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# Digestive Enzyme System of Larvae of Different Freshwater Teleosts and Its Differentiation during the Initial Phase of Exogenous Feeding

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**ABSTRACT**

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Activities of digestive enzymes and main histological characteristics of the intestine were investigated in larvae of three salmonid species (*Coregonus maraena*, *C. atterensis*, *Thymallus thymallus*), of burbot (*Lota lota*), and pikeperch (*Sander lucioperca*) at the onset of exogenous feeding (0 day degrees (°d)) and at 250°d thereafter. At the onset of exogenous feeding the activities of proteolytic, lipolytic, and carbohydrate splitting enzymes were detected in the intestines of all species. The enzymatic activities showed significant species specific differences indicating specializations in functionality and digestion ability. In *C. atterensis* and *L. lota* the activities of most enzymes were low in comparison to the other investigated species and therefore their digestive system was only poorly developed. In *S. lucioperca* it was moderately developed and in *T. thymallus* and *C. maraena* well-developed. After 250°d, the activities of the investigated enzymes changed in a very species specific way. Histologically, the intestines of the investigated species revealed no species specific differentiations at the onset of the first feeding with the exception of the absence of goblet cells in *L. lota* and *C. atterensis*. These differentiated after 250°d.

**Keywords**: digestive system; larvae; freshwater fish; digestive enzymes; histology

At the onset of exogenous feeding the digestive system of larvae of teleost fish species is an undifferentiated organ (Govoni et al. 1986; Zambonino Infante and Cahu 2001). During larval development buccopharynx, esophagus, and fore-, mid-, and hindgut differentiate and in a last step the stomach and the pyloric caeca (Govoni et al. 1986; Zambonino Infante and Cahu 2001). Also the activities of digestive enzymes change during development. Most information on this topic is available from studies on marine fish larvae (for review see Zambonino Infante et al. 2008). Enzyme activities are relatively low at the onset of exogenous feeding, change in a specific way, and are also influenced by the administered food (Zambonino Infante et al. 2008).

Only little information is available on the digestive enzyme system of larvae of freshwater fish (Torrissen 1984; Govoni et al. 1986; Lemieux et al. 2003; Furne et al. 2005). Data are still missing for species, which are considered as new candidates for intensive aquaculture or have been recently introduced in intensive culture, as e.g. *Thymallus thymallus*, *Coregonus sp.*, *Sander lucioperca*, and *Lota lota*. In *S. lucioperca* larvae differentiation of key digestive enzymes (Cuvier-Peres and Kestemont

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2001; Hamza et al. 2015) and effects of different diets and weaning conditions on selected digestive enzyme activities were investigated (Hamza et al. 2007, 2008). In *Coregonus lavaretus* larvae enzyme activities in different segments of the intestine were investigated by histochemical methods (Segner et al. 1989). An overall characterization of the various types of digestive enzymes, of their changes during larval development, and of species specific differentiations in functionality and activity is still lacking for the above listed species. However, these data provide an important basic knowledge for understanding early differentiation processes of teleost fish larvae and under practical considerations for developing new and sustainable feeding strategies.

Therefore, the present study was conducted. It investigates the activities of proteolytic enzymes (chymotrypsin, trypsin, and leucine aminopeptidase), of lipolytic enzymes (lipase, phospholipase), of disaccharide and polysaccharide splitting enzymes (α-glucosidase, amylase, chitinase), and of alkaline and acid phosphatase in the intestines of three salmonid species (*Coregonus maraena*, *C. atterensis*, *Thymallus thymallus*), of burbot (*Lota lota*), and pikeperch (*Sander lucioperca*) during the initial phase of exogenous feeding. Complementarily, also the morphological and cellular differentiations of the digestive tract are monitored using histological methods.

## MATERIAL AND METHODS

The investigation was performed on grayling, *Thymallus thymallus*, two coregonid species, *Coregonus maraena* and *C. atterensis*, pikeperch, *Sander lucioperca*, and burbot, *Lota lota*. The used water source was ground water with a temperature of 9 ± 1°C, pH of 7.85 ± 0.02, conductivity of 335.2 ± 8.4 µS/cm at 25°C, acid-neutralizing capacities of 3.38 ± 0.02 mval/l, $O_2$ concentration of 12.53 ± 0.19 mg/l, and $PO_4^{3-}$ and $NH_4^+$ concentrations ≤ 0.005 mg/l. Eggs of *T. thymallus*, *C. maraena*, *C. atterensis*, and *S. lucioperca* were obtained by stripping, eggs of *Lota lota* after natural spawning (Lahnsteiner et al. 2012a). Water hardened eggs were disinfected with 1000 ppm formalin for 20 min to reduce potential fungus and bacteria growth during egg incubation. Eggs of *T. thymallus* were incubated in flow through incubators at 9°C (Lahnsteiner and Kletzl 2014). Eggs of the other species were incubated in Zug chars, those of *C. maraena* and *C. atterensis* at 9 ± 1°C, those of *S. lucioperca* at 18 ± 1°C, and those of *L. lota* at 4°C (Lahnsteiner et al. 2012b; Lahnsteiner and Kletzl 2014, 2015). For heating of ground water to 18 ± 1°C a thermal heat pump in combination with a degassing column was used. Water was degassed to avoid its oversaturation with nitrogen gas which is harmful to eggs and larvae (Kolkovski et al. 2004). Cooling of ground water to 4°C was done with a cooling aggregate Aqua Medic Titan 2000 (Aqua Medic GmbH, Germany).

For hatching, eggs of *T. thymallus*, *C. maraena*, *C. atterensis*, and *S. lucioperca* were transferred into rearing tanks supplied with ground water of the same temperature as used for egg incubation. *L. lota* eggs were transferred into rearing tanks, too, and the water temperature was gradually raised to 9°C to shorten and unify the hatching period (Lahnsteiner et al. 2012b). Defined quantities of yolk sac larvae were transferred into new tanks for the rearing experiments. Larvae were quantified volumetrically after density determinations.

***Rearing of larvae and sampling procedure***. Feeding and sampling was done under standardized conditions as different parameters (e.g. administered food – Kamaszewski et al. 2010, infestation with intestinal parasites, sampling procedure – Izvekova et al. 2013) can affect the digestive enzyme activities in fish. All rearing experiments were done in duplicate. In each species 2 tanks with a volume of 250 l were stocked with 2500 fish, respectively, and tanks were supplied with ground water at a flow rate of 0.25 l/s. Fish remaining from the experiments were used for commercial fingerling production. In all species with the exception of *S. lucioperca* the rearing experiments of larvae were performed at 9 ± 1°C. In the latter species ground water was heated to 18 ± 1°C and degassed as described above. Larvae were fed with live freshwater zooplankton. This feeding method was developed by Einsele (1949) and continuously developed in unpublished experiments. Details about this feeding method were described by Lahnsteiner et al. (2012b). Briefly, freshwater zooplankton was twice daily collected from Lake Mondsee with plankton nets. The nets were dredged floating behind a boat at a depth of 10–15 m and at a cruising speed of 0.2 m/s. Sieve nets with 100 µm as the lower and 200 µm as the upper mesh size limit were used to collect zooplankton suitable for the first feeding of *S. lucioperca*, *L. lota*, and *C. atterensis* and sieve nets with a mesh size of

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200–400 µm for *C. maraena* and *T. thymallus* (Lahnsteiner et al. 2012b; Lahnsteiner and Kletzl 2015). The collected zooplankton organisms were washed out of the plankton nets into buckets, diluted to an organism density of approximately 100 000 individuals per litre, and quantities of 10–15 individuals per fish larvae were fed 4 times per day. These feed quantities depend on unpublished standardizations and were successfully applied for rearing of *T. thymallus*, *C. maraena, C. atterensis*, and *L. lota* in other studies (Lahnsteiner et al. 2012b; Lahnsteiner and Kletzl 2014, 2015). The size of the food organisms was gradually increased depending on the growth of the larvae. Zooplankton was stored for maximally 4 h at 8–9°C. Tanks were cleaned 2–3 times daily. Dead larvae were collected, counted, and recorded during the cleaning procedure. Photoperiod was 14 h light : 8 h darkness. Illumination was artificial using neon tubes with an emission spectrum in the red to orange range, i.e. 580–680 nm, and the light intensity was ca. 50 lx at the water surface of the tanks.

