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Suitability of Different Live Feed for First Feeding of Freshwater Fish Larvae

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Abstract: First feeding of many fish larvae depends on live feed. A comparative investigation on the effectiveness of different types of live feed is not available to our knowledge. Hence, we conducted a study to examine the effect of different types and combinations of live feed on the performance (survival rate, total length, body width, body mass, malformation rate) of pikeperch, *Sander lucioperca*, larvae. From day 0 (onset of exogenous feeding) to day 10, the saltwater rotifer *Brachionus plicatilis*, the freshwater rotifer *Brachionus* calyciflorus, the ciliate *Paramecium bursaria*, copepods (nauplii and copepodites) from a lake population, and *Artemia* nauplii were tested. Feeding with *B. plicatilis*, *B.* calyciflorus, and *P. bursaria* resulted in high survival rates of 80% and a homogenous and significant growth (increase in total length of 50% and in body width of 20%). As follow-up feed, copepod nauplii and *Artemia* nauplii were tested from day 11 to day 20. Copepod nauplii were superior to *Artemia* nauplii, as larvae fed with copepods showed higher survival rates (67–70% versus 38–47%) and a more homogeneous growth. A switch from seawater live feed to freshwater live feed or vice versa resulted in decreased survival rates. Therefore, a feeding regime consisting of *B.* calyciflorus or *P. bursaria* followed by copepods is considered optimal as first feed of pikeperch. The malformation rate was not affected by the tested feeding regimes. To investigate the wider applicability and transferability of these findings, complementary investigations were performed on burbot, *Lota lota*, and the freshwater whitefish *Coregonus atterensis*. The feeding regimes used for *S. lucioperca* larvae were also suitable for *Lota lota*. Moreover, *L. lota* could be fed with lake copepods from the onset of exogenous feeding. For *C. atterensis*, initial feeding with *B. plicatilis*, *B. calyciflorus*, or *P. bursaria* had no positive effects. Feeding with copepods from the onset of exogenous feeding was optimal considering survival rate and growth. Therefore, optimal first feeding regimes are very species specific and should be established for each new species.

Keywords: *Sander lucioperca*; *Lota lota*; *Coregonus atterensis*; rotifers; copepods; *Artemia*; larvae

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1. Introduction

Global production of fish in aquaculture has developed rapidly over the past decades [1]. Today, aquaculture accounts for almost half of the world's food fish and is expected to provide more fish for human consumption than capture fishery activity in the future [1]. Mass rearing of healthy fish larvae is a key step in the aquaculture production processes. Fish larvae of some species, mainly of the family of Salmonidae, can be fed with formulated dry feed right from the beginning of exogenous feeding [2]. The rearing of more sensitive freshwater and marine fish larvae depends heavily on live feed. As formulated dry feed has economic advantages, intense research efforts have been made to replace live feed by formulated dry feed. However, no reliable recipes are available at present [2]. This is mainly attributed to an unbalanced composition of nutrients and a low digestibility of feed particles due to the underdeveloped digestive tracts of fish larvae in early stages of development [2]. Additionally, the deterioration of water quality of the rearing tanks is another disadvantage

of the presently available formulated types of dry feed [2]. The saltwater rotifer *Brachionus plicatilis*, the freshwater rotifer *Brachionus calyciflorus*, and unicellular organisms such as *Paramecium* sp. are used as starter feed and successively replaced by brine shrimp, *Artemia*, in more advanced larval stages [3,4]. Nevertheless, the described live feeds do not meet all nutritional requirements of fish larvae, and enrichment procedures are often necessary to increase their quality [4,5]. Copepods are preferable compared to the aforementioned types of live feed due to their specific composition in unsaturated fatty acids, high astaxanthin levels, balanced levels of triglycerides and phospholipids, and high digestibility [6,7]. However, intensive culture methods for freshwater copepods are not available at present, and therefore they have to be collected continuously from wild populations [6].

Concerning economically relevant freshwater fish, the larviculture of pikeperch (*Sander lucioperca*), perch (*Perca fluviatilis*), burbot (*Lota lota*), and specific coregonid species (*Coregonus atterensis*) depends primarily on live feed. *B. calyciflorus* was used for first feeding of burbot, *Lota lota* [8,9], and pikeperch, *Sander lucioperca* [10]; *B. plicatilis* for *S. lucioperca* [11,12] and the coregonid species *Coregonus lavaretus* [13]; *Paramecium* sp. for perch, *Perca fluviatilis* [14]; and copepods for *L. lota* and *C. atterensis* [15,16]. In recent studies, *Sander lucioperca* has also been reared with a combination of *B. plicatilis* and *Artemia* nauplii [17] and with a feeding regime consisting of *Brachionus plicatilis* and *Apocyclops panamensis* [18].

However, no direct comparison has been made on the effectiveness of the different types of live feed until now. It is also unclear if there exists a species-specific preference to different types of live feed. This data is of great importance for on-growing fish farms to select the most efficient feed types and feeding regimes.

Therefore, the effect of different types and combinations of live feed (*B. plicatilis*, *B. calyciflorus*, *Paramecium bursaria*, copepod nauplii, *Artemia*) on performance (survival rate, weight gain, malformation rate) of *S. lucioperca* larvae during first feeding was tested. Based on these results, complementary investigations were performed on *L. lota* and *C. atterensis*. *S. lucioperca* is a freshwater and brackish-water piscivorous fish occurring in large, turbid rivers, lakes, and estuaries [19]. It is a valuable commercial and recreational fisheries species. *L. lota* is a demersal fish found in deep temperate lake bottoms and in slow-moving river bottoms [19] and a promising candidate as an emerging aquaculture species. *C. atterensis* is a pelagic species of alpine lakes living at depths of 10–30 m and feeding on zooplankton [19]. It has high economic importance in capture fisheries and fingerling production for restocking activities and therefore has a high commercial value for local aquaculture.

2. Material and Methods

2.1. Institutional Review Board Statement

The experimental procedures for fish were conducted in accordance with the guidelines of the Federal Agency of Water Management and with the Austrian regulations governing animal welfare and protection (Tierversuchsgesetz, BGBl. I Nr. 114/2012). All practices and procedures for the care and management of animals were based on current best practice under the supervision of skilled workers or scientists.

2.2. Species Involved in the Study

Eggs of *S. lucioperca* were collected after natural spawning in ponds. Eggs of *L. lota* were derived from a brood stock kept in the fish farm Kreuzstein, while eggs of *C. atterensis* were stripped from spawners caught in Lake Mondsee by commercial fishermen. *L. lota* broodstock fish had an age of 3–4 years, a mass of 1040 $\pm$ 25 g, and a total length of 52 $\pm$ 12 cm. As eggs of *S. lucioperca* were collected from ponds, no exact information on the body data of broodstock fish is available. The age ranged from 3 to 5 years, the mass from 2500 to 4000 g, and the total length from 40 to 60 cm. Likewise, no exact body data is available for the spawners of *C. atterensis*, as individual fish were not measured during capture. The caught fish had an approximate body mass of 200–350 g and a total length of 25–40 cm. Age data are not available for *C. atterensis*. Eggs and larvae of *S. lucioperca*

were kept at 17 °C, and those of *C. atterensis* at 10 °C. *L. lota* eggs were incubated at 4 °C, and the hatched larvae were transferred to 10 °C [15]. The used water source was 10 °C well water, which was cooled or heated to the required temperature. The water had a pH of 7.86 ± 0.01, a conductivity of 341 ± 8.0 µS/cm at 25 °C, and PO_4^{3-} and NH_4^+ concentrations ≤ 0.005 mg/L. O_2 concentration was 11.59 ± 0.21 mg/L for the 10 °C water and 7.91 ± 0.34 mg/L for the 17 °C water.

