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Live feed enrichments using microalgae for pikeperch (*Sander lucioperca*) larval culture

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Abstract

This trial aimed to customize pikeperch (*Sander lucioperca*) larval nutrition using live feed enrichments based on *Chlorella vulgaris* and *Trachydiscus minutus*. Pikeperch larvae were fed with rotifers and Artemia enriched with *C. vulgaris and T. minutus* during the first 17 days after exogenous feeding (started 4 days post-hatching [dph]) and only Artemia until 20 dph. Larvae were exposed to seven different enrichments: (a) *Nannochloropsis occulata* (Nanno 3600 reed Mariculture) (Control), (b) *C. vulgaris* cultured at 20°C in BG-117 medium (BG20), (c) *C. vulgaris* cultured at 30°C in BG-117 medium (BG30), (d) *T. minutus* cultured at 15°C (T15), (e) *T. minutus* cultured at 25°C (T25), (f) *C. vulgaris* cultured at 20°C in urea medium (U20), and (g) *C. vulgaris* cultured at 30°C in urea medium (U30). After 20 days, no significant differences were found between treatments on total length, standard length, myomere height, and eye diameter. On the contrary, significant differences were found in larval fatty acid composition after the trial period. Larvae from the BG30 treatment showed a significantly higher concentration of docosahexaenoic acid (5.61%), and

larvae from the T25 treatment had a higher concentration of eicosapentaenoic acid 12.95%. Furthermore, larvae from the U20 treatment had a significantly higher arachidonic acid concentration of 0.116%. Overall, regarding essential fatty acid concentration, a significant difference was observed between the control treatment (*Nannochloropsis*) and the other treatments. No adverse effects were found on growth or survival when Nannochloropsis-enriched live feed was replaced with the other enrichments. This trial's results will help optimize the pikeperch larvae's nutritional requirements and diversify the live feed enrichments used during the first feeding.

KEYWORDS
Chlorella, fatty acids, first feeding, fish larvae, rotifers, *Trachydiscus*

1 | INTRODUCTION

Pikeperch is a highly demanded new fish species (Policar et al., 2013) included in the list of species for diversification of freshwater-intensive aquaculture in Europe (Křištan et al., 2013; Pěnka et al., 2023). Its aquaculture technology has been developed in Europe for over 25 years (Policar et al., 2019). Controlled larval culture is critical for developing a reliable and stable pikeperch production, which involves optimizing exogenous feeding (Imentai et al., 2020), live feed enrichments (Yanes-Roca, Leclercq, et al., 2020; Yanes-Roca, Mráz, et al., 2020; Yanes-Roca et al., 2022), and a practical application (Imentai et al., 2019).

Just like in other aquaculture species, pikeperch larvae rely on live feeds, such as microalgae, rotifers, and *Artemia salina* (Yanes-Roca et al., 2018; Yanes-Roca, Leclercq, et al., 2020; Yanes-Roca, Mráz, et al., 2020), mainly because of their high nutritional composition and size (Hamackova et al., 2009; Imentai et al., 2020; Policar et al., 2007; Wocher et al., 2012; Yanes-Roca et al., 2018). These organisms are effectively added to the larval tanks for larval first exogenous feeding because of their free-swimming ability, which makes them the ideal prey. Such ability also stimulates a feeding response in larvae (Kinne, 1997; Watanabe et al., 1978).

Enrichment methods have been developed in marine fish species to improve the nutritional quality of rotifers and Artemia. Feeds such as microalgae (Morizane, 1991), baker's yeast (Lubzens et al., 1989), and commercial diets (Cavalin & Weirich, 2009) are commonly used in aquaculture hatcheries. Because of their filter-feeding ability, rotifers and Artemia are exposed to particular enrichment over a period (8–15 h), enhancing their biochemical composition (Klaoudatos et al., 2004). Commercial enrichment diets are customized to meet the larvae's nutritional needs (Ferreira et al., 2018; Mæhre et al., 2013; Olsen et al., 1993) but tend to be more costly. Nevertheless, microalgae can provide high nutritional value by manipulating temperature and using various culture media, so their nutritional composition can be adjusted to the needs of fish larvae (Ferreira et al., 2008, 2009), and therefore commercial diets could be partially replaced (Aragão et al., 2004; Fehér et al., 2013; Koiso et al., 2009; Lee et al., 2019; Mæhre et al., 2013; Srivastava et al., 2006). One of these microalgae is *Chlorella vulgaris*, a fast-growing unicellular green microalga (Chlorophyta) that is widely used as a human food supplement (Görs et al., 2010), being rich in omega-3 long-chain polyunsaturated fatty acids (LC-PUFA). This has been added to fish diets (Estudillo-del Castillo et al., 2009) and has been fed to species like Ayu (*Plecoglossus altivelis*), the Korean rockfish (*Sebastes schlegeli*) and

pikeperch (Bai et al., 2001; Nematipour et al., 1987; Yanes-Roca et al., 2018; Yanes-Roca, Leclercq, et al., 2020; Yanes-Roca, Mráz, et al., 2020).

Such nutritional value enhancement has a direct effect on growth, functional development, stress resilience, and survival rate, as shown in several species such as barramundi (*Lates calcarifer*), seabream (*Sparus aurata*), seabass (*Dicentrarchus labrax*), and pikeperch (Ferreira et al., 2018; Nyina-wamwiza et al., 2005; Thépot et al., 2016; Yanes-Roca, Leclercq, et al., 2020).

This study aimed to enhance pikeperch nutrition by providing larvae with a diet adjusted to pikeperch's requirements during the first 20 days post-hatching.

2 | MATERIALS AND METHODS

The experimental trial was run at the Facility of Fisheries and Protection of Waters (FFPW), University of South Bohemia in Vodňany, Czech Republic. Experimental larvae were obtained from six pairs of pikeperch broodstock (males TL = 527 ± 33 mm/W = 1441 ± 101 and females TL = 519 ± 33 mm/W = 1388 ± 163 g) obtained from an outdoors aquaculture pond at Fishery Nové Hrady Ltd. (Czech Republic). Broodstock were exposed to all year round to natural environment conditions and fed mostly on live prey fish (*Pseudorasbora parva*) available at the pond. Broodstock were sampled during the month of April in a weekly basis to assess the reproduction status (mostly triggered by the seasonal temperatures and photoperiod changes) (Křištan et al., 2014; Malinovskyi et al., 2018, 2019).

Spawning and hatching of experimental larvae were synchronized by hormonal treatment of both sexes with Chorulon preparation (HCG 500 IU.kg^{-1}), performed a by a single injection. Females were injected once stage 5 was reached (Blecha et al., 2016). After five spawnings, five nests with attached and fertilized eggs (89.3 fertilization rate and average diameter size 1.4 ± 0.42 mm) were moved from a small pond to five indoor tanks (each of 350 L) connected to an RAS at USB, FFPW. Ninety-five percent of hatching occurred after 7 days at a water temperature of 17 ± 0.2°C and under a 15L/9D photoperiod. In total, 12,600 larvae after 3 days post-hatching (dph) were stocked for this study after 3 dph.

Larvae were stocked with an initial density of 100 larvae per liter (Policar et al., 2019) into 21 experimental rearing tanks (6-L cuboidal tank, three tanks per group), which were part of one separated experimental RAS in USB FFPW. Rearing conditions were set up at 17 ± 0.4°C, salinity of 4 ± 0.4 ppt, and the same photoperiod as the incubation period (15L/9D) and at a light intensity of 300 lux. Dissolved oxygen was kept at over 75%. The concentration of total ammonia (TAN) and nitrite were monitored according to Pěnka et al. (2021) every 3 days and were kept within the following range, respectively: TAN = 0.19 ± 0.05 NO_2 = 0.20 ± 0.01 mg L^{-1}. Daily cleaning and maintenance were done in order to keep clean conditions within the experimental tanks.

In total, seven treatments were tested.

Rotifers and *Artemia*, were fed with the different microalgae. Concentration of such microalgae at the live feed culture vessels was kept at 600.000 cells per mL. Rotifers were cultured following a 7-day batch system in 50-L culture vessels at an average concentration of 567 rotifer per mL. Rotifer average size was 280 µm. Rotifers and *Artemia* were placed for 14 h and held at 15°C prior to feeding. *Artemia* nauplii's average size was 430 µm.

The first was the control treatment (Control), where larvae were offered rotifers (*Brachionus plicatilis*) fed with *Nannochloropsis occulata* (Nanno 3600TM Reed Mariculture, Campbell, CA), at a rate of 1 mL of paste per liter of culture twice a day during the first 11 days (4–14 dph), and *A. salina* fed with same the microalgae feeding as rotifers from 12th dph until the end of the trial (20 dph).