As *T. thymallus*, *C. maraena*, *C. atterensis*, and *Lota lota* were reared at water temperatures different to *S. lucioperca* temperature, related differences in development were compensated by sampling larvae in similar time spans in terms of day degrees (°d). Sampling procedure for morphometric, histological, and enzymatic analyses is shown in the scheme given below. It was done at 0, 125, and 250°d, i.e. after 0, 14, and 28 days in *T. thymallus*, *C. maraena*, *C. atterensis*, and *Lota lota* and after 0, 7, and 15 days in *S. lucioperca*. The first sampling was performed when the larvae yolk reserves were completely consumed (0°d, onset of exogenous feeding). Before sampling, fish were homogeneously distributed in the tanks by gentle mixing, preferably during the cleaning process.

Sampled larvae were killed by an over dose of MS-222 (Sigma-Aldrich, USA). MS-222 was used as it is the only fish anesthetic permitted by law in Austria. Furthermore, MS-222 is fast effective and fish are not deformed during killing. Larvae used for enzymatic and histological analysis were removed from the MS-222 solution and placed in 4°C cold water to reduce degenerative processes during sample processing.

***Enzymatic analysis***. From the individual larvae the whole digestive tract was removed by micro-preparatory techniques in a Motic stereomicroscope DM-39C-N9GO with integrated digital camera (Motic GmbH, Germany). Feces and remnants of feed were squeezed out with fine forceps. Then the intestine was separated from esophagus and bucopharynx and rinsed in 4°C 0.1 mol/l tris buffer (pH 7.5). Intestines of 5 larvae were pooled to one sample to obtain enzyme activities and sample volumes reliable for analysis. By this procedure 8 pooled samples were obtained (4 from each tank) in each species and at each sampling date.

The removed intestines were transferred into Eppendorf tubes containing 0.5 ml of 4°C cold tris buffer and homogenized using a Dounce type tissue homogenizer (VWR International, Austria). Insoluble particles were centrifuged at 1000 *g* for 5 min at 4°C and the supernatants were stored at –20°C.

Enzymatic assays were performed at 20 ± 1°C. All chemicals used for enzymatic analysis were of analytical grade and purchased from Sigma-Aldrich. Measurements were performed using an UV/Vis spectrophotometer UV-6300PC (VWR International) and a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, USA). Preliminary studies on the activities of phospho-

| | 0 Day degrees | | 125 Day degrees | | 250 Day degrees | |
|---|---|---|---|---|---|---|
| | Tank 1 | Tank 2 | Tank 1 | Tank 2 | Tank 1 | Tank 2 |
| Morphometry | 20 larvae | 20 larvae | – | – | 20 larvae | 20 larvae |
| Histology | 2 larvae | 2 larvae | – | – | 2 larvae | 2 larvae |
| Enzymatology | 40 larvae (5 intestines pooled to 1 sample respectively) | 40 larvae | 40 larvae (5 intestines pooled to 1 sample respectively) | 40 larvae | 40 larvae (5 intestines pooled to 1 sample respectively) | 40 larvae |

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lipase A, acid phosphatase, alkaline phosphatase, and trypsin revealed that a temperature range of 15–20°C yielded the highest enzyme activities in all investigated species. Acid phosphatase and alkaline phosphatase were determined with *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 $N_{\alpha}$-benzoyl-L-arginine 4-nitroanilide as substrate. The assay mixture contained 2 mmol/l EDTA, 2.5 mmol/l cysteine, and had a pH of 7.5 (Bergmeyer 1985). Chymotrypsin was determined 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). For the leucine aminopeptidase assay a colorimetrical assay was used with 1 mM L-leucine *p*-nitroanilide as substrate. The pH of the assay was 7.2 (Lin and Van Wart 1982).

Lipase was assayed in two ways: (a) colorimetrically with 0.25 mmol/l 4-nitrophenyl dodecanoate as non-micellar substrate (pH 8.2) and (b) by the liberation of fatty acids from a 0.5% triolein emulsion in triton X100 supplemented sodium phosphate buffer (pH 8.2) (Pinsirodom and Parkin 2001). In the latter assay liberated fatty acids were extracted into benzene and measured with the cupric acetate/pyridine reagent (Lowry and Tinsley 1976). The assay for total phospholipase activity contained 0.1% phosphatidylcholine as substrate and 10 mmol/l $CaCl_2$ (pH 8.0) (Pinsirodom and Parkin 2001). Also in this assay liberated fatty acids were extracted and measured using the method of Lowry and Tinsley (1976). Phospholipase A was investigated with 4-nitro-3-(octanoyloxy)benzoic acid as substrate at $CaCl_2$ concentrations of 10 mmol/l (pH 8.0) (Pinsirodom and Parkin 2001). Lipase with triolein as substrate revealed a significant correlation with lipase with nitrophenyl dodecanoate as substrate and total phospholipase with phospholipase A (data not shown). Therefore, only lipase with nitrophenyl dodecanoate as substrate and phospholipase A were analyzed after 250°d. Amylase was measured using 0.5% potato starch as substrate (pH of assay buffer: 6.9). Reducing groups released from starch were measured with the 3,5-dinitrosalicylic acid reagent (Bergmeyer 1985). α-Glucosidase was measured with 60 mmol/l sucrose as substrate (pH 6.0) by UV-spectrophotometrical determination of the liberated glucose (Bergmeyer 1985). For the chitinase assay 2 mmol/l 4-nitrophenyl N-acetyl-β-D-glucosamide was used as substrate and the pH was 6.0.

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 et al. 1951).

***Determination of larvae viability parameters and morphometric analysis***. The larvae survival rate was calculated as the number of larvae surviving the 250°d exogenous feeding period in relation to the total number of larvae. For morphometric analyses larvae were photographed in a stereomicroscope DM-39C-N9GO with integrated digital camera (Motic GmbH). The recording system was computer controlled using the Motic Image Plus program. The files were loaded in the Image J program and the larvae total length was measured. Measurements were calibrated using an object micrometer with a 20 mm scale and 0.1 mm steps (VWR International).

***Histological analysis***. Intestines were excised in a similar way as described for enzymatic analysis. Remnants of feces or food were not squeezed out, but the samples were immediately fixed in 7% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4) at 4°C for 3 h. The fixative was washed out, the samples were dehydrated in a graded series of ethanol, and embedded in Technovit 7100 according to manufacturer's instructions (Kulzer GmbH, Germany).

