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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

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Abstract

The present study investigates methods for larviculture and fingerling production in the European perch, *Perca fluviatilis*. Perch larvae in the stage of first feeding were sensitive to many manipulations necessary in fish culture. Lowering and increasing the tank water level as required for cleaning and water renewal, water flow and aeration led to disturbed buoyancy or mortality in a distinct percentage of larvae. *Paramecium caudatum*, wild zooplankton containing > 70% copeopds, and formulated dry feed were used for first feeding in combination with flow through tanks or static tanks. For first feeding *Paramecium caudatum* in combination with a static tank system was optimal resulting in survival rates of circa 90% at 15 days post hatch (dph). Wild zooplankton was no optimal starter feed as perch fed non-selectively on any feed particle available. This resulted in moderate survival rates of circa 50% at 15 dph. First feeding with formulated dry feed caused malformations (enlargement of swimbladders) and a high mortality of > 80%. Weaning from *Paramecium caudatum* to formulated dry feed and to zooplankton was tested on 8-16 dph larvae. Perch accepted the new food type within 3 days. The optimal time point for zooplankton weaning was 12 dph. In this age larvae had developed a selective feeding behaviour, and fed mainly on nauplii and copepodites. Weaning to formulated dry feed was impossible as larvae developed malformations resulting in high mortality as described above. Weaning from wild zooplankton to formulated dry feed was possible for larvae ≥ 29 dph. No cannibalism was observed in the experiments. The method was also tested in large scale experiments resulting in a survival rate of 65±4%, a total length of 45.0±7.2 mm, and a body weight of 1097±293 mg at 75 dph.

Keywords: *Perca fluviatilis*, larvae, *Paramecium caudatum*, zooplankton, formulated dry feed, malformation rate, survival rate, growth rate

1. Introduction

The European perch, *Perca fluviatilis*, is a species with high potential for aquaculture (Kestemont, Dabrowski, & Summerfelt, 2015). At present the production of fingerlings adapted to formulated dry feed is one of the major bottlenecks for the intensification of its culture (Policar, Samarin, & Mélard, 2015). The small larvae size, their sensitivity to different kinds of abiotic factors, and their dependence on specific high quality live food are considered as limiting factors (Policar, Samarin, & Mélard, 2015).

At present perch larvae are reared to fingerlings using three different methods (Policar, Samarin, & Mélard, 2015). (1) Pond culture with suitable-sized zooplankton is the traditional method. (2) Culture in mesocosm systems provides more controlled natural conditions. Feeding is performed starting with zooplankton (mainly rotifers) followed by *Artemia* nauplii and finally by formulated dry feed. (3) Under intensive conditions perch larvae are fed with *Artemia* nauplii or with formulated diets as starter feeds. Generally, survival rates of larvae to fingerling size are low for all three culture systems and range from 5 to 40% (Policar, Samarin, & Mélard, 2015).

Intensive culture of perch larvae under controlled conditions is an important goal to increase its production (Kestemont, Dabrowski, & Summerfelt, 2015). However, this culture method still faces several problems: Failure in swim bladder inflation may lead to development of abnormalities and to increased mortality

(Jacquemond, 2004). For first feeding *Artemia* nauplii are used (Mélard, Baras, Mary, & Kestemont, 1996a; Vlavonou, Masson, & Moreau, 1999; Kestemont, Xueliang, Hamza, Maboudou, & Toko, 2003; Król & Zieliński, 2015). However, due to their large size they are not optimal as they can be ingested only by a distinct percentage of larvae (Mélard, Baras, Mary, & Kestemont, 1996a; Vlavonou, Masson, & Moreau, 1999). Suboptimal feed results in decreased survival rates and size heterogeneity of developing fish. Further consequences are cannibalism, increased disease susceptibility, and highly variable culture success (Mélard, Baras, Mary, & Kestemont, 1996; Mélard, Kestemont, & Grignard, 1996; Król & Zieliński, 2015). Perch larvae accept also formulated dry feed, but survival and growth rates are significantly lower than those obtained with *Artemia* nauplii (Tamazouzt, Leray, Escaffre, & Terver, 1998; for review see Kestemont, Dabrowski, & Summerfelt, 2015). To improve the culture success optimizations were made in aspects of rearing conditions. Tamazouzt, Chatai, and Fontaine (2000) demonstrated that highest growth in weight and length was obtained in tanks with light grey and white walls, which were strongly illuminated. Contrary, according to Jentoft, Øxnevad, Aastveit, and Andersen (2006) perch larvae grew significantly faster in black tanks than in gray tanks due to better prey contrast. Ribi (1992) reported that perch larvae survive better in diluted sea water than in fresh water. Cannibalism is a critical factor in perch culture. It could be decreased by high stocking density due to a confusion effect when the predator is confronted to a large number of targets (Baras, Kestemont, & Mélard, 2003; Kestemont et al., 2003), by conditions resulting in homogeneous growth (Mélard, Baras, Mary, & Kestemont, 1996b), and by regular size-sorting (Mélard, Baras, Mary, & Kestemont, 1996b). Cannibalism was not related to the weaning age (Król & Zieliński, 2015).

In summary, a practical method for fingerling production could not be established under aspects of economy, animal welfare and sustainability. Therefore, the present study has the aim to extend the knowledge on larviculture and fingerling production in the European perch, *Perca fluviatilis*. It investigates the effect of different kinds of formulated dry feeds and live feeds in combination with different rearing systems on the survival rate, growth rate, feed uptake rate, and malformation rate. Complementary, malformations are histologically investigated and water quality in the tested tank systems is analyzed. By combining suitable feed types and rearing systems a method for perch fingerling production under intensive conditions is developed and also tested under hatchery relevant large scale conditions.

2. Material and Methods

Egg ribbons of perch, *Perca fluviatilis*, were collected after natural spawning in ponds. The eggs were disinfected with 1000 ppm formalin for 30 min, and incubated in ground water warmed to 18 °C by adequate heating units. Water parameters are reported in Table 5. After hatching larvae were collected with measuring cups and defined numbers were stocked in the experimental tanks having similar water conditions (for determination of larvae numbers see 2.7.-Fish investigations).

2.1 Collection and Processing of Zooplankton

Live zooplankton was collected from wild populations from lake Mondsee with plankton nets (AquaTech, Kitzbühl, Austria). The nets were dredged floating behind a boat in a depth of 10-15 m and at a cruising speed of 0.5 miles/h. The collection area and the dredging depth were adjusted depending on weather conditions and on occurrence of zooplankton organisms. Using a sieve netting procedure a < 200 µm size fraction and a 200-400 µm size fraction were collected. The collected zooplankton was washed out of the nets into 20 l buckets and diluted to a density of circa 100.000 organisms per litre. Five ml subsamples were collected in 2-day intervals and fixed in 4% formaldehyde. Zooplankton composition was determined by counting the number of nauplii, copepodites, *Daphnia* sp., *Cyclops* sp., *Diaptomus* sp. *Cypris* sp., rotifers, phytoplankton, and pollen (the latter occurred in zooplankton during the spring season, too). Organisms/particles are reported as percentile values of the total number of organisms/particles counted in the subsamples.

For preparation of zooplankton meal the 200-400 µm size fraction was used. The water was drained away, ascorbic acid and tocopherol were added as antioxidants in final concentrations of 80 mg/100 g dry weight and 10 mg/100 g dry weight, respectively. The zooplankton was spread in a thin layer of 2-4 mm on cooking trays, dried at 80 °C for 12 hours, and grinded to a powder.

