R was 85% and 66.5% in Group A. This result is once again caused by the higher viability of larvae fed with rotifers at the beginning of exogenous feeding. Because a higher share of larvae was feeding, more individuals were in good condition and were able to breach the water surface and successfully inflate their swim bladder. In this study, the use of rotifers increased the swim bladder inflation rate by 18.5%, which is nearly one-fifth of the whole production. No study aimed at the application of rotifers and their use for pikeperch culture has discussed SBI in intensive larval culture before.

Another variable is the presence of deformities among the larvae, which could be tracked from the larval stage (Kestemont et al. 2007). It is generally associated with nutritional deficiencies, the unavailability of nutrients (Zakes 2012) and noninflation of the swim bladder (Szkudlarek and Zakes 2007). Individuals with uninflated swim bladders end up with prehaemal lordosis, which is caused by upwards bending of the mid-posterior part of vertebrae (Steenfeldt 2015). In Group A, the

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overall deformity rate reached 33%. Lordosis as the most significant malformation added 62% of all malformed fish. In Group A, where the SBI was 66.5%, the high occurrence of lordosis was quite predictable compared to Group R where TDR was 15%, SBI 85% and lordosis was therefore present on only 40% of malformed fish. The high relative share of lordosis could be also caused by the higher survival of lordosis-suffering fish compared to other types of deformities. Fish with lordosis feeds on the food sinking to the bottom where the deformed fish sits. This process reduces energetically demanding swimming and increases survivability which may artificially increase the observed share of lordosis-impacted individuals, as suggested previously by Steenfeldt (2015).

The production cost to cultivate 6 million rotifers per day for 13 consecutive days to successfully feed the larvae was approximately 3.9 times more expensive than the cultivation of *Artemia* for 21 days. However, the use of rotifers for intensive culture of pikeperch larvae in RAS on a large scale was proven. The cost of one dry feed-adapted juvenile pikeperch with a body weight of 8–10 g normally ranges from 0.6–0.8 EUR (Policar et al. 2016b). Currently, rising costs all around the world has increased the production costs of this size of pikeperch juveniles up to 1.1–1.5 EUR per piece. These results render it economically unsustainable for future development of European pikeperch intensive aquaculture and its commercial production. Therefore, the reduction of production costs and simultaneous improvement of rearing efficacy and stability of production is crucial for the future development of this sector (Overton et al. 2015). This study presents an analysis of the economic aspects associated with pikeperch larvae culture, especially focusing on rotifers and *Artemia* utilization. This study serves as a valuable contribution to the field, providing a precise feeding protocol and economic insight previously infeasible in a comparative analysis.

The results from this study, when integrated into commercial-scale production, may bring significant benefits. The optimization of the feeding strategy provides an increased survival rate and higher quality of produced juveniles for future pikeperch culture phases. These results reduce production costs per produced pikeperch juvenile and enhance the overall profitability of intensive commercial pikeperch farming. As a result, the aquaculture industry could improve practices that would lead to sustainable and economically viable production of malformation-free weaned pikeperch juveniles for ongoing intensive culture phases.

CONCLUSION

In this study, the applicability of rotifer as the first exogenous feeding for pikeperch larvae under large-scale conditions was proven. The use of an established feeding protocol using rotifers during 5–12 DPH with following combination of rotifers with *Artemia* (13–17 DPH) and following weaning during 18–26 DPH significantly increases pikeperch juvenile production value, quality and economic profitability up to 65 DPH.

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Conflict of interest

The authors declare no conflict of interest.

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