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Weaning of Burbot, *Lota lota* (L.) from Live to Dry Feed Using NaCl as Dietary Attractant

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Abstract

Weaning of 150 days post hatch *Lota lota* from live feed (zooplankton) to formulated dry feed (FDF) was investigated. *L. lota* could not be forced from live feed to FDF. They refused FDF for periods up to 21 d. Body mass decreased for 20%, condition factor for 15%, and hepatosomatic index for 50%. In 21 days lasting co-feeding experiments with FDF and live feed *L. lota* selected exclusively the live feed organisms. NaCl in a concentration of 5% was a dietary feeding attractant for *L. lota*. When FDF was supplemented with 5% NaCl, *L. lota* could be abruptly weaned from live to dry feed. During a 21 d lasting experiment body mass increased for circa 40%, total length for 10%, condition factor for 5% and hepatosomatic index remained constant. These values were similar to live zooplankton feeding. However, increased mortality of > 20% was recorded for fish fed with the 5% NaCl containing FDF. To reduce mortality FDF was supplemented with zooplankton meal to upgrade its quality and extruded to optimize its density and sedimentation rate. With the adjusted FDF mortality rates were reduced to < 5%. Using the optimized dry feed easy and sustainable weaning protocols were developed where NaCl and zooplankton meal were gradually reduced to adapt fish to pure FDF.

Keywords: formulated dry feed, live feed, zooplankton, weaning, larvae, *Lota lota*, burbot

1. Introduction

In fish culture the term weaning describes the transition in larvae or juveniles feeding from live feed to formulated artificial diets. The burbot, *Lota lota*, is a new and promising candidate for aquaculture. At the onset of exogenous feeding its digestive system is only poorly developed (Lahnsteiner, 2016) and larvae require live feed as *e.g.* zooplankton (Lahnsteiner, Kletzl, & Weismann, 2012a) or *Artemia* (Wocher, Harsányi, & Schwarz, 2012; Palińska-Żarska, Żarski, Krejszeff, Kupren, Łączyńska, & Kucharczyk, 2015). Therefore, weaning to artificial dry feed is necessary when fish are reared for aquaculture purposes. Larvae and juveniles of *L. lota* are very sensitive during the weaning process and severe mortality can occur when the feeding procedure or the rearing conditions are not adequate (Wocher, Harsányi, & Schwarz, 2012; Vanheule, Nevejan, Chiers, Meeus, Harzevili, Van Den Broeck, De Charleroy, & Decostere, 2012; Trejchel, Żarski, Palińska-Żarska, Krejszeff, Dryl, Dakowski, & Kucharczyk, 2014; Rekecki, Meeus, Chiers, Adriaen, Boyen, Declercq, Van den Broeck, & Decostere, 2016; authors, unpublished data). Several studies have been conducted on the weaning of *L. lota* larvae. Palińska-Żarska, Żarski, Krejszeff, Nowosas, Bilas, Trejchel, Brylewski, Targonska, and Kucharczyk (2014) demonstrated that the weaning success expressed as survival rate was highest when fish were weaned at 47-54 days post hatch. However, the survival rate was variable ranging from 58% to 78% and the artificial dry feed optimal for weaning was not defined. Trabelsi, Gardeur, Teletchea, and Fontaine (2011) investigated the effect of 12 environmental and feeding related factors on *L. lota* weaning performances using fractional factorial design. Low water salinity, the mode of dry feed administration, and a long weaning period significantly increased the survival rate (Trabelsi, Gardeur, Teletchea, & Fontaine, 2011). Wocher, Harsányi, and Schwarz (2012) reported that weaning of *L. lota* was possible only with a specific feed type and the specific growth rate was 9.7% day^{-1} and the survival rate 13.5%. Own unpublished experiments indicated that *L. lota* refuse many types of FDF, starve, and become very sensitive to different kinds of diseases.

Therefore, the present study was conducted on the weaning process of *L. lota* larvae. As previous studies (Wocher, Harsányi, & Schwarz, 2012; authors unpublished) demonstrated that many FDFs were not acceped

various kinds of chemoattractans were tested to increase the acceptibility of the feed. Once a suitable chemoattractant had been defined the artificial dry feed was adapted for *L. lota* in aspects of composition and processing technology. Finally weaning protocols were developed and tested in large scale experiments. High sustainability in aspects of survival rate and animal welfare and specific growth rates suitable for aquaculture production were the applied criteria.

2. Material and Methods

2.1 Egg Collection, Embryo Incubation and Larvae Rearing

Lota lota eggs derived from a broodstock kept in the fish farm Kreuzstein. Spontaneous spawning was initiated by a decrease in water temperature from 5 °C to 1.5 °C in the beginning of February (Lahnsteiner, Kletzl, & Weismann, 2012b). Eggs were collected, disinfected, and incubated in Zug chars at ≤ 4 °C till the stage of ready to hatch embryos. For synchronization and shortening of the hatching period the temperature was raised to 9 °C (Lahnsteiner, Kletzl, & Weismann, 2012b). Larvae were drained out of the Zug charrs using PVC tubes and stocked in 5 m^3 rectangular tanks supplied with 9.0±0.5 °C ground water. Initially the tanks had a water height of circa 10 cm to facilitate up-swimming of larvae and inflation of the swimbladder. Five days after hatching was finished, the tanks were gradually filled to a height of circa 40 cm and the flow rate was adjusted to 0.5 L/sec. Initial stocking density was circa 50 larvae per l water.