Sections 2–3 µm in thickness were cut with a Thermo Scientific HM 325 Rotary Microtome (Thermo Fisher Scientific) and stained with methylene blue. Samples were analyzed on gross morphology and subcellular arrangement in a Motic BA310 biological light microscope with built-in 3.0 mega pixel digital camera (Motic GmbH). Micrographs were recorded from selected sections as described above. Epithelium height and dimensions of cells and cell organelles were measured on digitized micrographs using the Image J program.

***Statistical analysis***. SPSS software (Version 18.0, 2009) and SigmaPlot (Version 12.1, 2015) were used for statistical calculations.

*Enzymatic data.* Enzymatic data are presented as mean ± standard deviation and are referred to the protein concentration of the sample. Statistical

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analyses were performed with the log transformed data sets to reach the assumptions for normal distributions. Species specific and time specific differences in single enzyme activities were analyzed by ANOVA procedures (independent variables: species, development time; dependent variable: enzyme activities) with Dunnett's T3 test as a multiple comparison post hoc test.

To summarize the relatively complex enzymatic data set and to get a better overview about species specific differences, enzyme activities measured at the onset of exogenous feeding were grouped according to their functionality into 4 groups: Group 1: proteolytic enzymes (chymotrypsin, trypsin, peptidase, acid phosphatase); Group 2: lipolytic enzymes (lipase, phospholipase A, alkaline phosphatase); Group 3: carbohydrate splitting enzymes (α-glucosidase, chitinase, amylase); Group 4: total enzyme activity (all analyzed enzymes taken together). For the different groups, the overall mean of enzyme activities was calculated for each species. ANOVA with species as independent variable, enzyme functionality group as dependent variable, and Dunnett's T3 test as multiple comparison post hoc test was used to test enzyme functionality groups on species specific differences.

Enzyme data from the onset of exogenous feeding were also subjected to discriminate analysis. By this statistical analysis method it was investigated if the enzymatic pattern of individual larvae could be assigned to a certain species. Based on the entered data the discriminate analysis calculates discriminate functions, the number of which depending on the number of used variables and the number of groups. Data for the first two functions, which explained the highest degree of variance, were plotted in diagrams. Finally, the group centroids were calculated by Euclidean distances and plotted in the diagrams, too.

The changes (increase or decrease) in enzyme activities from 0 to 250°d of development were calculated based on an enzyme activity change factor (EACF). EACF was calculated as log transformed mean enzyme activity at 250°d divided by the log transformed mean enzyme activity at 0°d. The mean values of enzyme activities were used for EACF calculation as samples from 0 and 250°d were from different individuals and could not be assigned to each other. In each species EACFs were calculated for the single enzyme activities and for the four functionality groups. EACFs < 1.0 indicated a decrease in enzyme activity, EACF = 1 a constant activity, and EACFs > 1.0 an increase in activity. No statistical tests were performed on EACFs, as sample numbers were low due to utilization of mean values of enzyme activities.

*Morphometric and histological data.* Data on larvae total length and histological data are reported as absolute data. In each species larvae total lengths from 0 and 250°d were tested on significant differences using the Student's *t* test. Species specific and time specific differences in histological data were analyzed by ANOVA procedures using species and development time as independent variables and histological parameters as dependent variables. Dunnett's T3 test was used as a multiple comparison post hoc test.

## RESULTS

***Larvae digestive enzymes at the onset of exogenous feeding***. In the intestine of larvae of *T. thymallus*, *C. maraena*, *C. atterensis*, *L. lota*, and *S. lucioperca* activities of acid phosphatase, alkaline phosphatase, amylase, chitinase, α-glucosidase, lipase, leucine aminopeptidase, phospholipase A, and total phospholipase were detected (Table 1). With the exception of α-glucosidase the activities of enzymes were significantly different between the species (Table 1).

Enzyme activities measured at the onset of exogenous feeding were grouped according to their functionality into 4 groups – in proteolytic enzymes, lipolytic enzymes, carbohydrate splitting enzymes, and total enzymes. The log transformed results are shown in Table 2. Proteolytic enzymes were the lowest in *S. lucioperca*, followed by *C. atterensis*, *L. lota*, *C. maraena*, and *T. thymallus*. Lipolytic enzymes were the lowest in *L. lota*, followed *by C. atterensis*, *C. maraena*, *S. lucioperca*, and *T. thymallus.* Carbohydrate splitting enzymes were the lowest in *L. lota*, followed by *C. atterensis*, *S. lucioperca*, *C. maraena,* and *T. thymallus.* Total activity of digestive enzymes was the lowest in *C. atterensis* and *L. lota,* medium *in S. lucioperca,* and the highest in *C. maraena* and *T. thymallus.*

To determine if the enzymatic activity pattern of individual larvae could be assigned to a certain species, a discriminate analysis was performed. Four canonical variates functions resulted from the canonical variates analysis: variates function 1

doi: 10.17221/25/2016-CJAS

explained 52.6% of the observed variance, function 2 – 24.2%.

Data for the variates function 1:

$y = 0.001x_1 + 0.015x_2 - 0.125x_3 - 0.009x_4 + 0.296x_5 + 0.298x_6 - 0.030x_7 - 0.383x_8 - 0.003x_9 + 0.001x_{10} + 0.280$; Wilks-Lambda (W-L) = 0.005; $P = 0.000$

and data for the variates function 2:

$y = -0.001x_1 + 0.007x_2 + 0.018x_3 - 0.014x_4 + 0.253x_5 + 0.036x_6 - 0.506x_7 + 0.604x_8 + 0.014x_9 - 0.001x_{10} - 0.797$; W-L = 0.039; $P = 0.000$

together with the group centroids were plotted in a diagram and are shown in Figure 1. The pattern of digestive enzymes of *S. lucioperca* and *L. lota* was clearly separated from that of the three salmonid species. Also within the salmonid species a separation was found.

***Changes in larval digestive enzyme activities during exogenous feeding***. When larvae of *T. thymallus*, *C. maraena*, *C. atterensis*, *L. lota*, and *S. lucioperca* were fed with live zooplankton for 250°d, the survival rate was > 80% and the larvae total length increased significantly (Table 4). Enzyme activities changed species specifically during the 250°d feeding period (Table 1).

Enzyme activity change factors (EACF) were calculated for the four enzyme functionality groups (i.e. proteolytic, lipolytic, carbohydrate splitting, and total enzymes) (Table 3). In all species the EACFs for the four functionality groups were > 1.00.

Table 1. Enzyme activities in the intestine of larvae of different freshwater teleosts at the onset of exogenous feeding (0 degree days (°d)) and 250°d thereafter ($n$ = 8; units are μmol/min/g protein)