The second treatment, BG20, followed the same feeding protocol as the Control, but rotifers and *Artemia* supplied to the larvae were fed with *C. vulgaris* grown in BG-11 medium at 20°C (Hu, 2013). In treatment BG30, the live feed was fed with *C. vulgaris* cultured at 30°C in BG-117 medium. The fourth and fifth treatments (U20, U30) used *C. vulgaris* cultured at 20°C and 30°C, respectively, in urea medium as a feed for the rotifers and *Artemia*. The last two treatments were based on the eustigmatophyte *Trachydiscus minutus* cultured at 15 and 25°C (T-15, T25) in BG-117 medium. All

microalgae cultures were performed in an annular photobioreactor at the Institute of Microbiology in Centre Algatech (Třeboň, Czech Republic). The following protocol was used to produce such cultures: After reaching the stationary growth phase, cultures were transferred into the annular column photobioreactor (AC-PBR) and adjusted to a total volume of 30 L using BG11 medium, as detailed in Ranglová et al. (2022). The AC-PBR was continuously illuminated and agitated by bubbling air mixed with 1% CO2 (v/v) at a flow rate of 3 L/min. Initial biomass densities ranged from 0.5 to 0.8 g of dry weight per liter. It took 14 days for the cultures to reach the stationary phase.

Starting with an initial light intensity (LI) of 200 µmol photons m^{-2} s^{-1}, the light intensity was gradually increased based on penetration values, reaching a maximum of 1600 µmol photons m^{-2} s^{-1}. Transmitted light was manually measured using a spherical sensor within the culture. Temperature was maintained at the optimal level for each strain: 25°C for Chlorella and 27°C for Trachydiscus (Ranglová et al., 2022).

Rotifers were fed to the larvae thrice daily (08:00, 11:30, and 15:30 h), starting at 4 dph until 14 dph with an initial concentration of 10 individuals per mL (Table 1). *Artemia* feeding was applied in each experimental group from 12 dph until 20 dph, increasing density from 2 to 8 in. per mL. Prior to each feeding, residual counts were measured and feeding densities were steadily increased based on the counts (Table 1). From 17 to 20 dph, the final live feed density was applied as 0 rotifers mL^{-1} and eight artemia mL^{-1}. Live feed (rotifers and *Artemia*) culture for the experimental trial was performed onsite (Yanes-Roca, Leclercq, et al., 2020).

The water flow rate through the experimental tanks began at 100 $mL.min^{-1}$ and gradually increased according to the data presented in Table 1. In order to enhance the feeding efficiency of the larvae, the water flow was interrupted and then restarted 2 h after the live feeds were applied before each feeding session. On the 21st day following hatching, the final morphometric analysis was conducted using 102 larvae per treatment (34 for each tank repetition) which included measurements of TL, SL, MH, and ED. This analysis was carried out using the Olympus

TABLE 1 Experiment with husbandry schedule for pikeperch larvae.

DHP	Daily feed: rots-art/mL	Flow (mL/min)
3	No feeding	100
4	10-0	100
5	10-0	100
6	10-0	100
7	10-0	100
8	14-0	160
9	14-0	160
10	14-0	160
11	14-0	160
12	14-2	200
13	10-3	200
14	8-4	200
15	0-7	250
16	0-7	250
17	0-8	250
18	0-8	250
19	0-8	250
20	0-8	250
21	End of trial	250

Note: The amount of daily live feed offered and recirculation flow changes through tanks with time are shown.

cellSens imaging software (version 1.3). Additionally, for fatty acid (FA) analysis at 21 days post-hatch (dph), 60 larvae per treatment (20 for each tank repetition) were collected, shock-frozen, and stored at −80°C. Moreover, the FA composition of all rotifers, and Artemia utilized in each treatment during the trial was analyzed (3 mg).

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 & Radin, 1978).

This study was performed under RTD capacity permits issued to No. 58672/2020-MZE-18134 and No. 33446/2020-MZE-18134. Larvae during this trial were handled under national (the Czech National Directive Law against Animal Cruelty, No. 246/1992) and international animal welfare protection guidelines (EU-harmonized Animal Welfare Act of the Czech Republic). All samples were performed with the appropriate permission of the Departmental Expert Committee for the Authorization of Experimental Projects of the Ministry of Education, Youth, and Sports of the Czech Republic, permit no. MSMT-8155/2022-4 for project NAZV QK22020144.

2.2 | Statistical analysis

A series of tests using R Core platoon (2014), were conducted to assess the impact of various enrichment methods on the growth and fatty acid composition of fish larvae. Multiple response variables were used, including total length, standard length, myomere height, eye diameter, and fatty acid composition (LA, ALA, ARA, EPA, and DHA). Survival rates were also analyzed across different enrichment groups. Analyses were performed using direct mixed models (LMM) and generalized direct mixed models (GLMM) with binomial error structures (package auto, interpretation 2.1.2; Fox & Weisberg, 2011; LMM, package lme4, interpretation1.1- 7; Bates et al., 2014). The tank was included as an arbitrary effect, and multiple pairwise comparisons were conducted using Tukey's all-pairs and all-days comparisons. Bonferroni correction was applied to adjust the p-values for multiple comparisons (package multcomp, interpretation1.3- 3; Hothorn et al., 2008). All analyses were carried out in R, with statistical significance set at $p = 0.05$.

3 | RESULTS

3.1 | Survival

Survival rates were not significantly different among treatments (GLMM and pairwise comparisons $p > 0.005$); overall average survival from all treatments was 36.6 ± 1.9% after 17 days of culture at age 21 dph. Although not

significant, the highest survival rate (39.4 ± 2.7%) was from the T25 treatment, followed by the U20 treatment (38.6 ± 0.5%) and the U30 treatment (37.3 ± 1.1%). The survival rate in the control treatment was 36.7 ± 2.7%, followed by BG20 (35.1 ± 2.3%) and T15 (34.8% ± 0.9), and the lowest was from BG30 (34.4 ± 2.1%) (Figure 1).

3.2 | Larval growth

In terms of growth parameters, larvae fed from the BG30 treatment have higher values in total length (TL = 11.5 ± 1.35 mm) and myomere height (2.0 ± 0.02 mm), especially when compared with the control treatment but no significant differences were found between treatments.

Initial pikeperch larval total length and body weight at 3 dph was TL = 5.25 ± 0.5 mm and BW = 0.52 ± 0.1 mg. By the end of the trial (21 dph), the average total length was more significant in larval-fed BG30 (TL = 11.48 ± 1.35 mm) than in other treatments (control: TL = 9.9 ± 1.41 mm, BG20 = 11.0 ± 1.5 mm, U20 = 11.01 ± 1.3 mm, U30 = 11.35 ± 1.5 mm, T15 = 10.73 ± 1.6 mm, T25 = 11.3 ± 1.3 mm) (Figure 1). However, no significant treatment differences (LMM, *p*-value >0.05) were found.

A similar pattern was found in myomere height, with larvae from BG30 having the highest value (2.04 ± 0.31 mm), and the control treatment had the lowest MH = 1.55 ± 0.25 mm. However, no significant differences were detected (LMM, *p*-value >0.05) among treatments (BG20 = 2.10 ± 0.25 mm, U20 = 19.01 ± 0.3 mm, U30 = 1.96.35 ± 0.3 mm, T15 = 1.89 ± 0.3 mm, T25 = 2.02 ± 0.2 mm, Figure 1). When looking at the eye diameter, larvae from BG20 had the highest value (ED = 0.93 ± 0.08 mm), and the control treatment had the lowest values (ED = 0.59 ± 0.09 mm). Again, no significant differences were found (LMM, *p*-value >0.05) among the tested