Larvae were fed with live zooplankton collected from Lake Mondsee according to a previously published method (Lahnsteiner, Kletzl, & Weismann, 2012a). Sieve nets with 100 µm as lower and 200 µm as upper mesh size limit were used to collect zooplankton organisms suitable in size for 10-30 dph old fish (composition of zooplankton organisms: 40% nauplia, 55% copepodites, 5% others), nets with 200 to 400 µm for the 31-50 dph fish (60% copepodites, 30% copepods, 10% cladocerans), and nets with a mesh size of 400 µm for > 50 dph fish (60% copepods, 40% cladocerans) (Lahnsteiner, Kletzl, & Weismann, 2012a). The zooplankton organisms were washed out of the nets into 20 l buckets and diluted to a density of circa 100.000 animals per litre. Feeding frequency of *L. lota* was 3 times per day and feeding quantity 20-30 zooplankton organisms per fish per feeding.

2.2 Preparation of Feeds

The formulated dry feed (FDF) was an extruded feed for salmonid fry with a pellet size of 1.5 mm containing 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. Listed ingredients were fish meal, wheat gluten, wheat, hemoglobin powder, fish oil, and sun flower concentrate. No information was available about supplementation with dietary attractants.

Betaine, glutamate, glycine, alanine, and NaCl in concentrations of 2.5% and fish extract in a concentration of 5% were tested as dietary attractants. FDF was grinded to a powder, the dietary attractants were dissolved in water and mixed with the grinded FDF to a homogenous pastry. To prepare a fish extract, rainbow trout filets were minced to a fine pastry using an immersion blender. The pastry was pressed through a sieve, the filtrate was dissolved in water, and incorporated into the feed as described above.

Zooplankton was added to FDF in a concentration of 50% to test if it acted as dietary attractant and as a component increasing the quality of FDF (Lahnsteiner & Kletzl, 2014; Lahnsteiner & Kletzl, 2015). Before processing water was carefully drained from the organisms and 1 g/kg ascorbic acid and 0.25 g/kg tocopherol were added as antioxidants. Zooplankton was minced and mixed with the FDF powder in a ratio of 1:6.5 (= FDF powder:zooplankton wet weight) resulting in FDF:zooplankton ratios of 1:1 after drying. In experiment D NaCl in a final concentration of 5% was added to this type of feed mixture. All feeds were oven dried at 80 °C for 12-14 h and grinded to a particle size of 1-2 mm using a conical grinder mil.

As the zooplankton containing feed had a very low density and sedimentation rate an extrusion processing step was included in experiment D. Extrusion of the above described FDF-zooplankton-NaCl mixture was impossible due to the high water content. Therefore, zooplankton meal was produced for extrusion. For this reason it 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. This powder was mixed with FDF in a ratio of 1:1 (w:w), NaCl was added to a final concentration of 5% and water to at an volume of circa 35% water. The mixture was extruded using a Caleva variable density screw extruder with axial configuration. Used die hole diameter was 1 mm and die hole depth 10 mm. Extrusion was performed at 50 rpm. The extrudate was dried at 80 °C for 4 h. For the large scale weaning experiments also extruded FDF-zooplankton mixtures with 3% and 1% NaCl were used. They were prepared as described above, however the NaCl concentration was adapted.

2.3 Small Scale Experiments

A detailed summary of the conducted experiments is shown in Table 1. Small scale experiments were conducted with 140-148 dph fish in flow through incubators (length × width × depth: 70 cm × 40 cm × 20 cm) supplied with 9±1 °C ground water at a flow rate of circa 0.5 l/sec. Each experiment was done in triplicate. The small scale experiments had the following constant parameters: A number of 250 fish was stocked in the incubators, respectively. Feeding density was 20 zooplankton organisms per fish for live feed and 2% of the fish weight for FDF. Feeding frequency was 3 times per day and flow through incubators were cleaned two times daily.

Increased mortality rates were observed in experiment C, *i.e.* where ≥ 2.5% NaCl containing feed was administered to *L. lota* for 21 d. These may be explained by three hypothesis: (a) *L. lota* do not tolerate ≥ 2.5% NaCl concentrations in the feed, (b) the FDF is not fully digestible, and (c) the density of the feed is not optimal. The latter hypothesis was developed as the non extruded feed had a very low sinking rate and fish took the feed mainly from the water surface. In case of validity of hypothesis (a) the developed weaning method would be successless. To counteract the potentially low digestiblity of the dry feed (hypothesis b) zooplankton was incorporated in FDF as it was found to improve feed quality in previous studies (Lahnsteiner & Kletzl, 2014; Lahnsteiner & Kletzl, 2015). To increase the density of feed (hypothesis c) a feed extrusion step was included. These hypotheses were tested in experiment D.

Table 1. Small scale experiments design

Experiments	**Trials**
A. Direct weaning to FDF Fish age 148 dph, duration of experiment: 21 d, sampling: after 0, 7, 14, and 21 d	1. FDF 2. FDF, co-feeding with zooplankton every 7th day 3. Live zooplankton control
B. Effect of different feed attractants on weaning success Fish age: 140 dph, duration of experiment: 14 d, sampling: after 0 and 14 d	1. FDF with 2.5% betaine 2. FDF with 2.5% glutamate 3. FDF with 2.5% glycine and 2.5% alanine 4. FDF with 5% fish extract 5. FDF with 50% zooplankton 7. FDF with 2.5% NaCl 8. FDF without attractants 9. Live zooplankton control
C. Effect of incorporation of NaCl into FDF on weaning success Fish age: 145 dph, duration of experiment: 21 d, sampling: after 0 and 21 d	1.FDF without NaCl 2. FDF with 2.5% NaCl 3. FDF with 5% NaCl 4. FDF with 10% NaCl 5. Live zooplankton control
D. Effect of supplementation of dry feed with zooplankton and of feed extrusion on weaning success Fish age: 145 dph, duration of experiment: 21 d, sampling: after 0 and 21 d	1. FDF with 5% NaCl 2. FDF with 50% heat dried zooplankton and 5% NaCl 3. Extruded FDF with 50% zooplankton and 5% NaCl 4. Live zooplankton control

