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On-Feeding and Juvenile Production of Coregonid Species with Formulated Dry Feeds: Effects on Fish Viability and Digestive Enzymes

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Abstract

The present study describes a successful method for on-feeding and fingerling production of *C. atterensis* and *C. marena* using solely formulated dry feeds. Also the development and differentiation of the digestive system in relation to different feed types was investigated.

The tested commercially available starter feeds were not suitable for on-feeding of larvae of the two species. Only self-formulated starter feeds containing zooplankton or *Artemia* in defined quantities and organic acids as preservatives resulted in survival rates and growth rates similar to the live zooplankton control. For successful fingerling production resulting in survival rates and growth rates similar to a live feed control not only a suitable starter feed was necessary, but also specific feeding regimes where starter feed was replaced with intermediate feed and then with commercial feed in defined chronological sequences.

Trypsin, chymotrypsin, lipase, phospholipase A, and acid and alkaline phosphatase occurred in the digestive tract of larvae of *C. atterensis* and *C. marena*. At the onset of exogenous feeding the digestive system of *C. atterensis* was less developed than the digestive system of *C. marena*. In both species the activities of the investigated digestive enzymes depended strongly on the administered feed types. Inadequate feeds significantly decreased activities of the assayed digestive enzymes.

Keywords: *Coregonus*, larvae, fingerling, digestive enzyme, dry food, feeding regime

1. Introduction

In Europe, two major mtDNA lineages of coregonids are distinguishable, the Baltic-Scandinavian and the Alpine lineages (Østbye, Bernatchez, Næsje, Himberg, & Hindar, 2005). The lakes at the Northern rim of the Alps were originally inhabited by a species from the Alpine lineage, *Coregonus atterensis* (Kottelat & Freyhof, 2007). In the last century a coregonid species of the Baltic-Scandinavian lineage, *Coregonus maraena* (Kottelat & Freyhof, 2007), was first introduced in pond aquaculture and then also stocked in many prealpine lakes due to higher growth rates. Interestingly, the two coregonid populations did not mix as temporarily different spawning times limit hybridization (Ritterbusch-Nauwerck & Lahnsteiner, 2005; Pamminger-Lahnsteiner, Weiss, Winkler, & Wanzenböck, 2009).

Both species are excellent food fish. Under aspects of product quality, *C. atterensis* is considered as superior to *C. marena*, mainly as it is a typical, local fish. To increase the competing power of fish famers, concepts were developed for diversification in the food fish sector and for production of local, high quality goods. Neither *C. marena* and nor *C. atterensis* have been introduced in intensive aquaculture until now. Presently, *C. marena* is produced under extensive conditions in pond aquaculture and *C. atterensis* is solely harvested from wild populations.

Methods for fingerling production are an important first step to introduce the two species in aquaculture. Formulated dry feed is preferred to live feed as the latter is cost-intensive and the production techniques are available only for a limited number of fish farmers. Several studies have been conducted to rear larvae of different coregonid species with formulated dry feed in intensive culture systems (Dabrowski & Kaushik, 1984; Harris &

Hulsmann, 1991; Luczynski, Majkowski, Bardega, & Dabrowski, 1990; Poczyczyński, Dąbrowski, Kucharska, Mamcarz, & Kozłowski, 1990; Leithner & Wanzenböck, 2015). A practical method for fingerling production could not be established under aspects of economy, animal welfare and sustainability as mortality rates and disease susceptibility are high for fish reared solely on dry food.

The present study has the intention to develop a method for first-feeding and fingerling production of *C. atterensis* and *C. marena* using solely formulated dry feeds. The suitability of commercially available fry feeds and self-made feeds was tested and feeding regimes were developed to adapt the fish from special starter feed to commercial feeds. Fish survival rate, fish total length, and weight were used as viability characteristics. To get better knowledge about the suitability and digestibility of different feed types, the development and differentiation of the digestive system during feeding with live feed and formulated dry feed were monitored. Investigated enzymes were trypsin, chymotrypsin, lipase, phospholipase A, and acid and alkaline phosphatases.

2. Method

2.1 Broodfish, Egg Collection and Larvae Production

Mature *Coregonus maraena* (spawning time: middle of November) derived from a pond reared population in Heidenreichstein (Lower Austria), *Coregonus atterensis* (spawning time: end of December) were caught from a wild population from Lake Mondsee. In both species, semen and eggs were stripped and artificial insemination was performed. Fertilized eggs were transported to the fish farm Kreuzstein, disinfected with 1000 ppm formalin for 20 min and incubated in flow through incubators (*C. atterensis*) or zug jars (*C. maraena*) until hatching. Newly hatched larvae were stocked in the appropriate tanks for the experiments.

Ground water source used for egg incubation and the experiments had a temperature of 9.1 ± 0.6, a pH of 7.85 ± 0.02, a conductivity of 335.2 ± 8.4 µS/cm at 25 °C, acid-neutralizing capacities of 3.38 ± 0.02 mval/l, a O_2 concentration of 12.53 ± 0.19 mg/l and PO_4^{3-} and NH_4^+ concentrations $\leq$ 0.005 mg/l.

2.2 Tested Live Food and Its Processing

Fresh water zooplankton was collected from Lake Mondsee or Lake Wallersee with plankton nets (mesh size 200 µm for fish < 30 d old and 400 µm for fish $\geq$ 30 d old) (Lahnsteiner & Kletzl, 2015). The collected zooplankton was washed out of the plankton nets into 20 l buckets. For fish larvae that were fed with live zooplankton, the collected zooplankton was diluted to 100,000 individuals per litre and quantities of 10 - 15 individuals per fish larvae were fed in the tanks.

For fish larvae that were fed with self-formulated starter feeds containing zooplankton, water was drained from the collected zooplankton and homogenized using an immersion blender, incorporating 1 g/kg ascorbic acid and 0.25 g/kg tocopherol as antioxidants.

Artemia was cultured according to a standard protocol in 3% seawater (pH 8.5) at 27 ± 1 °C under continuous aeration. Nauplii and copepodites were collected from the cultivation vessels using plankton nets (mesh size 200 µm) 36 h to 48 h after onset of incubation. *Artemia* was not used as life feed, but only incorporated in formulated feeds. It was processed in a similar way as zooplankton, however, it was rinsed for several minutes in fresh water before processing started. The residual water content of *Artemia* was similar to zooplankton and amounted 85 ± 3% as determined in preliminary experiments.