2.3. Live Food Used for Feeding of Fish Larvae

2.3.1. Collection of Lake Zooplankton

Zooplankton in a size fraction < 200 µm was collected from Lake Mondsee using a sieve netting procedure [15,16]. Sieve nets had a lower mesh size of 100 µm and an upper one of 200 µm. The nets were dredged floating behind a boat at a depth of 10–15 m and a cruising speed of 0.5 miles/h. The dredging depth was adjusted depending on weather conditions and season. With this procedure, 0.1–0.15 kg zooplankton (organism mass separated from water) could be collected per hour. The collected zooplankton was still alive after the capture procedure. It was washed out of the nets into buckets and diluted to a density of circa 100,000 organisms per litre. As lake zooplankton is inhomogeneous in aspects of species composition, subsamples fixed in 4% buffered formaldehyde (*v*/*v*) were analyzed. The number of different organisms was counted and reported as percentile value of the total number of counted organisms.

2.3.2. Microalgae Culture

The seawater algae *Nannochloropsis* sp. was obtained from a commercial plankton breeder, while the freshwater algae *Chlorella* sp. was obtained from BEST-Bioenergy and Sustainable Technologies GmbH (Tulln, Austria). The microalgae *Nannochloropsis* sp. was used as feed source for *B. plicatilis*, and *Chlorella* sp. for *B. calyciflorus. Chlorella* sp. was also necessary as symbiont for *P. bursaria. Nannochloropsis* sp. was cultured in Guillard's F2 medium, and *Chlorella* sp. in BG11 liquid medium according to standard procedures [20,21].

2.3.3. Paramecium and Rotifer Culture

Starter cultures of *P. bursaria*, *B. plicatilis*, and *B. calyciflorus* were obtained from commercial suppliers. Primary subcultures were kept in 250 mL Erlenmeyer flasks and transferred to larger tanks (final volume: 20 L) as organism density increased.

P. bursaria was fed with bacteria. Bacteria cultures were produced using a rice/alfalfa medium, and *P. bursaria* and *Chlorella* sp. were added to the culture substrate [22]. The pH of the suspension was maintained between 7 and 7.5 to prevent the formation of ammonia.

B. plicatilis was cultured in saltwater with a salinity of 20 ppm [23], and *B. calyciflorus* in well water [24]. For both species, culture temperature was 25 ± 1 °C, illumination 150 lux, and water was constantly aerated using aquarium air-stones. The rotifers were fed with microalgae in an amount to keep the culture solution slightly green colored. For feeding of fish larvae, *P. bursaria* and rotifers were collected using a 50 µm mesh netting procedure. During this procedure, 25–50% of the tank water was also renewed. When the rotifers were not used for feeding, circa 50% of the tank water was renewed in 3–4 day intervals.

2.3.4. *Artemia* Culture

Micro *Artemia* cysts (obtained from Ocean Nutrition, Belgium) were hatched in 30 ppt saltwater at 25 ± 1 °C, according to the instructions of the supplier, and fed within 24 h after hatching.

2.3.5. Morphometric Investigations

All types of live feed were photographed in a stereomicroscope at 10- to 20-fold magnification, and dimensions were measured in the Image J program (Version 1.53k) [25]. The precision of the measurements was ± 5 µm.

2.4. Larvae First Feeding Experiments

Previous studies demonstrated that *S. lucioperca* and *L. lota* larvae at the stage of first feeding are sensitive to manipulations necessary in fish culture [14,15]. Lowering and increasing the tank water level as required for cleaning and water renewal, water flow, and aeration can disturb buoyancy or injure the larvae due to turbulences and mechanical forces [14]. Therefore, in *S. lucioperca* and *L. lota* first feeding experiments were conducted in rectangular tanks with a volume of 200 L under static conditions without aeration and water renewal. Aeration of tanks stocked with *L. lota* and *S. lucioperca* was started when oxygen saturation dropped to 75% (15 d from the first day of feeding). *C. atterensis* larvae were kept in similarly sized tanks. However, tanks were aerated from the onset of the experiments due to the high oxygen demand of the larvae (oxygen concentration: 10.8–11.6 mg/L). All tanks were illuminated with full spectrum lights with circa 150 lux for 18 h. Yolk sac larvae were stocked at a density of five animals/l in the experimental tanks, and all experiments were performed in triplicates. Hatching of fish larvae is not a strictly synchronous process but takes several hours (*S. lucioperca*) to days (*L. lota*, *C. atterensis*). Therefore, the day of hatch (0 dph) was defined as the time point when 50% of the larvae had hatched. Feeding was initiated when the yolk sac was completely absorbed in 50% of the larvae. Yolk sac absorption was determined in fish larvae not involved in the experiment but kept under similar conditions. They were removed from the rearing units, anaesthetized with 0.2% MS222, and investigated in a stereomicroscope. Yolk sac absorption was defined to be completed when no yolk sac was visible, but yolk compounds were still detectable in the digestive tract. In *S. lucioperca* this was 4 dph (days post hatch), and in *L. lota* and *C. atterensis* 10 dph. For better comparability of the data between the species, the date of first feeding was referred to as day 1 of the experiment. The tested feeding regimes are shown in Section 3.3 together with the results. *P. bursaria*, *B. plicatilis*, and *B. calyciflorus* were added to the tanks two times per day to maintain final concentrations of 500–1000 organisms/l water. The <200 µm fraction of the lake zooplankton and *Artemia* nauplii were fed two times daily in concentrations of 25–50 organisms/l tank water. As a switch from initial feeding with freshwater live feed (*B. calyciflorus*, *P. bursaria)* to saltwater follow-up feed (*Artemia* nauplii) resulted in decreased survival rates in preliminary experiments and in the main experiment, the reverse experiment (switch from initial feeding with saltwater live feed to freshwater follow up feed) was not tested. Body parameters were determined at the onset and at the end of the experiment, and survival rate and malformation rate at the end of the experiment.

Fish were kept on live feed for 20 d from the first day of feeding as these time periods were considered suitable to detect differences between different feeding regimes. Fish suffering from malnutrition show either heightened mortality or significantly decreased growth rates after this time. Loss of fish due to cannibalism might falsify feed-related survival rates, but during this period no cannibalism was observed in *L. lota* and *S. lucioperca.* However, the time period of the experiments did not coincide with the transition from one developmental stage to another. Theoretically, *S. lucioperca* might be switched to dry feed after 15 d [11,12] and *C. atterensis* after 10 d [16].

In static systems without aeration and water change, water quality might decrease to conditions lethal to fish larvae. Therefore, oxygen saturation, conductivity, and NH_4 concentration were regularly measured. A portable multi-parameter meter (Multi 3630 IDS, WTW, Germany) was used to measure oxygen concentration and conductivity, and a colorimetric rapid test system (JLB GmbH) was used to determine NH_4 concentration. In aerated tanks stocked with *C. atterensis*, oxygen saturation was $\geq$ 100%; in non-aerated tanks stocked with *S. lucioperca* and *L. lota*, oxygen saturation decreased during the experiment. When it reached a threshold of 75% saturation (i.e., 7.3 mg/L for *S. lucioperca* and 8.3 mg/L for *L. lota*), aeration was started. In tank systems continuously fed with marine rotifers or *Artemia*, conductivity increased to 1.55 $\pm$ 0.54 mS/cm (= 0.78 $\pm$ 0.26 ppt) (mean $\pm$ S.D for all measurements) after 10 d feeding and to 3.32 $\pm$ 1.23 mS/cm (= 1.74 $\pm$ 0.61 ppt) after 20 days. Therefore, salinity did not exceed the tolerance level of larvae [11,26,27]. NH_4

concentration was < 1 mg/L during the course of the experiment for all tank systems and in the optimal range for the investigated fish species.

2.5. Fish Investigations

For determination of the survival rate, all individuals were collected from a tank with a fine-meshed hand net and counted. The survival rate was calculated as number of larvae surviving at the end of the experiment in relation to the number of larvae stocked at the onset of the experiment. For determination of body length, body depth, and body mass, larvae were killed by prolonged exposure to 0.2 % (*w*/*v*) MS222. Thirty larvae in total were randomly sampled at the onset of the experiment (i.e., day 1), and thirty larvae per tank (90 in total) at the end of the experiment. They were photographed in a stereomicroscope Visiscope SZT360-6 coupled with a video camera (VisiCam 3 Plus) or with a cell phone camera (Galaxy XCover 5) depending on their size. Larval total length and body depth were measured from digitized pictures in the Image J program [25]. Measurements were calibrated using an object micrometer. The precision of the measurements was ± 0.2 µm. Larvae body mass was determined using an analytical balance after the water had been removed with tissue paper at a precision of ± 0.5 mg. At day 1 in *L. lota*, body mass could not be determined as larvae fragility due to small size allowed no accurate analysis.