2.4 Large Scale Experiments for Testing Different Weaning Protocols

Large scale experiments were conducted in duplicate with a number of 2000 fish (145 dph) per tank. The tanks were circular, had a volume of 250 l, and were supplied with ground water at a flow rate of 0.5 l/sec. Feed was administered with automatic fish feeders at a rate of 2% of the body weight and the duration of the experiment was 70 d. Sampling was performed in 7 d intervals in a similar way as described for the small scale experiments and 10 fish were taken in each sampling. The tested weaning protocols are shown in Table 2.

Table 2. Large scale experiments design

Feeding duration	Feeding regime 1	Feeding regime 2	Feeding regime 3
0-7 d	Zooplankton containing FDF with 5% NaCl	Zooplankton containing FDF with 5% NaCl	Zooplankton containing FDF with 5% NaCl
8-14 d			
15-21 d	Zooplankton containing FDF with 3% NaCl		
22-28 d	Zooplankton containing FDF with 1% NaCl	FDF	
29-35 d	Zooplankton containing FDF		
36-42 d	FDF		
43-70 d			

2.5 Sampling and Determination of Fish Viability Parameters

Dead fish were daily counted and recorded and mortality rates were calculated. Body mass, total length, condition factor, hepatosomatic index, and the content of the digestive tract were determined in the small scale experiments. Hepatosomatic index is beside of body mass and condition factor an important parameter determining the nutrition status of fish as it decreases in starving fish (Azodi, Ebrahimi, & Motaghi, 2015; Lahnsteiner unpublished data). In the large scale experiments only body mass, total length and condition factor were evaluated.

Before sampling fish were homogenously distributed in the tanks and 10 fish were randomly taken from each tank at each sampling date. Fish samples from the small scale experiments were killed by an over dose of MS-222. Total length was determined to the nearest 0.2 mm in a stereomicroscope and fish weight to the nearest mg with an analytical balance. Using these parameters the condition factor was calculated (condition factor = body mass/total length^3 × 100). Liver and intestine were excised. Liver mass was determined to the nearest 0.1 mg and the hepatosomatic index was calculated (hepatosomatic index = liver mass/body mass × 100). The digestive tract was analyzed in a stereomicroscope on the following parameters: presence or absence of feed, presence of FDF visible as a brownish mass, presence of zooplankton visible as remnants of crustacean chitin shells.

Fish sampled from the large scale experiments were anaesthetized in 0.2% MS 222 for circa 10 min, and body mass and total length were determined. After processing fish were restocked in the tanks.

2.6 Statistics

The mortality rate of fish was expressed as percentage of dead fish in relation to the total number of fish and reported as mean ± standard deviation. Morphometric data and indices were presented as mean ± standard deviation, too. Before statistical analysis data were tested on normality. Percentage data were transformed by angular transformation ($\arcsin\sqrt{P}$) and morphometric data by a logarithmic transformation to reach the assumptions of normal distribution. To determine whether viability parameters differed significantly between treatments (experiment B, C, D), one way analysis of variance (ANOVA) with subsequent Dunnett's T3 test was used (independent variable treatment procedure, dependent variable viability parameters). Time specific differences in viability parameters (*i.e.* experiment A and large scale weaning experiments) were analyzed by ANOVA procedures, too, using species and development time as independent variables and viability parameters as dependent variables. Dunnett's T3 test was used as multiple comparison posthoc test.

3. Results

3.1 Abrupt Weaning to FDF (Figure 1)

When the diet of fish, which had been fed with live zooplankton for 148 dph, was changed to FDF, the body mass, condition factor, and hepatosomatic index decreased significantly and continuously during the duration of the experiment (21 days) (Figures 1a, 1c, and 1d). The fish total length remained constant (Figure 1b). At the onset of the experiment the digestive tracts of 97±6% of the investigated fish (n = 3, representing 3 tanks) contained feed, but after 7, 14, and 21 days they were empty in 100% of the fishes.

When the fish were co-fed with live zooplankton every 7th day, the body mass, condition factor, and hepatosomatic index decreased significantly, too, (Figures 1a, 1c, and 1d), however the decrease was less drastically. Also in this experiment the fish total length remained constant (Figure 1b). On day 7, 14, and 21 the digestive tract of 97±6%, 93±5%, and of 97±6% of the investigated fish, respectively, contained feed which consisted of remnants of crustaceans. Additional fish investigations on day 10 and 18 revealed empty digestive tracts.

In control fish which were fed with live zooplankton, body mass and total length increased significantly (Figures 1a and 1b), while condition factor and hepatosomatic index remained constant (Figures 1c and 1d). Percentage of fish with feed in the digestive tract was 97±6% on day 7, 100±0% on day 14, and 97±6% on day 21. Fish mortality was ≤ 5% in all experiments and not significantly different between the treatments (data not shown).