2.2 Paramecium caudatum Cultures

A starter culture of *Paramecium caudatum* was obtained from a commercial supplier. Alfalfa medium was used as culture substrate (Detrich, Zon, & Westerfield, 2009). Primary subcultures were made in 5 l water. These were used for hatchery relevant follow-up subcultures in 50 l tanks. All culture tanks were covered to avoid potential contamination with microorganisms. At a temperature of 20-23 °C it took 4-5 days to grow healthy cultures. Criteria for healthy cultures were *Paramecium caudatum* concentrations of 5-10 cells/ml culture medium and

clear water conditions not smelling rotten. Opposite would have indicated imbalanced bacterial growth due to high quantities of substrate. Circa 5 l of the healthy cultures were daily used to start new subcultures. The rest of the *Paramecium caudatum* culture was harvested for larvae feeding. Tanks were illuminated with full spectrum lights with circa 300 lux and within 1 h *Paramecium caudatum* concentrated in clouds in the upper water areas. These clouds were sampled using measuring cups with a volume of 5 l and added in the larvae rearing tanks. Alternatively the cells were collected also with 100 µm plankton nets and added in the rearing tanks.

2.3 Preparation of Formulated Dry Feed

Two self-made starter feeds for fish larvae were tested. Both starter feeds consisted of low temperature fish meal, lactalbumin, salmon fish oil, soya lecithin, vitamin mix, and mineral mix (Lahnsteiner & Kletzl, 2015a, 2015b). Additionally, starter feed 1 contained krill meal, starter feed 2 self-prepared zooplankton meal. The components were mixed together with water, and the dough mixture was extruded using a Caleva variable density screw extruder with axial configuration. Used die hole diameter was 1 mm, die hole depth 10 mm, and extrusion temperature 75 °C. Extrusion was performed at 50 rpm. The extrudate was dried at 80 °C for 4 h and grinded in a corn mill to a particle size of 0.2 mm. The declaration of the two starter feeds is shown in Table 1.

Table 1. Composition of the formulated dry feed tested for first feeding of perch larvae

	FDF1	FDF2
Protein, %	69.1	72.7
Lipids, %	13.9	8.5
Carbohydrate, %	4.9	4.9
Calcium, %	1.2	1.2
Phosphor, %	0.7	0.7
Magnesium, %	0.3	0.3
Fatty acid composition		
∑ saturated fatty acids, g/100 g DW	4.6	2.8
∑ non saturated fatty acids, g/100 g DW	8.2	4.6
∑ total fatty acids, g/100 g DW	12.8	7.4
∑ ω3 fatty acids, g/100 g DW	4.2	1.9
∑ ω6 fatty acids, g/100 g DW	0.3	0.8
Ratio ω3:ω6	13.7	2.3

Note. DW: dry weight.

2.4 Effect of Manipulations on Larvae Viability

Larvae in the stage of first feeding were used and all experiments were made in triplicate. Forty larvae were stocked in 25 liter glass aquaria in 18 °C ground water: (1) In the control experiments the larvae were kept in aquaria without manipulations for 24 h. (2) In the water change experiment larvae were kept in aquaria for 24 h, too, but 50% of the tank water was changed 1 h after stocking. The water was drained away using a plastic tube with an inner diameter of 3 mm and filled up again using a similar tube. (3) In the tank aeration experiment larvae were kept in aquaria aerated with air stones via aquarium pumps for 24 h. Aeration intensity was adjusted, that the air stones gave rise to 80-120 air bubbles/min which had a diameter < 2 mm. (3) In the recirculation system experiment larvae were kept in aquaria tanks equipped with an under gravel filter system for 24 h. The filter plates were covered with a 20-30 mm gravel layer with a particle diameter of 3-4 mm and connected to an external filter canister. The water flow within the tank was 0.01 m/min.

At the end of the experiments the number of live larvae without behavioral abnormalities, of live larvae with behavioral abnormalities, and of dead larvae was recorded. Larvae with behavioral abnormalities were also investigated on morphological abnormalities in a stereomicroscope at 20-40-fold magnification. Percentage of live larvae without behavioral abnormalities, percentage of live larvae with behavioral abnormalities, and percentage of dead larvae were calculated relative to the number of fish stocked in the tanks.

2.5 Larvae First Feeding Experiments

Feeding experiments of larvae started 4 days post hatch (dph) and were terminated 16 dph. Two types of formulated dry feed (Table 1) and the < 200 µm size fraction of zooplankton were tested in circular flow through tanks. *Paramecium caudatum* feeding was tested in static tanks. Initial stocking density was 5 larvae/l water and

tanks were illuminated with full spectrum lights with circa 200 lux for 16 hours in all experiments. All experiments were performed in triplicate.

The circular flow through tanks had a volume of 200 l. The water inlet and outlet were laterally at opposite sides of the tanks, the inlet at the bottom and the drainage at the surface. When supplied with 0.1 l/sec water the average water flow inside the tank was 0.01 m/min. The complete water renewal in the tank took circa 30 min. The tanks were cleaned 2 times daily by draining away remnants of feed and dead larvae. The static systems were rectangular tanks with a volume of 200 l. Water was not changed and tanks were not aerated or cleaned.

Formulated dry feed was fed in quantities of 3 g/day using a band feeder. The < 200 µm zooplankton fraction was fed 2 times daily in concentrations of 15-30 organisms/l tank water. *Paramecium caudatum* was added to the tanks in final concentrations of 500-1000 cells/l water. Its concentration was controlled daily and readjusted when necessary.

Larvae total length and body depth were measured at the onset and at the end of the experiments. The larvae feed uptake rate was determined 3 days after the start of the experiments. The larvae survival rate and larvae malformation rate were determined at the end of the experiment.

2.5 Weaning of Larvae

All weaning experiments were made in triplicate. The experimental setup is shown in Figure 1. In experiment 1 the weaning from *Paramecium caudatum* to the self-produced formulated dry feed 2 was tested (Figure 1a). This feed was successfully used for first feeding of larvae with undifferentiated digestive tracts in previous experiments (Lahnsteiner & Kletzl, 2015a, 2015b, 2017). In experiment 2 the weaning from *Paramecium caudatum* to zooplankton was tested (Figure 1b), and in experiment 3 the weaning from *Paramecium caudatum* to zooplankton and finally to formulated dry feed (Figure 1c). In experiment 3 a commercially available starter feed for salmonid fry with a particle size of 300 µm was used. As 24-34 dph perch have a fully differentiated digestive tract with stomach, pyloric caecae and intestine (Kestemont, Dabrowski, & Summerfelt, 2015) this type of food was considered to be suitable for weaning. It contained 53% crude protein, 18% crude fat, 1% crude fibers, 1.15% phosphorus, 15000 I.E. vitamin A, 3000 I.E. vitamin D3, 300 mg vitamin E, and immunostimulants. The digestible energy was 18.0 MJ.

The static system was a 200 l tank equipped with an under gravel filter. It consisted of filter plates which were covered with a circa 2-3 cm thick gravel layer (gravel size 3-6 mm) and which could be connected with an external canister filter. During *Paramecium caudatum* feeding and during feeding the < 200 µm zooplankton fraction the under gravel filter was disconnected from the external canister filter to obtain static conditions. Canister filters were operated in tanks free of fish for full biological maturation of filter material. At the time points indicated in Figure 1 (change from *Paramecium caudatum* to formulated dry feed, change from the < 200 µm zooplankton fraction to the 200-400 µm zooplankton fraction) the under gravel filter plates were connected to the canister filters and water flow inside the tanks was adjusted to approximately 0.01 m/min. For weaning from zooplankton to formulated dry feed fish were transferred into 150 l circular tanks with the water inlet laterally at the surface and the water outlet centrally at the bottom. Water supply was 0.2 l/sec and the average flow rate inside the tank 0.02-0.04 m/min.