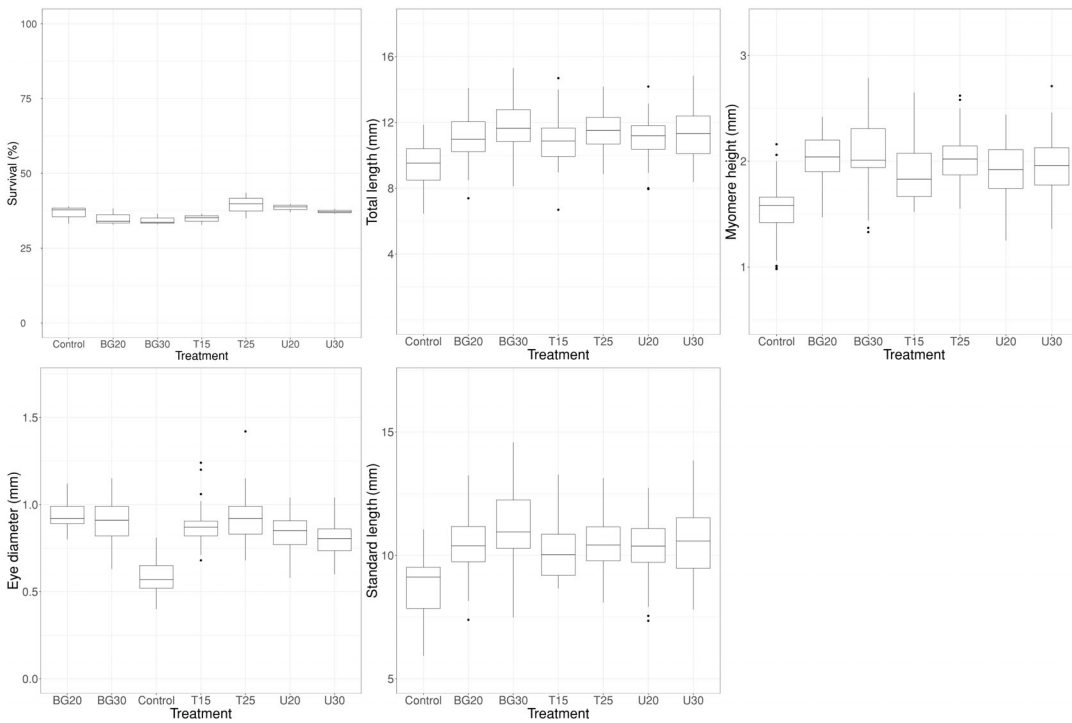

FIGURE 1 Larval growth parameters and survival from seven diet treatments at 21 dph ($n = 100$). Survival, ED, eye diameter; MH, myomere height; SL, standard length; TL, total length. Dots show mean values and whiskers indicate standard error.

treatments (BG20 = 0.93 ± 0.07 mm, U20 = 0.82 ± 0.1 mm, U30 = 8.80 ± 0.1 mm, T15 = 0.88 ± 0.1 mm, T25 = 0.92 ± 0.1 mm, Figure 1).

3.3 | Fatty acids

Larvae from the BG30 treatment showed a significantly higher concentration of docosahexaenoic acid (DHA) (5.61%) (Figure 2). The larvae from the T15 treatment had a higher concentration of eicosapentaenoic acid (EPA; C20:5n-3) (12.945%) and the larvae from the treatment U20 treatment had significant (LMM, *p*-value <0.05) higher concentration of arachidonic acid (ARA) (0.12%) among all treatments (Figure 2).

The fatty acid composition of Artemia and rotifers enriched with the different microalgae diets and Nanno 3600, Reed Mariculture, Campbell, CA, are shown in Tables 2a, 2b and 3a, 3b. Rotifers fed on the Chlorella BG30 and BG20 had 2.03 and 1.87 times higher LA levels than rotifers fed on Nannochloropsis (Control). In comparison, the a-linolenic acid (ALA) levels were 1.23 and 0.96 times higher (Table 3a). On the contrary, DHA levels were 4.78 times and 3.87 times lower in the rotifer group fed on the BG30 and U30 diets than the rotifers fed on Nannochloropsis (Control) (Table 3a, 3b). However, rotifers fed on BG20 and T25 had 1.43 and 2.11 times higher ARA values than the Control. When looking at EPA values, rotifers fed on T15 and T25 had 1.77 and 1.84 times higher concentrations than those rotifers fed on Nannochloropsis (Table 3b) (LMM analyses, all with *p*-value <0.001). The fatty acid profile of the artemia groups fed on the identical diets as the rotifers showed the same pattern, where the control treatment has the highest concentration of DHA (1.81 ± 0.07%) compared with the other treatments (Tables 2a, 2b and 3a, 3b). Larvae from the BG30 treatment showed significantly higher concentration of docosahexaenoic acid (DHA) (5.61%) (Figure 2). The larvae from the T15 treatment had a higher concentration of

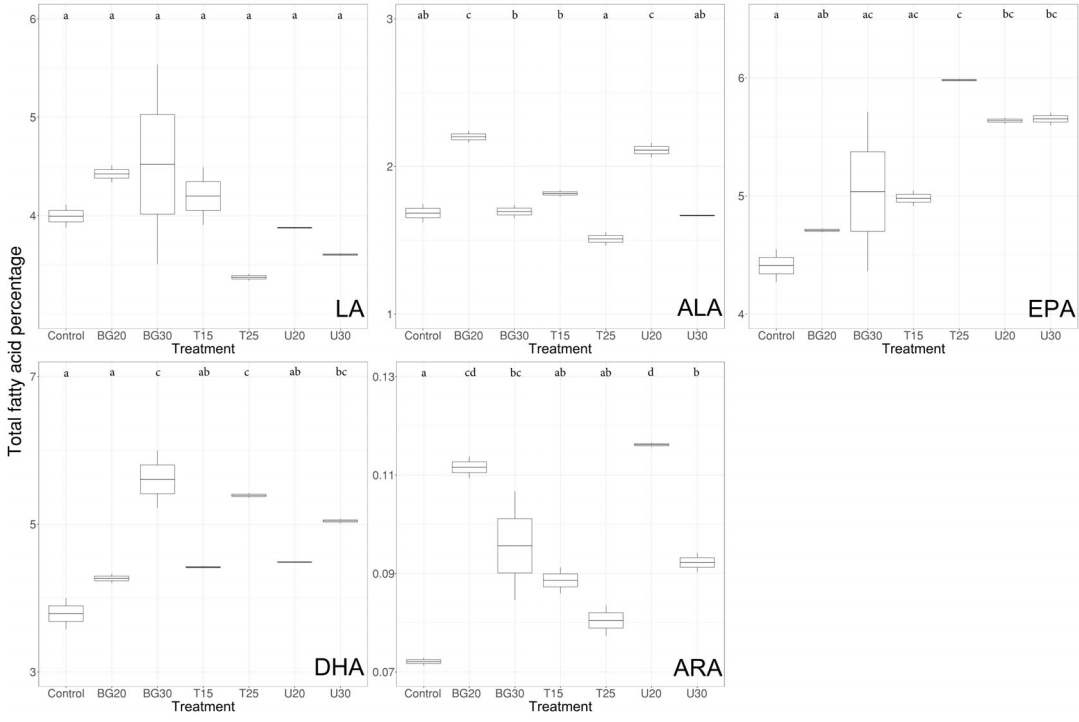

FIGURE 2 Larval essential fatty acids composition (a, LA, b, ALA, c, EPA, d, DHA, e, ARA) 21 days post-hatching. Statistically significant differences between treatments at 21 dph are marked with an asterisk.

TABLE 2a Artemia fatty acid composition after enrichment and pikeperch larvae total fatty acids percentage composition and standard deviation (±), from the Control (*Nannochloropsis*) and Chlorella treatments BG20, BG30, and U20 after 21 days post-hatching (NF, no values detected).