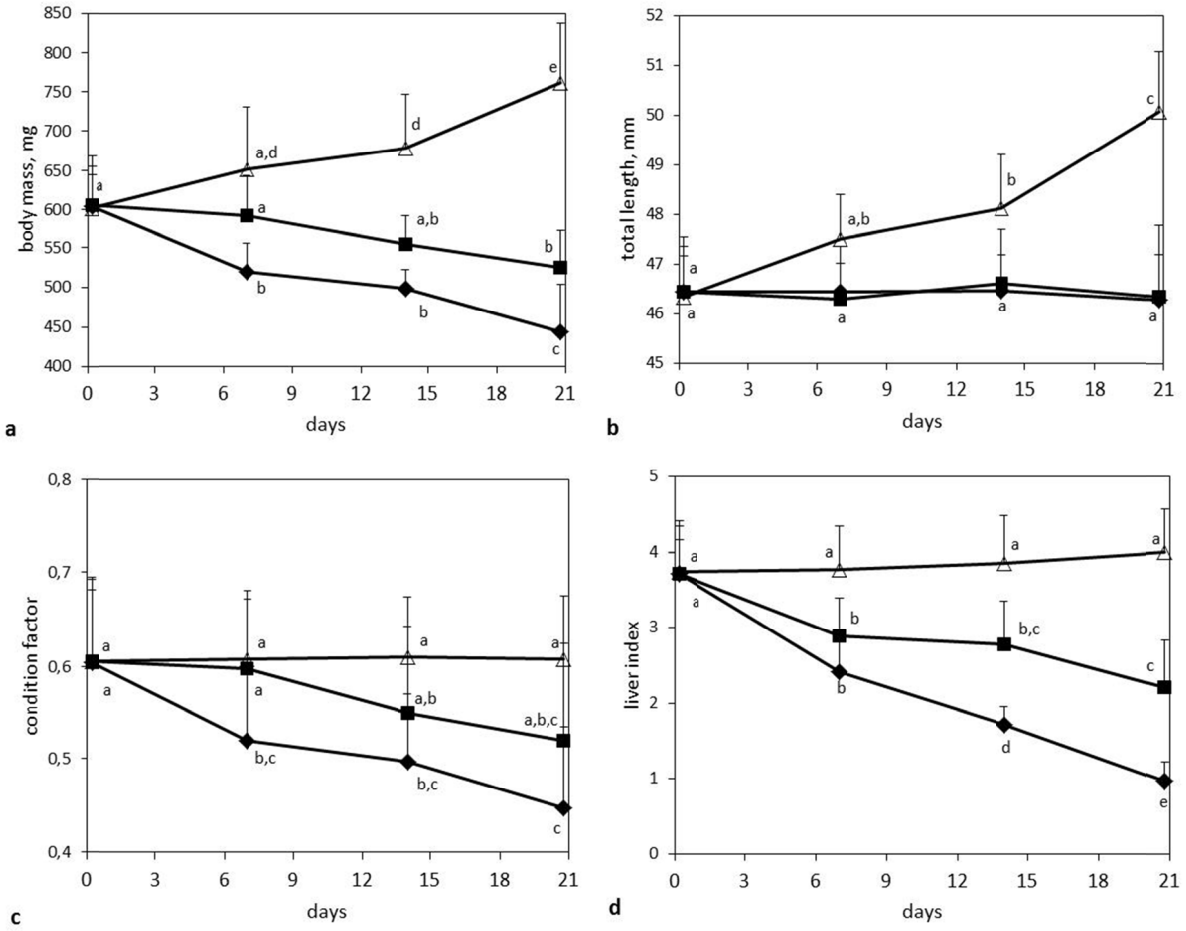

Figure 1. Effect of weaning of 148 dph *L. lota* from live zooplankton to formulated dry feed (FDF) on body mass, total length, condition factor, and hepatosomatic index

Note. ◆ FDF, ■ FDF and co-feeding with live zooplankton every 7th day, Δ live zooplankton control. Sample number = 30 (10 fish from 3 different tanks, respectively). Data superscripted by different letters are significantly different, $P < 0.05$.

3.2 Effect of Different Feed Attractants on Weaning Success (Table 3)

In this experiment the diet of fish, which had been fed with live zooplankton for 140 dph, was changed to FDF supplemented with different chemo-attractants. Body mass, condition factor, and hepatosomatic index of fish fed with FDF containing 2.5% betaine, glutamate, glycin or alanine, 50% zooplankton, or 5% fish extract were significantly decreased after 14 days while the total length showed no significant changes. At the onset of the experiment (day 0) the digestive tract of the 90% of the investigated fish contained remnants of crustaceans, while no feed was detectable after 14 days.

In fish fed with FDF containing 2.5% NaCl for 14 d, the body mass, total length and condition factor were similar to the onset of the experiment, the hepatosomatic index was significantly decreased. A percentage of 77±6% of the investigated fish had feed in the intestine.

Highest body mass and total length were recorded in zooplankton fed fish. Most fish (97±3%) had feed in the intestine. The condition factor and hepatosomatic index of zooplankton fed fish was unchanged to the onset of the experiment.

Fish mortality was ≤ 5% in all experiments and not significantly different between the treatments (data not shown).