The survival rate, the malformation rate, the total length, and the body depth or body weight (depending on the age of the fish-see below) were determined at the beginning and at the end of the weaning procedure. The larvae feed uptake rate was determined 3 days after the start of weaning and at the end of weaning.

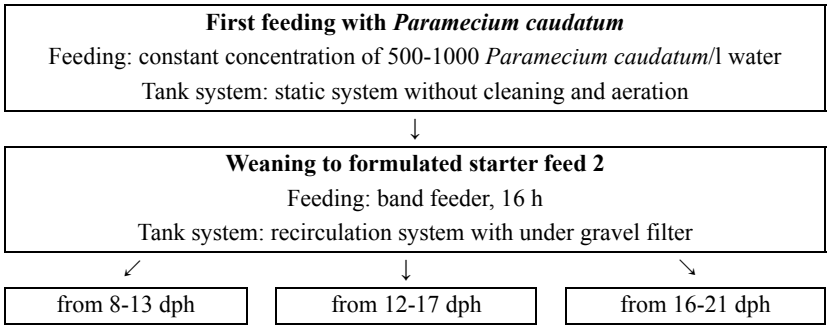

Figure 1a. Experiment 1: Weaning from *Paramecium caudatum* to formulated dry feed 2

Note. dph: days post hatch.

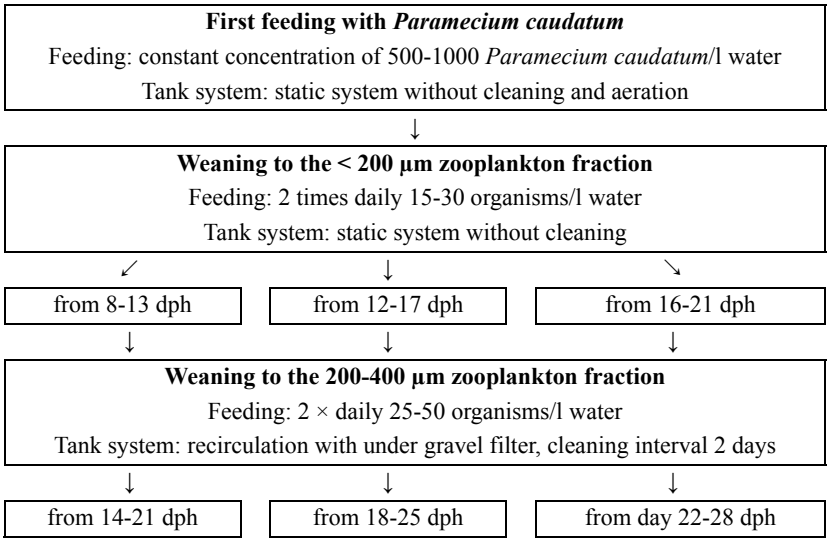

Figure 1b. Experiment 2: Weaning from *Paramecium caudatum* to zooplankton

Note. dph: days post hatch.

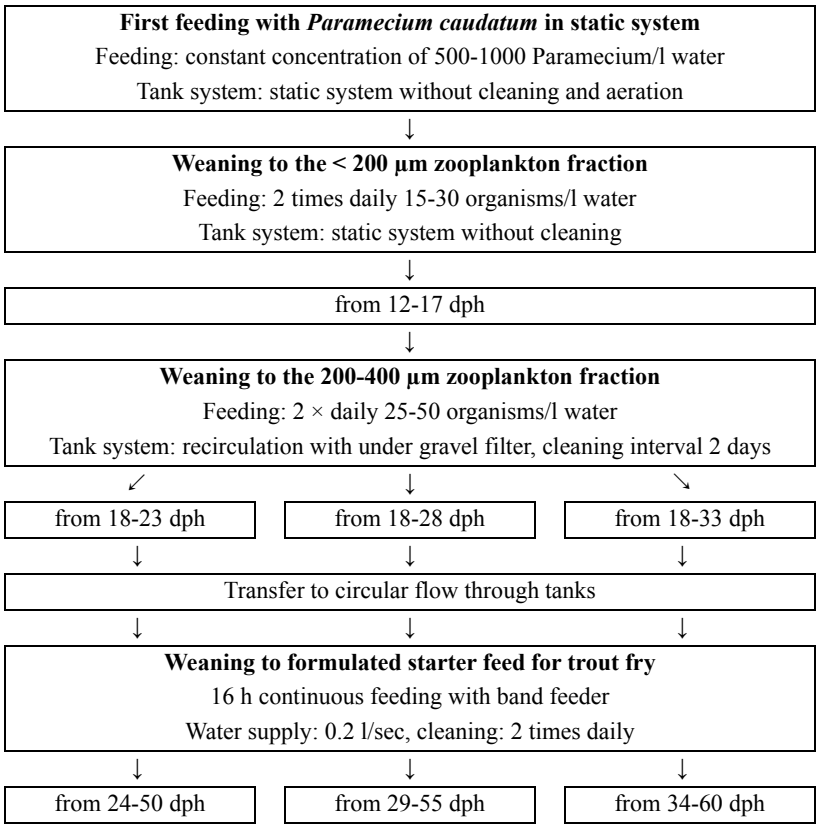

Figure 1c. Experiment 3: Weaning from *Paramecium caudatum* to zooplankton and then to formulated dry feed

Note. dph: days post hatch.

Content of the digestive tract was investigated in larvae weaned from *Paramecium caudatum* to the < 200 µm zooplankton fraction 3 days after weaning. Analysis procedure is described in section 2.7.

2.6 Hatchery Relevant Large Scale Experiments

The developed rearing method was tested in a large scale experiment. This experiment was conducted in duplicate. Rectangular tanks (5 × 1.6 × 0.6 m, length × width × height) were used which had the water inlet at one

broadside and the water outlet at the other one. Tanks were filled to a height of 20 cm resulting in a water volume of 1200 l and water was tempered to 17±2 °C using thermostat regulated heating coils. Tanks were stocked with quantities of 5 larvae/l (circa 6,000 larvae in total) and fed with *Paramecium caudatum* (cell concentration: 500-1000 cells/l water) from 3-11 dph. From 12-17 dph larvae were fed with the < 200 µm zooplankton size fraction (organism concentration: 15-30 organisms /l water, respectively) and from 18-28 dph with the 200-400 µm size fraction (organism concentration: 25-50 organisms /l water). Starting at 15 dph, tanks were supplied with 0.05 l/sec water and the water supply was gradually increased to 0.2 l/sec at 28 dph. At 29 dph larvae were transferred into 200 l circular tanks with the water inlet laterally at the surface and the water outlet centrally at the bottom. In the circular tanks the water supply was 0.2 l/sec, the average flow rate inside the tank was 0.02-0.04 cm/min and the stocking density 1000 fish (5 fish/l water). Starter feed for salmonid fry as described above was administered continuously using an automatic feeder for a period of 16 h/day. Initially it was administered in quantities of 0.75% of the body weight and from 35 dph on in quantities of 1.25% of the body weight. Tanks were cleaned 2 times daily by draining away remnants of feed and dead larvae. The experiment was terminated at 75 dph. The larvae survival rate and the larvae total length and body weight were determined at 75 dph.