2.3 Tested Formulated Feed Types and Their Preparation

Two commercial starter feeds for fish larvae (commercial starter feed A, commercial starter feed B), 4 self-formulated starter feeds (self-formulated starter feeds 1, 2, 3, and 4), and an intermediate feed were tested. The composition of the different feeds is shown in the scheme below. All types of self-formulated starter feeds consisted of low temperature fish meal, lactalbumin, salmon fish oil, soya lecithin, vitamin mix, and mineral mix (Lahnsteiner, Kletzl, & Weismann 2012; Lahnsteiner & Kletzl, 2015). These components were mixed in a defined ratio constant in weight and the mixture was termed component mixture. Homogenized zooplankton or *Artemia* was added in different ratios to the component mixture and all ingredients were mixed well for approximately 5 min using an immersion blender.

An intermediate feed was formulated as it was not possible to adapt fish from self-formulated starter feeds to commercial feed (see section 3.2). The commercial fry feed B was ground in a corn mill (Fidibus 21 basic, Komo-Koidl KG, Austria) to a particle size < 300 µm and homogenized zooplankton was added as indicated in the scheme below. For the self-formulated starter feeds 3 and 4 and for the intermediate feed, there was a risk of spoiling during the drying process due to the high initial water content of the zooplankton and *Artemia*. Therefore, propionic acid in a concentration of 1% was used to preserve the feed due to easy spoilage. This type

of organic acid was determined as the most efficient preservative in comparison to other tested organic acids (0.5 – 2% citric acid, acetic acid, benzoic acid). Finally, the mixtures were spread in an approximately 0.5 cm thick layer on baking trays and dried for 12 h at 80 °C. For the measurement of enzymatic activities, one portion of self-formulated starter feed 3 was also dried at 40 °C for 24 h. Dried feeds were ground to a required particle size (300–400 µm for the first 20 d of feeding, gradual increase in size for older fish) using a corn mill. The self-formulated starter feeds were sealed in vacuum bags and stored at -20 °C until use.

Table 1. Tested formulated feed types

Feed type	Main composition
Commercial starter feed A	Extruded starter feed for sensible fish larvae with 62% protein, 11% fat, 8% carbohydrates, 0.8% fibres. Listed ingredients: LT fish meal, fish oil, wheat, soya lecithin, cod oil, shrimp meal, fish bouillon. 19 MJ/kg digestible energy
Commercial starter feed B	Extruded feed for salmonid fry with 64% protein, 9% fat, 0.5% fibers, and immune-stimulants. Listed ingredients: fish meal, wheat gluten, wheat, and fish oil. 15 MJ/kg digestible energy
Self-formulated starter feed 1	Component mixture : homogenized zooplankton = 9 : 1 (dry mass : dry mass)
Self-formulated starter feed 2	Component mixture : homogenized zooplankton = 1 : 1 (dry mass : dry mass)
Self-formulated starter feed-3	Component mixture : homogenized zooplankton = 1 : 1 (dry mass : dry mass) and 1% propionic acid (final concentration in dry food)
Self-formulated starter feed 4	Component mixture : homogenized artemia = 1 : 1 (dry mass : dry mass) and 1% propionic acid (final concentration in dry food)
Intermediate feed	Commercial feed B : homogenized zooplankton = 1 : 1 (dry mass : dry mass) and 1% propionic acid (final concentration in dry food)

2.4 First Feeding Experiment

The on-feeding experiment lasted 25 d. A number of 5,000 fish was stocked in circular tanks (2 tanks per treatment) with a volume of 250 l, respectively. Tanks were supplied with ground water at a flow rate of 0.25 l/sec. Each experiment was conducted 2 times. In *C. marena* commercial starter feeds A and B and self-formulated starter feeds 1, 2, 3 and 4 were tested, in *C. atterensis* commercial starter feed B and self-formulated starter feeds 1, 3, and 4. Dry feed was administered with automatic fish feeders for 16 h per day. Live zooplankton (200 – 400 µm size fraction) at a density of 10 - 15 individuals per fish larvae (200 – 300 individuals per liter water) was used as control. It was fed to the fish larvae 4 times per day. Tanks were cleaned two times daily and dead fish were removed and counted.

2.5 Fingerling Production Experiment

The tested feeding regimes I – VII are shown in Table 2:

Table 2. The tested feeding regimes I – VII

	0 - 10 d	**11 - 20 d**	**21 - 30 d**	**31 - 40 d**	**> 40 d**
Feeding regime I	Commercial B	Commercial starter feed B	Commercial starter feed B	Commercial starter feed B	Commercial starter feed B
Feeding regime II	Self-formulated starter feed 3	Self-formulated starter feed 3	Commercial starter feed B	Commercial starter feed B	Commercial starter feed B
Feeding regime III	Self-formulated starter feed 3	Self-formulated starter feed 3	Self-formulated starter feed 3	Commercial starter feed B	Commercial starter feed B
Feeding regime IV	Self-formulated starter feed 3	Intermediate feed	Intermediate feed	Intermediate feed	Commercial starter feed B
Feeding regime V	Self-formulated starter feed 3	Self-formulated starter feed 3	Intermediate feed	Intermediate feed	Commercial starter feed B
Feeding regime VI	Self-formulated starter feed 3	Self-formulated starter feed 3	Self-formulated starter feed 3	Intermediate feed	Commercial starter feed B
Feeding regime VII	Live zooplankton	Live zooplankton	Live zooplankton	Live zooplankton	Live zooplankton

All seven feeding regimes were tested on *C. marena*. Based on the results obtained in *C. marena*, feeding regimes I, II and III were excluded for *C. atterensis*. The duration of the experiments lasted for 60 d. The best feeding regime, i.e. no. V, was monitored for 90 d in both species. After 60 d the water temperature was increased to 13.0 ± 1.5 °C for better growth. A number of 10,000 fish was stocked in circular tanks (2 tanks per treatment) with a volume of 500 l. Similar as in the on-feeding experiment, tanks were supplied with ground water at a flow rate of 0.25 l/sec and each experiment was conducted 2 times. Tank cleaning procedure, evaluation of dead fish and food administration was also similar as described for the on-feeding experiment. After 20 – 30 d particle size of dry feed and zooplankton were increased to 400 – 500 µm and > 400 µm, respectively depending on the species and growth of the fish.