Table 3. Effect of weaning of 140 dph *L. lota* from live zooplankton to formulated dry feed (FDF) containing different chemo-attractants on fish viability parameters

	Total length, mm	Body mass, mg	Condition factor	Hepato-somatic index	% feeding fish
Onset of experiment (0 d)					
Control	44.6±3.6 [a]	530±41 [a]	0.598±0.079 [a]	3.2±1.2 [a]	90±10 [a]
After 14 d feeding with FDF containing					
2.5% betaine	44.2±3.3 [a]	445±41 [b]	0.515±0.059 [b]	1.6±0.3 [b]	0±0 [b]
2.5% glutamate	43.3±2.9 [a]	429±28 [b]	0.526±0.061 [b]	1.6±0.5 [b]	0±0 [b]
2.5% glycine & 2.5% alanine	42.9±1.6 [a]	409±26 [b]	0.517±0.072 [b]	1.5±0.7 [b]	0±0 [b]
5% fish extract	43.7±2.1 [a]	428±37 [b]	0.511±0.060 [b]	1.4±0.5 [b]	0±0 [b]
50% zooplankton	45.3±3.5 [a,b]	444±35 [b]	0.509±0.089 [b]	1.4±0.7 [b]	0±0 [b]
2.5% NaCl	45.0±3.6 [a,b]	522±63 [a]	0.599±0.103 [a]	2.4±1.9 [c]	77±6 [c]
no attractants	43.4±2.3 [a]	428±49 [b]	0.524±0.041 [b]	1.5±0.8 [b]	0±0 [b]
After 14 d feeding with live feed					
Zooplankton	48.5±1.9 [b]	679.9±61 [d]	0.595±0.056 [a]	3.3±1.1 [a]	97±3 [a]

Note. % feeding fish: percentage of fish with feed in the intestine. Fish mortality was < 5% and not sgnificantly different between the treatments. Sample number (n) = 30 (10 fish from 3 different tanks, respectively) for metrical data, n = 3 (data for the 3 different tanks) for percentage data. Data superscripted by different letters are significantly different, $P < 0.05$.

3.3 Effect of Incorporation of NaCl Concentrations into FDF on the Weaning Success (Table 4)

L. lota, which had been fed with live zooplankton for 145 dph, were adapted to FDF supplemented with different NaCl concentrations. After 21 days of feeding with FDF containing 5% NaCl, body mass was significantly increased in comparison to the onset of the experiment (0 d control). The increase was similar to live zooplankton. Feed with 2.5 and 10% NaCl resulted in no growth as the body mass was similar to the 0 d control. Administering FDF without NaCl for 21 d resulted in a significant reduction of body mass.

Also condition factor and hepatosomatic index were affected by the feeding regimes. When feeding FDF without NaCl, the condition factor was decreased in comparison to the 0 d control, when feeding FDF with 2.5% or 10% NaCl it was similar to the 0 d control, when feeding FDF with 5% NaCl or live zooplankton it was increased. The hepatosomatic index was decreased in comparison to the 0 d control with 0%, 2.5%, or 10% NaCl, but similar to zooplankton with 5% NaCl. No significant changes in total length were observed in this experiment.

Mortality rates were significantly increased at 2.5%-10% NaCl concentrations, with a maximum at 5-10%. Mortality occurred 14-21 days after the onset of the experiment. 2-4 days before the fish died, their body cavity became enlarged, they started to swim at the water surface and were unable to descend in deeper water layers. However, they did not stop feeding. Dissections of dead fish revealed that they had severely enlarged swim bladders. They differed neither in total length, body mass, condition factor, hepatosomatic index, nor content of the digestive tract from live fish. Unpublished examinations on ecto- and endoparasites and common fish bacteria were negative. Increased NaCl concentrations might have affected the kidney function. However, unpublished histological investigation revealed no pathological alterations in comparison to healthy fish.

Table 4. Effect of weaning of 145 dph *L. lota* from live zooplankton to formulated dry feed (FDF) containing different NaCl concentrations on fish viability parameters

	Total length, mm	Body mass, mg	Condition factor	Hepato-somatic index	% feeding fish	% mortality
Onset of experiment (0 d)						
Control	42.2±7.8 [a]	448±56 [a]	0.596±0.110 [a,c]	4.4±1.2 [a]	93±6 [a]	-
After 21 d feeding with FDF containing						
0% NaCl	42.3±9.2 [a]	344±51 [b]	0.458±0.115 [b]	1.7±0.7 [b]	0±0 [b]	1±1 [a]
2.5% NaCl	42.3±6.8 [a]	464±39 [a]	0.568±0.069 [a]	2.6±0.9 [c]	90±0 [a]	7±4 [a]
5% NaCl	46.6±8. 3 [a]	648±45 [d]	0.642±0.070 [c]	4.0±0.8 [a]	93±6 [a]	23±5 [b]
10% NaCl	43.0±7.9 [a]	460±35 [a]	0.579±0.062 [a]	2.3±0.7 [c]	96±5 [a]	26±4 [b]
After 21 d feeding with live feed						
Zooplankton	46.6±8.8 [a]	642±39 [d]	0.635±0.068 [c]	4.4±1.0 [a]	93±6 [a]	1±1 [a]

Note. % feeding fish: percentage of fish with feed in the intestine. Sample number = 30 (10 fish from 3 different tanks, respectively) for metrical data, n = 3 (data for the 3 different tanks) for percentage data. Data superscripted by different letters are significantly different, $P < 0.05$.

3.4 Effect of Supplementation of FDF with Dried Zooplankton and of Feed Extrusion on Weaning Success (Table 5)

Fish fed with live zooplankton for 145 days were used in the experiment. Feeding *L. lota* with FDF containing 5% NaCl (FDF-5NaCl), with a mixture of 50% FDF, 50% dried zooplankton and 5% NaCl (FDF-Zoo-5NaCl), or with the extruded FDF-Zoo-5NaCl for 21 days resulted in a significant increase in body mass similar to live zooplankton. Total length increased non-significantly. Condition factor and hepatosomatic index were similar to the zooplankton control. A percentage of > 90% of the fish had feed in the digestive tract. However, fish mortality was significantly different between the 3 feed types. For FDF-5NaCl and FDF-Zoo-5NaCl it was significantly highest (15%-26%). For the extruded FDF-Zoo-5NaC the mortality rates were < 5% and not significantly different from the zooplankton control.