2.7 Fish Investigations

For determination of the survival rate 2 methods were used. For fish < 20 dph five liters of tank water were collected after the larvae had been homogenously mixed in the tanks. The number of larvae was counted. Counting was repeated 2 times and the mean value of the determinations was taken to calculate the density of fish per liter water. Larvae numbers were extrapolated to the total water volume of the tanks. For fish ≥ 20 dph 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 the malformation rate, the feed uptake rate, and for measurement of the total length and body depth 20 live fish were sampled. They were killed by prolonged exposure to 0.2% MS222. Larvae were photographed in a Motic stereomicroscope equipped with a digital camera at 20 to 40 fold magnification. Using the digitized micrographs the following parameters were evaluated: larvae with abnormalities in development (abnormalities in organs and vertebral column), larvae with food in the digestive tract (*Paramecium caudatum* visible as amorphous almost transparent mass, zooplankton visible as remnants of crustacean chitin shells, formulated dry feed visible as a brownish mass). The malformation rate was expressed as the number of larvae with malformations in relation to the total number of investigated fish and the food uptake rate as the number of fish with food in the intestine in relation to the total number of fish investigated.

For fish < 20 dph the digitized micrographs were loaded in the Image J program and the larvae total length and body depth were measured. Details for measurement of body depth are shown in Figure 2a. Measurements were calibrated using an object micrometer. For fish ≥ 20 dph the total length was determined with a ruler to the nearest 0.5 mm and the total weight with an analytical balance to the nearest mg.

Relative growth in total length (RGTL) was calculated according to the formula,

$$RGTL = \frac{TL_e - TL_i}{TL_i} \times 100 \quad (1)$$

Where, TL_e: mean final total length of 20 fish in a tank; TL_i: mean initial total length of 20 fish in a tank.

Relative growth in body weight (RGBW) was calculated according to the formula,

$$RGBW = \frac{W_e - W_i}{W_i} \times 100 \quad (2)$$

Where, W_e: mean final weight of 20 fish in a tank; W_i: mean initial weight of 20 fish in a tank.

Relative growth in body depth (RGBD) was calculated according to the formula,

$$RGBD = \frac{BWI_e - BWI_i}{BWI_i} \times 100 \quad (2)$$

Where, BWI_e: mean final body depth of 20 fish in a tank; BWI_i: mean initial body depth of 20 fish in a tank.

Wild zooplankton is inhomogeneous in aspects of organism/particle composition. Therefore the content of the digestive tract was analyzed to determine which organisms/particles were ingested by perch. Analysis was performed on 7 dph perch fed with the < 200 µm zooplankton size fraction and on 10 dph perch weaned from *Paramecium caudatum* to the < 200 µm zooplankton size fraction (start of weaning at 8 dph). From the first feeding experiment 10 live larvae and 10 freshly dead larvae were sampled from each of the 3 tanks. From the

weaning experiment 10 live larvae were sampled from each of the 3 tanks. Larvae were killed as described, the digestive tract was removed by micro-preparatory techniques, cut open in its longitudinal direction and the main content of the digestive tract (food organisms/particles occurring in a frequency > 50%) was investigated in a stereomicroscope at 40-fold magnification. Food particles occurring in the digestive tract in a frequency > 50% were defined as main food type ingested by the investigated fish. The content of the digestive tract was defined as indifferent when no food organisms/particles occurred in a frequency > 50%, or when the organisms/particles could not be classified. The percentage of larvae with nauplii, copepodites, ostracodes (*Cypris* sp.), algae, rotifers, or pollen as main food type in the digestive tract was calculated in relation to the total number of investigated larvae.

Malformations of perch were also analyzed histologically. Ten malformed fish were sampled from the first feeding experiment with self-produced formulated dry feed 2 after 10 dph and fixed in 7% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4). The fixative was washed out, the samples were decalcified in 0.4 mol/l EDTA solution for 48 h, dehydrated in a graded series of ethanol, and embedded in Technovit 7100 according to manufacturer instructions. 3-4 µm thick sections were cut with a microtome (Thermo Scientific HM325) and stained with 0.1% toluidine blue in 0.1 mol/l phosphate buffer (pH 7.4). Sections were investigated in a light microscope at 400-1000-fold magnification.

2.8 Water Analysis

At 15 dph 1.5 l water was collected from each of the tanks used for the first feeding experiments. pH was measured electrochemically with a standard electrode. O_2, NH_4^+, and $KMnO_4$ consumption were measured with chemical standard methods using adequate blanks and standard curves (Quevauviller & Thompson, 2006).

Water bacteria were cultured on general purpose culture media (tryptic soy broth), on culture media specific for intestinal and fecal bacteria (MacConkey broth, eosin methylene blue broth), on cetrimide broth (specific for *Pseudomonas* sp.) and on *Aeromonas* enrichment medium (specific for *Aeromonas* sp.). Assay conditions were standardized in preliminary experiments in a way that microbiological growth was in the logarithmic growth phase at the time point of sampling. This was achieved by inoculating 1500 µl tryptic soy broth with 10 µl sample for 18 h and 1500 µl of the other types of culture media with 100 µl sample for 32 h. Samples were aerobically inoculated in the growth media at 37 °C under constant agitation. Bacterial densities were measured turbimetrically in a photometer at 540 nm versus a blank sample. Bacteria numbers were estimated using a standard curve with freeze dried *Micrococcus luteus* suspended in 0.75% NaCl solution. Bacterial number determined in the specific culture media was added up and is reported as total bacteria concentration of the water samples. *In Paramecium caudatum* culture water were anaerobic conditions. Therefore bacteria were not investigated on the above described aerobic culture media.

2.9 Statistics

Percentage values used for statistical analysis were arcsin transformed. Metric data were tested for normality and processed without further transformation. Data from the first feeding experiments were analyzed with one way ANOVA. The rearing condition was the independent variable and the fish viability parameters were the dependent variables. Also data from the weaning experiment were analyzed with one way ANOVA. In this analysis weaning conditions were used as independent variable and viability and growth parameters of fish as dependent variables. Newman-Keuls method was used as post hoc test. Student t-test was used to analyze differences between live and dead larvae fed with the < 200 µm zooplankton size fraction. SPSS software (Version 18.0) (USA) and SigmaPlot (Version 12.1) (U.S.A.) were used for statistical calculations.

3. Results

3.1 Effects of Manipulation on Larvae Survival Rates

All tested manipulations, *i.e.* tank water changes, tank aeration, and tank filtration caused a significant decrease in the percentage of live larvae without behavioral abnormalities and a significant increase in the percentage of larvae with behavioral abnormalities and in the percentage of dead larvae (Table 1). Larvae without behavioral abnormalities were distributed in the whole tank and swam in jerky movements. Larvae with behavioral abnormalities had reduced swimming activity, swam just beyond the water surface, and did not move in deeper water layers. However, they revealed no morphological abnormalities when investigated in the stereomicroscope. Dead larvae from the tank aeration, and tank filtration experiments had no morphological abnormalities, too. In the water renewal experiment 5% of the dead larvae had lesions in the musculature of the tail region.

Table 2. Effect of different manipulation procedures on the viability of 3 dph perch larvae

Manipulation procedure	% live larvae without behavioral abnormalities	% live larvae with behavioral abnormalities	% dead larvae
Static aquaria without any manipulation for 24 h (control)	97±2 [a]	0±0 [a]	3±1 [a]
Static aquaria for 24 h with a water change after 1 h	69±14 [b]	17±9 [b]	14±8 [b]
Static aquaria with aeration for 24 h	82±8 [b]	10±9 [b]	8±3 [c]
Static aquaria with under-gravel re-circulation filter systems for 24 h	76±8 [b]	9±6 [b]	15±6 [b]

Note. Data are mean±standard deviation. N = 3 deriving from different tank replicates. Data superscripted by different letters are significantly different ($P \leq 0.05$).