| | °d | *L. lota* | *S. lucioperca* | *T. thymallus* | *C. maraena* | *C. atterensis* |
|---|---|---|---|---|---|---|
| Chymotrypsin | 0 | 174 ± 230$^{a}$ | 427 ± 202$^{b}$ | 642 ± 158$^{c}$ | 661 ± 295$^{c}$ | 341 ± 176$^{d}$ |
| | 250 | 455 ± 120$^{b}$ | 917 ± 83$^{e}$ | 791 ± 192$^{f}$ | 682 ± 96$^{c}$ | 589 ± 83$^{c}$ |
| Trypsin | 0 | 0.85 ± 0.33$^{a}$ | 0.20 ± 0.14$^{b}$ | 9.32 ± 7.31$^{c}$ | 7.38 ± 4.62$^{c}$ | 0.93 ± 0.91$^{a}$ |
| | 250 | 0.92 ± 0.32$^{a}$ | 5.01 ± 0.67$^{d}$ | 22.50 ± 2.62$^{e}$ | 9.38 ± 2.21$^{c}$ | 6.03 ± 2.2$^{c,d}$ |
| Peptidase | 0 | 5.10 ± 1.12$^{a}$ | 0.08 ± 0.12$^{b}$ | 3.42 ± 1.44$^{c}$ | 3.54 ± 1.33$^{c}$ | 0.54 ± 0.23$^{d}$ |
| | 250 | 11.53 ± 2.37$^{e}$ | 22.93 ± 4.54$^{f}$ | 15.28 ± 3.37$^{g}$ | 6.06 ± 1.93$^{a}$ | 12.03 ± 2.29$^{g}$ |
| Lipase, NPL | 0 | 1.69 ± 1.04$^{a}$ | 23.27 ± 11.68$^{b}$ | 10.84 ± 4.71$^{c}$ | 0.30 ± 0.12$^{d}$ | 0.24 ± 0.16$^{d}$ |
| | 250 | 2.02 ± 0.45$^{a}$ | 36.88 ± 4.20$^{e}$ | 12.97 ± 3.23$^{c}$ | 0.78 ± 0.15$^{d}$ | 5.45 ± 1.79$^{f}$ |
| Lipase, triolein | 0 | 13.23 ± 0.14$^{a}$ | 97.24 ± 49.49$^{b}$ | 58.01 ± 38.40$^{c}$ | 3.41 ± 1.84$^{d}$ | 1.13 ± 0.61$^{d}$ |
| | 250 | | | n.i. | | |
| Phospholipase A | 0 | 0.49 ± 0.30$^{a}$ | 1.50 ± 1.07$^{b}$ | 41.04 ± 19.35$^{c}$ | 3.28 ± 1.66$^{d}$ | 2.45 ± 0.56$^{b,d}$ |
| | 250 | 1.41 ± 0.26$^{b}$ | 42.20 ± 3.00$^{c}$ | 59.02 ± 9.13$^{e}$ | 3.84 ± 1.53$^{d}$ | 1.24 ± 0.66$^{b}$ |
| Phospholipase, total | 0 | 1.01 ± 0.87$^{a}$ | 2.89 ± 1.99$^{b}$ | 86.18 ± 40.63$^{c}$ | 6.56 ± 3.23$^{d}$ | 5.94 ± 2.83$^{d}$ |
| | 250 | | | n.i. | | |
| Acid phosphatase | 0 | 2.99 ± 0.52$^{a}$ | 3.18 ± 1.14$^{a}$ | 2.44 ± 1.10$^{a}$ | 2.53 ± 1.61$^{a}$ | 1.23 ± 0.56$^{b}$ |
| | 250 | 3.10 ± 0.57$^{a}$ | 3.80 ± 1.52$^{a}$ | 2.98 ± 0.86$^{a}$ | 2.62 ± 0.45$^{a}$ | 2.47 ± 0.32$^{a}$ |
| Alkaline phosphatase | 0 | 0.05 ± 0.04$^{a}$ | 2.99 ± 0.84$^{b}$ | 3.31 ± 1.51$^{b}$ | 5.31 ± 3.21$^{c}$ | 1.42 ± 0.68$^{d}$ |
| | 250 | 0.18 ± 0.12$^{e}$ | 5.89 ± 1.44$^{c}$ | 3.29 ± 1.13$^{b}$ | 5.98 ± 2.12$^{c}$ | 5.67 ± 0.91$^{c}$ |
| α-Glucosidase | 0 | 0.08 ± 0.06$^{a}$ | 0.09 ± 0.08$^{a}$ | 0.12 ± 0.10$^{a}$ | 0.09 ± 0.04$^{a}$ | 0.07 ± 0.06$^{a}$ |
| | 250 | 0.09 ± 0.05$^{a}$ | 0.07 ± 0.07$^{a}$ | 0.12 ± 0.07$^{a}$ | 0.11 ± 0.06$^{a}$ | 0.10 ± 0.08$^{a}$ |
| Chitinase | 0 | 40.01 ± 14.53$^{a}$ | 40.42 ± 11.04$^{a}$ | 47.45 ± 21.42$^{a}$ | 27.96 ± 11.72$^{b}$ | 9.03 ± 5.07$^{c}$ |
| | 250 | 42.23 ± 15.65$^{a}$ | 77.1 ± 30.31$^{d}$ | 138.60± 60.20$^{e}$ | 46.89 ± 13.08$^{a}$ | 37.51 ± 8.36$^{a}$ |
| Amylase | 0 | 56.05 ± 29.07$^{a}$ | 51.46 ± 10.68$^{a}$ | 28.48 ± 13.33$^{b}$ | 6.67 ± 3.16$^{c}$ | 6.53 ± 3.01$^{c}$ |
| | 250 | 25.22 ± 14.12$^{b}$ | 39.42 ± 27.45$^{d}$ | 23.83 ± 1.37$^{b}$ | 7.08 ± 0.72$^{c}$ | 5.73 ± 0.41$^{c}$ |

lipase, NPL = lipase with nitrophenyl dodecanoate as substrate; lipase, triolein = lipase with triolein as substrate; peptidase = leucine aminopeptidase; n.i. = not investigated

data are mean ± standard deviation

$^{a–g}$values from the same parameter with the same superscript symbol are not significantly different ($P > 0.05$)

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Table 2. Species specific differences in activities of proteolytic enzymes, lipolytic enzymes, carbohydrate splitting enzymes, and total enzymes at the onset of exogenous feeding

| | Log transformed enzyme activities of | | | |
|---|---|---|---|---|
| | proteolytic enzymes ($n$ = 48)[1] | lipolytic enzymes ($n$ = 32)[2] | carbohydrate splitting enzymes ($n$ = 24)[3] | overall ($n$ = 80) (all listed enzymes) |
| *L. lota* | 2.83 ± 0.69[a] | 1.54 ± 0.48[a] | 2.16 ± 0.52[a] | 2.39 ± 0.41[a] |
| *C. atterensis* | 2.58 ± 0.61[a,b] | 1.97 ± 0.43[b] | 2.19 ± 0.44[a] | 2.41 ± 0.37[a] |
| *S. lucioperca* | 2.33 ± 0.67[b] | 2.68 ± 0.42[c] | 2.43 ± 0.32[b] | 2.59 ± 0.33[b] |
| *C. maraena* | 3.16 ± 0.75[c] | 2.24 ± 0.37[d] | 2.53 ± 0.41[b] | 2.86 ± 0.41[c] |
| *T. thymallus* | 3.17 ± 0.61[c] | 3.07 ± 0.45[e] | 2.79 ± 0.54[c] | 3.12 ± 0.41[d] |

[1]chymotrypsin, trypsin, peptidase, acid phosphatase; [2]lipase NPL, phospholipase A, alkaline phosphatase; [3]α-glucosidase, chitinase, amylase

single enzyme activities were log transformed, grouped to four listed functionality groups, and the mean ± standard deviation was calculated

[a–e]values from the same parameter with the same superscript symbol are not significantly different ($P$ > 0.05)