Skeletal malformations were determined at the end of the experiments on the 90 sampled larvae. Larvae were fixed in 4% buffered formalin (*v*/*v*) and stained with the Alcian blue and Alizarin red method for cartilage and bone [28]. Larvae were photographed with a cell phone camera, and pictures were analyzed on the following malformations potentially occurring in fish larvae [29]: (1) increased or decreased vertebra number, (2) fused or deformed vertebral bodies, (3) kyphosis, (4) lordosis, (5) saddleback, (6) lack of fins or supernumerary fins, (7) anormal opercula or opercula reduction, (8) lower jaw reduction or elongation, (9) pug-headedness. Malformations were expressed as percentage data in relation to the total number of investigated larvae.

2.6. Statistics

Survival rates are reported as mean ± standard deviation for the tank repetitions, and metric data as mean ± standard deviation for the total number of analyzed fish. Percentage data were angular transformed ($\arcsin\sqrt{p}$). Metric data revealed normal distribution as determined by Shapiro–Wilk test. Data were analyzed with one-way ANOVA. The feeding regime was the independent variable, and the fish length, body width, body mass, survival rate, and malformation rate were the dependent variables. Tukey post hoc test was used for fish length, body width, and body mass, and Games–Howell test for survival rates. Malformation rates are percentage data for the total number of fish analyzed per feeding trial and no statistical tests were performed. Statistical analysis was performed with the open-source program JASP (Version 0.17.1), and diagrams were made in Microsoft Excel.

3. Results

3.1. Morphometric Characterization Larvae

Among the three species, *L. lota* larvae were the smallest, followed by *S. lucioperca* and *C. atterensis* (Figure 1, Table 1). Morphometric parameters including body width, mouth width, and mouth length are shown in Table 1.

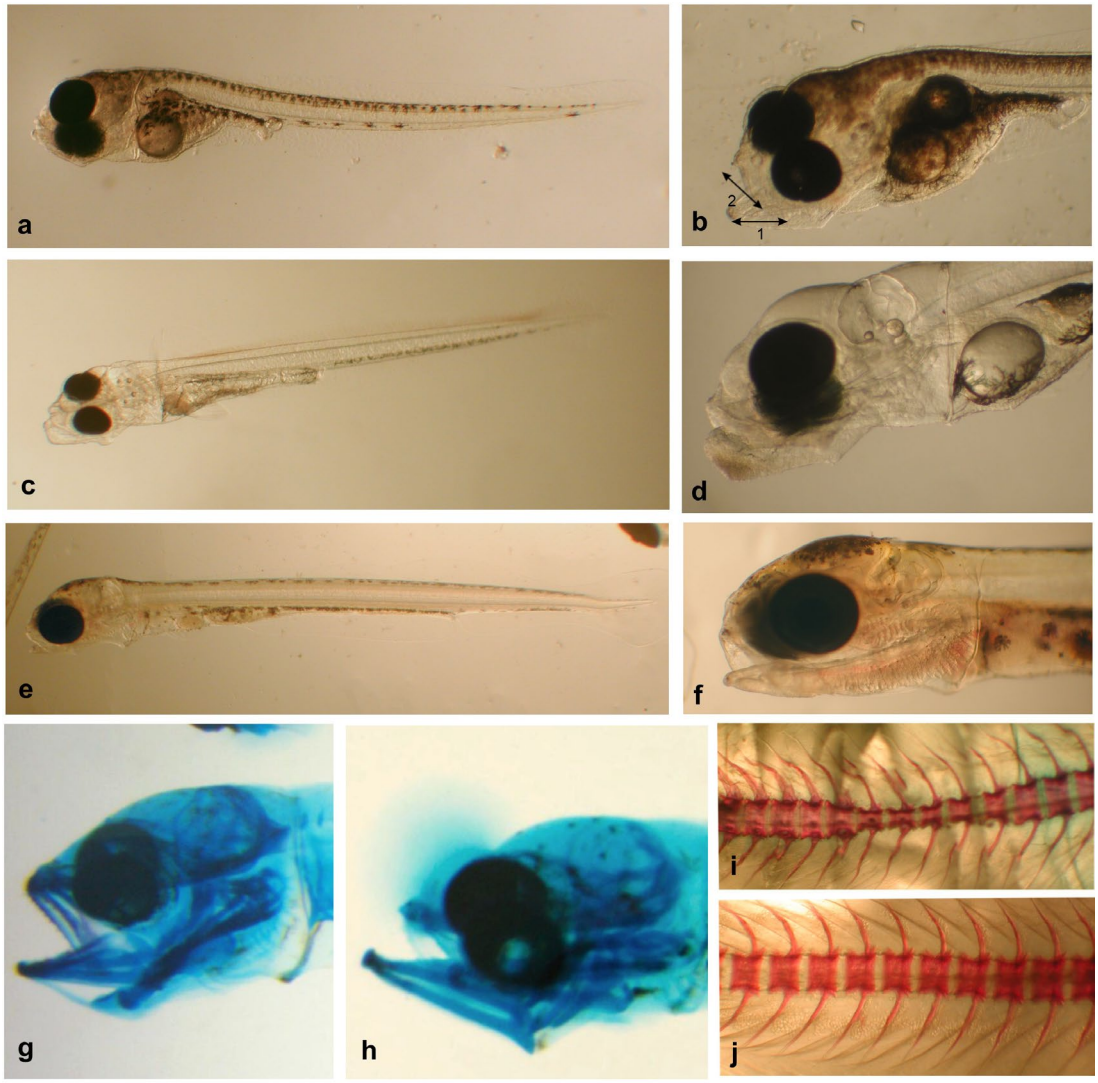

Figure 1. Morphology of the investigated fish larvae: (**a**) *L. lota* (magnification: ×25); (**b**) detail of its head (×35); (**c**) *S. lucioperca* (×15); (**d**) detail of its head (×40); (**e**) *C. atterensis* (×5); (**f**) detail of its head (×10); (**g**) *S. lucioperca* with normal jaw (×45) and (**h**) with prolonged lower jaw (×45); (**i**) deformed vertebra bodies (×60) and (**j**) normal vertebra bodies in *C. atterensis* (×60). 1—mouth length, 2—mouth width.

Table 1. Morphometric parameters of larvae of *L. lota*, *S. lucioperca*, and *C. atterensis* at the onset of first feeding. Sample number = 30, data with different superscripts across the different species are significantly different ($p < 0.05$).

Measurements [mm]	***L. lota***	***S. lucioperca***	***C. atterensis***
Total length	3.6 ± 0.1^{a}	5.0 ± 0.2^{b}	13.1 ± 0.7^{c}
Body width	0.3 ± 0.0^{a}	0.6 ± 0.1^{b}	0.9 ± 0.1^{c}
Mouth width	0.23 ± 0.05^{a}	0.42 ± 0.03^{b}	0.90 ± 0.10^{b}
Mouth length	0.15 ± 0.04^{a}	0.32 ± 0.06^{b}	0.59 ± 0.16^{b}

3.2. Morphometric Characterization of the Tested Live Feed

The fraction of the sieve-netted zooplankton used in this experiment was 100–200 µm in size. This fraction contained 51 ± 12% copepodites in the CI stage and 46 ± 8% nauplii in the NI–NVI stage (for nomenclature, see [30,31]) and 3 ± 3 % freshwater rotifers. Based

on the nauplii and copepodites stages, the species cannot be determined exactly with microscopic techniques. The >200 µm fraction of the zooplankton was collected as well. It was not used in the experiments but for feeding of more developed fish larvae and juvenile stages in the routine production of the fish farm. This fraction contained the adult organisms, and the species composition was 32 ± 13% *Cyclops* sp., 45 ± 23% *Diaptomus* sp., and 33 ± 22% *Daphnia* sp. Morphometric features of the 100–200 µm fraction of the lake zooplankton and of the cultured live feed are shown in Table 2.