3.2 First Feeding of Perch Larvae

3.2.1 Dry Feed

When feeding perch larvae with formulated dry feed 1 or 2 the feed uptake rate was > 80% on the 5th dph (Table 2). At the end of the experiment (15 dph) the larvae survival rate was 12-16%, the malformation rate > 75% and the relative increase in total length and in body depth was 20-30% (Table 2). No significant differences in viability and growth parameters were detectable between the two feed types. Malformed larvae revealed enlarged swim bladders and a compressed intestine. Malformed larvae and normally developed larvae are shown in Figures 2a and 2b. Malformed larvae swam just beneath the water surface and were unable to move in deeper water regions. Histological investigations revealed detachment of the swim bladder epithelium from the surrounding tissue (normally developed swimbladders see Figures 2c and 2e, enlarged swimbladders see Figures 2d and 2f). Numerous spherical granules were found adhering to the swim bladder epithelium, in the swim bladder and in the surrounding tissue (Figure 2f). They had a diameter of 0.9±0.2 µm and were interpreted as bacteria.

Under behavioral aspects the normally developed larvae concentrated in the outermost peripheral regions of the tank. When drifting in more central regions they exhibited a high swimming activity and were often swirled around due to turbulences. Larvae needed time periods of 5-10 min to reach again the peripheral regions.

3.2.2 Live Zooplankton Feeding

The < 200 µm zooplankton fraction used for first feeding of perch larvae varied in its composition from day to day. Copepodites and nauplii contributed to circa 70% of the individual number (copepodites: 36.4±6.7 [mean±S.D., n = 7], nauplii: 35.2±9.2%). The samples contained also *Cypris* sp. (9.3±12.2%), rotifers (4.3±4.7%), phytoplankton (diatoms 6.5±7.9%, green algae: 5.1±4.7%), and a low percentage of pollen (3.2±3.0%).

When feeding the < 200 µm zooplankton fraction to perch larvae the feed uptake rate was > 80% on the 5th dph (Table 2). The digestive tract of a larva without feed is shown in Figure 2g. After 15 dph, the larvae survival rate was 50%, the larvae feed uptake rate > 90%, and the malformation rate 12% (Table 3). Malformations concerned the enlargement of swim bladders as described above. Relative increase in total length and in body depth was the significantly higher than with formulated dry feed 1 or 2, or with *Paramecium caudatum* (Table 2). The swimming behavior of perch larvae was similar as described in section 3.2.1 for formulated dry feed.

Analysis of food organisms/particles in the digestive tract revealed a significant discrepancy between live and dead fish (Table 4). While the digestive tract of live fish contained > 50% nauplii and copepodites, the digestive tract of dead larvae contained > 50% *Cypris* sp., algae, pollen, and indifferent material (Figure 2h).

Table 3. Effect of different feed types in combination with different tank systems on growth and viability parameters of perch larvae

Larvae parameters	Flow through, FDF 1	Flow through, FDF 2	Flow through, Zooplankton	Static, *Paramecium caudatum*
RGTL at 15 dph (%)	30.3±7.2[a]	27.3±7.0[a]	81.9 ±10.7[b]	42.6±5.9[c]
RGBD at 15 dph (%)	21.1±11.9[a]	20.0±9.2[a]	165.6±14.0[b]	27.8±5.0[a]
Survival rate at 15 dph (%)	16±5[a]	12±9[a]	48±7[b]	90±4[c]
Malformation rate at 15 dph (%)	71±8[a]	75±11[a]	12±4[b]	0±0[c]
Food uptake rate at 5 dph (%)	86±6[a]	85±4[a]	83±8[a]	88±4[a]

Note. dph: days post hatch, 3 dph = date of first exogenous feeding. RGTL relative growth in total length, RGBD relative growth in body depth. Data are mean±standard deviation, n = 3 deriving from different tank replicates. Data within a row superscripted by different letters are significantly different ($P \leq 0.05$).

Table 4. Main food type in the digestive tract of 7 dph perch larvae fed with the < 200 µm zooplankton size fraction

Main food type in the digestive tract	% larvae with a specific main food type in the digestive tract	
	Live larvae	Dead larvae
Nauplii	64±7[a]	1±1[b]
Copepodites	21±5[a]	2±1[b]
Cypris sp.	0±0[a]	48±7[b]
Algae	0±1[a]	13±4[b]
Pollen	0±0[a]	12±5[b]
Indifferent material	15±4[a]	24±4[b]

Note. Data represent percentage of larvae with a specific main food type in the digestive tract and are mean±standard deviation, n = 3 from different tank replicates. Data superscripted by different letters are significantly different ($P \leq 0.05$).

3.3 Weaning Experiments

3.3.1 Weaning From *Paramecium caudatum* to Formulated Dry Feed

When 8-16 dph perch larvae were weaned from *Paramecium caudatum* to self-produced formulated dry feed 2 > 80% of the larvae accepted dry feed within 3 days after the start of weaning (Table 6). Survival rate after a 6 days lasting weaning period was very low and not extending 10% (Table 6). Therefore, the experiment stopped thereafter. Most of the larvae revealed malformations, *i.e.* significantly enlarged swim bladders as described above in the first feeding experiment with dry feed. For the normally developed larvae the relative increase in total length was 30-40% and the relative increase in body depth 15-20%.

3.3.2 Weaning From *Paramecium caudatum* to Zooplankton

The < 200 µm zooplankton fraction which was used as food during the first 6 days of the experiment had the following species composition: copepodites: 43.6±8.1 [mean±S.D., n = 3], nauplii: 25.5±10.4, ostracodes (7.4±6.5%), rotifers (5.0±3.4%), diatoms (9.4±4.3%), green algae (7.0±3.5%), and pollen (2.1±1.5%). The 200-400 µm fraction used for the following 8 days of the experiment contained 12.3±8.4% copepodites, 40.8±33.6% *Daphnia* sp., 26.1±23.7%, *Cyclops* sp. and 20.9±10.9% *Diaptomus* sp.