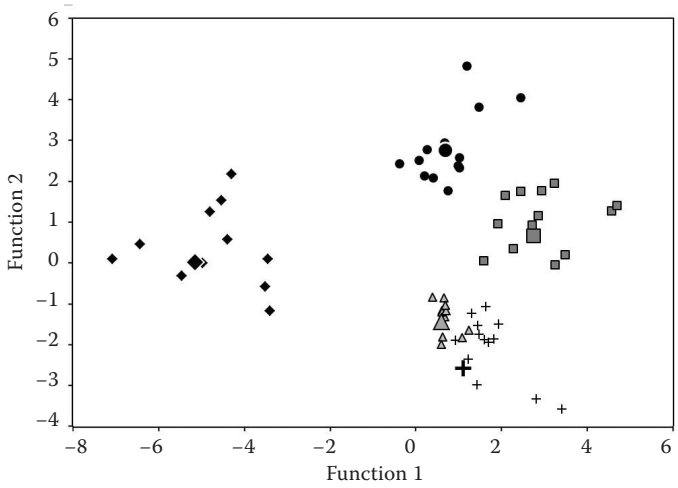


Figure 1. Scatterplot of the standardized canonical variates functions 1 and 2 (for the equations see Results) for the larval digestive enzymes at the onset of exogenous feeding

● *L. lota*, ◆ *S. lucioperca*, ■ *T. thymallus*, + *C. maraena*, △ *C. atterensis* (small symbols: actual data, large symbols: group centroids)

Table 3. Enzyme activity change factors (EACFs) for four functionality groups (proteolytic, lipolytic, carbohydrate splitting, and total enzymes) after a 250 degree day (°d) on-feeding period

| | Enzyme activity change factors | | | |
|---|---|---|---|---|
| | proteolytic enzymes ($n$ = 4)[1] | lipolytic enzymes ($n$ = 3)[2] | carbohydrate splitting enzymes ($n$ = 3)[3] | overall ($n$ = 10) (all listed enzymes) |
| *L. lota* | 1.21 ± 0.21 | 1.34 ± 0.29 | 0.85 ± 0.16 | 1.13 ± 0.24 |
| *C. atterensis* | 1.68 ± 0.53 | 1.65 ± 0.38 | 1.29 ± 0.40 | 1.59 ± 0.51 |
| *S. lucioperca* | 2.07 ± 1.09 | 1.65 ± 0.30 | 1.02 ± 0.13 | 1.69 ± 0.82 |
| *C. maraena* | 1.09 ± 0.10 | 1.11 ± 0.19 | 1.14 ± 0.19 | 1.12 ± 0.12 |
| *T. thymallus* | 1.30 ± 0.27 | 1.08 ± 0.08 | 1.19 ± 0.15 | 1.22 ± 0.22 |

[1]chymotrypsin, trypsin, peptidase, acid phosphatase; [2]lipase NPL, phospholipase A, alkaline phosphatase; [3]α-glucosidase, chitinase, amylase

data are reported as log transformed mean ± standard deviation; for details on the calculation procedure see the statistics section; no statistical tests were performed due to low sample numbers

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For proteolytic enzymes the EACF was the lowest for *C. maraena*, followed by *L. lota*, *T. thymallus*, *C. atterensis*, and the highest for *S. lucioperca*. For lipolytic enzymes, the lowest EACFs were found in *T. thymallus* and *C. maraena*, a medium one in *L. lota*, and the highest in *C. atterensis* and *S. lucioperca*. Concerning the carbohydrate splitting enzymes, EACF values were the lowest in *C. maraena*, followed by *T. thymallus*, *L. lota*, *S. lucioperca*, and *C. atterensis*. The overall EACF was minimal in *C. maraena* and *T. thymallus*, medium in *L. lota*, and maximal in *C. atterensis* and *S. lucioperca*.

***Morphometric and histological characteristics of the intestine of T. thymallus, C. maraena, C. atterensis, L. lota, and S. lucioperca.*** In *C. maraena*, *C. atterensis*, and *T. thymallus* the intestine was an undifferentiated tube, in *L. lota* and *S. lucioperca*

Table 4. Total lengths and histological characteristics of the intestine of larvae of *L. lota*, *S. lucioperca, T. thymallus*, *C. maraena, and C. atterensis* at 0 and 250 degree days (°d)

| | *L. lota* | *S. lucioperca* | *T. thymallus* | *C. maraena* | *C. atterensis* |
|---|---|---|---|---|---|
| **Larvae total length** | | | | | |
| 0°d | 4.6 ± 0.1[a] | 4.6 ± 0.2[a] | 14.9 ± 1.7[a] | 9.0 ± 1.1[a] | 7.5 ± 0.8[a] |
| 250°d | 7.3 ± 0.8[b] | 8.2 ± 0.2[b] | 27.8 ± 1.9[b] | 16.8 ± 4.1[b] | 12.8 ± 1.0[b] |
| **Epithelium** | | | | | |
| Characterization[SI] | monolayered, folded, epithelium with cylindrical cells | | | | |
| Height of folds at 0°d | 49.9 ± 16.7[a] | 65.1 ± 33.3[a] | 67.3 ± 19.2[a] | 56.9 ± 17.9[a] | 55.8 ± 15.1[a] |
| Height of folds at 250°d | 75.9 ± 17.2[b] | 95.4 ± 34.8[b] | 115.5 ± 55.3[b] | 104.2 ± 38.6[b] | 96.2 ± 34.5[b] |
| Height of epithelium[SI] | 27.3 ± 5.8[a] | 27.6 ± 5.3[a] | 33.0 ± 7.5[a] | 35.8 ± 6.3[a] | 30.5 ± 4.6[a] |
| **Nucleus** | | | | | |
| Shape[SI] | ovoid | ovoid | elongated, irregular | ovoid | ovoid |
| Position in cell[SI] | middle | basal or middle | basal | basal | basal or middle |
| Length (μm)[SI] | 8.8 ± 1.4[a] | 12.4 ± 1.0[b] | 9.1 ± 0.5[a] | 9.9 ± 1.6[a,b] | 10.5 ± 0.8[a,b] |
| Width (μm)[SI] | 3.5 ± 0.5[a] | 6.7 ± 0.3[b] | 2.6 ± 0.2[a] | 3.7 ± 0.6[a] | 5.5 ± 0.6[a,b] |
| **Nucleolus** | | | | | |
| Shape[SI] | spherical or irregular | spherical | irregular | spherical | spherical or irregular |
| Number (*n*)[SI] | 1 | 1 | 1–2 | 1–2 | 1 |
| Diameter (μm)[SI] | 2.2 ± 0.5[a] | 1.5 ± 0.3[b] | 1.5 ± 0.2[b] | 2.0 ± 0.2[a,b] | 1.9 ± 0.2[a,b] |
| **Cytoplasm** | | | | | |
| Structure[SI] | with granular inclusions and irregularly shaped vacuoles apically of nucleus | | | | |
| Diameter of small vacuoles[SI] | 1.1 ± 0.6[a] | 1.6 ± 0.4[b] | 0.9 ± 0.3[a] | 2.4 ± 0.5[b] | 1.2 ± 0.3[a] |
| Heterophagic vacuoles occurrence at 0°d | absent | absent | absent | absent | absent |
| Heterophagic vacuoles occurrence at 250°d | present | present | present | present | present |
| Diameter | 6.1 ± 2.1[a] | 5.3 ± 2.8[a] | 6.2 ± 1.2[a] | 7.6 ± 1.2[a] | 8.1 ± 2.2[a] |
| **Goblet cells** | | | | | |
| Occurrence at 0°d | absent | present | present | present | absent |
| Occurrence at 250°d | present | present | present | present | present |
| Length (μm) | 16.3 ± 3.2[a] | 15.1 ± 1.7[a, SI] | 18.3 ± 1.2[a, SI] | 18.0 ± 1.7[a, SI] | 18.4 ± 1.7[a] |
| Width (μm)[SI] | 6.6 ± 0.7[a] | 6.4 ± 1.0[a, SI] | 7.6 ± 0.9[a, SI] | 6.7 ± 1.0[a, SI] | 7.7 ± 1.0[a] |
| **Brush border layer** | | | | | |
| Width (μm)[SI] | 1.7 ± 0.2[a] | 1.4 ± 0.1[a] | 2.7 ± 0.3[b] | 3.2 ± 0.6[b] | 1.8 ± 0.5[a] |

morphometric data are mean ± standard deviation; [SI]similarity at 0 and 250°d; $n = 20$ for total length, $n = 40$ for histological data (10 measurements from 4 different larvae samples, respectively); histological data from 0 and 250°d were combined when not significantly different ($n = 80$)