FA [%]	Organism	Nannochloropsis	BG20	BG30	U20
14:00	Artemia	2.011 ± 0.01	3.366 ± 0.01	3.560 ± 0.01	2.558 ± 0.01
	Larvae	0.792 ± 0.02	0.731 ± 0.00	0.774 ± 0.26	0.654 ± 0.00
14:1	Artemia	2.926 ± 0.00	2.700 ± 0.00	3.307 ± 0.00	3.913 ± 0.00
	Larvae	0.409 ± 0.02	0.404 ± 0.02	0.194 ± 0.20	0.369 ± 0.02
16:0	Artemia	16.032 ± 0.41	15.644 ± 0.26	14.759 ± 0.78	13.907 ± 0.26
	Larvae	16.767 ± 0.45	17.010 ± 0.30	18.561 ± 0.82	17.403 ± 0.30
16:1	Artemia	9.270 ± 0.36	4.783 ± 0.16	5.226 ± 0.01	5.542 ± 0.16
	Larvae	7.434 ± 0.40	6.891 ± 0.21	5.774 ± 0.03	6.206 ± 0.21
18:0	Artemia	3.801 ± 0.43	8.296 ± 0.15	7.006 ± 0.79	5.159 ± 0.15
	Larvae	8.577 ± 0.47	8.546 ± 0.19	8.343 ± 0.83	8.915 ± 0.19
18:1n-9	Artemia	3.510 ± 0.36	3.198 ± 0.00	3.409 ± 3.66	3.089 ± 0.20
	Larvae	18.414 ± 0.40	18.003 ± 0.04	19.503 ± 3.71	17.232 ± 0.04
18:1n-7	Artemia	22.217 ± 0.30	8.503 ± 0.02	9.906 ± 1.55	19.115 ± 0.02
	Larvae	13.444 ± 0.34	12.815 ± 0.06	10.834 ± 1.60	12.156 ± 0.06
18:2n-6	Artemia	8.481 ± 0.12	18.859 ± 0.08	17.339 ± 1.39	10.091 ± 0.08
	Larvae	3.995 ± 0.16	4.425 ± 0.12	4.522 ± 1.43	3.877 ± 0.01
18:3n-3	Artemia	8.499 ± 0.05	11.302 ± 0.01	10.782 ± 0.02	12.682 ± 0.01
	Larvae	1.684 ± 0.16	2.201 ± 0.12	1.695 ± 1.43	2.111 ± 0.12
20:0	Artemia	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.00
	Larvae	0.225 ± 0.09	0.221 ± 0.06	0.209 ± 0.06	0.218 ± 0.06
20:1n-9	Artemia	1.116 ± 0.00	1.653 ± 0.00	1.758 ± 0.00	1.617 ± 0.00
	Larvae	0.472 ± 0.02	0.457 ± 0.01	0.589 ± 0.00	0.418 ± 0.01
20:2	Artemia	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.00
	Larvae	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.00
20:4n-6	Artemia	1.411 ± 0.07	2.072 ± 0.02	2.146 ± 1.21	1.566 ± 0.10
	Larvae	0.072 ± 0.01	0.112 ± 0.01	0.096 ± 0.27	0.116 ± 0.01
20:3n-3	Artemia	NF ±	NF ±	NF ±	NF ±
	Larvae	6.204 ± 0.00	6.137 ± 0.00	6.089 ± 0.02	6.601 ± 0.00
22:0	Artemia	NF ±	NF ±	NF ±	NF ±
	Larvae	0.302 ± 0.09	0.288 ± 0.14	0.208 ± 2.23	0.241 ± 0.14
22:1n-9	Artemia	0.420 ± 0.01	0.768 ± 0.01	0.531 ± 0.01	1.376 ± 0.01
	Larvae	0.324 ± 0.01	0.383 ± 0.01	0.350 ± 0.03	0.387 ± 0.01
20:5n-3	Artemia	6.134 ± 0.15	4.224 ± 0.02	4.567 ± 0.91	3.608 ± 0.30
	Larvae	12.624 ± 0.12	12.337 ± 0.02	11.483 ± 1.25	12.849 ± 0.02
22:5n-3	Artemia	6.134 ± 0.15	4.224 ± 0.02	4.567 ± 0.91	3.608 ± 0.30
	Larvae	4.410 ± 0.01	4.710 ± 0.00	5.037 ± 0.02	5.638 ± 0.00
22:6n-3	Artemia	1.818 ± 0.12	0.541 ± 0.03	0.275 ± 0.03	0.581 ± 0.04
	Larvae	3.789 ± 0.20	4.265 ± 0.03	5.609 ± 0.95	4.487 ± 0.03
24:0	Artemia	1.043 ± 0.26	0.372 ± 0.05	0.628 ± 0.51	0.828 ± 0.05
	Larvae	0.063 ± 0.30	0.066 ± 0.09	0.079 ± 0.55	0.085 ± 0.09

TABLE 2b Artemia total fatty acid composition after enrichment and pikeperch larvae total fatty acids percentage composition and standard deviation (±), from the Control (*Nannochloropsis*), the Chlorella treatment U30, and the Trachydiscus treatments T15 and T25 after 21 days post-hatching (NF, no values detected).

FA [%]		Nannochloropsis	U30	T15	T25
14:00	Artemia	2.797 ± 0.01	4.852 ± 0.01	6.111 ± 0.01	2.797 ± 0.01
	Larvae	0.593 ± 0.01	0.758 ± 0.02	0.624 ± 0.02	0.593 ± 0.01
14:1	Artemia	3.955 ± 0.00	2.223 ± 0.00	1.574 ± 0.00	3.955 ± 0.00
	Larvae	0.318 ± 0.00	0.381 ± 0.02	0.308 ± 0.01	0.318 ± 0.00
16:0	Artemia	12.516 ± 0.24	13.252 ± 0.11	15.549 ± 0.16	12.516 ± 0.24
	Larvae	17.913 ± 0.24	17.790 ± 0.15	18.401 ± 0.20	17.913 ± 0.24
16:1	Artemia	5.448 ± 0.15	7.161 ± 0.12	5.796 ± 0.51	5.448 ± 0.15
	Larvae	5.670 ± 0.08	6.507 ± 0.16	5.592 ± 0.03	5.670 ± 0.08
18:0	Artemia	5.398 ± 0.13	3.932 ± 0.36	5.687 ± 0.01	5.398 ± 0.13
	Larvae	9.295 ± 0.03	9.039 ± 0.40	9.247 ± 0.05	9.295 ± 0.03
18:1n-9	Artemia	2.946 ± 0.23	3.346 ± 0.33	2.794 ± 0.06	2.946 ± 0.23
	Larvae	17.129 ± 0.19	17.590 ± 0.37	16.614 ± 0.10	17.129 ± 0.19
18:1n-7	Artemia	15.448 ± 0.01	11.787 ± 0.16	5.104 ± 0.27	15.448 ± 0.01
	Larvae	12.017 ± 0.28	12.230 ± 0.21	11.541 ± 0.03	12.017 ± 0.28
18:2n-6	Artemia	12.652 ± 0.07	9.765 ± 0.37	13.595 ± 0.01	12.652 ± 0.07
	Larvae	3.604 ± 0.02	4.199 ± 0.41	3.375 ± 0.05	3.604 ± 0.02
18:3n-3	Artemia	13.343 ± 0.00	7.546 ± 0.75	10.061 ± 0.02	13.343 ± 0.00
	Larvae	1.668 ± 0.02	1.817 ± 0.41	1.509 ± 0.05	1.668 ± 0.02
20:0	Artemia	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.00
	Larvae	0.217 ± 0.01	0.231 ± 0.03	0.242 ± 0.06	0.217 ± 0.01
20:1n-9	Artemia	1.612 ± 0.00	1.779 ± 0.00	1.725 ± 0.00	1.612 ± 0.00
	Larvae	0.417 ± 0.00	0.430 ± 0.01	0.411 ± 0.03	0.417 ± 0.00
20:2	Artemia	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.00
	Larvae	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.00
20:4n-6	Artemia	1.961 ± 0.21	2.814 ± 0.01	3.130 ± 0.03	1.961 ± 0.21
	Larvae	0.092 ± 0.01	0.089 ± 0.01	0.080 ± 0.00	0.092 ± 0.01
20:3n-3	Artemia	NF ±	NF ±	NF ± 0.00	NF ±
	Larvae	6.928 ± 0.00	6.309 ± 0.00	7.037 ± 0.00	6.928 ± 0.00
22:0	Artemia	NF ±	NF ± 0.00	NF ±	NF ±
	Larvae	0.231 ± 0.14	0.264 ± 0.07	0.224 ± 0.03	0.231 ± 0.14
22:1n-9	Artemia	0.871 ± 0.01	1.482 ± 0.01	0.826 ± 0.01	0.871 ± 0.01
	Larvae	0.343 ± 0.01	0.378 ± 0.02	0.345 ± 0.01	0.343 ± 0.01
20:5n-3	Artemia	4.807 ± 0.42	12.147 ± 0.05	12.552 ± 0.03	4.807 ± 0.42
	Larvae	12.778 ± 0.00	12.520 ± 0.05	12.945 ± 0.01	12.778 ± 0.00
22:5n-3	Artemia	4.807 ± 0.42	12.147 ± 0.05	12.552 ± 0.03	4.807 ± 0.42
	Larvae	5.653 ± 0.01	4.981 ± 0.02	5.981 ± 0.00	5.653 ± 0.01
22:6n-3	Artemia	0.372 ± 0.01	0.550 ± 0.03	0.381 ± 0.04	0.372 ± 0.01
	Larvae	5.045 ± 0.08	4.418 ± 0.09	5.390 ± 0.01	5.045 ± 0.08
24:0	Artemia	1.447 ± 0.03	1.861 ± 0.04	0.675 ± 0.00	1.447 ± 0.03
	Larvae	0.068 ± 0.04	0.071 ± 0.02	0.081 ± 0.04	0.068 ± 0.04

TABLE 3a Rotifer total fatty acid composition after enrichment and standard deviation (±), from the Control (*Nannochloropsis*) and the Chlorella treatments BG20, BG30, and U20 after 21 days post-hatching (NF, no values detected).