2.6 Fish Viability Parameters

Dead larvae were counted and recorded two times daily after cleaning the tanks. Sampling of larvae for determination of total length and weight was performed in 10 d intervals. In the results only end points (25 d for on-feeding experiment, 60 d and 90 d, respectively, for fingerling production experiment) are reported. For sampling purpose, fish were homogenously distributed in the tanks by gentle mixing preferably during cleaning process. Thereafter, 20 fish were randomly taken from each tank and killed by an over dose of MS-222. Fish were inspected macroscopically on malformations and total length was determined to the nearest 0.1 mm in stereomicroscope and fish weight to the nearest mg with an analytical balance.

2.7 Investigation of Digestive Enzymes

Trypsin, chymotrypsin, lipase, phospholipase A, acid and alkaline phosphatase activity were investigated in the digestive tract of *C. atterensis* and *C. marena* larvae sampled on the day before the first feeding and after a feeding period of 25 d with live zooplankton. The described enzyme activities were also investigated in *C. atterensis* and *C. marena* larvae which had been fed for 30 d with self-formulated starter feed 3 or commercial starter feed B in comparison to fish fed on live zooplankton. Fish were killed by an overdose of MS-222 and the whole digestive tract was removed by micro-preparatory techniques. Feces and remnants of feed were squeezed out with fine forceps and the intestine was rinsed in 0.1 mol/l tris buffer (pH 7.5). Five digestive tracts were pooled to one sample, respectively, to obtain enzyme activities and sample volumes reliable for analysis. Seven samples were prepared for each experiment and for each species.

The occurrence of digestive enzymes was also investigated in selected food types, i.e. fresh zooplankton and fresh *Artemia*, in self–formulated starter feed 3 dried at 40 °C or 80 °C, and in commercial fry feed B. Three different samples of live feeds and 3 different charges for dry feed were investigated, respectively. Aliquots of approximately 200 mg homogenized *Artemia*, zooplankton, or dry feeds were used per sample.

All samples were diluted with the tris buffer described above, homogenized with a pestle homogenizer. Insoluble particles were centrifuged at 1000 g for 5 min at 4 °C and the supernatants were stored at -20 °C. All enzymatic assays were performed at 20 ± 1 °C. Acid phosphatase and alkaline phosphatase were determined with fixed time methods and *p*-nitrophenyl phosphate as substrate (70 mmol/l at pH 4.5 for acid phosphatase and 50 mmol/l at pH 9.5 for alkaline phosphatase) (Bergmeyer, 1985). For trypsin determination a colorimetrical assay was used with 1 mmol/l N_{α}-benzoyl-L-arginine 4-nitroanilide as substrate in the presence of 2 mmol/l EDTA and 2.5 mmol/l cysteine (pH 7.5) (Bergmeyer, 1985). Chymotrypsin was determined UV-spectrophomometrically with 0.5 mmol/l N-benzoyl-L-tyrosine ethyl ester as substrate and at a $CaCl_2$ concentration of 4 mmol/l (pH 7.8) (Bergmeyer, 1985). Lipase was assayed in two ways, colorimetrically with 0.25 mmol/l 4-nitrophenyl dodecanoate as non-micellar substrate (pH 8.2) and by the liberation of fatty acids using a 0.5% triolein emulsion in triton X100 supplemented sodium phosphate buffer (pH 8.2) as substrate (Pinsirodom & Parkin, 2001). Liberated fatty acids were extracted into benzene and measured with the cupric acetate/pyridine reagent (Lowry & Tinsley, 1976). Phospholipase A was investigated with 4-nitro-3-octanoyloxy benzoic acid at $CaCl_2$ concentrations of 10 mmol/l at pH 8.0 (Pinsirodom & Parkin, 2001). Adequate blanks were run for each assay to exclude interactions from other enzymes in the crude extract and to exclude background reaction due to dissociation of chromogenic substrates. Protein was determined with the Lowry procedure (Lowry, Rosenbrough, Farr, & Randall, 1951).

2.8 Statistics

The survival rate of fish was expressed as percentage of surviving fish, respectively, relative to the total number of fish and reported as mean ± standard deviation. Morphometric data are 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. For survival rate the sample number was 2 resulting from two different tanks. To determine

whether these parameters differed significantly between treatments, analysis of variance (ANOVA) with subsequent Waller–Duncan post hoc test was used. For fish weight and fish length the sample number was 40 resulting from 20 fish, respectively, from the two different tanks. To determine significant differences between these parameters, analysis of variance (ANOVA) with subsequent Tukey's *b*- post hoc test was used.

3. Results

3.1 On-Feeding Experiments of C. marena and C. lavaretus Larvae (Table 3)

The initial weight and total length were significantly higher for *C. marena* than *C. atterensis* larvae. After 25 d of feeding, more than 80% of *C. marena* larvae survived with commercial fry feed B, approximately 50% with commercial fry feed A. Contrary, survival rates for *C. atterensis* were less than 10% with the two commercial fry feeds.

Self-formulated starter feeds 3 and 4 resulted in high survival rates of approximately 80% in *C. marena* and *C. atterensis*. These feeds contained the formulated component mixture and homogenized zooplankton or *Artemia* in a ratio of 1 : 1 (dry mass : dry mass) and 1% propionic acid as preservative. Similar self-formulated starter feeds without propionic acid (no. 2) and self-formulated starter feeds with lower amounts of homogenized zooplankton (no. 1) resulted in lower survival rates not exceeding 50%.

Table 3. Effect of different dry feeds on survival rate, weight, and total length of *Coregonus maraena* and *C. atterensis* larvae after an on-feeding period of 25 d

	Survival (%)		**Weight** (mg)		**Length** (mm)	
	C. marena	*C. atterensis*	*C. marena*	*C. atterensis*	*C. marena*	*C. atterensis*
Day of first feeding	-	-	9.2 ± 0.6^{a}	7.3 ± 0.6^{a}	12.6 ± 0.5^{a}	10.4 ± 0.6^{a}
After 25 d feeding with:						
Commercial fry food A	58 ± 7^{a}	0^{a}	12.8 ± 1.6^{b}	-	14.5 ± 0.8^{b}	-
Commercial fry food B	83 ± 7^{b}	8 ± 7^{b}	20.1 ± 4.3^{c}	9.0 ± 0.7^{b}	18.1 ± 1.4^{c}	12.8 ± 1.2^{b}
Self-formulated feed 1	$36 \pm 11^{a,c}$	8 ± 6^{b}	15.8 ± 2.0^{b}	8.9 ± 0.9^{b}	15.4 ± 0.9^{b}	12.6 ± 1.1^{b}
Self-formulated feed 2	45 ± 10^{a}	22 ± 8^{c}	16.2 ± 3.2^{b}	9.0 ± 1.8^{b}	15.8 ± 1.0^{b}	12.9 ± 1.4^{b}
Self-formulated feed 3	82 ± 6^{b}	81 ± 6^{d}	20.7 ± 2.3^{c}	11.5 ± 0.9^{c}	17.6 ± 1.1^{c}	14.0 ± 1.1^{c}
Self-formulated feed 4	85 ± 6^{b}	78 ± 9^{d}	20.9 ± 1.9^{c}	11.5 ± 1.0^{c}	17.9 ± 1.1^{c}	14.2 ± 1.2^{c}
Live zooplankton	84 ± 9^{b}	84 ± 9^{d}	27.1 ± 6.0^{d}	12.6 ± 0.8^{d}	19.9 ± 1.1^{d}	15.4 ± 1.0^{d}