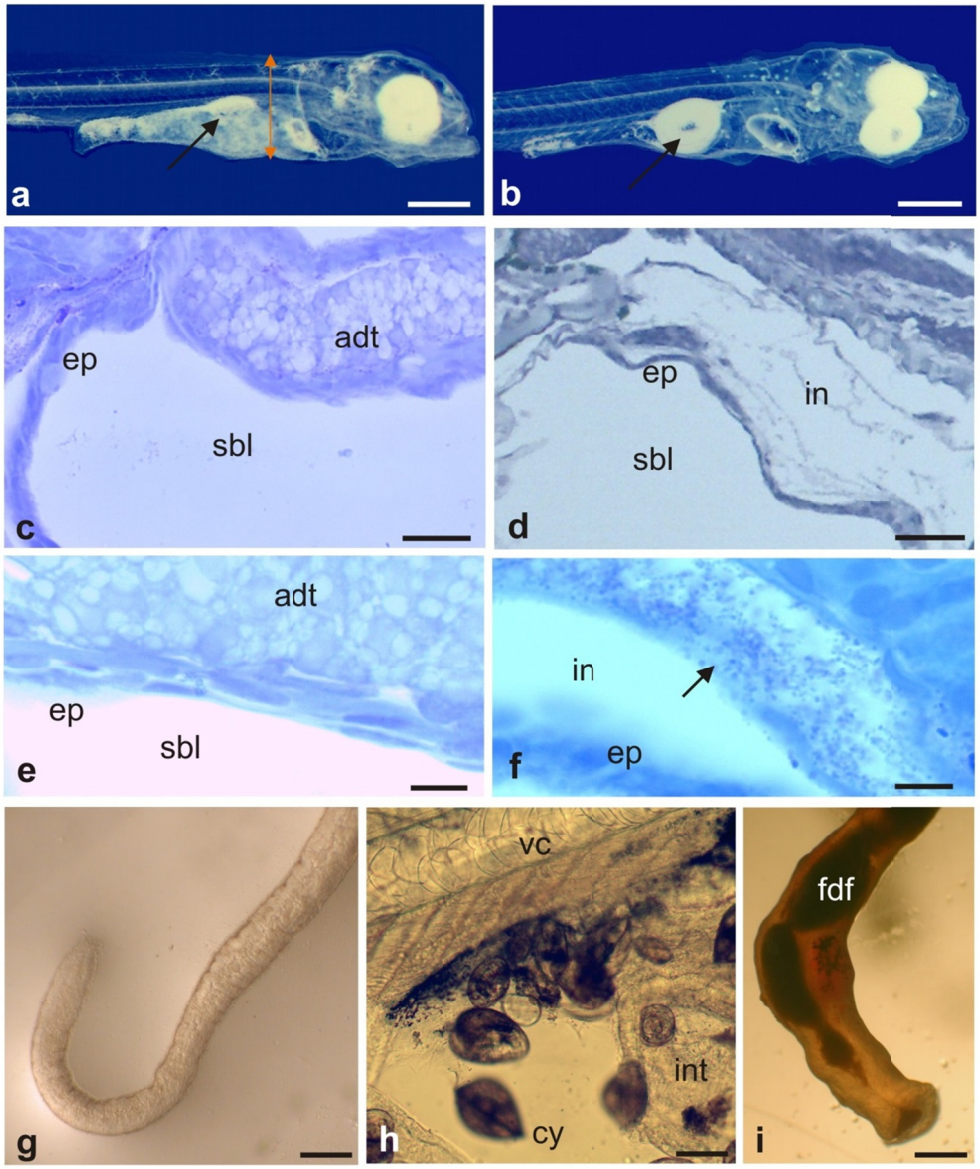

Figure 2. Stereomicroscopic and histological investigations on perch during the rearing experiments

Note. For Figures 2a and 2b a digital clean-up was used to remove disturbing objects from the background and for structure enhancement.

Figure 2a: Normal swimbladder (arrow) of a 7 dph perch fed with *Paramecium caudatum*. Scale bar = 0.5 mm. Orange arrow indicates the measurement points for determination of body depth; Figure 2b: Overinflated swim bladder (arrow) of a 7 dph perch fed with formulated dry feed. Scale bar = 0.6 mm; Figure 2c: Histology of an intact swimbladder showing the epithelium (ep) closely attached to the adipose tissue (adt). Sbl swim bladder lumen. Scale bar = 15 µm; Figure 2d: Histology of an enlarged swimbladder. The swim bladder epithelium (ep) is detached from the surrounding tissue. in interstitium, sbl swim bladder lumen. Scale bar = 35 µm; Figure 2e: Detail of the epithelium (ep) of a normal swim bladder. adipose tissue (adt), sbl swim bladder lumen. Histological section. Scale bar = 3.5 µm; Figure 2f: Detail of the epithelium (ep) of an enlarged swim bladder. sbl swim bladder lumen. Arrow shows spherical granules in the interstitium (in) Histological section. Scale bar = 3.5 µm; Figure 2g: Digestive tract of a 7 dph perch without food. Scale bar = 150 µm; Figure 2h: Digestive tract of a 7 dph perch larvae containing *Cypis* sp. (feeding: < 200 µm size fraction of zooplankton). Scale bar = 120 µm. cy *Cypris*, int intestine, vc vertebral column; Figure 2i: Digestive tract of a 26 dph perch containing formulated dry feed. Weaning from zooplankton to formulated dry feed (fdf) was started at 24 dph. Scale bar = 100 µm.

When 8-16 dph perch larvae were weaned from *Paramecium caudatum* to zooplankton > 80% of the fish accepted the feed within 3 days (Table 7). Organisms ingested by larvae were nauplii (55±7%), copepodites (27±5%), and green algae (2±1%). No ostracodes and pollen were found in the digestive tract. Perch larvae weaned to zooplankton had survival rates > 70% after a feeding period of 14 days (Table 7). Survival rates were highest when the 12 dph larvae were weaned to zooplankton and significantly lower for younger (8 dph) or older (16 dph) larvae (Table 7). The malformation rate was < 5% and the only type of malformation observed was the above described swim bladder enlargement.

The relative increase in total length was similar for larvae weaned from *Paramecium caudatum* to zooplankton at 8, 10, and 12 dph (Table 7). The relative increase in body depth was significantly higher for larvae weaned at 10 dph than for larvae weaned at 8 and 12 dph (Table 7).

Table 5. Chemical parameters of fresh ground water, of *Paramecium caudatum* culture water, and of water from the different combinations of tank and food systems used for first feeding of larvae

	pH	O_2, mg/l	NH_4^+, mg/l	$KMnO_4$ consumption, mg/l	Total bacteria mass, cells × 10^9/ml
Ground water (control)	7.85±0.02 [a]	10.7±0.2 [a]	0.002±0.001 [a]	4.7±0.2 [a]	0.1±0.1 [a]
P. caudatum culture water	7.55±0.20 [a]	0.0 [b]	11.10±4.37 [b]	236.5±45.0 [b]	n.i.
Flow through, FDF 1 (after 15 dph)	7.98±0.02 [b]	8.6±0.2 [a]	0.040±0.005 [c]	12.3±0.6 [c,d]	3.81±0.54 [c]
Flow through, FDF 2 (after 15 dph)	8.00±0.01 [b]	8.5±0.2 [c]	0.047±0.012 [d,c]	11.5±0.7 [c]	3.91±0.63 [c]
Flow through, zooplankton (after 15 dph)	8.02±0.04 [b]	8.7±0.2 [c]	0.041±0.005 [c]	10.5±0.8 [c]	3.35±0.43 [c]
Static, *P. caudatum* (after 15 dph)	8.21±0.03 [c]	8.1 ±0.3 [c]	0.054±0.006 [d]	14.9±3.1 [d]	3.03±0.49 [c]

Note. Data are mean±standard deviation, n = 3 (from 3 tank replicates). N.i. not investigated. Total bacteria mass is the sum of bacteria mass grown on Eosin Methylene blue, McConcey, Cetrimide, Tryptic soy, and Aeromonas broth. Data superscripted by different letters are significantly different ($P \leq 0.05$).

Table 6. Weaning of 8-16 dph perch from *Paramecium caudatum* to formulated dry feed and its effect on viability and growth parameters

Larvae parameters	Duration of experiment		
	8-13 dph	12-17 dph	16-21 dph
RGTL at the end of the experiment (%)	37.6±3.8	34.3±5.8	35.6±6.3
RGBD at the end of the experiment (%)	17±2.1	20.0±1.7	18.2±2.0
Survival rate at the end of the experiment (%)	10.2±6.5	6.2±6.5	4.3±4.0
Malformation rate at the end of the experiment (%)	95±5	91±8	96±4
Food uptake rate 3 d after start of weaning (%)	83±8	85±7[a]	82±8

Note. dph: days post hatch. Data are mean±standard deviation, n = 3 deriving from different tank replicates. RGTL relative growth in total length, RGBD relative growth in body depth. Data are not significantly different ($P > 0.05$).

3.3.3 Weaning From Zooplankton to Formulated Dry Feed

When 24-34 dph perch larvae were weaned from zooplankton to formulated dry feed > 85% of the larvae accepted dry feed within 3 days (Table 8, Figure 2i). The survival rate at the end of the experiment was significantly lower when perch were weaned to formulated dry feed after 27 dph than when they were weaned after 29 or 34 dph (Table 8). Malformation rate was < 2% in all experiments. The relative increase in total length and in body weight was significantly higher for larvae weaned at 29 dph and at 34 dph than for larvae weaned at 24 dph (Table 8).