FA [%]	Nannochloropsis	BG20	BG30	U20
14:0	2.144 ± 0.01	3.498 ± 0.00	3.692 ± 0.25	2.691 ± 0.00
14:1	3.059 ± 0.00	2.832 ± 0.00	3.440 ± 0.19	4.045 ± 0.00
16:0	17.669 ± 0.44	17.281 ± 0.29	16.396 ± 0.81	15.544 ± 0.29
16:1	10.907 ± 0.39	6.420 ± 0.19	6.863 ± 0.02	7.179 ± 0.19
18:0	3.933 ± 0.46	8.428 ± 0.18	7.139 ± 0.82	5.291 ± 0.18
18:1n-9	3.642 ± 0.39	3.331 ± 0.02	3.542 ± 3.69	3.221 ± 0.02
18:1n-7	23.854 ± 0.33	10.140 ± 0.05	11.543 ± 1.58	20.752 ± 0.05
18:2n-6	10.118 ± 0.15	20.496 ± 0.11	18.976 ± 1.42	11.728 ± 0.11
18:3n-3	10.136 ± 0.08	12.939 ± 0.04	12.419 ± 0.05	14.319 ± 0.04
20:0	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.00
20:1n-9	1.249 ± 0.00	1.785 ± 0.00	1.891 ± 0.25	1.750 ± 0.00
20:2	NF ± 0.00	0.502 ± 0.00	0.533 ± 0.00	NF ± 0.00
20:4n-6	1.544 ± 0.10	2.205 ± 0.01	2.278 ± 1.24	1.698 ± 0.01
20:3n-3	NF ± 0.08	0.464 ± 0.13	0.539 ± 2.21	0.486 ± 0.13
22:0	NF ± 0.00	0.279 ± 0.00	0.273 ± 0.02	0.481 ± 0.00
22:1n-9	0.553 ± 0.00	0.900 ± 0.00	0.663 ± 0.01	1.508 ± 0.00
20:5n-3	7.771 ± 0.18	5.861 ± 0.01	6.204 ± 0.94	5.245 ± 0.01
22:5n-3	1.605 ± 0.00	1.151 ± 0.00	1.903 ± 0.00	1.7848 ± 0.00
22:6n-3	1.950 ± 0.15	0.674 ± 0.00	0.408 ± 0.00	0.7132 ± 0.00
24:0	1.175 ± 0.29	0.504 ± 0.08	0.761 ± 0.54	0.961 ± 0.08
24:1	0.643 ± 0.00	0.309 ± 0.00	0.538 ± 0.00	0.601 ± 0.00

eicosapentaenoic acid (EPA; C20:5n-3) (12.945%), and the larvae from the treatment U20 treatment had significant (LMM, *p*-value <0.05) higher concentration of arachidonic acid (ARA) (0.12%) among all treatments (Figure 2).

Rotifers fed on the *Chlorella* BG30 and BG20 had 2.03 and 1.87 times higher LA levels, respectively, than rotifers fed on *Nannochloropsis* (Control) while the levels of α-linolenic acid (ALA) were 1.23 and 0.96 times higher (Table 3a). On the contrary, DHA levels were 4.78 times and 3.87 times lower than in the rotifer group fed on the BG30 and U30 diets than the rotifers fed on *Nannochloropsis* (Control) (Table 3a, 3b). However, rotifers fed on BG20 and T25 had 1.43 and 2.11 times higher ARA values than the control. When looking at EPA values in rotifers fed on T15 and T25 had 1.77 and 1.84 times higher concentrations than those rotifers fed on *Nannochloropsis* (Table 3b) (LMM analyses, all with *p*-value <0.001). The fatty acid profile of the artemia groups fed on the same diets as the rotifers showed the same pattern, where the control treatment has the highest concentration of DHA (1.81 ± 0.07%) compared with the other treatments (Tables 2a, 2b and 3a, 3b). The fatty acid composition of artemia and rotifers enriched with the different microalgae diets and Nanno 3600, Reed Mariculture, Campbell, CA, is shown in Tables 2a, 2b and 3a, 3b. Additional data containing the fatty acid composition of the different microalgae can be found in Tables A1 and A2 in Appendix A.

After 21 days post-hatching, larvae from the BG20 treatments had 1.11 and 1.37 times significantly higher LA values (Figure 2) than larvae from the control treatments and the T25 treatment (LMM, *p*-value <0.001). No significant differences were found between larvae from BG20, BG30, U20, U30, and T25 in their levels of EPA (LMM, *p*-

TABLE 3b Rotifer total fatty acid composition after enrichment and standard deviation (±), from the Control (*Nannochloropsis*), the Chlorella treatment U30, and the Trachydiscus treatments T15 and T25 after 21 days post-hatching. (NF, no values detected).

FA [%]	Nannochloropsis	U30	T15	T25
14:0	2.144 ± 0.01	2.929 ± 0.00	4.984 ± 0.01	6.243 ± 0.01
14:1	3.059 ± 0.00	4.087 ± 0.00	2.356 ± 0.01	1.706 ± 0.00
16:0	17.669 ± 0.44	14.153 ± 0.27	14.889 ± 0.14	17.186 ± 0.19
16:1	10.907 ± 0.39	7.085 ± 0.18	8.798 ± 0.15	7.433 ± 0.01
18:0	3.933 ± 0.46	5.530 ± 0.16	4.065 ± 0.39	5.820 ± 0.04
18:1n-9	3.642 ± 0.39	3.078 ± 0.01	3.479 ± 0.36	2.927 ± 0.09
18:1n-7	23.854 ± 0.33	17.085 ± 0.04	13.424 ± 0.19	6.741 ± 0.02
18:2n-6	10.118 ± 0.15	14.289 ± 0.10	11.402 ± 0.40	15.232 ± 0.04
18:3n-3	10.136 ± 0.08	14.980 ± 0.03	9.183 ± 0.02	11.698 ± 0.05
20:0	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.01
20:1n-9	1.249 ± 0.00	1.744 ± 0.00	1.911 ± 0.00	1.857 ± 0.00
20:2	NF ± 0.00	0.000 ± 0.00	NF ± 0.00	NF ± 0.00
20:4n-6	1.544 ± 0.10	2.094 ± 0.00	2.946 ± 0.04	3.262 ± 0.00
20:3n-3	NF ± 0.08	0.000 ± 0.11	NF ± 0.06	0.346 ± 0.02
22:0	NF ± 0.00	0.000 ± 0.00	NF ± 0.01	0.394 ± 0.00
22:1n-9	0.553 ± 0.00	1.004 ± 0.00	1.615 ± 0.00	0.959 ± 0.00
20:5n-3	7.771 ± 0.18	6.444 ± 0.00	13.784 ± 0.08	14.189 ± 0.00
22:5n-3	1.605 ± 0.00	2.400 ± 0.00	3.295 ± 0.00	2.214 ± 0.00
22:6n-3	1.950 ± 0.15	0.504 ± 0.00	0.682 ± 0.00	0.513 ± 0.00
24:0	1.175 ± 0.29	1.579 ± 0.06	1.993 ± 0.01	0.807 ± 0.03
24:1	0.643 ± 0.00	1.015 ± 0.00	1.194 ± 0.00	0.473 ± 0.00

value >0.05) when comparing treatments. However, a significant difference was found between T15 and the other treatments (LMM, *p*-value <0.001).

When looking at ALA concentrations, larvae fed BG20 and U20 had significantly (LMM, *p*-value <0.001) higher values than the other five treatments.

Furthermore, larvae from treatment BG30 and T25 showed 1.47 and 1.42 times higher DHA values than larvae fed with *Nannochloropsis* (Control), which was significantly different (LMM, *p*-value <0.001). A significant difference between treatments was found regarding ARA levels (Figure 2), where larvae from the Control had the lowest levels on day 21 postexposure, and larvae fed BG20 and U20 had the highest levels, which were significantly different compared with the other treatments. Pairwise comparisons were generally with LMM results.

4 | DISCUSSION

Finding larval nutritional requirements and the optimal way to provide them with such nutrition is often one of the first challenges faced when developing rearing protocols for new aquaculture species (Ghan & Sprules, 1993; Izquierdo, 1996; Kestemont & Henrotte, 2015).