Table 2. Size of the investigated live feed specimens. Lake zooplankton is the 100–200 µm fraction collected by a sieve netting procedure. Sample number = 20, data with different superscripts are significantly different ($p < 0.05$).

Live Feed	Length of Organism [mm]	Width of Organism [mm]
Nauplii and copepodites (lake zooplankton)	0.21 ± 0.03 ^{a}	0.10 ± 0.01 ^{a}
Artemia nauplii	0.46 ± 0.04 ^{c}	0.18 ± 0.05 ^{a}
P. bursaria	0.15 ± 0.04 ^{d}	0.08 ± 0.02 ^{a}
B. plicatilis	0.20 ± 0.03 ^{a}	0.11 ± 0.01 ^{a}
B. calyciflorus	0.18 ± 0.03 ^{f}	0.10 ± 0.01 ^{a}

3.3. Feeding Trials

3.3.1. *S. lucioperca*

Survival rate, total length, and body width of *S. lucioperca* larvae was similar when fed with feeding regime (A), (B), or (C) for 10 d (Table 3). Survival rate was significantly reduced, and total length and body width were significantly increased with feeding regimes (D) and (E) (Table 3).

Table 3. Survival rate, total length, and body width of *S. lucioperca* larvae after 10 d feeding with *B. plicatilis*, *B. calyciflorus*, *P. bursaria*, *Artemia*, or copepod nauplii/copepodites (lake zooplankton). Control: larvae starting exogenous feeding. Lake zooplankton is the 100–200 µm fraction collected by a sieve netting procedure and consists of nauplii of stages I–VI and copepodites of stage CI. Data on survival rate = 3, body data = 30 for control and 90 for feeding trials (30 samples per tank). Data with different superscripts are significantly different ($p < 0.05$).

Feeding Regime	Survival Rate [%]	Total Length [mm]	Body Width [mm]
Larvae starting exogenous feeding	-	5.0 ± 0.2 ^{a}	0.6 ± 0.1 ^{a}
(A) *B. plicatilis* for 10 d	79.0 ± 7.0 ^{a}	7.7 ± 0.4 ^{b}	0.7 ± 0.1 ^{b}
(B) *B. calyciflorus* for 10 d	84.0 ± 5.0 ^{a}	7.7 ± 0.4 ^{b}	0.7 ± 0.1 ^{b}
(C) *P. bursaria* for 10 d	80.0 ± 9.0 ^{a}	7.3 ± 0.9 ^{b}	0.8 ± 0.1 ^{b}
(D) *Artemia* nauplii for 10 d	17.0 ± 16.0 ^{b}	10.7 ± 2.7 ^{c}	1.0 ± 0.3 ^{c}
(E) Lake zooplankton for 10 d	33.0 ± 14.0 ^{c}	10.0 ± 3.0 ^{c}	1.1 ± 0.2 ^{c}

Feeding regimes (H) and (J) yielded significantly higher survival rates, while growth parameters (total length, body width, body mass) were medium (Table 4). Feeding regimes (F) and (K) resulted in medium survival rates and high growth parameters (Table 4). With feeding regimes (G) and (I), medium survival rates and medium growth were obtained (Table 4). The percentage of misshaped larvae did not differ between the tested feeding regimes. Overall, 5–10% of the investigated larvae revealed elongated lower jaws. Kyphosis and vertebral deformities were detected in < 5% of the investigated larvae (Figure 2a).

Table 4. Survival rate, total length, body width, and body mass of *S. lucioperca* larvae fed for 20 d with different combinations of live feed. Lake zooplankton is the 100–200 µm fraction collected by a sieve netting procedure and consists of nauplii of stages I–VI and copepodites of stage CI. Data on survival rate = 3, body data = 30 for control and 90 for feeding trials (30 samples per tank). Data with different superscripts are significantly different ($p < 0.05$).

	Feeding Regime		Survival Rate	Body Mass	Total Length	Body Width
	Day 1–10	Day 11–20	[%]	[mg]	[mm]	[mm]
	Larvae starting exogenous feeding		-	2.0 ± 0.3^{a}	5.0 ± 0.2^{a}	0.6 ± 0.1^{a}
(F)	*B. plicatilis*	*Artemia* nauplii	44.0 ± 3.0^{a}	23.1 ± 13.1^{b}	12.0 ± 2.1^{b}	1.6 ± 0.4^{b}
(G)	*B. calyciflorus*	*Artemia* nauplii	38.0 ± 5.0^{a}	7.6 ± 1.3^{c}	9.7 ± 1.9^{c}	0.9 ± 0.2^{c}
(H)	*B. calyciflorus*	Lake zooplankton	67.5 ± 11.0^{b}	7.4 ± 1.5^{c}	8.6 ± 0.7^{c}	1.1 ± 0.2^{c}
(I)	*P. bursaria*	*Artemia* nauplii	47.0 ± 9.0^{a}	9.0 ± 2.8^{c}	8.5 ± 1.8^{c}	1.0 ± 0.2^{c}
(J)	*P. bursaria*	Lake zooplankton	70.3 ± 4.5^{b}	9.0 ± 2.8^{c}	7.9 ± 1.4^{c}	0.9 ± 0.2^{c}
(K)	Lake zooplankton	Lake zooplankton	36.0 ± 6.0^{a}	18.0 ± 5.4^{b}	11.6 ± 1.0^{b}	1.5 ± 0.4^{b}

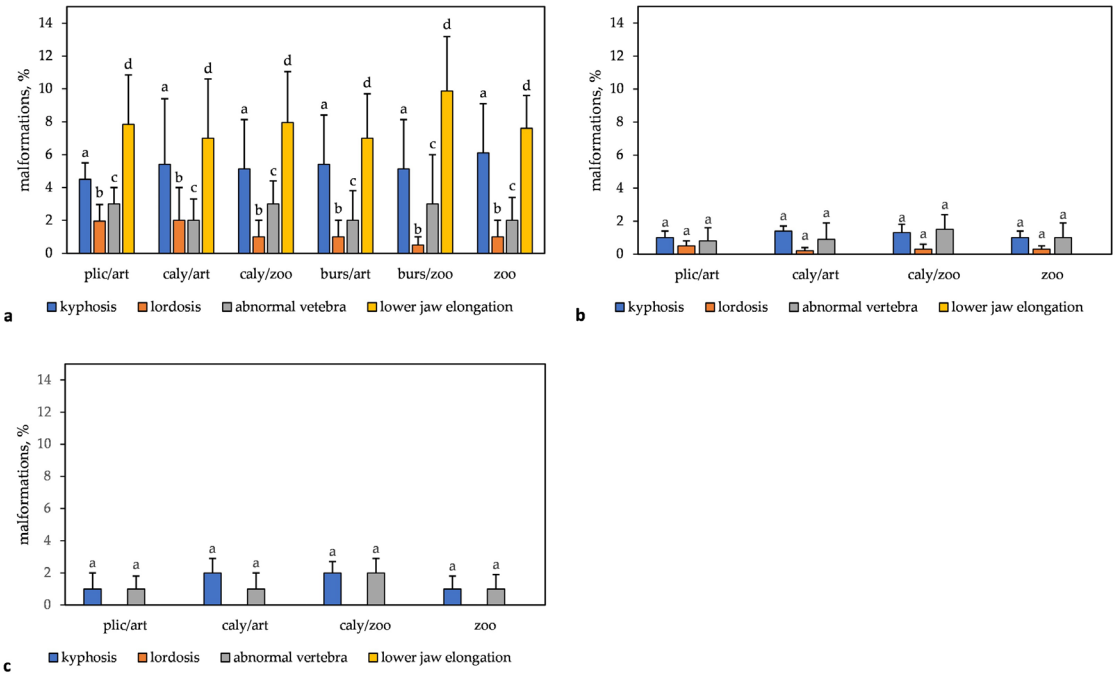

Figure 2. Frequency of malformations during different feeding regimes in *Sander lucioperca* (**a**), *Lota lota* (**b**), and *Coregonus atterensis* (**c**) larvae. plic/art: 10 d *B. plicatilis* + 10 d *Artemia* nauplii; caly/art: 10 d *B. calyciflorus* + 10 d *Artemia* nauplii; caly/cop: 10 d *B. calyciflorus* + 10 d lake zooplankton; burs/art: 10 d *P. bursaria* + 10 d *Artemia* nauplii, burs/cop: 10 d *P. bursaria* + 10 d lake zooplankton; cop: 20 d lake zooplankton. Lake zooplankton is the 100–200 µm fraction collected by a sieve netting procedure and consists of nauplii of stages I–VI and copepodites of stage CI. Data on malformation rate = 3. Data with different superscripts are significantly different ($p < 0.05$).