3.4 Large Scale Experiment on Rearing

In a large scale experiment the survival rate of perch was 61±4% at 75 dph. At the end of the experiment perch had a total length of 45.0±7.2 mm and a body weight of 1097±293 mg.

Table 7. Weaning of 8-16 dph perch from *Paramecium caudatum* to live zooplankton and its effect on larvae viability

Larvae parameters	Duration of experiment		
	8-21 dph	12-25 dph	16-29 dph
RGTL at the end of the experiment (%)	62.2±7.0[a]	70.7±7.4[a]	67±6.7[a]
RGBD at the end of the experiment (%)	330.0±13.5[a]	350.0±14.4[b]	327.3±13.3[a]
Survival rate at the end of the experiment (%)	65.1±6.4[a]	83.3±8.2[b]	62.6±8.2[a]
Malformation rate at the end of the experiment (%)	4±4[a]	3±3[a]	2±2[a]
Food uptake rate 3 d after start of weaning (%)	86±5[a]	89±4[a]	84±8[a]

Note. Larvae were fed with the 100-200 µm zooplankton fraction for 6 days and thereafter with the 200-400 µm fraction for 8 days. dph: days post hatch, RGTL relative growth in total length, RGBD relative growth in body depth. Data are mean±standard deviation, n = 3 deriving from different tank replicates. Data superscripted by different letters are significantly different ($P \leq 0.05$).

Table 8. Weaning of 24-34 dph perch from live zooplankton feed to formulated dry feed and its effect on larvae viability

Larvae parameters	Duration of experiment		
	24-50 dph	29-55 dph	34-60 dph
RGTL at the end of the experiment (%)	89.5±9.4[a]	96.8±10.4[a]	100.4±9.5[a]
RGBW at the end of the experiment (%)	316.7±15.0[a]	398.2±14.9[b]	388.8±12.6[a]
Survival rate at the end of the experiment (%)	53±12[a]	69±6[b]	67±11[b]
Malformation rate at the end of the experiment (%)	0.7±0.4[a]	1.5±0.5[b]	1.3±0.2[b]
Food uptake rate 3 d after start of weaning (%)	85±8[a]	87±8[a]	87±7[a]

Note. dph: days post hatch, RGTL relative growth in total length, RGBW relative growth in body weight. Data are mean±standard deviation, n = 3 deriving from different tank replicates. Data superscripted by different letters are significantly different ($P \leq 0.05$).

4. Discussion

The study demonstrated that perch larvae in the stage of first feeding are sensitive to many manipulations necessary in fish culture. Lowering and increasing the tank water level as required for cleaning and water renewal, water flow and aeration can disturb larvae buoyancy or cause injury or death of larvae possibly due to changes in hydrostatic pressure, turbulences, or mechanical forces. The perch is a physoclistous fish and the initial inflation of the swimbladder and the closure of the pneumatic duct occur at 6-12 dph (Eglof, 1996; Policar, Samarin, & Mélard, 2015). Woolley and Qin (2010) suggested that larvae might be especially vulnerable to changes in hydrostatic pressure during the period of swimbladder development and differentiation. Rapid changes in hydrostatic pressure might occur when larvae are caught in upwelling water flows and drawn to the surface (Woolley & Qin, 2010). Also in nature perch larvae select habitats with minimal water flow and are sensitive to turbulences and increased water flow (Treasurer, 1990; Kratochvil et al., 2008). On the other hand small-scale water turbulences might be advantageous in food-limited environments as they enhance the contact rate between fish larvae and their prey (Lewis & Pedley, 2001).

First feeding of perch larvae is a critical step under intensive culture conditions (Hamre, Yúfera, Rønnestad, Boglione, Conceição, & Izquierdo, 2013). The present study demonstrated that for first feeding a static water system without aeration and a homogenous, small-sized live food are optimal. This hypothesis bases on the following two observations. (1) Only in the static system larvae showed their natural swimming behaviour. They swam free in the water in jerky movements as also observed in their natural habitats (Treasurer, 1990; Kratochvil et al., 2008). Contrary, in the flow through tanks larvae crowded peripherally close to the tank walls. These peripheral regions probably have the lowest water flow due to friction forces between tank wall and fluid (Munson, Huebsch, & Rothmayer, 2012). (2) Larvae did not tolerate formulated dry feed as starter feed. Also wild zooplankton was not optimal. Zooplankton is a mixture of different organisms and particles and larvae fed non-selectively on any organism/particle available. From these experiments it was concluded that a homogenous live feed was necessary as starter feed for perch larvae. *Paramecium caudatum* fulfilled the necessary criteria as it had an adequate size (length 120-220 µm, width 60-120 µm-Fokin, 1997) to be ingested by perch larvae,

which mouth size is 0.35 mm (Policar, Samarin, & Mélard, 2015). However, growth of perch larvae fed with *Paramecium caudatum* was moderate in comparison to zooplankton, indicating a decreased nutritional value. *Paramecium caudatum* has been used successfully for first feeding of different species of ornamental fish larvae with a mouth size too small to ingest crustacean species (Copepoda, Anostraca, Cladocera) (Borla, Palecek, Budick, & O'Malley, 2002; Andrews, 2011) and for experimental fish (*e.g. Danio rerio*—Detrich, Zon, & Westerfield, 2015). Müller et al. (2012) raised hybrids between *Sander lucioperca* and *Sander volgensis* successfully with live *Paramecium* sp. as starter feed. As *Paramecium caudatum* survives in the fish tanks for unlimited time periods no cleaning and manipulation procedures are necessary (Andrews, 2013). As a disadvantage also bacteria may be introduced into the fish tanks together with the culture medium (Peterson, Ferguson, Watral, Mutoji, Ennis, & Kent, 2013) and the culture water is inadequate for fish larvae in aspects of O_2 and NH_4 concentrations. Therefore, accurate monitoring of tank water quality is necessary when using *Paramecium caudatum* as larvae feed. Rotifers are an adequate, homogenous small sized live feed for fish larvae, too (Lubens, Zmora, & Barr, 2001; Shiri Harzevili, De Charleroy, Auwerx, Vught, Van Slycken, Dhert, & Sorgeloos, 2003; Park, Puvanendran, Kellett, Parris, & Brown, 2006). However, rotifers are elaborative to culture and require microalgae as food organisms (Lubens, Zmora, & Barr, 2001; Shiri Harzevili, De Charleroy, Auwerx, Vught, Van Slycken, Dhert, & Sorgeloos, 2003; Park, Puvanendran, Kellett, Parris, & Brown, 2006). Also organic loads and bacterial concentrations can be high in rotifer cultures (Dhont, Dierckens, Støttrup, Van Stappen, Wille, & Sorgeloos, 2013).