3.5 Large Scale Weaning Experiments (Figure 2)

Feeding regime 1 (feeding zooplankton containing FDF with 5% NaCl for 14 d, gradual decrease to FDF from d 22-35) resulted in a continuous and significant increase in body mass and total length. Condition factor increased steadily but not significantly. Increased standard deviation in body mass, total length and condition factor indicated the necessity for sorting the fish according to size classes after ≥ 63 d.

When applying feeding regime 2 (feeding zooplankton containing FDF with 5% NaCl for 21 d, thereafter abrupt change to NaCl free feed) body mass and total length stagnated from d 21 to d 42. Therafter, the two paramters increased continously and significantly. Condition factor remained unchanged. The final body mass, total length and condition factor at the end of the experiment were significantly reduced in comparison to feeding regime 1.

With feeding regime 3 (permanent feeding FDF with 5% NaCl and 50% zooplankton) growth stagnated after ≥ 42 d. Therefore the experiment was stopped after 56 d. Mortality was < 5% in all experiments.

Table 5. Effect of supplementation of FDF with dried zooplankton and of feed extrusion on the weaning success in *L. lota*

	Total length, mm	Body mass, mg	Condition factor	Hepato-somatic index	% feeding fish	% mortality
0 d control, onset of experiment						
Control	42.7±7.8 [a]	471±42[a]	0.605±0.101[a]	4.3±1.1 [a]	96±3 [a]	-
After 21 d feeding with						
FDF with 5% NaCl	47.4±8.0 [b]	675±46[b]	0.634±0.074[a]	4.3±1.4 [a]	100±0 [a]	26±6 [a]
FDF with 50% zooplankton and 5% NaCl	47.6±8.3 [b]	691±42[b]	0.641±0.076[a]	4.2±1.0 [a]	96±3 [a]	15±7 [a]
Extruded FDF with 50% zooplankton and 5% NaCl	47.8±7. 5 [b]	686±44[b]	0.628±0.091[a]	4.2±1.1 [a]	93±6 [a]	4±3 [b]
After 21 d feeding with live feed						
Zooplankton	48.0±8.8 [b]	697±41[b]	0.630±0.102[a]	4.4±1.0 [a]	96±3 [a]	2±2 [b]

Note. % feeding fish: percentage of fish with feed in the intestine. Sample number = 30 (10 fish from 3 different tanks, respectively) for metrical data, n = 3 (data for the 3 different tanks) for percentage data. Data superscripted by different letters are significantly different, $P < 0.05$.

4. Discussion

The present study investigated the weaning of *L. lota* from live zooplankton feed to FDF 140-150 days post hatch. The time point for weaning was selected based on the following considerations. In this life stage fish had a fully functional digestive tract (Palińska-Żarska, Żarski, Krejszeff, Nowosad, Biłas, Trejchel, Brylewski, Targońska, & Kucharczyk, 2014) and due to their size were robust to handling, experimental procedures and insensitive to diseases. These aspects are important to obtain an accurate and solid data base on weaning parameters of *L. lota*. In previous studies where *Artemia* was used as starter feed, weaning was tested in earlier life stages (35-50 dph – Wocher, Harsányi, & Schwarz, 2012; Palińska-Żarska, Żarski, Krejszeff, Kupren, Łączyńska, & Kucharczyk, 2015) as this type of live feed is suitable for nutriton of larvae only for limited periods (Karlsen, van der Meeren, & Rønnestad, 2015). Weaning time points as tested in the present study are practicable when high quality and low cost live feeds are used. This is the case when fish are pond reared with natural feed or reared under semi-intensive conditions with live zooplankton as described in the present study.

The present study demonstrated that 140-150 dph *L. lota* refused the tested FDF. Also other types of formulated starter feeds were refused as found in unpublished experiments. When FDF and zooplankton were administered together in co-feeding regimes, *L. lota* selected exclusively the zooplankton, while FDF was refused, too. From the experiments it is concluded that *L. lota* could not be forced to feed on FDF. Generally, the weaning success depends also on the development stage of fish as most species have an optimal period for weaning (Qin, 2008). Once fish pass this specific period, they adapt to live feed and acceptation of FDF becomes very difficult (Qin, 2008). *L. lota* did not start feeding FDF even after extended starving periods of up to 14 days when body weight, hepatosomatic index, and condition factor had significantly decreased. Starving periods longer than 14 d resulted in increased susceptibility to diseases and mortalities and therefore were inacceptable for a sustainable weaning process.