As described by Kestemont and Henrotte (2015), pikeperch falls in the category of such "new" species, and developing new larval rearing protocols encompasses the challenges mentioned above, leading to an insufficient

production. Recent developments in pikeperch larvae rearing have started addressing such issues, and promising results have been obtained (Imentai et al., 2019, 2020; Yanes-Roca et al., 2018; Yanes-Roca, Leclercq, et al., 2020; Yanes-Roca, Mráz, et al., 2020). Such breakthroughs were mainly focused on the early introduction of rotifers during first feeding, addressing the prey size issue and the lack of nutrients during such a period. Nevertheless, further developments are in need.

This trial, focused on supplying the larvae with adequate fatty acids from a sustainable source (microalgae), so production costs can be reduced and more sustainable enrichment methods can be promoted. Although no significant, growth results showed a slight improvement from all treatments compared with the control. Such outcomes were most likely because of the significant differences found in fatty acid composition, matching conclusions in Pikeperch by Lund and Steenfeldt (2011) and Lund et al. (2012).

The trial's overall larval survival averaged over 37% after 21 days, and no significant differences between treatments were found. Larvae from T25 had the highest survival over the other treatments. The lack of differences in survival found between treatments shows no adverse effects on utilizing other microalgae and can be used as a baseline for future work on this topic. Such lack of significance in survival is likely because of the possible negligible amounts between treatments of dietary DHA required for normal development fish larvae, which relate to a predominance of EPA rather than DHA found on other species (Villalta et al., 2005). A DHA deficit during larval stage has been directly linked to low survival (Tocher, 2010), likely because of the importance that DHA has over the nervous and ocular system development (Izquierdo et al., 2000; Villalta et al., 2008). During this study, DHA concentration in larvae after 21 dph reached recommended values (Izquierdo et al., 2000) and was not significantly different between treatments, except for larvae from BG20. When looking at differences in growth between treatments, no significant differences were found, and this could be explained by the differences found in some monounsaturated fatty acids, particularly 18:1n-9, which are easily catabolized in fish to produce energy, while DHA is not easily catabolized via h-oxidation (Sargent et al., 2002). Dietary DHA content may not be crucial in determining pikeperch growth rate during the larval stage when baseline concentrations are met. Such conclusions were also reached by Morshedi et al. (2020) when looking at yellow-tail seabream (*Acanthopagrus latus*) in line with other species such as striped bass (*Morone saxatilis*), gilthead (*S. aurata*), red porgy (*Pagrus pagrus*), striped trumpeter (*Latris lineata*), and Senegalese sole (*Solea senegalensis*) (Bransden et al., 2004; Harel et al., 2002; Morais et al., 2004; Mourente & Tocher, 1993; Roo et al., 2019). Subsequently, increased dietary DHA and reduced monounsaturated fatty acids, particularly 18:1n-9, may have reduced energy availability and growth.

However, several fish species have been reported to have a different effect, where growth performance was enhanced, such as in amberjack larvae (*Seriola lalandi*) and other marines species such as the Japanese flounder (*Paralichthys olivaceus*), gilthead seabream (*S. aurata*), meager (*Argyrosomus regius*), and greater amberjack (*Seriola dumerili*) (Campoverde et al., 2017; Matsunari et al., 2013; Person-Le Ruyet & Verillaud, 1980; Roo et al., 2019), among others.

The difference could lie in the positive effects that higher concentrations of LA and ALA had on the overall larval performance and quality because fatty acids are stored in muscle tissue to meet physiological needs and are closely related to growth (Jardine et al., 2020), matching conclusions reached by other studies such as Malzahn et al. (2022).

5 | CONCLUSIONS

Feasible alternatives of live feed enrichment strategies for pikeperch larval rearing have been tested with positive results. Using various microalgae selected for their fatty acid profile has helped optimize the larval nutrient requirements and promoted sustainable large-scale aquaculture practices. The wide diversity of microalgae species and the ability to modify the fatty acid profile by altering culture conditions make them potential candidates for further investigations and development of zooplankton-fish larvae rearing. This study shows that *C. vulgaris* and *T. minutus*

cultured under various conditions can have such potential. Further trials on other microalgae species to improve pikeperch larvae's fatty acid and protein requirements are suggested.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Appelqvist, L.-A. (1968). Lipids in Cruciferae. III. Fatty acid composition of diploid tetraploid seeds of *Brassica campestris* and *Sinapis alba* grown under two climatic extremes. *Physiologia Plantarum*, *21*, 615–625. https://doi.org/10.1111/j.1399-3054.1968.tb07286.x

Aragão, C., Conceição, L. E. C., Fyhn, H.-J., & Dinis, M. T. (2004). Estimated amino acid requirements during early ontogeny in fish with different life styles: Gilthead seabream (*Sparus aurata*) and Senegalese sole (*Solea senegalensis*). *Aquaculture*, *242*, 589–605. https://doi.org/10.1016/j.aquaculture.2004.09.015

Bai, S. C., Koo, J., Kim, K., & Kim, S. (2001). Effects of *Chlorella* powder as a feed additive on growth performance in juvenile Korean rock, *Sebastes schlegeli* (Hilgendorf). *Aquaculture Research*, *32*(S1), 92–98.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

Blecha, M., Samarin, A. M., Kristan, J., & Policar, T. (2016). Benefits of hormone treatment of both sexes in semi-artificial reproduction of pikeperch (*Sander lucioperca* L.). *Czech Journal of Animal Science*, *61*(5), 2016–2203.

Bransden, M. P., Cobcroft, J. M., & Battaglene, S. C. (2004). Dietary arachidonic acid alters tissue fatty acid profile, whole body eicosanoid production and resistance to hypersaline challenge in larvae of the temperate marine fish, striped trumpeter (*Latris lineata*). *Fish Physiology and Biochemistry*, *30*, 241–256. https://doi.org/10.1007/s10695-005-8245-4

Campoverde, C., Rodriguez, C., Perez, J., Gisbert, E., & Estévez, A. (2017). Early weaning in meagre *Argyrosomus regius*: Effects on growth, survival, digestion and skeletal deformities. *Aquaculture Research*, *48*(10), 5289–5299.

Cavalin, F. G., & Weirich, C. R. (2009). Larval performance of aquacultured Florida pompano (*Trachinotus carolinus*) fed rotifers (*Brachionus plicatilis*) enriched with selected commercial diets. *Aquaculture*, *292*(1), 67–73. https://doi.org/10.1016/j.aquaculture.2009.03.042

Estudillo-del Castillo, C., Gapasin, R. S., & Leaño, E. M. (2009). Enrichment potential of HUFA-rich thraustochytrid *Schizochytrium mangrovei* for the rotifer *Brachionus plicatilis*. *Aquaculture*, *293*(1–2), 57–61.

Fehér, M., Baranyai, E., Simon, E., Bársony, P., Szűcs, I., Posta, J., & Stündl, L. (2013). The interactive effect of cobalt enrichment in *Artemia* on the survival and larval growth of barramundi, *Lates calcarifer*. *Aquaculture*, *414–415*, 92–99.

Ferreira, M., Cortina-Burgueño, Á., Freire, I., & Otero, A. (2018). Effect of nutritional status and concentration of *Nannochloropsis gaditana* as enrichment diet for the marine rotifer *Brachionus* sp. *Aquaculture*, *491*, 351–357. https://doi.org/10.1016/j.aquaculture.2018.03.024

Ferreira, M., Coutinho, P., Seixas, P., Fábregas, J., & Otero, A. (2009). Enriching rotifers with "Premium" microalgae. *Nannochloropsis gaditana*. *Marine Biotechnology*, *11*(5), 585–595. https://doi.org/10.1007/s10126-008-9174-x

Ferreira, M., Maseda, A., Fábregas, J., & Otero, A. (2008). Enriching rotifers with "premium" microalgae. *Isochrysis* aff. *galbana* clone T-ISO. *Aquaculture*, *279*(1), 126–130. https://doi.org/10.1016/j.aquaculture.2008.03.044

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression* (2nd ed.). Sage.

Ghan, D., & Sprules, W. G. (1993). Diet, prey selection, and growth of larval and juvenile burbot *Lota lota* (L.). *Journal of Fish Biology*, *42*(1), 47–64.

Görs, M., Schumann, R., Hepperle, D., & Karsten, U. (2010). Quality analysis of commercial *Chlorella* products used as dietary supplement in human nutrition. *Journal of Applied Phycology*, *22*(3), 265–276.