Note. Data are mean ± standard deviation. For survival rate sample number (n) = 2 resulting from 2 different tanks; for weight and length n = 40 deriving from 20 fish, respectively, from 2 tanks. Values from the same parameter and species and with the same superscript symbol are not significantly different ($P > 0.05$).

After a feeding period of 25 d the weight and total length increase was highest with live zooplankton, followed by self-formulated feed 3 and 4 in both species. Generally, the growth was higher for *C. marena* (weight increase approximately 200% for live zooplankton and 120% for dry feed, length increase approximately 55% for live zooplankton and 40% for dry feed) than for *C. atterensis* (weight increase 65% for live zooplankton and 45% for dry feed, length increase 50% for live zooplankton and 35% for dry feed).

3.2 Experiments for Fingerling Production in C. marena and C. lavaretus Larvae (Table 4)

When feeding *C. marena* exclusively with commercial fry feed B (feeding regime no. I) high mortality occurred after 30 to 40 d, similar as with feeding regimes II and III (self-formulated starter feed 3 for 20 or 30 d and commercial fry feed B thereafter).

Feeding regime number V (self-formulated starter feed 3 from d 0 to 20, intermediate feed from day 21 to 40, commercial fry feed B after 40 d) resulted in a survival rate similar to the live zooplankton control (= feeding regimes VII). When the initial feeding period with self-formulated starter feed 3 was reduced to 10 d (feeding regime III) or prolonged to 30 d (feeding regime VI), the survival rates were decreased, too. The fish weight and total length were reduced in feeding regime III and VI.

Also in *C. atterensis* feeding regime V resulted in a survival rate similar to the live zooplankton control (feeding regimes VII). The increase in weight and total length was similar to zooplankton fed fish, too. Feeding regimes III and VI resulted in lower survival rates, while the growth of fish was not affected. Feeding regime VI reduced

survival rates, fish weight and length. Feeding regimes I, II, and III were not tested in this species.

Feeding regime V was followed for 90 d in both species. From d 60 to 90 mortality rate was < 1% in *C. atterensis* and *C. marena*. After 90 d *C. marena* had reached a weight of 362 ± 28 mg and a length of 31 ± 4 mm and *C. atterensis* a weight of 143 ± 21 mg and a length of 25 ± 3 mm.

Table 4. Effect of 60 d lasting feeding regimes on the survival rate, body weight, and total length of *Coregonus marenus* and *C. atterensis.*

Feeding regime	**Survival** (%)		**Weight** (mg)		**Length** (mm)	
	C. marena	*C. atterensis*	*C. marena*	*C. atterensis*	*C. marena*	*C. atterensis*
No. I	5 ± 5^{a}	n.i.	n.i.	n.i.	n.i.	n.i.
No. II	35 ± 5^{b}	n.i.	131 ± 35^{a}	n.i.	23.4 ± 5.5^{a}	n.i.
No. III	30 ± 7^{b}	n.i.	115 ± 15^{b}	n.i.	20.2 ± 2.3^{b}	n.i.
No. IV	65 ± 5^{c}	36 ± 1^{a}	126 ± 25^{a}	42 ± 10^{a}	24.8 ± 2.4^{a}	18.5 ± 1.4^{a}
No. V	80 ± 5^{d}	70 ± 4^{b}	128 ± 24^{a}	57 ± 12^{b}	24.3 ± 2.3^{a}	22.0 ± 1.5^{b}
No. VI	50 ± 5^{e}	25 ± 11^{a}	113 ± 28^{b}	43 ± 9^{a}	19.5 ± 2.0^{b}	18.7 ± 1.4^{a}
No. VII	80 ± 5^{d}	68 ± 5^{b}	129 ± 33^{a}	57 ± 8^{b}	24.9 ± 3.5^{a}	21.8 ± 1.5^{b}

Note. Data are mean ± standard deviation. For survival rate n = 2 and resulting from 2 different tanks; for weight and length n = 40 deriving from 20 fish, respectively, from two tanks. n.i. not investigated. Values belonging to the same parameter and species with the same superscript symbol are not significantly different ($P > 0.05$).

3.3 Development of Digestive Enzymes of C. marena and C. atterensis Larvae (Table 5)

Table 5. Enzyme activities of *Coregonus maraena* and *Coregonus atterensis* larvae at the onset of external food uptake (0 d) and 25 d after first feeding with live zooplankton

	Enzyme activities (μmol/min/g protein)			
	C. marena (0 d)	***C. atterensis*** (0 d)	***C. marena*** (25 d)	***C. atterensis*** (25 d)
Chymotrypsin	661.7 ± 295.0^{a}	302.5 ± 211.8^{b}	682.4 ± 95.6^{a}	569.3 ± 83.1^{a}
Trypsin	7.38 ± 4.62^{a}	1.05 ± 1.05^{b}	9.23 ± 3.21^{a}	6.03 ± 2.20^{a}
Lipase, NPL	0.16 ± 0.12^{a}	0.38 ± 0.37^{a}	0.27 ± 0.15^{a}	5.45 ± 1.7^{b}
Lipase, Triolein	1.42 ± 1.36^{a}	2.06 ± 0.64^{a}	3.43 ± 1.53^{a}	14.6 ± 5.5^{b}
Acid phosphatase	2.53 ± 1.61^{a}	0.86 ± 0.41^{b}	2.21 ± 0.35^{a}	2.47 ± 0.55^{a}
Alkaline phosphatase	5.31 ± 3.21^{a}	1.68 ± 0.77^{b}	6.48 ± 3.64^{a}	5.07 ± 3.05^{a}
Phospholipase A	3.28 ± 1.66^{a}	2.73 ± 0.76^{a}	1.24 ± 0.53^{b}	1.39 ± 0.26^{b}

Note. For lipase triolein and *p*-nitrophenyl laurate (NPL) were used as substrates. Data are mean ± standard deviation. Sample number = 7, values within a line with the same superscript symbol are not significantly different ($P > 0.05$).