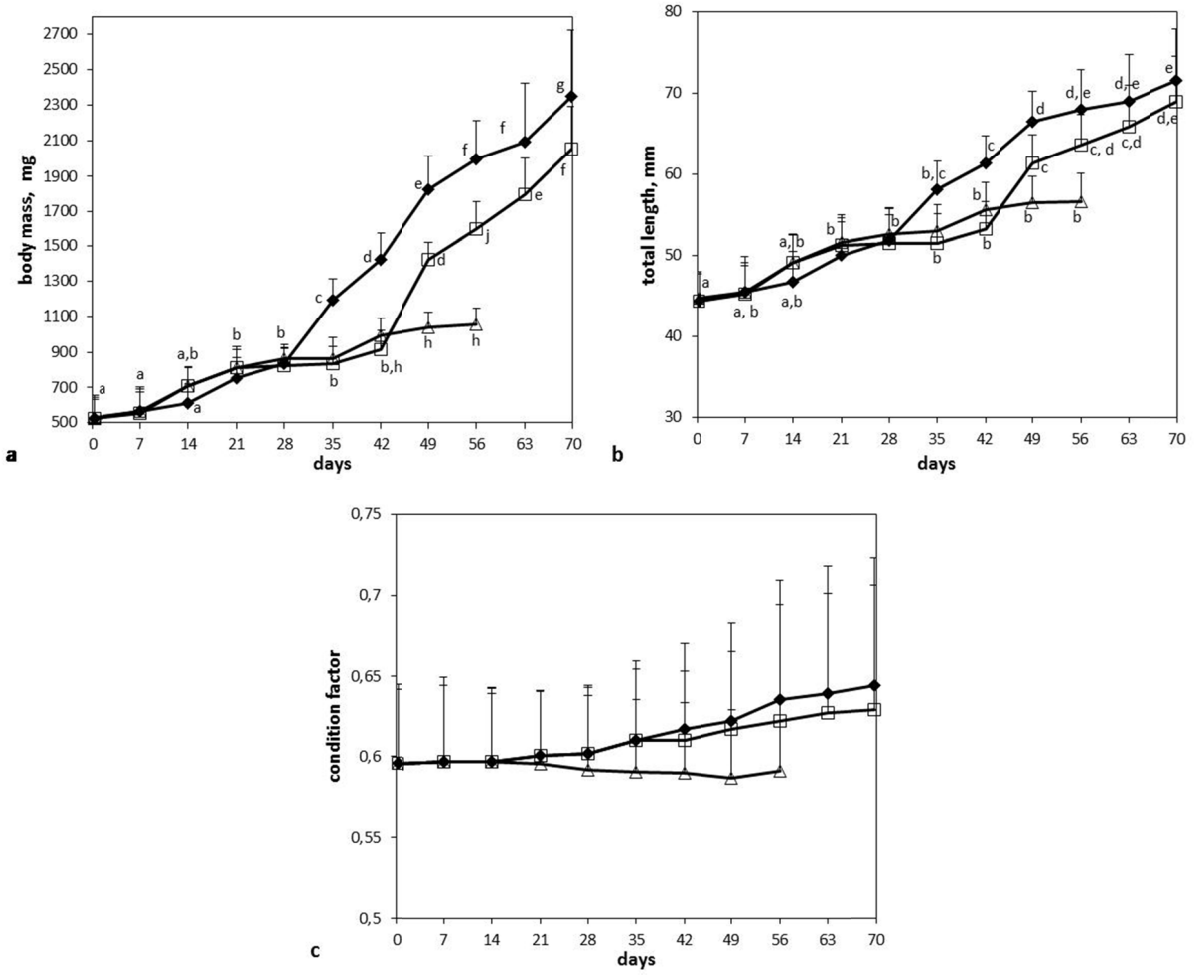

Figure 2. Effect of weaning of 148 dph *L. lota* from live zooplankton to commercial formulated dry feed (FDF) on body mass, total length, and condition factor

Note. ◆ feeding regime 1 (stepwise adaption from zooplankton containing FDF with 5% NaCl to pure FDF), □ feeding regime 2 (abrupt change from zooplankton containing FDF with 5% NaCl to pure FDF after 21 d), Δ feeding regime 3 (continuous feeding with zooplankton containing FDF with 5% NaCl). All feed mixtures were extruded. Sample number = 30 (10 fish from 3 different tanks, respectively). Data superscripted by different letters are significantly different, $P < 0.05$. No significant changes were found in condition factor.

Copepods and cladocerans, the main organismic groups of the used zooplankton had a high attractiveness for *L. lota*. It is likely that their attractiveness was not due to visual triggers but to chemical ones as the organisms were selectively picked out of wet feed mixtures containing also FDF (unpublished experiments). When zooplankton was incorporated into FDF in form of a meal, chemoattraction was lost possibly due to the drying process indicating the heat instability of the involved molecules. Nothing is known about molecules making copepods or cladocerens attractive to fish. However, several studies demonstrated intensive intraspecific and interspecific chemical interactions in copepods (Heuschele & Selander, 2014). Such messenger substances might also be recognized by fish.

Betaine, L-alanine, L-glutamic acid, and glycine are highly water soluble substances, able to stimulate the olfactory bulb of fish, and therefore they are used as dietary feeding attractants for various fresh water and marine fish species (Hara, 2006; Olsén & Lundh, 2016). However, in the conducted experiments these substances had no effect in *L. lota*. Also extract of fish filets which contains trimethylamines detectable in very low concentrations by chemoreceptors of teleost fish (Hussain, Saraiva, & Korsching, 2009) was no attractant for *L. lota*. Only NaCl stimulated feed uptake in *L. lota*, indicating that this fish species can localize and detect electrolytes. No recent

information is available how electrolytes stimulate chemoreceptor activity in teleost fish and specifically in *L. lota*.