[a,b]values from the same parameter with the same superscript symbol are not significantly different ($P > 0.05$)

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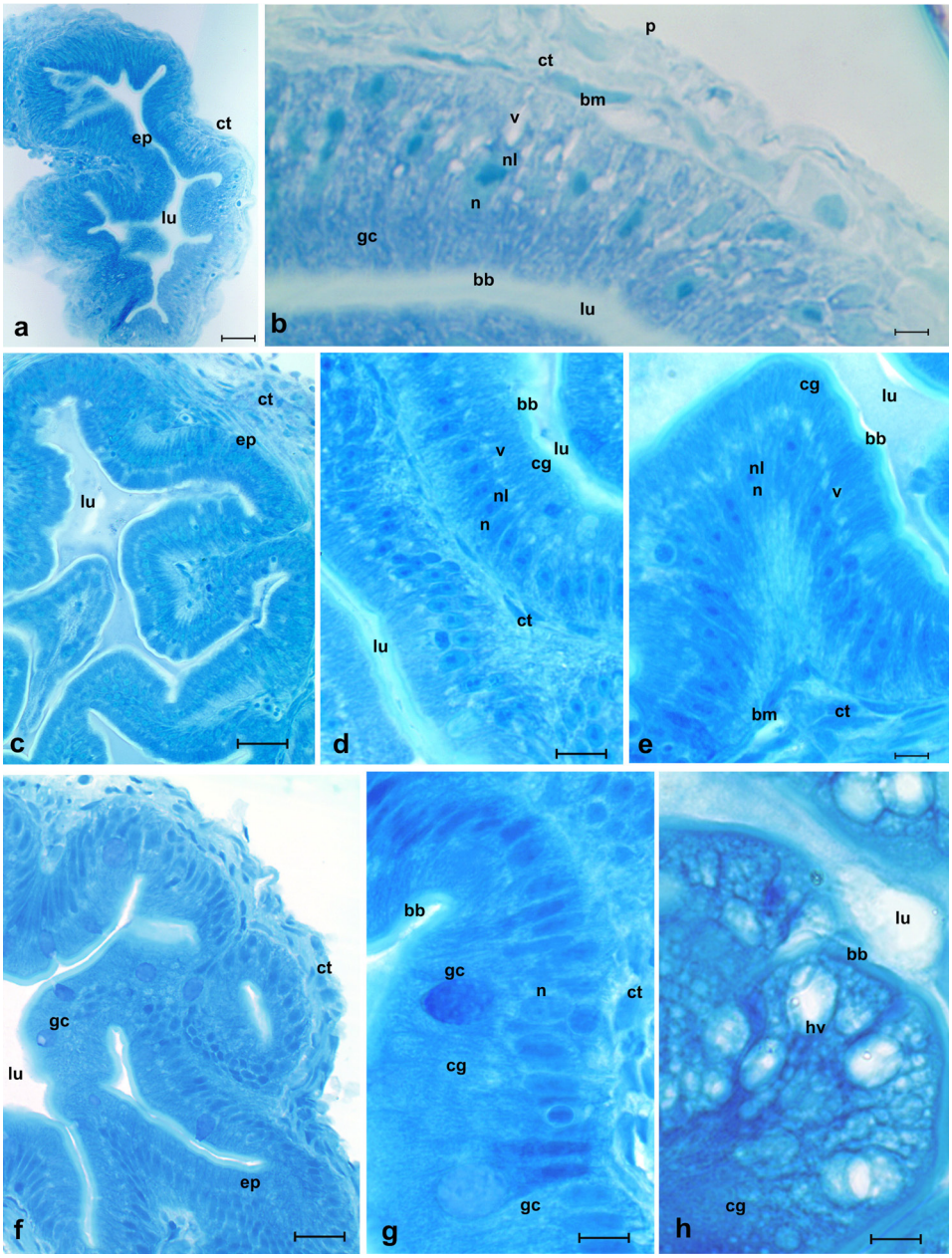


Figure 2. Intestine of larvae of *L. lota*, *S. lucioperca*, and *T. thymallus*

bb = brush border layer, bm = basal lamina, cg = cytoplasm with granular inclusions, ct = connective tissue layer, ep = epithelium, gc = goblet cell, hv = heterophagic vacuole, lu = lumen, n = nucleus, nl = nucleolus, p = peritoneum, v = vacuoles

(**a**) intestine of *Lota lota* larvae at the start of exogenous feeding at degree day 0 (0°d); general arrangement, cross section, scale bar = 35 µm; (**b**) intestinal epithelium of *Lota lota* larvae at the start of exogenous feeding (0°d); note vacuoles (v) in the basal and middle portions of the cells, scale bar = 5 µm; (**c**) intestine of *Sander lucioperca* larvae at the start of exogenous feeding (0°d); cross section, overview, scale bar = 40 µm; (**d**) intestinal epithelium of *S. lucioperca* larvae at the start of exogenous feeding (0°d); vacuoles (v) are located apically of the nucleus, scale bar = 10 µm; (**e**) arrangement of an intestinal fold in *S. lucioperca* larvae at the start of exogenous feeding (0°d); scale bar = 12 µm; (**f**) intestine of *T. thymallus* larvae at the start of exogenous feeding (0°d); cross section, overview, scale bar = 25 µm; (**g**) goblet cells (gc) in the intestinal epithelium of *T. thymallus* larvae at the start of exogenous feeding (0°d); scale bar = 7 µm; (**h**) intestinal epithelium of *T. thymallus* larvae after 250°d; note large heterophagic vacuoles (hv), scale bar = 7 µm