Activities of chymotrypsin, trypsin, acid and alkaline phosphatase, lipase, and phospholipase A were detected in the digestive tract of both species at the onset of feeding. With exception of lipase all enzyme activities were significantly higher in *C. marena* than in *C. atterensis*. In *C. marena* the activities of chymotrypsin, trypsin, acid phosphatase, and alkaline phosphatase remained constant after a feeding period of 25 d with live zooplankton, while phospholipase A activity decreased. In *C. atterensis* all assayed enzyme activities with exception of phospholipase A increased during a feeding period of 25 d and reached values similar to *C. marena,* lipase activity even higher than in *C. marena*. Activities of phospholipase A decreased.

3.4 Effect of Different Feed Types on Digestive Enzymes in C. marena and C. atterensis Larvae (Table 6)

In *C. marena* fed with self-formulated starter feed 3 or with commercial fry feed B specific enzyme activities were decreased in comparison to larvae fed with live zooplankton and the decrease was more drastically for commercial dry feed than for self-formulated starter feed 3. In fish fed with self-formulated starter feed 3, the activities of lipase, alkaline phosphatase, and phospholipase A were decreased. In *C. marena* fed with

commercial fry feed B the activities of all assayed enzymes with exception of acid phosphatase were decreased.

In *C. atterensis* fed with self-formulated starter feed 3, trypsin and phospholipase A activities were decreased, while all other enzymes remained constant. Also in this species all enzyme activities were significantly decreased after feeding commercial feed B.

Table 6. Effect of different food types on the activities of digestive enzymes in *C. marena* and *C. atterensis*

	C. marena			***C. atterensis***		
	Live zooplankton	Self-formulated mixture 3	Commercial fry food B	Live zooplankton	Self-formulated mixture 3	Commercial fry food B
Chymotrypsin	625 ± 149[a]	610 ± 98[a]	281 ± 120[b]	596 ± 73[a]	584 ± 171[a]	175 ± 56[c]
Trypsin	9.9 ± 2.5[a]	8.6 ± 2.6[a]	5.2 ± 2.6[b]	8.0 ± 1.2[a,b]	0.99 ± 0.92[c]	0.25 ± 0.62[c]
Lipase, NPL	0.23 ± 0.07[a]	0.17 ± 0.03[c]	0.02 ± 0.01[b]	5.4 ± 1.1[d]	4.2 ± 1.4[d]	0.9 ± 0.3[e]
Lipase, Triolein	4.2 ± 1.9[a]	2.0 ± 2.5[c]	0.9 ± 0.5[b]	16.3 ± 0.8[d]	13.1 ± 6.1[d]	1.5 ± 1.0[e]
Acid phosphatase	2.8 ± 0.5[a]	2.6 ± 1.0[a]	2.3 ± 0.1[a]	2.3 ± 0.6[a]	2.2 ± 0.7[a]	1.1 ± 0.3[b]
Alk. phosphatase	5.2 ± 0.1[a]	1.9 ± 0.4[b]	1.6 ± 0.6[b]	4.9 ± 1.5[a]	5.6 ± 1.8[a]	1.9 ± 0.6[b]
Phospholipase A	3.1± 1.3[a]	2.7± 0.7[b]	1.4 ± 1.0[c]	1.6 ± 0.7[c]	0.8 ± 0.3[d]	0.2 ± 0.1[e]

Note. Units are μmol/min/g protein. For lipase triolein and *p*-nitrophenyl laurate (NPL) were used as substrates. Data are mean ± standard deviation. Sample number = 7, each sample is a pool of different digestive tracts. Values with the same superscript symbol are not significantly different ($P > 0.05$).

3.5 Enzyme Activities in Different Feeds (Table 7)

Zooplankton and *Artemia* had similar activities of chymotrypsin, trypsin, and alkaline and acid phosphatase, while activities of lipase and phospholipase A were lower in *Artemia* than in zooplankton. There were no differences in enzymatic activities between self-formulated starter feed 3 dried at 80 °C or at 40 °C. In comparison to fresh *Artemia* and fresh zooplankton in self-formulated starter feed 3 the activities of chymotrypsin, trypsin, and phospholipase A were significantly reduced. Lipase activity was reduced in comparison to zooplankton but similar to *Artemia*, activities of acid and alkaline phosphatase revealed no significant differences. In the self-formulated starter feed 1 all measured enzyme activities were lower than in *Artemia*, zooplankton, and self-formulated starter feed 3. In commercial feed B no enzyme activities were detectable.

Table 7. Enzyme activities in fresh zooplankton, fresh *Artemia*, self-formulated mixture 3 dried at 40 or 80 °C and in commercial starter feed B

	Enzyme activities (μmol/min/g protein)					
	Live zoo-plankton	Live *Artemia*	Self-formulated mixture 3 DT: 80 °C	Self-formulated mixture 3 DT: 40°C	Self-formulated mixture 1 DT: 80 °C	Commercial starter feed B
Chymotrypsin	261.8 ± 35.0[a]	242.2 ± 34.3[a]	103.5 ± 10.6[b]	94.7 ± 12.4[b]	10.7 ± 3.9[c]	n.d.
Trypsin	4.9 ± 0.7[a]	4.2 ± 0.2[a]	2.2 ± 0.5[b]	2.3 ± 0.8[b]	0.6 ± 0.6[c]	n.d.
Lipase, NPL	3.1 ± 0.5[a]	0.5 ± 0.1[b]	0.8 ± 0.1[b]	0.6 ± 0.34[b]	0.1 ± 0.1[c]	n.d.
Lipase, Triolein	8.7 ± 1.8[a]	1.5 ± 0.1[b]	2.1 ± 0.3[b]	1.8 ± 0.6[b]	0.5 ± 0.5[c]	n.d.
Acid phosphatase	6.3 ± 0.4[a]	7.1 ± 0.6[a]	4.6 ± 1.8[b]	4.7 ± 1.0[b]	0.8 ± 0.8[a]	n.d.
Alkaline phosphatase	4.9 ± 1.5[a]	5.2 ± 0.9[a]	4.1 ± 1.8[a]	4.0 ± 0.9[a]	1.9 ± 0.6[b]	n.d.
Phospholipase A	3.2 ± 0.7[a]	2.0 ± 0.77[b]	1.8 ± 0.6[b]	1.9 ± 0.7[b]	0.2 ± 0.1[c]	n.d.