3.3.2. *L. lota*

Survival rate, body width, body mass, and total length were highest when *L. lota* larvae were fed with feeding regime (G), (H), or (L) (Table 5). Feeding regime (F) resulted in reduced survival rate and larval growth (body width, body mass, total length) (Table 5). Similar results were obtained with feeding regime (G) (Table 5). Malformation rate was low and did not differ between the tested feeding regimes. Kyphosis and vertebral deformities were detected in <5% of the investigated larvae (Figure 2b).

Table 5. Survival rate, total length, body width, and body mass of *L. lota* larvae fed for 20 d with different feeding regimes. Lake zooplankton is the 100–200 µm fraction collected by a sieve netting procedure and consists of nauplii of stages I–VI and copepodites of stage CI. Data on survival rate = 3, body data = 30 for control and 90 for feeding trials (30 samples per tank). On day one, body mass could not be determined due to small size of the larvae. Data with different superscripts are significantly different ($p < 0.05$).

	Feeding Regime		Survival Rate	Body Mass	Total Length	Body Width
	Day 1–10	Day 11–20	[%]	[mg]	[mm]	[mm]
Larvae starting exogenous feeding			-	-	3.6 ± 0.1^{a}	0.3 ± 0.0^{a}
(F)	*B. plicatilis*	*Artemia* nauplii	44.0 ± 3.0^{a}	3.0 ± 0.8^{a}	4.9 ± 0.3^{b}	0.9 ± 0.1^{b}
(G)	B. *calyciflorus*	*Artemia* nauplii	38.0 ± 5.0^{a}	2.2 ± 0.8^{b}	4.8 ± 0.5^{b}	0.8 ± 0.1^{b}
(J)	*P. bursaria*	Lake zooplankton	74.0 ± 6.0^{b}	3.5 ± 0.9^{c}	5.5 ± 0.0^{b}	1.3 ± 0.5^{c}
(H)	*B. calyciflorus*	Lake zooplankton	77.0 ± 11.0^{b}	3.7 ± 0.6^{c}	5.6 ± 0.4^{b}	1.6 ± 0.4^{c}
(L)	Lake zooplankton	Lake zooplankton	83.0 ± 9.0^{b}	4.2 ± 1.1^{c}	5.8 ± 1.2^{b}	1.7 ± 0.5^{c}

3.3.3. *Coregonus atterensis*

Larvae of *Coregonus atterensis* fed with feeding regime (L) (Table 6) had the highest survival rate, followed by larvae fed with regime (H) (Table 6). Regime (F) resulted in a significantly lower survival rate. The larvae growth (body width, body mass) was significantly higher when feeding copepods or *Artemia* for 20 d (L, M) (Table 6). Total length showed no significant differences in dependence of the feeding regimes (Table 6). The percentage of malformations was <2% and did not differ between the feeding regimes.

Table 6. Survival rate, total length, body width, and body mass of *C. atterensis* larvae fed for 20 d with different feeding regimes. Lake zooplankton is the 100–200 µm fraction collected by a sieve netting procedure and consists of nauplii of stage I–VI and copepodites of stage CI. Data on survival rate = 3, body data = 30 for control and 90 for feeding trials (30 samples per tank). Data with different superscripts are significantly different ($p < 0.05$).

	Feeding Regime		Survival Rate	Body Mass	Total Length	Body Width
	Day 1–10	Day 11–20	[%]	[mg]	[mm]	[mm]
Larvae starting exogenous feeding			-	6.6 ± 1.2^{a}	13.1 ± 0.7^{a}	0.9 ± 0.1^{a}
(F)	*B. plicatilis*	*Artemia* nauplii	59.2 ± 11.9^{a}	12.5 ± 4.8^{b}	15.1 ± 0.6^{b}	2.0 ± 0.5^{b}
(H)	*B. calyciflorus*	Lake zooplankton	73.4 ± 8.3^{b}	12.8 ± 2.6^{b}	14.9 ± 0.9^{b}	2.2 ± 0.2^{b}
(L)	Lake zooplankton	Lake zooplankton	86.4 ± 7.9^{c}	24.1 ± 6.9^{c}	15.2 ± 0.7^{b}	2.8 ± 0.4^{c}
(M)	*Artemia* nauplii	*Artemia* nauplii	51.0 ± 7.3^{a}	27.8 ± 8.1^{c}	15.8 ± 1.9^{b}	2.8 ± 0.5^{c}

4. Discussion

The study demonstrates that *B. calyciflorus*, *B. plicatilis*, and *P. bursaria* were suitable for first feeding of *S. lucioperca* larvae as survival rates were high (80%) and significant growth (increase in total length of 50% and in body width of 20%) was obtained. The nutritional content of the rotifer species has been discussed in several papers [3,5,32]. The concentration of highly unsaturated fatty acids, their respective ratios, and the vitamin levels are limiting parameters in their nutritional value [5], but they can be modified and improved by different enrichment procedures. Although no nutritional information is available for *P. bursaria*, it is considered a valuable live feed as one *P. bursaria* cell contains up to 700 symbiotic *Chlorella* sp. cells [33]. These symbiotic cells supply the host with photosynthetic products, mainly maltose, while the host provides the algae with nitrogenous compounds and carbon dioxide [33]. *Paramecium caudatum* is a food organism that is less elaborate to culture as only bacteria are necessary for its culture. It was successfully used for first feeding of larvae of *P. fluviatilis* [14] and of *S. lucioperca* hybrids [34]. However, the culture water of *P. caudatum* contains low oxygen and high ammonium concentrations and is inadequate for fish larvae [14]. Under practical aspects, the utilization of freshwater live feed has clear

advantages for freshwater fish larvae: the organisms survive in the fish tanks for unlimited time periods, and no salts are introduced into the tanks, resulting in stable tank conditions and water quality.

Artemia nauplii and copepod nauplii/copepodites were not suitable for first feeding of pikeperch larvae as survival rates were low and growth was heterogenous. Morphometric measurements showed that *Artemia* nauplii were too large for pikeperch larvae (0.46 × 0.18 mm in comparison to larvae mouth size of 0.42 × 0.32 mm), while the used lake zooplankton was smaller (copepodites and nauplii 0.21 × 0.10 mm) and might be ingested more easily. However, the locomotor activity of the prey also plays a role for the capture and ingestion success. Copepod nauplii and copepodites have a specific motion consisting of sequences of sinking, swimming, and jumping events, and they show escape reactions in response to predators [35,36]. This might reduce the capture success of larvae in the first feeding stage. In nature, ciliates can contribute 40–60% of the total consumed carbon biomass of *S. lucioperca* larvae; this is similar for other Percidae larvae (*P. fluviatilis*, *Gymnocephalus cernua*) [37,38]. In turbot, *Psetta maxima*, capture success of copepod nauplii was <50% during the flexion stage and increased to 73% in the post-flexion stage [39]. In contrast, *Artemia* nauplii have no effective escape response [3]. Besides capture success, the biochemical composition of the prey may play an important role for the performance of fish larvae, as it differs significantly between ciliates, rotifers, and copepods [3,5]. Moreover, copepods contain a chitinous exoskeleton, which might be difficult to digest by larvae [3,5].