This is the first time that NaCl was identified as a dietary feeding attractant for fish. In our experiments the lowest effective dose was 2.5%. At this concentration feed uptake was suboptimal but sufficient to keep body weight and condition factor stable, i.e. at a value similar to the onset of the experiment. The optimal NaCl concentration for weaning was 5% as at this concentration feed uptake was high and the growth similar to that obtained with zooplankton. Elevated NaCl concentrations of 10% and prolonged feeding with diets containing 5% NaCl (see large scale weaning experiments) were also suboptimal as they led to stagnation in growth. Data on the organismic response to dietary salt uptake are available for rainbow trout (Salman & Eddy, 1988; James-Curtis & Wood, 1992). For trout fed a dietary NaCl concentration of 5% branchial Na^+ influx rates were reduced to compensate for the NaCl uptake across the digestive tract. All of the dietary NaCl was reabsorbed by the kidney tubules, indicating that this organ removed NaCl from the urine, regardless of the NaCl concentration (Salman & Eddy, 1988; James-Curtis & Wood, 1992). NaCl concentrations > 5% induced an elevation in plasma Na^+ concentration, increased gill efflux and decreased branchial Na^+ uptake indicating disturbance in osmotic equilibrium (Salman & Eddy, 1988; James-Curtis & Wood, 1992). The latter factors might be responsible for growth stagnation observed in the present study.

Generally, fish larvae can detect feed via a wide range of chemical, visual and mechanical stimuli (Carr, Netherton, Gleeson, & Derby, 1996; Kasumyan & Døing, 2003). In most fish, the basic sensory organs develop rapidly in the course of the first few days after hatching that prey can be detected at first feeding (Carr, Netherton, Gleeson, & Derby, 1996; Kasumyan & Døing, 2003). The timing and sequence of further development of sensory tissues and organs is species specific and depends on the feeding behaviour (Kasumyan & Nikolaeva, 2002; Kasumyan & Døing, 2003). Therefore, it has to be tested in future studies if NaCl is a dietary attractant for *L. lota* also in earlier life stages.

Once *L. lota* were conditioned to FDF by means of NaCl, its concentration could be gradually decreased to 0% and fish proceeded to accept the FDF resulting in fish farm practicable weaning protocols. Contrary, abrupt reduction of NaCl resulted in growth stagnation for circa 20 d and therefore is considered as not practicable for fish farms. The behavioral and sensory adaption mechanisms underlying this process are unknown (Kasumyan & Nikolaeva, 2002) and not part of this study. The here presented method is an easy and sustainable way to adapt *L. lota* from live feed to dry feed.

Composition and quality of FDF was also a critical parameter for weaning of *L. lota* as supplementation of FDF with zooplankton meal was necessary to obtain survival rates similar to live zooplankton feed. Generally, supplementation of FDF with zooplankton meal or *Artemia* meal is a possibility to increase its quality (Lahnsteiner & Kletzl, 2014; Lahnsteiner & Kletzl, 2015). *Thymallus thymallus* and *Coreognus maraena* larvae and juveniles reared exclusively on FDF frequently exhibited increased percentages of malformations, disturbed buoyancy, and increased mortality rates, negative effects which could be compensated by addition of zooplankton or *Artemia* meal (Lahnsteiner & Kletzl, 2014; Lahnsteiner & Kletzl, 2015). FDF of different composition might be better suited for weaning of *L. lota*. Wocher, Harsányi, and Schwarz (2012) found that suitability of FDF for weaning of *L. lota* differed greatly. Generally, it is unknown which components of zooplankton have positive impacts on fish nutrition. Feeding studies conducted on cod larvae with copepod nauplii in comparison to rotifers revealed that copepod fed fish performed better (Karlsen, van der Meeren, & Rønnestad, 2015). Biochemical analyses of the feed showed that from all analyzed compounds protein, taurine, astaxanthin and zinc were higher on a dry weight basis in copepods than in rotifers and might be components responsible for better performance (Karlsen, van der Meeren, & Rønnestad, 2015). Also chitin, a main component of cladocerans and copepods, might play a role either as nutrient or as dietary fibre (Gutowska, Drazen, & Robison, 2004).

Also the feed processing had a significant effect on the weaning success. *L. lota* fed with non-extruded FDF optimized in aspects of composition (supplementation with zooplankton meal and NaCl) developed enlarged swim bladders after circa 20 days of feeding, showed disturbed buoyancy, and subsequently also increased susceptibility to diseases, parameters which finally caused increased fish mortality. Non extruded feed had a low density and a low sedimentation rate and *L. lota* were feeding mainly from the water surface (unpublished data), factors which might have affected gas regulation of the swim bladder. However, there is no casual proof for a relation. Also other still unknown parameters in feed digestibility may have been affected by the extrusion process. Swim bladder enlargement was not associated with bacterial diseases as investigations of swim bladder tissue on potentially gas producing bacteria (*Clostridium perfringens*, *C. difficile*) were negative (unpublished data). Swimbladder hyperinflation was also observed during onfeeding of *L. lota* larvae (Rekecki, Meeus, Chiers, Adriaen, Boyen, Declercq, Van den Broeck, & Decostere, 2016). Similar as in the present study the cause remained uncertain but

was not due to common bacterial diseases. *L. lota* is a physoclistous species having a swim bladder with a gas gland to regulate buoyancy (Iasinski & Kilarski, 1964). In natural environment the gas gland of *L. lota* may adapt in size to environmental conditions (Cott, Guzzo, Chapelsky, Milne, & Blanchfield, 2015).

In summary, the present results demonstrate that 150 dph *L. lota* could not be forced to feed on dry feed. They did not accept FDF and starved to death. NaCl was a dietary feeding attractant. When FDF was supplemented with 5% NaCl it was accepted and *L. lota* could be abruptly weaned from live to dry feed. Once the fish had been conditioned to FDF its concentration had to be gradually decreased to 0%. Also the quality of FDF and its density had a significant effect on the weaning success. Based on the conducted experiments and on the collected data weaning protocols were developed sustainable in aspects of fish health and practicable in fish farms.

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