Note. DT – drying temperature, n.d. - not detectable. For lipase triolein and *p*-nitrophenyl laurate (NPL) were used as substrates. Sample number (n) = 3 deriving from different feed preparations. Values within a line with the same superscript symbol are not significantly different ($P > 0.05$).

4. Discussion

Larvae of *Coregonus atterensis* and *Coregonus maraena* differ in body size, growth rates, and differentiation of

digestive enzymes. The smaller size and growth rate and the less developed digestive system of *C. atterensis* larvae aggravates handling and manipulation procedures necessary in intensive aquaculture and results in lower ability to digest formulated dry feeds. To our knowledge the present study is the first one demonstrating that *Coregonus atterenis* and *Coregous marena* can be reared to fingerling size solely on dry feed under intensive culture conditions relevant in aquaculture.

4.1 On-Feeding Experiments

The commercially available starter feeds were not suitable for on-feeding of larvae of the two species. Mortality was probably caused by malformations and inflammations of gill lamellae as frequently observed in fish fed with commercial dry feed (unpublished data). Generally, malformation, inflammations, and decreased resistance to diseases are factors favored by a poor nutrition status of fish (Hamre et al., 2013). Comparable observations were recently made on grayling solely fed on dry feed (Lahnsteiner & Kletzl, 2015). Also self-formulated starter feed number 1 which has been successfully used for on-feeding of grayling larvae (Lahnsteiner & Kletzl, 2015) was not suitable for rearing of *C. atterensis* and *C. marena* larvae, as it resulted only in low to medium survival rates. This indicates that the feed demands of grayling and coregonids differ greatly. Only self-formulated starter feeds 3 and 4 containing high quantities of zooplankton or artemia (ratio of component mixture : live feed = 1 : 1 [dry weight : dry weight]) and adequate preservatives (1% propionic acid) resulted in survival rates and growth rates similar to the live zooplankton control. There was no difference in the survival and growth rates of larvae when either *Artemia* or zooplankton was incorporated in the feed, indicating that natural zooplankton can be replaced by artificially cultured live feed.

Although zooplankton is the natural starter feed of fish larvae (Fulton, 1984; Nielsen & Munk, 1998) and routinely used in extensive pond culture (Mitra, Mukhopadhyay, & Ayyappan, 2007; Cunha et al., 2010) and in several intensive fish farms (Evejemo, Reitan, & Olsen, 2003; Lahnsteiner et al. 2012), it is considered as inadequate for fish culture due to the risk of pathogen transfer in many studies (Maugeri, Carbone, Fera, Irrera, & Gugliandolo, 2004, Caceres, Tessier, Duffy, & Hall, 2014). Generally, those ingredients of live feed which are essential for nutrition of fish larvae are not fully understood until now. Previous studies stressed the importance of an optimal composition and pattern of amino acids (Li, Mai, & Trushenski, 2008; Conceição, Aragão, & Rønnestad, 2010) and of unsaturated fatty acids (Takeuchi, 1997, Navarro-Guillén et al., 2014). As discussed below live feed (also in the dried and conserved form) may be a source of digestive enzymes for fish larvae. Live food organisms contain also intestinal neuropeptides and nutritional growth factors which could enhance digestion in fish larvae (Kolkovski, 2001).

4.2 Adaption of Fish to Commercial Dry Feed and Rearing to Fingerling Size

Our results show that not only a suitable starter feed is necessary for successful rearing *C. marena* and *C. atterensis* to juveniles, but also specific feeding regimes where starter feeds are replaced with intermediate feed and then with commercial feed in defined chronological sequences. Chronological sequences depend on the development stage of fishes and therefore on the water temperature. As demonstrated in the present experiments and also in earlier ones (Lahnsteiner & Kletzl, 2015) the self-formulated starter feeds are suitable only during the early development phase of fish larvae. After prolonged feeding with starter feed, fish stagnate in weight or even lose weight (Lahnsteiner & Kletzl, 2015). Possibly feed composition is not suitable for more advanced fish stages. Our experiments showed that it was impossible to adapt *C. marena* and *C. atterensis* from starter feed to commercial dry feed as this direct adaptation resulted in increased fish mortality. To increase the digestibility of commercial feed and provide other potentially essential feed components, zooplankton was incorporated in the commercial feed. Co-feeding starter feed and commercial fry feed for a distinct time period may be an alternative method which was not tested here. Co-feeding is the most practical method for adaption of fish larvae from live to dry diets (Hamre et al., 2013).

4.3 Enzymes

In the digestive tract of larvae of *C. atterensis* and *C. marena* trypsin, chymotrypsin, lipase, phospholipase A, i.e. the main digestive enzymes secreted by the pancreas, and acid and alkaline phosphatase could be proved at the onset of first feeding. Alkaline phosphatase is an enzyme located in the brush border layer (Kozaric, Kužir, Nejedli, Petrinec, & Sreboèan, 2004) regulating lipid absorption into enterocytes, participating in bicarbonate secretion and controlling bacterial endotoxin-induced inflammation (Lallès, 2010). Acid phosphatase is located in the lysosomes of the enterocytes (Kozaric et al., 2004) and could support the digestion of specific nutrients in the intestine. E.g. in nursing humans, proteins remain intact until they reach the intestinal lumen, where they are intracellularly digested by support of endosomes and lysosomes (Matter, Stieger, Klumperman, Ginsel, & Hauri, 1990; Zhang, Wick, Haas, Seetharam, & Dahms, 1995). Similar enzymatic patterns were also found in the

intestine of larvae of other teleosts whereby published data are available mainly for larvae of marine species (Kolkovski, 2001; Zambonino Infante, & Cahu 2001).

The present study demonstrates that at the onset of exogenous feeding, the digestive system of *C. atterensis* was less developed than the digestive system of *C. marena.* Under optimal feeding conditions the digestive system of *C. atterensis* differentitated, as enzyme activities increased and after 25 d no significant changes could be detected in the activities of chymotrypsin, trypsin, acid phosphatase, and alkaline phosphatase between the two species. Lipase activity was approximately 5 times higher in *C. atterensis* than in *C. marena* after the 25 d lasting on-feeding period which might be explained by increased lipid utilization. Phospholipase A activity decreased during the 25 d on-feeding period in both species probably indicating that phospholipids are important nutrients only in the initial phase of larvae development.