The present study demonstrated that the aforementioned feed items used after the initial 10 days had a significant influence on larval survival rate and growth parameters of *S. lucioperca*. *Artemia* feeding resulted in significantly lower survival rates and in more heterogeneous growth than copepod feeding. Low survival rates and heterogeneous growth are disadvantages in larviculture. They reduce the number of produced fish, favor the development of cannibalism, and require sorting/manipulation procedures of larvae/juveniles in early life stages still sensitive to manipulations. Low performance of *S. lucioperca* larvae fed with *Artemia* nauplii may be due to its larger size, as discussed. Additionally, the nutritional profile of *Artemia* might be suboptimal. Performance of *S. lucioperca* larvae (survival rate, growth, stress resistance) can be improved when *Artemia* is enriched with highly unsaturated fatty acids and vitamin C [40]. According to [41], the calcium-to-phosphor ratio and the concentrations of highly unsaturated fatty acids and vitamins C and E are key nutritional factors influencing development of *S. lucioperca*. Using copepods as follow-up feed for *S. lucioperca* larvae resulted in high survival rates and homogenous growth as indicated by low standard deviations in total length, body width, and body mass. Today there is a general agreement that copepods are an optimal food for raising marine fish larvae [42–44] and freshwater fish larvae [14,15,45,46]. They have a specific and balanced lipid composition, optimal astaxanthin and free amino acid levels, and high digestibility [6,7]. From this data, it becomes obvious that the development of freshwater copepod culture systems is an important goal for the improvement of freshwater fish larvae culture. Independent from the feeding regime, a percentage of 10–20% of pikeperch larvae revealed malformations in the present study, predominately lower jaw elongation. This could be related to the pikeperch strain or to rearing conditions. Further investigations are necessary on this topic.

A switch from initial feeding with freshwater live feed (*B. calyciflorus*, *P. bursaria)* to saltwater follow-up feed (*Artemia* nauplii) resulted in decreased survival rates and is therefore not recommended for hatchery practice. The exact reasons are unclear. It might possibly depend on taste changes.

Results of comparative feeding experiments on *L. lota* larvae were mainly similar to *S. lucioperca*. Feeding regimes consisting of combinations of *B. calcyflorus* or *P. bursaria* and copepods were preferable compared to feeding regimes consisting of *B. plicatilis* and *Artemia* nauplii and of *B. calyciflorus* and *Artemia* nauplii. One result was contradictory to *S. lucioperca*: although the mouth size of *L. lota* larvae is smaller than that of *S. lucioperca*, *L. lota* could be reared with lake copepods from the onset of first feeding. This data is

in accordance with a previously conducted study [15]. It might depend on differences in capture and swimming behavior between the two species. In nature, newly hatched *L. lota* larvae live in the pelagic phase and feed on copepods and cladocerans during the daytime [47,48]. Feeding *L. lota* larvae from the onset of first feeding with *Artemia* nauplii resulted in reduced survival rates of 30–45% [49,50]. Initial feeding of *L. lota* larvae with *B. calyciflorus* resulted in survival rates up to 90% [8], which also conforms with the present study.

Larvae of *C. atterensis* are bigger and more robust than those of *S. lucioperca* and *L. lota* and have a better developed digestive system [51]. Consequently, they also tolerate specifically formulated dry feed [16,52]. The present study demonstrated that *C. atterensis* larvae could be reared with lake copepods or *Artemia* nauplii from the onset of first feeding. As in the other experiments, feeding of lake copepods was preferable for *Artemia* nauplii as it resulted in higher survival rates and more homogenous growth. In contrast, feeding regimes using a combination of *B. plicatilis* and *Artemia* nauplii or of *B. calyciflorus* and lake copepods had no positive effects on larval performance.

To overcome the limitations in live feed availability and reduce production costs for live feed, larvae of many freshwater species (e.g., Cyprinidae, Percidae, Siluridae) are cultured under extensive or semi-extensive conditions in nursery ponds [53,54]. For rearing of marine fish larvae, a combination of *B. plicatilis* and *Artemia* is presently used as the routine method (e.g., seabass, *Dicentrarchus labrax* [55]; gilthead seabream, *Sparus aurata* [56]; turbot, *Scophthalmus maximus* [57]; Atlantic cod, *Gadus morhua* [58]). Copepods are not routinely used in marine larviculture but are being investigated presently on an experimental scale (e.g., Atlantic cod, *G. morhua* [59]; seabream, *S. aurata* [60]; spotted rose snapper, *Lutjanus guttatus* [61]).

Human workload and potential costs for production are relatively similar for *B. calyciflorus*, *B. plicatilis*, and *P. bursaria*: microalgae must be cultured as food organisms for rotifers and as symbionts for *P. bursaria*. Further, it is necessary to maintain stable water quality in the rotifer and protozoan populations to avoid their collapse. *Artemia* production is less elaborate as it requires no food organisms. Culture techniques have been described in detail [3,4]. Collection of copepods from wild zooplankton populations is a special technique with several requirements. The fish farm has to be located near a zooplankton source to avoid long transportation of food organisms. Adequate equipment and permits are necessary as well. In the required densities, copepods can be stored only for limited time periods of approximately 24 h. Therefore, regular collection is necessary. In our setup, it is possible to collect approximately 10 kg of zooplankton (wet weight without water) in time periods over 1–2 h depending on the productivity of the lake and the weather conditions. Under these conditions, the zooplankton collection is less elaborate than the production of comparable quantities of cultured live feed.

Another important aspect that needs to be considered with live feed is their role as potential carriers for pathogens. Both cultured rotifers and *Artemia* have been shown to be potential vectors [62,63]. Nevertheless, as these organisms are grown under controlled conditions, adequate hygienic concepts can minimize the risk of pathogen transfer to fish larvae. In addition, copepods collected from wild populations may be sources of pathogens themselves. It is well known that distinct copepod species are fish parasites or serve as intermediate hosts for parasites (e.g., for cestodes) [46,64]. On a practical scale, copepods collected from a lake population have been used at the study site of the fish farm Kreuzstein for intensive culture of several million larvae of different freshwater fish species for seven decades. According to unpublished hatchery protocols, copepod feeding was never associated with the outbreak of massive fish diseases. However, there is a risk that distinct percentages of fish become infested with specific parasites during particular times of the year [65].

5. Conclusions

The saltwater rotifer *B. plicatilis*, the freshwater rotifer *B. calyciflorus*, and the ciliate *P. bursaria* were suitable for first feeding of *S. lucioperca* larvae for 10 d. The same three types of live feed as well as lake copepods were applicable for first feeding of *L. lota* larvae. In *C. atterensis*, first feeding with *B. plicatilis*, *B. calyciflorus*, or *P. bursaria* had no positive effects on larval performance, while copepods were the optimal feeding regime. As follow-up feed for *S. lucioperca* and *L. lota*, copepods were superior to *Artemia*. Larvae fed with *Artemia* had lower survival rates and more inhomogeneous growth in general. This study highlights the importance of testing different live feed for different freshwater fish larvae, as different species have very diverging needs and capabilities regarding their first feeding.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The author declares that all data supporting the findings of this study are included within the article. The datasets of the current study are available on reasonable request.