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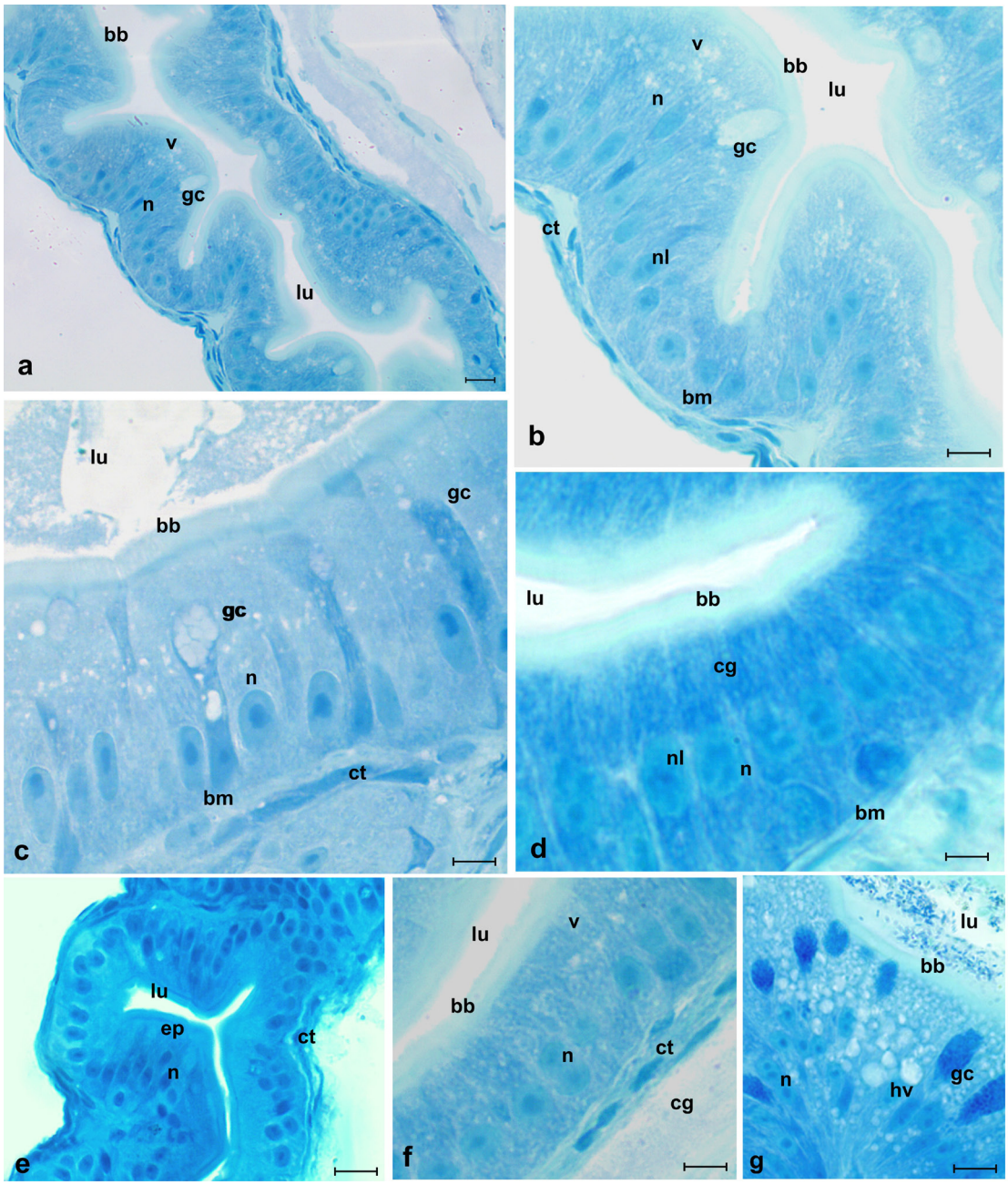


Figure 3. Intestine of larvae of *C. maraena* and *C. atterensis*

bb = brush border layer, bm = basal lamina, cg = cytoplasm with granular inclusions, ct = connective tissue layer, ep = epithelium, gc = goblet cell, hv = heterophagic vacuole, lu = lumen, lv = large vacuoles, n = nucleus, nl =nucleolus, p = peritoneum, v = vacuoles

(**a**) intestine of *C. maraena* larvae at the start of exogenous feeding at degree day 0 (0°d); longitudinal section showing general arrangement, scale bar = 20 µm; (**b**) intestinal fold of *C. maraena* larvae at the start of exogenous feeding (0°d); note prominent goblet cell (gc), scale bar = 10 µm; (**c**) detail of the intestinal epithelium of *C. maraena* larvae at the start of exogenous feeding (0°d); scale bar = 5 µm; (**d**) epithelium cell of *C. maraena* larvae at the start of exogenous feeding (0°d) showing brush border layer (bb), nucleus (n), and cytoplasm with granular inclusions (cg); scale bar = 5 µm; (**e**) intestine of *C. atterensis* larvae at the start of exogenous feeding (0°d); transversal section showing general arrangement, scale bar = 25 µm; (**f**) detail of intestinal epithelium of *C. atterensis* larvae at the start of exogenous feeding (0°d); scale bar = 8 µm; (**g**) detail of intestinal epithelium of *C. atterensis* larvae after 250°d; large heterophagic vacuoles (lv) are found in the epithelial cells, scale bar = 11 µm

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its anterior portion was enlarged to a sac-like structure. Histological features of the intestine of *L. lota* are shown in Figure 2a, b, of *S. lucioperca* in Figure 2c–e, of *T. thymallus* in Figure 2f–h, of *C. maraena* in Figure 3a–d, and of *C. atterensis* in Figure 3e, f. Histological data are summarized in Table 5. In all investigated species the intestine revealed no histological differences along its length. It had a folded, monolayered epithelium adhering to a basal lamina. Its luminal side was bordered by a brush border seam. The cells were approximately cylindrically shaped with a nucleus in their basal or middle region. The cytoplasm contained granular inclusions and small vacuoles without inclusions. Species specific differences existed in the shape of the nucleus, in the shape and number of nucleoli, in the size of the vacuoles, and in the occurrence of goblet cells. Also the height of the epithelium and the dimensions of the nuclei and nucleoli and of the vacuoles differed significantly between the species. After 250°d the height of the epithelial folds was significantly increased in comparison to 0°d in all species and large heterophagic vacuoles were found frequently in the epithelial cells of all investigated species. Goblet cells had differentiated in *L. lota* and *C. atterensis* (Figure 2h, Figure 3g, Table 4).

## DISCUSSION

***Larvae digestive enzymes***. Larvae of *T. thymallus*, *C. maraena*, *C. atterensis*, *L. lota*, and *S. lucioperca* are capable of protein, lipid, and carbohydrate digestion at the onset of exogenous feeding as indicated by the occurrence of proteolytic enzymes (chymotrypsin, trypsin, and leucine aminopeptidase), lipolytic enzymes (lipase, phospholipase), and of disaccharide and polysaccharide splitting enzymes (α-glucosidase, amylase, chitinase). Chitinase activities reflect the ability of larvae to digest the exoskeletons of zooplankton organisms. Chitin might play an important role in the food web of larvae of freshwater teleosts, because it is present in high concentrations in zooplankton organisms (Gutowska et al. 2004). As chymotrypsin, trypsin, lipase, phospholipase A, and amylase are pancreatic enzymes (Mata-Sotres et al. 2016), this gland must be considered to be functional in larvae of *T. thymallus*, *C. maraena*, *C. atterensis*, *L. lota*, and *S. lucioperca* at the onset of the first feeding similar as in other fish larvae (Govoni et al. 1986; Hamza et al. 2015). Whether chitinase is of pancreatic or intestinal origin is still disputed (Hansen et al. 2013). Alkaline phosphatase, leucine aminopeptidase, and maltase are typical intestinal enzymes (Zambonino Infante et al. 2008; Gisbert et al. 2013). Alkaline phosphatase is located in the brush border layer (Kozaric et al. 2004) regulating lipid absorption into enterocytes, participating in bicarbonate secretion and controlling bacterial endotoxin-induced inflammation (Lalles 2010). Acid phosphatase is located in the lysosomes of enterocytes (Kozaric et al. 2004) and could support intracellular digestion (Zhang et al. 1995).