In both coregonid species the activities of the investigated digestive enzymes depended strongly on the administered feed types. Commercial fry feed B significantly decreased activities of all assayed enzymes (with exception of acid phosphatase in *C. marena*) indicating that the potential for digestion was reduced and specific feed components could be not or only partly digested. This may be one possible explanation for high mortality rates when using commercial fry feed for on-feeding of *C. marena* and *C. atterensis* larvae. Also self-formulated starter feed had negative effects on activities of the investigated digestive enzymes. However, the effect was moderate as the activity decrease was less significant than for commercial fry feed. In *C. marena* only activities of lipase, alkaline phosphatase, and phospholipase A decreased, and in *C. atterensis* activities of trypsin and phospholipase A. A significant effect of feeds on the digestive enzymes of fish larvae was also described in previous studies (Cousin, Baudin-Laurencin, & Gabaudan, 1987; Kolkovski, 2001; Kamarudin, Otoiand, & Saad, 2011). Analysis of enzyme activities in different types of feeds indicated that commercial fry feeds lacked any enzyme activities while live feeds and self-formulated starter feeds contained activities of digestive enzymes, the activities depending on the quantities of added zooplankton. From these data it can be concluded that zooplankton is a source of enzymes even in the dried form when carefully processed under moderate conditions. The self-formulated starter feeds 3 and 4 might have been superior for on-feeding of coregonid larvae due to high enzyme activities resulting in a better digestibility. However, this hypothesis is speculative at present as the different feed types differed also in other parameters allowing no clear conclusions. Kolkovski (2001) suggested that live food organisms consumed by the fish larvae assist the digestion process by means of their digestive enzymes.

In summary, the present data indicate that raising of *C. marena* and *C. atterensis* with formulated fry feed is possible under intensive conditions practicable in hatcheries. However, the effort is higher than for other fresh water fishes (e.g. *Oncorhynchus mykiss*, *Salvelinus fontinalis*, *Salmo trutta*) produced presently in intensive culture as specifically formulated fry feeds and intermediate feeds are necessary. These fry feeds and intermediate feeds use carefully processed zooplankton or *Artemia* as a main compound as these organisms contain still unknown but essential factors for development of fish larvae and juveniles.

References

Bergmeyer, H. U. (Ed.). (1985) *Methods of enzymatic analysis*. Weinheim, Germany: VCH Verlagsgesellschaft.

Caceres, C. E., Tessier, A. J., Duffy, M. A., & Hall, S. R. (2014). Disease in freshwater zooplankton: what have we learned and where are we going? *Journal of Plankton Research, 36*, 326-333. http://dx.doi.org/10.1093/plankt/fbt136

Conceição, L. E. C., Aragão, C., & Rønnestad, I. (2010). Protein Metabolism and Amino Acid Requirements in Fish Larvae. In L. E. Cruz-Suarez, D. Ricque-Marie, M. Tapia-Salazar, M. G. Nieto-López, D. A. Villarreal-Cavazos, & J. Gamboa-Delgado (Eds.), *Avances en Nutrición Acuícola X – Memorias del DécimoSimposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N. L., México* (pp. 250-263). Monterrey, México: Universidad Autónoma de Nuevo León.

Cousin, J. C. B., Baudin-Laurencin, F., & Gabaudan, J. (1987). Ontogeny of enzymatic activities in fed and fasting turbot, *Scophtalmus maximus* L. *Journal of Fish Biology, 30*, 15-33. http://dx.doi.org/10.1111/j.1095-8649.1987.tb05728.x

Cunha, M. E., Quental-Ferreirah, H., Boglione, C., Palamara, E., Gavaia, P., & Pousão-Ferreira, P. (2010). Rearing fish larvae for extensive and semi-intensive aquaculture – the "natural" mesocosms system. *Aquaculture Europe, 35*, 23-26.

Dabrowski, K., & Kaushik, S. (1984). Rearing of coregonid (*Coregonus schinzi palea* Cuv. Et Val.) larvae using dry and live food: II. Oxygen consumption and nitrogen excretion. *Aquaculture, 41*, 333-344.

http://dx.doi.org/10.1016/0044-8486(84)90201-1

Evejemo, J. O., Reitan, K. I., & Olsen, Y. (2003). Copepods as live food organisms in the larval rearing of halibut larvae (*Hippoglossus hippoglossus* L.) with special emphasis on the nutritional value. *Aquaculture, 227*, 191-210. http://dx.doi.org/10.1016/S00448486(03)00503-9

Fulton, R. S. (1984). Predation, production and the organization of an estuarine copepod community. *Plankton Research, 6*, 399-415. http://dx.doi.org/10.1093/plankt/6.3.399

Hamre, K., Yufera, M., Rønnestad, I., Boglione, C., Conceiçãao, L. E. C., & Izquierdo, M. (2013). Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. *Reviews in Aquaculture*, 5, S26–S58. http://dx.doi.org/10.1111/j.1753-5131.2012.01086.x

Harris, K. C., & Hulsmann, P. F. (1991). Intensive culture of lake whitefish (*Coregonus clupeaformis*) from larvae to yearling size using dry feeds. *Aquaculture, 96*, 255-268. http://dx.doi.org/10.1016/0044-8486(91)90156-2

Kamarudin, M. S., Otoiand, S., & Saad, C. R. (2011). Changes in growth, survival and digestive enzyme activities of Asian redtail catfish, Mystusnemurus, larvae fed on different diets. *African Journal of Biotechnology, 10*, 4484-4493. http://dx.doi.org/10.5897/AJB09

Karjalainen, J. S. (1991). Survival, growth and feeding of vendace, *Coregonus albula* (L.), larvae in net enclosures. *Journal of Fish Biology, 38*, 905-919. http://dx.doi.org/10.1111/j.1095-8649.1991.tb03630.x

Kolkovski, S. (2001). Digestive enzymes in fish larvae and juveniles—implications and applications to formulated diets. *Aquaculture, 200*, 181-201. http://dx.doi.org/10.1016/S0044-8486(01)00700-1

Kottelat, M., & Freyhof, J. (Eds.). (2007) *Handbook of European Freshwater Fishes*. Berlin, Germany: Kottelat, Cornol, Switzerland and Freyhof.