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References

1. Tacon, A.G.J. Trends in global aquaculture and aquafeed production: 2000–2017. *Rev. Fish. Sci. Aquacul.* **2020**, *28*, 43–56. [CrossRef]
2. Hamre, K.; Yufera, M.; Rønnestad, I.; Boglione, C.; Conceição, L.; Izquierdo, M. Fish larval nutrition and feed formulation: Knowledge gaps and bottlenecks for advances in larval rearing. *Rev. Aquacult.* **2013**, *5*, S26–S58. [CrossRef]
3. Dhont, J.; Dierckens, K.; Støttrup, J.G.; Stappen, G.; Wille, M.; Sorgeloos, P. Rotifers, *Artemia* and copepods as live feeds for fish larvae in aquaculture. In *Advances in Aquaculture Hatchery Technology*; Allan, G., Burnell, G., Eds.; Elsevier: Amsterdam, The Netherlands, 2013; pp. 157–202.
4. Pan, Y.J.; Dahms, H.U.; Hwang, J.S.; Souissi, S. Recent trends in live feeds for marine larviculture: A mini review. *Front. Mar. Sci.* **2022**, *9*, 864165. [CrossRef]
5. Kotani, T. Enrichment of rotifers and its effect on the growth and survival of fish larvae. In *Rotifers*; Hagiwara, A., Yoshinaga, T., Eds.; Springer: Berlin/Heidelberg, Germany, 2017; pp. 47–62.
6. Støttrup, J.G. Production and nutritional value of copepods. In *Live Feeds in Marine Aquaculture*; Støttrup, J.G., McEvoy, L.A., Eds.; Blackwell Science: Oxford, UK, 2013; pp. 145–205.
7. Busch, K.E.T.; Peruzzi, S.; Tonning, F.; Falk-Petersen, I.B. Effect of prey type and size on the growth, survival and pigmentation of cod (*Gadus morhua*, L.) larvae. *Aquacult. Nutr.* **2011**, *17*, 595–603. [CrossRef]
8. Harzevili, A.; De Charleroy, D.; Auwerx, J.; Vught, I.; Slycken, J.; Dhert, P.; Sorgeloos, P. Larval rearing of burbot (*Lota lota* L.) using *Brachionus calyciflorus* rotifer as starter food. *J. App. Ichthyol.* **2003**, *19*, 84–87. [CrossRef]
9. Harzevili, A.S.; Dooremont, I.; Vught, I.; Auwerx, J.; Quataert, P.; De Charleroy, D. First feeding of burbot, *Lota lota* (Gadidae, Teleostei) larvae under different temperature and light conditions. *Aquac. Res.* **2004**, *35*, 49–55. [CrossRef]
10. Bischoff-Lang, A.; Kubitz, M.; Wranik, C.; Ballesteros-Redondo, L.; Fink, P.; Palm, H. The effect of *Brachionus calyciflorus* (Rotifera) on larviculture and fatty acid composition of pikeperch (*Sander lucioperca* (L.) cultured under pseudo-green water conditions. *Sustainability* **2022**, *14*, 6607. [CrossRef]
11. Imentai, A.; Yanes-Roca, C.; Steinbach, C.; Policar, T. Optimized application of rotifers *Brachionus plicatilis* for rearing pikeperch *Sander lucioperca* L. larvae. *Aquacult. Internat.* **2019**, *27*, 1137–1149. [CrossRef]

12. Yanes-Roca, C.; Mráz, J.; Born-Torrijos, A.; Holzer, A.S.; Imentai, A.; Policar, T. Introduction of rotifers (*Brachionus plicatilis*) during pikeperch first feeding. *Aquaculture* **2018**, *497*, 260–268. [CrossRef]
13. Mahmoudzadeh, H.; Ahmadi, M.; Shamsaei, M. Comparison of rotifer *Brachionus plicatilis* as a choice of live feed with dry feed in rearing *Coregonus lavaretus* fry. *Aquacult. Nutr.* **2009**, *15*, 129–134. [CrossRef]
14. Lahnsteiner, F.; Kletzl, M. A method for rearing perch, *Perca fluviatilis*, larvae using *Paramecium caudatum*, followed by wild zooplankton and formulated dry feed in combination with adequate tank systems. *J. Agricult. Sci.* **2018**, *10*, 26–42. [CrossRef]
15. Lahnsteiner, F.; Kletzl, M.; Weismann, T. Rearing of burbot, *Lota lota* (Pisces, Teleostei), larvae with zooplankton and formulated microdiets. *J. Agricult. Sci.* **2012**, *4*, 269–277. [CrossRef]
16. Lahnsteiner, F.; Kletzl, M. On-feeding and juvenile production of coregonid species with formulated dry feeds: Effects on fish viability and digestive enzymes. *J. Agricult. Sci.* **2015**, *7*, 48–58. [CrossRef]
17. Ballesteros-Redondo, L.; Palm, H.W.; Bährs, H.; Wacker, A.; Bischoff, A.A. Pikeperch larviculture (*Sander lucioperca* [L., 1758]) with *Brachionus plicatilis* (Mueller, 1786) (Rotifera) and *Apocyclops panamensis* (Marsh, 1913) (Copepoda). *J. World Aquacult. Soc.* 2023; *early view*.
18. Imentai, A.; Rašković, B.; Steinbach, C.; Rahimnejad, S.; Yanes-Roca, C.; Policar, T. Effects of first feeding regime on growth performance, survival rate and development of digestive system in pikeperch (*Sander lucioperca*) larvae. *Aquaculture* **2020**, *529*, 735636. [CrossRef]
19. Kottelat, M. European freshwater fishes. *Env. Biol. Fish.* **1998**, *51*, 116. [CrossRef]
20. Guillard, R.R.L.; Ryther, J.H. Studies of marine planktonic diatoms: I. Cyclotella nana Husted, and Detonula confervacea (Cleve). *Can. J. Microbiol.* **1962**, *8*, 229–239. [CrossRef]
21. Allen, M.M.; Stanier, R.Y. Growth and Division of Some Unicellular Blue-green Algae. *J. Gen. Microbiol.* **1968**, *51*, 199–202. [CrossRef]
22. Hoshina, R. Size of *Paramecium bursaria* individuals under cold and dark conditions. *Biologia* **2014**, *69*, 1018–1022. [CrossRef]
23. Suantika, G.; Dhert, P.; Nurhudah, M.; Sorgeloos, P. High-density production of the rotifer *Brachionus plicatilis* in a recirculation system: Consideration of water quality, zootechnical and nutritional aspects. *Aquacult. Engin.* **2000**, *21*, 201–213. [CrossRef]
24. Park, H.; Lee, K.W.; Cho, S.; Kim, H.; Jung, M.M.; Kim, H.S. High density culture of the freshwater rotifer, Brachionus calyciflorus. *Hydrobiologia* **2001**, *446*, 369–374. [CrossRef]
25. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; et al. Fiji: An open-source platform for biological-image analysis. *Nat. Meth.* **2012**, *9*, 676–682. [CrossRef] [PubMed]
26. Wolnicki, J.; Leszek Myszkowski, L.; Kamiński, R. The influence of water temperature on the growth, survival, condition and biological quality of juvenile burbot, Lota lota (L.). *Arch. Pol. Fish.* **2001**, *9*, 79–86.
27. Albert, A.; Vetemaa, M.; Saat, T. Effects of salinity on the development of Peipsi whitefish *Coregonus lavaretus maraenoides* Poljakow embryos. *Annales Zool. Fenn.* **2004**, *41*, 85–88.
28. Walker, M.B.; Kimmel, C.B. A two-color acid-free cartilage and bone stain for zebrafish larvae. *Biotech. Histochem.* **2007**, *82*, 23–28. [CrossRef] [PubMed]
29. Boglione, C.; Gisbert, E.; Gavaia, P.; Witten, P.; Moren, M.; Fontagné-Dicharry, S.; Koumoundouros, G. Skeletal anomalies in reared European fish larvae and juveniles. Part 2: Main typologies, occurrences and causative factors. *Rev. Aquacult.* **2013**, *5*, 121–167. [CrossRef]
30. Peterson, W.T. Patterns in stage duration and development among marine and freshwater calanoid and cyclopoid copepods: A review of rules, physiological constraints, and evolutionary significance. *Hydrobiologia* **2001**, *453*, 91–105. [CrossRef]
31. Mittmann, B.; Ungerer, P.; Klann, M.; Stollewerk, A.; Wolff, C. Development and staging of the water flea *Daphnia magna* (Straus, 1820; Cladocera, Daphniidae) based on morphological landmarks. *EvoDevo.* **2014**, *5*, 12. [CrossRef]
32. Udit, U.; Biswal, A.; Mane, A.; Sinha, V.; Hussan, A.; Munilkumar, S. Culture of *Brachionus calyciflorus* as fish food organisms: An approach to improve larval survival of freshwater fish. *J. Exp. Zool. India* **2020**, *23*, 313–321.
33. Kodama, Y.; Fujishima, M. Endosymbiotic *Chlorella variabilis* reduces mitochondrial number in the ciliate *Paramecium bursaria*. *Scient. Rep.* **2022**, *12*, 8216. [CrossRef]
34. Müller, T.; Pál, L.; Bódis, M.; Kucska, B.; Wágner, L.; Bercsényi, M.; Husvéth, F.; Szabó, G.; Molná, T. Effect of dietary fat replacement on body composition of intensively reared hybrids of pikeperch and Volga pikeperch. *Isr. J. Aquacult. Bam.* **2012**, *64*, 1–7. [CrossRef]
35. Paffenhöfer, G.A.; Strickler, J.R.; Lewis, K.D.; Richman, S. Motion behavior of nauplii and early copepodid stages of marine planktonic copepods. *J. Plankt. Res.* **1996**, *18*, 1699–1715. [CrossRef]
36. Titelman, J.; Kiørboe, T. Motility of copepod nauplii and implications for food encounter. *Mar. Ecol. Prog. Ser.* **2003**, *247*, 123–135. [CrossRef]
37. Zingel, P.; Paaver, T.; Karus, K.; Agasild, H.; Nõges, T. Ciliates as the crucial food source of larval fish in a shallow eutrophic lake. *Limn. Oceano.* **2012**, *57*, 1049–1056. [CrossRef]
38. Zingel, P.; Agasild, H.; Karus, K.; Buholce, L.; Nõges, T. Importance of ciliates as food for fish larvae in a shallow sea bay and a large shallow lake. *Europ. J. Prot.* **2019**, *67*, 59–70. [CrossRef]
39. Bruno, E.; Mahjoub, M.; Hansen, B.; Munk, P.; Støttrup, J.G. Feeding behavior and capture success of turbot *Psetta maxima* larvae during the transition from upright to tilted swimming position. *Aquat. Liv. Res.* **2017**, *30*, 35. [CrossRef]