Hamackova, J., Prokes, M., Kozak, P., Penaz, M., Stanny, L. A., Policar, T., & Barus, V. (2009). Growth and development of vimba bream (*Vimba vimba*) larvae in relation to feeding duration with live and/or dry starter feed. *Aquaculture*, *287*, 158–162.

Hara, A., & Radin, N. S. (1978). Lipid extraction of tissues with a low-toxicity solvent. *Analytical Biochemistry*, *90*, 420–426.

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*(1-4), 347–362.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363.

Hu, Q. (2013). Photosynthesis in microalgae. In A. Richmond & Q. Hu (Eds.), *Handbook of microalgal culture: Applied phycology and biotechnology* (2nd ed., pp. 114–122). Wiley Blackwell.

Imentai, A., Rašković, B., Steinbach, C., 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. https://doi.org/10.1016/j.aquaculture.2020.735636

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*(4), 1137–1149. https://doi.org/10.1007/s10499-019-00420-3

Izquierdo, M. S. (1996). Essential fatty acid requirements of cultured marine fish larvae. *Aquaculture Nutrition*, *2*, 183–191. https://doi.org/10.1111/j.1365-2095.1996.tb00058.x

Izquierdo, M. S., Socorro, J., Arantzamendi, L., & Hernández-Cruz, C. M. (2000). Recent advances in lipid nutrition in fish larvae. *Fish Physiology and Biochemistry*, *22*, 97–107. https://doi.org/10.1023/A:1007810506259

Jardine, T. D., Galloway, A. W. E., & Kainz, M. J. (2020). Unlocking the power of fatty acids as dietary tracers and metabolic signals in fishes and aquatic invertebrates. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*, 20190639. https://doi.org/10.1098/rstb.2019.0639

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* (pp. 539–564). Springer Netherlands.

Kinne, O. (Ed.). (1997). *Cultivation of animals. Cultivation part 2* (Vol. 3, pp. 579–1293). Wiley.

Klaoudatos, S. D., Iakovopoulos, G., & Klaoudatos, D. S. (2004). *Pagellus erythrinus* (Common Pandora): A promising candidate species for enlarging the diversity of aquaculture production. *Aquaculture International*, *12*(3), 299–320. https://doi.org/10.1023/B:AQUI.0000036186.31318.4a

Koiso, M., Yoshikawa, M., Kuwada, H., & Hagiwara, A. (2009). Effect of maternal diet on survival and life history parameters of next generations in the rotifer *Brachionus plicatilis* sp. complex. *Nippon Suisan Gakkaishi*, *75*(5), 828–833. https://doi.org/10.2331/suisan.75.828

Křištan, J., Alavi, T., Stejskal, S. M., & Policar, T. (2013). Hormonal induction of ovulation in pikeperch (*Sander lucioperca* L.) using human chorionic gonadotropin (hCG) and mammalian GnRH analogue. *Aquaculture International*, *21*(4), 811–818.

Křištan, J., Stara, J., Polgesek, A., Drasovean, M., Kolarova, A., Priborsky, J., Blecha, M., Svacina, P., Policar, T., & Velisek, T. (2014). Efficacy of different anaesthetics for pikeperch (*Sander lucioperca L.)* in relation to water temperature. *Neuroendocrinology Letters*, *35*(2), 81–85.

Lee, M.-C., Park, J. C., Yoon, D.-S., Choi, H., Shin, K.-H., & Kim, H.-J. (2019). Lipid metabolism modulation by five different food types in the monogonont marine rotifer *Brachionus koreanus*. *Aquaculture*, *503*, 596–601. https://doi.org/10.1016/j.aquaculture.2018.12.043

Lubzens, E., Tandler, A., & Minkoff, G. (1989). Rotifers as food in aquaculture. *Hydrobiologia*, *186–187*(1), 387–400.

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.). *Aquaculture Nutrition*, *17*(2), 191–199.

Lund, I., Vilhelm, P., & Winding, B. (2012). Dietary supplementation of essential fatty acids in larval pikeperch (*Sander lucioperca*); short and long term effects on stress tolerance and metabolic physiology. *Comparative Biochemistry and Physiology. Part A*, *162*(4), 340–348.

Mæhre, H. K., Hamre, K., & Elvevoll, E. O. (2013). Nutrient evaluation of rotifers and zooplankton: Feed for marine fish larvae. *Aquaculture Nutrition*, *19*(3), 301–311. https://doi.org/10.1111/j.1365-2095.2012.00960.x

Malinovskyi, O., Veselý, L., Blecha, M., Křišťan, J., & Policar, T. (2018). The substrate selection and spawning behaviour of pikeperch *Sander lucioperca* L. broodstock under pond conditions. *Aquaculture Research*, *49*(11), 3541–3547.

Malinovskyi, O., Veselý, L., Křišťan, J., & Policar, T. (2019). Behavior and physiological status of pond-cultured pikeperch (*Sander lucioperca*) broodstock effected by sexual interactions throughout semi-artificial reproduction. *Aquaculture International*, *27*(4), 1093–1107.

Malzahn, A. M., Ribičić, D., Hansen, B. H., Sarno, A., Kjørsvik, E., Norberg-Aase, A., Alves Musialak, L., García-Calvo, L., & Hagemann, A. (2022). First feed matters: The first diet of larval fish programmes growth, survival, and metabolism of larval ballan wrasse (*Labrus bergylta*). *Aquaculture*, *561*, 738586. https://doi.org/10.1016/j.aquaculture.2022.738586

Matsunari, H., Hashimoto, H., Oda, K., Masuda, Y., Imaizumi, H., Teruya, K., Furuita, H., Yamamoto, T., Hamada, K., & Mushiake, K. (2013). Effects of docosahexaenoic acid on growth, survival and swim bladder inflation of larval amberjack (*Seriola dumerili*, Risso). *Aquaculture Research*, *44*, 1696–1705.

Morais, S., Narciso, L., Dores, E., & Pousão-Ferreira, P. (2004). Lipid enrichment for Senegalese sole *(Solea senegalensis)* larvae: Effect on larval growth, survival and fatty acid profile. *Aquaculture International*, *12*(3), 281–298. https://doi.org/10.1023/b:aqui.0000036184.13187.6b

Morizane, T. (1991). A review of automation and mechanization used in the production of rotifer in Japan. Rotifer and microalgae culture systems. In *Proceeding of US–Asia workshop* (pp. 79–88). The Oceanic Institute.

Morshedi, V., Torfi Mozanzadeh, M., Hamedi, S., Naserifard, I., Ebrahimi, H., Agh, N., Nafisi, M., Azodi, M., & Rashidian, G. (2020). Enrichment of live feed with very low level of docosahexaenoic acid (DHA) is enough for yellowtail sea bream (*Acanthopagrus latus*) larvae. *Aquaculture Reports*, *26*, 101310. https://doi.org/10.1016/j.aqrep.2022.101310

Mourente, G., & Tocher, D. R. (1993). Incorporation and metabolism of ^{14}C-labelled polyunsaturated fatty acids in juvenile gilthead sea bream *Sparus aurata* L. in vivo. *Fish Physiology and Biochemistry*, *10*, 443–453.

Nematipour, G. R., Nakagawa, H., Nanba, K., Kasahara, S., Tsujimura, A., & Akira, K. (1987). Effect of *Chlorella*-extract supplement to diet on lipid accumulation of ayu. *Nippon Suisan Gakkaishi*, *53*(9), 1687–1692.

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. *Aquaculture Research*, *36*, 486–492.

Olsen, Y., Inge Reitan, K., & Vadstein, O. (1993). Dependence of temperature on loss rates of rotifers, lipids, and ω3 fatty acids in starved *Brachionus plicatilis* cultures. *Hydrobiologia*, *255*(1), 13–20. https://doi.org/10.1007/bf00025815

Pěnka, T., Malinovskyi, O., Imentai, A., Kolářová, J., Kučera, V., & Policar, T. (2023). Evaluation of different feeding frequencies in RAS-based juvenile pikeperch (*Sander lucioperca*) aquaculture. *Aquaculture*, *562*, 738815. https://doi.org/10.1016/j.aquaculture.2022.738815

Pěnka, T., Malinovskyi, O., Křišťan, J., Imentai, A., & Policar, T. (2021). Effect of density and mixed culture of largemouth bass (*Micropterus salmoides*) and pikeperch (*Sander lucioperca*) on growth, survival and feed conversion rate in intensive culture. *Czech Journal of Animal Science*, *66*(10), 428–440. https://doi.org/10.17221/59/2021-CJAS

Person-Le Ruyet, 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*(4), 351–370.

