

Digestive Enzyme Activity in Eurasian Perch (*Perca Fluviatilis*) and Arctic Charr (*Salvelinus Alpinus*)

Markus Langeland, Jan Erik Lindberg and Torbjörn Lundh*

Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences P.O. Box 7024, SE-750 07 Uppsala, Sweden

Abstract

The activity of the digestive enzymes lipase, α -amylase and disaccharidases (sucrase, maltase, isomaltase and trehalase) was studied in slow-growing and fast-growing groups of farmed Eurasian perch (*Perca fluviatilis*) of different ages. The activity of carbohydrases (disaccharidases and α -amylase), lipase, trypsin and chymotrypsin were also compared in Eurasian perch and Arctic charr (*Salvelinus alpinus*). No effect was noted of growth rate and age on the digestive enzyme activities in Eurasian perch. Total lipase activity was higher than total combined carbohydrase activity in both species (142.0 vs. 6.2 and 111.0 vs. 2.5 U mg⁻¹ protein for Eurasian perch and Arctic charr respectively; $P < 0.001$ for both). Compared with Arctic charr, Eurasian perch had higher lipase activity in the pancreas and lower lipase activity in the pyloric caeca and mid-intestine. Total chymotrypsin activity was higher than total trypsin activity in both species, whereas total chymotrypsin activity was higher for Arctic charr than for Eurasian perch (192.8 and 110.2 U mg⁻¹ sample respectively; $P < 0.001$). The high lipase and protease activity, and low carbohydrase activity in both species can be linked to their carnivorous feeding habits. Moreover, Eurasian Perch had a higher total carbohydrase activity than Arctic charr, suggesting a greater capacity for digesting carbohydrates, especially starch.

Keywords: Digestive enzymes; Eurasian perch; Arctic charr; Carbohydrases; Lipase; Protease

Introduction

The utilisation of nutrients depends on the presence and quantity of appropriate digestive enzymes. There are several enzymes involved in the digestion of feed into subunits appropriate for absorption in the gastrointestinal (GI) tract of fish, and there are variations in enzyme activity between species [1-3]. Fish are commonly divided according to their feeding habits into carnivorous, omnivorous and herbivorous, and it is generally believed that this is reflected in digestive enzyme activities [2,4-6]. However, this is not always the case. In some studies the relationships between feeding habits and proteolytic and α -amylase activities were unclear [7,8], and it has been shown that carnivorous fish have α -amylase activity despite the lack of starch in their natural diet [9,10]. In practice, species-specific data on the presence and activity of digestive enzymes can be used in diet ingredient selection [11] and can result in a formulation of diets that match the digestive capacity of the fish [12]. For example, studies on digestive enzymes in fish have helped define limits for the dietary inclusion of protein and carbohydrates [12-14].

Both Arctic charr (*Salvelinus alpinus*) and Eurasian perch (*Perca fluviatilis*) are considered to be facultatively piscivorous [15,16], but Eurasian perch has also been described eating benthos [2]. However, there are differences between the GI tracts in perch and charr. The number of pyloric appendages differs between the species [17]. In Eurasian perch the pyloric caeca consists of only three appendages, while Arctic charr has 20-74 appendages. Moreover, the intestine in Eurasian perch is S-shaped and longer than the straight intestine found in Arctic charr. Nevertheless, the morphological structure of the digestive tract does not necessarily match its manner of feeding [18].

Studies have been performed on the activity of some of the digestive enzymes in early life stages of both Eurasian perch [19] and Arctic charr [20,21], but little is known about the digestive enzyme activities of adult individuals. Kuzmina [2] found that carbohydrase activity varied more than protease and alkaline phosphatase activities with age in wild-caught Eurasian perch, but no correlation with growth rate was performed. Fish [7] reported on the activity of digestive enzymes in

perch, but provided no information on the origin and size of the fish used.