Kozaric, Z., Kužir, S., Nejedli, S., Petrinec, Z., & Sreboèan, E. (2004). Histochemical distribution of digestive enzymes in hake, *Merluccius merluccius* L. 1758. *Veterinary Archives, 74*, 299-308.

Lahnsteiner, F., Kletzl, M., & Weismann, T. (2012). Rearing of burbot, *Lota lota (Pisces,* Teleostei), larvae with zooplankton and formulated microdiets. *Journal of Agricultural Science, 4*, 269-277. http://dx.doi.org/10.5539/jas.v4n9p269

Lahnsteiner, F., & Kletzl, M. (2015). Suitability of different food types for on-feeding and juvenile production of European grayling, *Thymallus thymallus*, under intensive farming conditions. *Journal of Agricultural Science, 7*, 161-168. http://dx.doi.org/10.5539/jas.v7n1p16

Lallès, J. P. (2010). Intestinal alkaline phosphatase: Multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. *Nutrition Review, 68*, 323-32. http://dx.doi.org/10.1111/j.1753-4887.2010.00292.x

Leithner, S., & Wanzenböck, J. (2015). Rearing larvae of different strains of *Coregonus lavaretus* under cold water conditions: comparison of a special cold-water line with a standard agglomerated microdiet. *Journal of Agricultural Science, 7*, 28-36. http://dx.doi.org/10.5539/jas.v7n5p28

Li, P., Mai, K., & Trushenski, J. (2008). New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids, 37*, 43-53. http://dx.doi.org/10.1007/s00726-008-0171-1

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *The Journal of Biological Chemistry, 193*, 265-275. http://dx.doi.org/10.1007/bf02636814

Lowry, R. R., & Tinsley, I. J. (1976). Rapid colorimetric determination of free fatty acids. *Journal of the American Oil Chemists' Society, 53*, 1558-9331.

Luczynski, M., Majkowski, P., Bardega, R., & Dabrowski, K. (1986). Rearing of larvae of four coregonid species using dry and live food. *Aquaculture, 56*, 179-185. http://dx.doi.org/10.1016/0044-8486(86)90333-9

Maugeri, T. L., Carbone, M., Fera, M. T., Irrera, G. P., & Gugliandolo, C. (2004). Distribution of potentially pathogenic bacteria as free living and plankton associated in a marine coastal zone. *Journal of Applied Microbiology, 97*, 354-361. http://dx.doi.org/10.1111/j.1365-2672.2004.02303.x

Matter, K., Stieger, B., Klumperman, J., Ginsel, L., & Hauri, H. P. (1990). Endocytosis, recycling, and lysosomal delivery of brush border hydrolases in cultured human intestinal epithelial cells (Caco-2). *Journal of Biological Chemistry, 265*, 3503-3512.

Mitra, G., Mukhopadhyay, P. K., & Ayyappan, S. (2007). Biochemical composition of zooplankton community grown in freshwater earthen ponds: Nutritional implication in nursery rearing of fish larvae and early

juveniles. *Aquaculture, 272*, 346-360. http://dx.doi.org/10.1016/j.aquaculture.2007.08.026

Navarro-Guillén, C., Engrola, S., Castanheira, F. N., Hachero-Cruzado, I., Tocher, D. R., Conceição, L. E., & Morais, S. (2014). Effect of varying dietary levels of LC-PUFA and vegetable oil sources on performance and fatty acids of Senegalese sole post larvae: puzzling results suggest complete biosynthesis pathway from C18 PUFA to DHA. *Comparative Biochemistry Physiology B: Biochemistry and Molecular Biology, 167*, 51-58. http://dx.doi.org/10.1016/j.cbpb.2013.10.001

Nielsen, T. G., & Munk, P. (1998). Zooplankton diversity and the predatory impact by larval and small juvenile fish at the Fisher Banks in the North Sea. *Journal of Plankton Research, 20*, 2313-2332. http://dx.doi.org/10.1093/plankt/20.12.2313

Østbye, K., Bernatchez, L., Næsje, T. F., Himberg, K.-J. M., & Hindar, K. (2005). Evolutionary history of the European whitefish Coregonus lavaretus (L.) species complex as inferred from mtDNA phylogeography and gill-raker numbers. *Molecular Ecology, 14*, 4371-4387. http://dx.doi.org/10.1111/j.1365-294X.2005.02737.x

Pamminger-Lahnsteiner, B., Weiss, S., Winkler, K. A., & Wanzenböck, J. (2009). Composition of native and introduced mtDNA lineages in Coregonus sp. In two Austrian lakes: Evidence for spatio-temporal segregation of larvae. *Hydrobiologia, 632*, 167-175. http://dx.doi.org/10.1007/s10750-009-9836-3

Pinsirodom, P., & Parkin, K. L. (2001). Lypolytic Enzymes. In R. E. Wrolstad, T. E. Acree, H. An, E. A. Decker, M. H. Penner, D. S. Reid, S. J. Schwartz, ... P. Sporns (Eds.), *Current Protocols in Food Analytical Chemistry* (pp. C3.1.1-C3.1.13). Hoboken, New York: John Wiley & Sons, Inc.

Poczyczyński, P., Dąbrowski, K., Kucharska, K., Mamcarz, A., & Kozłowski, J. (1990). Laboratory rearing of whitefish (*Coregonus lavaretus*) – comparative studies of Polish and foreign experimental starter diets. *Polskie Archiwum Hydrobiologii, 37*, 397-411.

Ritterbusch-Nauwerck, B., & Lahnsteiner, F. (2005). Effects of stocking on morphological and meristic characteristics of native coregonid populations in four Austrian lakes. *Zeitschrift für Fischkunde, 7*, 101-111.

Takeuchi, T. (1997). Essential fatty acid requirements of aquatic animals with emphasis on fish larvae and fingerlings. *Reviews in Fisheries Science, 5*, 1-25. http://dx.doi.org/10.1080/10641269709388592

Zambonino Infante, J. L., & Cahu, C. L. (2001). Ontogeny of the gastrointestinal tract of marine fish larvae. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 130*, 477-487. http://dx.doi.org/10.1016/S1532-0456(01)00274-5

Zhang, Y., Wick, D. A., Haas, A. L., Seetharam, B., & Dahms, N. M. (1995). Regulation of lysosomal and ubiquitin degradative pathways in differentiating human intestinal Caco-2 cells. *Biochimica et Biophysica Acta, 1267*, 15-24. http://dx.doi.org/10.1016/0167-4889(95)00027-P