40. Kestemont, P.; Xueliang, X.; Hamza, N.; Maboudou, J.; Toko, I. Effect of weaning age and diet on pikeperch larviculture. *Aquaculture* **2007**, *264*, 197–204. [CrossRef]
41. El Kertaoui, N.; Lund, I.; Assogba, H.; Domínguez, D.; Izquierdo, M.S.; Baekelandt, S.; Cornet, V.; Mandiki, S.N.M.; Montero, D.; Kestemont, P. Key nutritional factors and interactions during larval development of pikeperch (*Sander lucioperca*). *Sci. Rep.* **2019**, *9*, 7074. [CrossRef]
42. Ajiboye, O.O.; Yakubu, A.F.; Adams, T.E.; Olaji, E.D.; Nwogu, N.A. A review of the use of copepods in marine fish larviculture. *Rev. Fish Biol. Fish* **2010**, *21*, 225–246. [CrossRef]
43. Hansen, B.W. Advances using copepods in aquaculture. *J. Plankt. Res.* **2017**, *39*, 972–974. [CrossRef]
44. Rasdi, N.W.; Qin, J. Copepod supplementation as a live food improved growth and survival of Asian seabass *Lates calcarifer* larvae. *Aquacult. Res.* **2018**, *49*, 3606–3613. [CrossRef]
45. Chakrabarti, R.; Sharma, J.G. Influence of management protocols on carp growth under nursery conditions: Relative importance of food and water quality. *Aquac. Int.* **1998**, *6*, 293–301. [CrossRef]
46. Piasecki, W.; Goodwin, A.E.; Eiras, J.C.; Nowak, B.F. Importance of copepoda in freshwater aquaculture. *Zool. Stud.* **2004**, *43*, 193–205.
47. Ghan, D.; Sprules, W.G. Diet, prey selection, and growth of larval and juvenile burbot *Lota lota* (L.). *J. Fish Biol.* **1993**, *42*, 47–64. [CrossRef]
48. Donner, M.T.; Eckmann, R. Diel vertical migration of larval and early-juvenile burbot optimises survival and growth in a deep, pre-alpine lake. *Freshw. Biol.* **2011**, *56*, 916–925. [CrossRef]
49. Żarski, D.; Sasinowski, W.; Kucharczyk, D.; Kwiatkowski, M.; Krejszeff, S.; Targońska, K. Mass initial rearing of burbot *Lota lota* (L.) larvae under controlled conditions. *Pol. J. Nat. Sci.* **2009**, *24*, 76–84. [CrossRef]
50. Wocher, H.; Harsányi, A.; Schwarz, J.F. Larviculture of burbot (*Lota lota* L.): Larval rearing using *Artemia* and weaning onto dry feed. *Aquac. Res.* **2013**, *44*, 106–113. [CrossRef]
51. Lahnsteiner, F. Digestive enzyme system of larvae of different freshwater teleosts and its differentiation during the initial phase of exogenous feeding. *Czech J. Animal Sci.* **2017**, *62*, 403–416. [CrossRef]
52. Esmaeilzadeh-Leithner, S.; Wanzenböck, J. Suitability of two agglomerated commercial microdiets for rearing larvae of different strains of *Coregonus lavaretus* under cold-water conditions. *Aquac. Nutr.* **2017**, *24*, 260–268. [CrossRef]
53. Billard, R. *The Carp: Biology and Culture*; Springer: Berlin/Heidelberg, Germany, 1999.
54. Mitra, G.; Mukhopadhyay, P.K.M.; Ayyappan, S. Biochemical composition of zooplankton community grown in freshwater earthen ponds: Nutritional implication in nursery rearing of fish larvae and early juveniles. *Aquaculture* **2007**, *272*, 346–360. [CrossRef]
55. Zaki, M.I.; Saad, H. Comparative study on growth and survival of larval and juvenile *Dicentrarchus labrax* rearing on rotifer and *Artemia* enriched with four different microalgae species. *Afr. J. Biotech.* **2010**, *9*, 3676–3688.
56. Fernández-Diaz, C.; Pascual, E.; Yúfera, M. Feeding behaviour and prey size selection of gilthead seabream, *Sparus aurata*, larvae fed on inert and live food. *Marine Biol.* **1994**, *118*, 323–328. [CrossRef]
57. Støttrup, J.G.; Attramadal, Y. The influence of different rotifer and *Artemia* enrichment diets on growth, survival and pigmentation in turbot (*Scophthalmus maximus* L.) larvae. *J. World Aquacult. Soc.* **2007**, *23*, 307–316. [CrossRef]
58. Hamre, K.; Ted, A.; Mollan, T.A.; Sæle, Ø.; Erstad, B. Rotifers enriched with iodine and selenium increase survival in Atlantic cod (*Gadus morhua*) larvae. *Aquaculture* **2008**, *284*, 190–195. [CrossRef]
59. Karlsen, Ø.; van der Meeren, T.; Rønnestad, I.; Mangor-Jensen, A.; Galloway, T.F.; Kjørsvik, E.; Hamre, K. Copepods enhance nutritional status, growth and development in Atlantic cod (*Gadus morhua* L.) larvae—Can we identify the underlying factors? *PeerJ.* **2015**, *3*, e902. [CrossRef]
60. Mona, M.; Rizk, E.T.; El-feky, M.; Elawany, M. Effect of nutritional quality of rotifers and copepods on sea bream (*Sparus aurata*) fry fish productivity. *Egypt. J. Exp. Biol.* **2019**, *15*, 135–142. [CrossRef]
61. Puello-Cruz, A.; Flores-Rojas, A.; Almazán-Rueda, P.; García-Ortega, A. Use of copepods during early feeding stages of spotted rose snapper larvae in a scale-up system. *North Am. J. Aquacult.* **2015**, *77*, 230–238. [CrossRef]
62. Turgay, E.; Steinum, T.M.; Eryalçın, K.M.; Yardımcı, R.E.; Karataş, S. The influence of diet on the microbiota of live-feed rotifers (*Brachionus plicatilis*) used in commercial fish larviculture. *FEMS Microbiol Lett.* **2020**, *367*, fnaa020. [CrossRef]
63. Vázquez-Salgado, L.; Marsella, A.; Biasini, L.; Buratin, A.; Pretoo, T.; Melchiotti, E.; Bandín, I.; Toffan, A. Role of rotifers in betanodavirus transmission to European sea bass larvae. *Front. Vet. Sci.* **2022**, *9*, 932327. [CrossRef]
64. Johnson, S.; Treasurer, J.; Bravo, S.; Nagasawa, K.; Kabata, Z. A review of the impact of parasitic copepods on marine aquaculture. *Zool. Stud.* **2004**, *43*, 229–243.
65. Lahnsteiner, F.; Kletzl, M.; Weismann, T. The risk of parasite transfer to juvenile fishes by live copepod food with the example Triaenophorus crassus and Triaenophorus nodulosus. *Aquaculture* **2009**, *295*, 120–125. [CrossRef]