Growth capacity can be restricted by several environmental and physiological factors [22]. In Atlantic cod (*Gadus morhua*) [23] and Atlantic salmon (*Salmo salar*) [24], trypsin activity has been shown to have an effect on growth rate. In rainbow trout (*Oncorhynchus mykiss*), amylase, maltase and protease activity has been found to be higher in fast-growing fish [25]. In intensive production of Eurasian perch, growth heterogeneity in the population is a major restriction [26] and the same problem exists in the production of yellow perch (*P. flavescens*) [27]. In this study, the objective was to compare digestive enzyme activities in slow and fast-growing groups of Eurasian perch of different ages to evaluate if differences in growth rate could be related to digestive enzyme activity. Furthermore, differences in digestive enzyme activity in Eurasian perch and Arctic charr were studied. The starting hypotheses were that fast-growing perch have higher digestive enzyme activity than slow-growing perch and that there are differences in digestive enzyme activity along the GI tract in Eurasian perch and in Arctic charr.

Materials and Methods

Fish and housing

Fingerlings of Eurasian perch (mean weight 6.0 g) were obtained from a commercial fish hatchery (Östgöds AB, Söderköping, Sweden)

*Corresponding author: Torbjörn Lundh, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences PO Box 7024, SE-750 07 Uppsala, Sweden, Tel:+46 18 67 2137; E-mail: torbjorn.lundh@slu.se

Received October 21, 2013; Accepted November 28, 2013; Published December 04, 2013

Citation: Langeland M, Lindberg JE, Lundh T (2013) Digestive Enzyme Activity in Eurasian Perch (*Perca Fluviatilis*) and Arctic Charr (*Salvelinus Alpinus*). J Aquac Res Development 5: 208 doi:10.4172/2155-9546.1000208

Copyright: © 2013 Langeland M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

and brought to the Swedish University of Agricultural Sciences, Uppsala. The fish were stored in a 1,000-L flow-through tank. Feed waste was removed daily, while every week approximately 70% of the water in the tank was flushed out and replaced with fresh water, and the walls of the tank cleaned. The average water temperature was 20°C, dissolved oxygen concentration ranged from 6.42 to 8.7 mg L⁻¹ (HQ 40d, Hach Lange GmbH, Berlin, Germany), pH fluctuated from 7.5 to 8.0, and ammonium-nitrogen (N) and nitrate-N were below detection limits (Sera Aqua-test box, Heinsberg, Germany). The photoperiod was set to 12 h light and 12 h darkness. The tank was provided with an artificial plant to provide shelter for the fish.

Seven months before sampling, 100 Arctic charr (mean weight 30.5 g) of the selected strain 'Arctic superior' [28] raised at the Kälärne research station, Sweden, were placed in a 1,000 L flow-through tank at the research station. The average water temperature was 4°C and the photoperiod was set to 12 h light and 12 h darkness. The experiment was performed in compliance with laws and regulations on procedures and experiments on live animals in Sweden, which are overseen by the Swedish Board of Agriculture.

Feeding

The Eurasian perch and Arctic charr were fed commercial iso-nitrogenous fish feeds, formulated to match the nutrient requirements of each species. The nutrient composition was therefore not identical with respect to lipid and nitrogen-free extractives (NFE). The nutrient composition (g kg⁻¹) was crude protein (CP): 500, lipid: 157, NFE: 200, crude fibre: 8 and ash: 78 for the Eurasian perch and CP: 500, lipid: 220, NFE: 115, crude fibre: 4 and ash: 96 for the Arctic charr. Fish were fed to apparent satiation twice daily (corresponding to approximately 1.7% and 2.0% of biomass in the tank for perch and charr respectively) by automatic feeders.

Sampling

To compare the effect of age and growth rate on lipase and carbohydrase activity in the GI tract of Eurasian perch, fish of two ages (approximately 12 and 18 months old) were visually sorted into small and large fish. From these four groups, 12 young small (PYS), six young large (PYL), six old small (POS) and six old large (POL) fish were randomly selected. In order to compare the lipase and carbohydrase activity in the GI tract in Eurasian perch and Arctic charr, six Arctic charr comparable in size to PYL were selected for sampling. The fish were starved for 24 hours prior to sampling and their weight and length recorded (Table 1). Before handling, all the fish were anaesthetised (tricaine methanesulphonate, MS 222; 124 Western Chemical Inc., Ferdale, WA, USA), and then finally killed by cutting the brachial arches. The liver (LI), stomach (ST), pancreas (PA), pyloric caeca (PC), mid-intestine (MI) and distal intestine (DI) were dissected, and the ST, MI and DI opened longitudinally and rinsed in ice-cold saline. The samples