Policar, T., Kozák, P., Hamáčková, J., Lepičová, A., Musil, J., & Kouřil, J. (2007). Effects of short-time Artemia spp. feeding in larvae and different rearing environments in juveniles of common barbel (*Barbus barbus*) on their growth and survival under intensive controlled conditions. *Aquatic Living Resources*, *20*(2), 175–183. https://doi.org/10.1051/alr:2007029

Policar, T., Kristan, J., & Malinovskyi, O. (2019). Recent progress in European percid fish culture production technology—tackling bottlenecks. *Aquaculture International*, *27*, 1151–1174.

Policar, T., Stejskal, V., 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. *Aquaculture International*, *21*(4), 869–882.

R Core Team. (2014). R: A language and environment for statistical computing [Online]. Accessed March 01, 2018. https://scholar.google.com/citations?user=yvS1QUEAAAAJ&hl=en&oi=sra

Ranglová, K., Bureš, M., & Manoel, J. C. (2022). Efficient microalgae feed production for fish hatcheries using an annular column photobioreactor characterized by a short light path and central LED illumination. *Journal of Applied Phycology*, *34*, 31–41. https://doi.org/10.1007/s10811-021-02647-1

Roo, J., Hernández-Cruz, C. M., Mesa-Rodriguez, A., Fernández-Palacios, H., & Izquierdo, M. S. (2019). Effect of increasing n-3 HUFA content in enriched *Artemia* on growth, survival and skeleton anomalies occurrence of greater amberjack *Seriola dumerili* larvae. *Aquaculture*, *500*, 651–659.

Sargent, J. R., Tocher, D. R., & Bell, J. G. (2002). The lipids. In J. E. Halver & R. W. Hardy (Eds.), *Fish nutrition* (pp. 181–257). Academic Press.

Srivastava, A., Rathore, R. M., & Chakrabarti, R. (2006). Effects of four different doses of organic manures in the production of *Ceriodaphnia cornuta*. *Bioresource Technology*, *97*(8), 1036–1040. https://doi.org/10.1016/j.biortech.2005.04.044

Thépot, V., Mangott, A., & Pirozzi, I. (2016). Rotifers enriched with a mixed algal diet promote survival, growth and development of barramundi larvae, *Lates calcarifer* (Bloch). *Aquaculture Reports*, *3*, 147–158.

Tocher, D. R. (2010). Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research*, *41*(5), 717–732.

Villalta, M., Estévez, A., Bransden, M. P., & Bell, G. (2005). The effect of graded concentrations of dietary DHA on growth, survival and tissue fatty acid profile of Senegal sole (*Solea senegalensis*) larvae during the *Artemia* feeding period. *Aquaculture*, *249*(1–4), 353–365. https://doi.org/10.1016/j.aquaculture.2005.03.037

Villalta, M., Estevez, A., Bransden, M. P., & Bell, J. G. (2008). Effects of dietary eicosapentaenoic acid on growth, survival, pigmentation and fatty acid composition in Senegal sole (*Solea senegalensis*) larvae during the *Artemia* feeding period. *Aquaculture Nutrition*, *14*(3), 232–241.

Watanabe, T., Oowa, F., Kitajima, C., & Fujita, S. (1978). Nutritional quality of brine shrimp, *Artemia salina*, as a living feed from the viewpoint of essential fatty acids for fish. *Bulletin of the Japanese Society Scientific Fisheries*, *44*, 1115–1121. https://doi.org/10.2331/suisan.44.1115

Wocher, H., Harsányi, A., & Schwarz, F. J. (2012). Larviculture of burbot (*Lota lota* L.): Larval rearing using *Artemia* and weaning onto dry feed. *Aquaculture Research*, *44*, 106–113.

Yanes-Roca, C., Galindo, A., Holzer, A. S., Veselý, L., Policar, T., Rodríguez, C., & Pérez, J. A. (2022). Taurine supplement improved growth performance and digestive capacity of pikeperch larvae. *Aquaculture Research*, *53*(14), 5105–5114.

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*(2), 238.

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. https://doi.org/10.1016/j.aquaculture.2018.08.004

Yanes-Roca, C., Mráz, J., Vesely, L., Malinovskyi, O., Holzer, A. S., & Policar, T. (2020). Improvements on live feed enrichments for pikeperch (*Sander lucioperca*) larval culture. *Animals*, *10*(3), 401.

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APPENDIX A

TABLE A1 Comparison of fatty acid composition (% ±SD) determined in microalgae diets and rotifers after enrichment from the Control (*Nannochloropsis*) and *Chlorella* diet U30 and *Trachydiscus* diets T15 and T25 after 21 days post-hatching (NF, no values detected).

FA [%]		Control	U30	T15	T25
14:00	Microalgae	27.610 ± 2.36	0.045 ± 0.03	28.779 ± 1.62	27.615 ± 2.36
C14:1	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C16:0	Microalgae	8.010 ± 0.09	1.205 ± 0.12	6.988 ± 0.11	8.005 ± 0.09
C16:1	Microalgae	NF ± NF	0.229 ± 0.08	NF ± NF	NF ± NF
C18:0	Microalgae	0.590 ± 0.06	0.210 ± 0.04	0.539 ± 0.05	0.591 ± 0.06
C18:1n-9	Microalgae	1.740 ± 0.04	0.103 ± 0.01	1.589 ± 0.08	1.736 ± 0.04
C18:1n-7	Microalgae	0.320 ± 0.12	0.003 ± 0.00	0.244 ± 0.12	0.315 ± 0.12
C18:2n-6	Microalgae	6.180 ± 0.22	0.888 ± 0.04	6.323 ± 0.28	6.181 ± 0.22
C18:3n-3	Microalgae	0.480 ± 0.06	0.124 ± 0.03	0.330 ± 0.12	0.484 ± 0.06
C20:0	Microalgae	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.00
C20:1n-9	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C20:2	Microalgae	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.00
C20:4n-6	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C20:3n-3	Microalgae	1.300 ± 0.07	NF ± NF	1.65 ± 0.04	1.30 ± 0.07
C22:0	Microalgae	NF ±	NF ±	NF ± 0.00	NF ±
C22:1n-9	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C20:5n-3	Microalgae	31.880 ± 2.03	NF ± NF	31.01 ± 1.95	31.88 ± 2.03
C22:5n-3	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C22:6n-3	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C24:0	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF

TABLE A2 Comparison of fatty acid composition (% ±SD) determined in microalgae diets and rotifers after enrichment from the Control (*Nannochloropsis*) and *Chlorella* diets BG20, BG30 and U20 after 21 days post-hatching (NF, no values detected).

FA [%]		Control	BG20	BG30	U20
14:00	Microalgae	27.610 ± 2.36	0.520 ± 0.08	1.121 ± 0.71	0.040 ± 0.01
C14:1	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C16:0	Microalgae	8.010 ± 0.09	19.720 ± 0.14	22.703 ± 1.28	1.243 ± 0.10
C16:1	Microalgae	NF ± NF	1.450 ± 0.16	1.457 ± 0.05	0.252 ± 0.03
C18:0	Microalgae	0.590 ± 0.06	2.690 ± 0.60	2.480 ± 0.15	0.310 ± 0.04
C18:1n-9	Microalgae	1.740 ± 0.04	1.410 ± 0.29	1.609 ± 0.08	0.127 ± 0.05
C18:1n-7	Microalgae	0.320 ± 0.12	0.010 ± 0.01	0.006 ± 0.01	NF ± NF
C18:2n-6	Microalgae	6.180 ± 0.22	24.660 ± 0.06	21.433 ± 0.63	0.641 ± 0.08
C18:3n-3	Microalgae	0.480 ± 0.06	0.900 ± 1.09	2.109 ± 1.75	0.164 ± 0.04
C20:0	Microalgae	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.00
C20:1n-9	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C20:2	Microalgae	NF ± 0.00	NF ± 0.00	NF ± 0.00	NF ± 0.00
C20:4n-6	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C20:3n-3	Microalgae	1.300 ± 0.07	NF ± NF	NF ± NF	NF ± NF
C22:0	Microalgae	NF ±	NF ±	NF ±	NF ±
C22:1n-9	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C20:5n-3	Microalgae	31.880 ± 2.03	NF ± NF	NF ± NF	NF ± NF
C22:5n-3	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C22:6n-3	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF
C24:0	Microalgae	NF ± NF	NF ± NF	NF ± NF	NF ± NF