In *T. thymallus*, *C. maraena*, *C. atterensis*, *L. lota*, and *S. lucioperca* the pattern of digestive enzyme activities revealed great species specific differences at the onset of exogenous feeding. This was indicated by the facts that (a) single enzyme activities and (b) enzyme activities of the four functional groups (proteolytic, lipolytic, carbohydrate splitting, and total enzyme activity) differed between the species, and that (c) the enzymatic pattern of specific larvae samples could be assigned to a certain species at a high probability level when using discriminate analysis. The herein presented data give also indications about functionality differences of the digestive system at the onset of exogenous feeding: in *C. atterensis* and *L. lota* proteolytic, lipolytic, and carbohydrate splitting enzymes were low. Therefore, it can be suggested that these larvae depend on a very specific food composition or even on external sources of digestive enzymes. Live food organisms as e.g. copepods, cladocerans, or Artemia might serve as external sources of enzymes (Kolkovski 2001). Larvae of *S. lucioperca* had low activities of proteolytic enzymes, high activities of lipolytic enzymes, and medium range activities of carbohydrate splitting enzymes. Thus could indicate the specialization of *S. lucioperca* larvae on a lipid rich diet which is also established by results obtained in larvae feeding experiments (Hamza et al. 2008). The highest activities of proteolytic, lipolytic, and carbohydrate splitting enzymes were observed in *C. maraena* and *T. thymallus* which is interpreted in favour of a well-developed digestive system capable of digesting a broad range of nutrients. In summary, these data indicate that *C. atterensis* and *L. lota* have a poorly developed digestive system at the onset of exogenous feeding, *S. lucioperca*

doi: 10.17221/25/2016-CJAS

a medium developed one, and *C. maraena* and *T. thymallus* a relatively well developed one. Species specific patterns of larval digestive enzymes and differences in the functionality are described for the first time in this study. They have also consequences in practice for formulating artificial feeds, as species specific demands in food composition and digestion ability of larvae must be considered. The data are also in accordance with practical experience in fish farms. It is established that larvae of *T. thymallus* and *C. maraena*, which have a well-developed digestive system, can be reared (at least at a survival rate of ca. 50%) with formulated dry feed (Lahnsteiner and Kletzl 2014, 2015), but *C. atterensis*, *L. lota*, and *S. lucioperca*, the species with a poorly or moderately developed digestive system, only with live feed of high quality (Hamza et al. 2008; Lahnsteiner et al. 2012b; Lahnsteiner and Kletzl 2015).

Also the changes of digestive enzymes during the period of the first feeding were species specific as three types of differentiation could be distinguished: type 1 was found in *T. thymallus* and *C. maraena*, species with a well-developed digestive system at the onset of exogenous feeding. They revealed a low increase (= low EACFs) for enzyme activities of all four functionality groups (overall ECAF: 1.12–1.22). Type 2 was assigned to *S. lucioperca* and *C. atterensis,* i.e. larvae species, which had a poorly or moderately developed digestive system at the onset of exogenous feeding, and which revealed a considerable increase (= high EACFs) in enzyme activities for all four functionality groups (overall ECAF: 1.59–1.69). Type 3 was found in *Lota lota*. Its digestive system was poorly developed at the beginning of exogenous feeding and the four enzyme functionality groups showed no or a low activity increase during the 250°d feeding period (overall EACF: 1.13).

The data on activity changes of larval digestive enzymes during the early development presented herein are conform to previous studies. *Perca fluviatilis* larvae showed an increase in activities of trypsin, chymotrypsin, amylase, alkaline phosphatase, leucine-aminopeptidase, and maltase during the period of the first exogenous feeding (Cuvier-Peres and Kestemont 2001) and *S. lucioperca* larvae an increase in activities of trypsin, amylase, leucine-alanine peptidase, alkaline phosphatase, and leucine-aminopeptidase (Hamza et al. 2015). Also in larvae of marine teleosts an increase in activities of pancreatic enzymes and of alkaline phosphatase was found during the period of the first feeding while the changes in activities of leucine aminopeptidase and amylase were species specific (*Diplodus puntazzo* – Savona et al. 2011; *Sciaenops ocellatus*, *Dicentrarchus labrax*, *Solea senegalensis* – Zambonino Infante and Cahu 2001).

***Cellular and subcellular structure of the intestine***. The histological results demonstrate that the intestines of *T. thymallus*, *C. maraena*, *C. atterensis*, and *S. lucioperca* reveal no epithelial differentiations at the onset of exogenous feeding, similar as the intestine of larvae of other teleost species (Govoni et al. 1986; Zambonino Infante and Cahu 2001; Ostaszewska 2005). Species specific differences were found only in the occurrence of goblet cells. They were present in *T. thymallus*, *C. maraena*, and *S. lucioperca*, but undeveloped in *C. atterensis* and *L. lota*. Goblet cells synthesize mucoproteins and mucopolysaccharides, which act as protective layer of the epithelium (Govoni et al. 1986; Zambonino Infante et al. 2008). For mammals it was reported that the mucus layer interacts with the intestinal microflora and with pathogens and has significance in innate and adaptive immunity (Kim and Ho 2010). Therefore, absence of goblet cells may be considered as sign of immaturity of the intestine. Histological investigations on different marine fish larvae indicated that the time point of differentiation of goblet cells is species specific (Zambonino Infante et al. 2008). In some species as e.g. *Solea solea*, *Pleuronectes ferruginea,* and *Paralabrax maculatofasciatus*, goblet cells are developed already at the onset of exogenous feeding, while in other species as e.g. *Sparus aurata*, *Paralichthys californicus*, *Dentex dentex*, and *Melanogrammus aeglefinus*, they differentiate at latter stages of development (Zambonino Infante et al. 2008). The supranuclear vacuoles found in the intestinal epithelium of all species investigated in the present study might be indicative of absorption processes of lipids, proteins or other macromolecules by pinocytotic processes via the brush border layer as suggested by Govoni et al. (1986) based on electron microscopical studies.

During the 250°d on-feeding period only few histological changes were observed. Goblet cells differentiated in *C. atterensis* and *L. lota*, which can be considered as maturation sign of the intestine. The height of the epithelial folds increased, being indicative of an enlargement of the inter-

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nal digestive surface. However, as the intestine of the investigated species grows approximately isometrically to the larvae total length (unpublished data), it is uncertain if the intestinal surface increases also in relation to the size of the organ. This topic would require more detailed morphometric investigations. Large vacuoles were frequently observed in the epithelial cells which are interpreted as heterophagic vacuoles. They can be considered as functional differentiation in response to food adsorption. Generally for fish larvae, development of stomach and pyloric caeca and differentiation of the intestine in three different regions (anterior-median or prevalvular segment, postvalvular segment, rectal zone) occur in more advanced development stages (Govoni et al. 1986; Ostaszewska 2005). Contrarily, the pancreas is already functional at the onset of feeding (Govoni et al. 1986; Ostaszewska 2005; Hamza et al. 2015).

In summary, the present data show that the pattern of digestive enzymes in fish larvae is species specific at the onset of the first feeding. Based on the activity levels of digestive enzymes and on histological data, the species could be distinguished as those having a poorly developed (*C. atterensis* and *L. lota*), a moderately developed (*S. lucioperca*), and a well-developed digestive system (*T. thymallus*, *C. maraena*). Also the differentiation of digestive enzymes during the period of the first feeding differed in a species specific way. Histologically, only one species specific differentiation was detected at the onset of the first feeding, i.e. the occurrence of goblet cells. Also histological differentiations during the 250°d feeding period were only minor.

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