Formulated dry feed 2 was successfully used for first feeding of different salmonid species which could not be reared with commercially available starter feed (*e.g. Thymallus thymallus*, *Coregonus maraena*, *Coregonus atterensis*) (Lahnsteiner & Kletzl, 2015a, 2015b). Formulated dry feed 1 was modified in fatty acid composition and in the ratio of ω3:ω6 unsaturated fatty acids as these are species specific parameter in fish larvae nutrition (Hamre, Yúfera, Rønnestad, Boglione, Conceição, & Izquierdo, 2016). When feeding these formulated dry feeds to larvae, they developed enlarged swimbladders leading to highly positive buoyancy. Histologically, the swim bladder revealed tissue destructions and indications for the occurrence of coccus-like bacteria. However, tank water did not significantly differ from other rearing regimes in aspects of qualitative and quantitative composition of bacteria when using tryptic soy broth, MacConkey broth, eosin methylene blue broth, cetrimide broth and *Aeromonas* enrichment medium as culture media. Theoretically, the formulated dry feed might favor the growth of specific species of pathogenic bacteria leading to disturbance of gas regulation and finally to swimbladder overinflation. Also the indigestibility of formulated dry feed might be related with swimbladder overinflation. However, these suggestions are speculative at present and require further investigations. A similar incidence of swim bladder overinflation was observed in larvae and juveniles of *Lota lota* and also in this species the reasons remained unknown (Rekecki et al., 2016). Failure in swim bladder inflation has been reported also for larvae of several marine species as *Gadhus morhua* (Grotmol, Kryvi, & Totlan, 2005), *Latris lineata* (Trotter, Pankhurst, & Hart, 2001) and *Dicentrarchus labrax* (Peruzzi, Westgaard, & Chatain, 2007) under intensive culture conditions. In *Gadhus morhua* it has been claimed that larvae are hypersensitive to moderate levels of gas super-saturation (< 119% total gas pressure) (King & Nardi, 2002), a parameter which can be excluded in the present experiments as fish fed with live feed did not develop swim bladder hyperinflation and gas oversaturation was not detected in unpublished measurement. Also in previous experiments survival rates were low when using formulated dry feed for first feeding of perch larvae similar as for pikeperch larvae (Ostaszewska, Dabrowski, Czumińska, Olech, & Olejniczak, 2005; for review see Kestemont, Dabrowski, & Summerfelt, 2015).

Copepod species and in particular their juvenile forms (nauplii and copepodites) have been demonstrated to be a nutritionally adequate live prey for rearing the larvae of many teleost fish species (Støttrup, 2003; Busch, Falk-Petersen, Peruzzi, Rist, & Hamre, 2010; Busch, Peruzzi, Tonning, & Falk-Petersen, 2011). Although the zooplankton used for feeding of perch larvae in the present study contained circa 70% nauplii and copepodites, analysis of the content of the digestive tract demonstrated that larvae fed non-selectively on any available particle which could be swallowed. Live larvae with significant growth fed mainly on nauplii and copepodites, while dead larvae fed on *Cypris* sp., algae, pollen, and indifferent material. *Cypris* sp. seemed indigestible for perch larvae as they were structurally still intact and alive in the terminal portion of the digestive tract. Live passage of ostracodes through teleost fish, amphibian and mammalian digestive tracts has been described in different studies (Aarnio & Mattila, 2000; Lopez, Gonçalves, Mantovani, & Rios, 2002). Algae, pollen, and indifferent material must be assumed to have inadequate nutritional value. Many experiments and culture protocols use *Artemia* nauplii for first feeding of perch larvae. However, larvae survival rate is variable and growth inhomogeneous, as *Artemia* nauplii are too large and can be ingested only by 60-70% of the larvae (Mélard, Baras, Mary, & Kestemont, 1996a; Vlavonou, Masson, & Moreau, 1999). *Artemia* has also a limited life span of 4-6 h in fresh water (Browne, Sorgeloos, & Trotman, 1991) and therefore tank manipulations and

cleaning procedures are necessary. In nature rotifers are the dominant first food of perch larvae and thereafter they feed on copepods (Treasurer, 1990; Kratochvil et al., 2008; Skrzypczak, Mamcarz, Kujawa, Kucharczyk, & Furgala-Selezniow, 1998). Rotifers could not be detected in the digestive tract in the present study. Possibly they could not be identified and represent the food components reported as indifferent material. Also in other species as *Elacatinus lori*, and *E. colini* the *Artemia* nauplii were not a suitable prey, but feeding with rotifers followed by wild zooplankton was optimal in aspects of survival and growth of larvae (Majoris, Francisco, Atema, & Buston, 2018).

The weaning experiments demonstrated that perch larvae did not refuse a new food type or had to be trained to a new food type by co-feeding procedures as described for different other fish species (*Dicentrarchus labrax*—Cahu & Zambonino Infante, 1994; *Elacatinus puncticulatus*—Pedrazzani, Pham, Lin, & Neto, 2014; *Sparus aurata*—Pantazis, Benekos, & Papadomichelakis, 2014; *Lota lota*—Lahnsteiner & Kletzl, 2017). Perch in an age of 8-34 dph accepted a new food type within 3 days. However, weaning from *Paramecium caudatum* to formulated dry feed was impossible. Similar as discussed above for the first feeding experiments, larvae developed strongly positive buoyancy due to enlarged swimbladders and finally died. Contrary, perch larvae could be successfully weaned from *Paramecium caudatum* to zooplankton as 10-18 dph larvae had developed a selective feeding behaviour and fed mainly on nauplii and copepodites. The experiments demonstrated that too early weaning was associated with increased mortality. Possibly, the selective feeding behavior was not fully developed at this time point. Also too late weaning resulted in increased mortality. This may be due to the fact that prolonged feeding with *Paramecium caudatum* resulted in growth stagnation probably as *Paramecium caudatum* was inadequate for more developed fish. Subsequently, prolonged *Paramecium caudatum* feeding might result in weakened fish with nutrition deficient which cannot be fully compensated after weaning.

Weaning from zooplankton to formulated dry feed was possible for perch $\geq$ 29 dph. In this age feeding with formulated dry feed did not induce swimbladder overinflation as in earlier development stages. Fish had differentiated a functional stomach, pyloric caecae, and a fully functional intestine which might facilitate digestion of dry feed (Kestemont, Dabrowski, & Summerfelt, 2015). Flow through tanks used for weaning experiments had also higher water flow and cleaning efficiency than tanks used for first feeding experiments. This might reduce the growth of potentially pathogenic bacteria which could be associated with swimbladder enlargement. When younger fish (24 dph) were weaned from zooplankton to formulated dry feed mortality was increased. Possible reasons might be the immaturity of the digestive tracts or the inability to tolerate flow through conditions.

Published weaning methods for perch and pike perch differ: Król and Zieliński (2015) weaned perch from *Artemia* to formulated dry feed after 14 days and Jentoft, Øxnevad, Aastveit, and Andersen (2006) after 28 days. Policar, Samarin, and Mélard (2015) described weaning as extended period of co-feeding *Artemia* and formulated dry feed from 7-30 dph during which *Artemia* is progressively replaced by formulated dry feed. According to the method of Szkudlarek and Zakęś (2007) for first feeding of pike perch a mixed feed of *Artemia nauplii* and artificial feed is useful which is replaced solely by dry feed after 14 dph. Kestemont, Xueliang, Hamza, Maboudou, and Toko (2007) described that weaning of pike perch from *Artemia* to formulated dry feed is possible between 12 and 19 dph with a varying mortality rate due to cannibalism.

The present large scale experiments demonstrated that the here described perch rearing method was also applicable in practice with final survival rates of 61±4% at 75 dph. These are one of the highest survival rates ever reported for intensive rearing of perch. Finally, it should be stressed that no cannibalism was observed in the present experiments as described in earlier studies. Fish develop cannibalistic behavior when growth is inhomogeneous (Baras & Jobling, 2002). This could be avoided by the feeding sequence with *Paramecium caudatum*, zooplankton, and formulated dry feed. For culture purposes adaptions of the method in aspects of tank form, special tank refuge structures for larvae, and heating systems might be beneficial to obtain still more robust methods. Future studies might also bring a solution for direct weaning from *Paramecium caudatum* to formulated dry feed which could significantly facilitate the costs and labor of perch larvae production.

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