Group	n	Weight (g)	Length (cm)
PYS	20	29.3 ± 3.9	14.0 ± 0.6
PYL	10	62.6 ± 7.6	18.4 ± 0.8
POS	10	84.3 ± 20.3	20.2 ± 1.4
POL	10	289.5 ± 26.2	28.3 ± 0.9
Charr	10	57.5 ± 9.8	17.4 ± 12.0
Perch (trypsin & chymotrypsin)	10	264.6 ± 26.1	-
Charr (trypsin & chymotrypsin)	10	82.7 ± 11.0	-

Data is presented as means ± standard deviation

Table 1: Numbers, weights and lengths of the sampled Eurasian perch and Arctic charr.

were frozen in liquid nitrogen and stored at -80°C until analysis. The pancreas was defined as the connective tissues surrounding the gut, including the diffuse pancreas. The intestines were sectioned as follows: in Eurasian perch the MI was taken to be the section distal to the most distal pyloric caecum and behind the second bend of the intestine and decrease in intestinal diameter, and the DI was the section posterior to the distal end to the anus; in Arctic charr, the MI was taken to be the anterior part between the pyloric caeca and the ileo-rectal valve, and the DI was the posterior part from the ileo-rectal valve to the anus.

In addition, ten Eurasian perch and ten Arctic charr were sampled in order to measure the trypsin and chymotrypsin activity in the intestinal chyme from PC, MI and DI. These fish were not starved prior to sampling. The fish were anaesthetised before killing. The PC was frozen as sampled, while the MI and DI were cut open longitudinally and the chyme gently removed and immediately frozen in liquid nitrogen and stored at -80°C.

Preparation of homogenate

For lipase, α-amylase and disaccharidase activity assays and protein determination, tissue samples were thawed, cut into small pieces and suspended (1:20) in ice-cold malic acid buffer (pH 5.4) and homogenised in an electrical homogeniser (Ultra turrax tube dispenser, IKA Werke GMBH & Co.KG, Staufen, Germany). The homogenate was then centrifuged for 10 minutes at 15,800×g and the supernatant stored at -80°C for later analysis. The protease inhibitor phenylmethylsulfonyl fluoride (PMSF; 0.5 mM; Sigma no. P7626, Sigma-Aldrich Sweden AB, Stockholm, Sweden) was added before the homogenate was analysed for lipase and α-amylase activity and protein content.

For trypsin and chymotrypsin activity assays, the PC with chyme was cut into small pieces and suspended (1:20) in ice-cold buffer (0.2 M Tris HCl, 0.05 M CaCl₂, pH 8.0) containing enterokinase solution (25.0 µg enterokinase mL⁻¹). Samples were homogenised in an electric homogeniser (Ultra turrax tube dispenser, IKA Werke GMBH & Co.KG), and the PC incubated for about 16 hours at 4°C to let enterokinase convert trypsinogen to trypsin, thereby activating chymotrypsinogen. The samples were then centrifuged for 10 minutes at 15,800×g. The supernatant was immediately analysed for trypsin and chymotrypsin activity. Chyme from MI and DI were suspended and homogenised as described for PC above, but without the addition of enterokinase solution to the buffer since trypsin was present and therefore converting trypsinogen to trypsin. The samples were centrifuged for 10 minutes at 15,800×g, and the supernatants were stored at -80°C for later analysis.

Enzyme assays

Preliminary tests were performed for each enzymatic assay during which various dilutions of the homogenates were tested, as well as different pH and temperatures, to determine which parameters resulted in the greatest initial rectilinear change in absorbance in a certain time. The enzyme activity of α-amylase and disaccharidases was determined with a microplate reader (Multiscan FC; Thermo Fisher Scientific Inc., IL, USA) and, for lipase, trypsin and chymotrypsin, a spectrophotometer (UV-1800, Shimadzu Europa GmbH, Duisburg, Germany) was used.

Alpha-amylase activity was determined with a commercial kit (Ceralpha Method, AOAC Official Method 2002.01., Megazyme International Ireland Ltd., Wicklow, Ireland) using benzylidene end-blocked p-nitrophenyl-α-D-maltoheptaoside as substrate. The test tube, containing 100 µL homogenate and 20 µL amylase high-range reagent solution, was incubated for 20 minutes at room temperature.

The reaction was stopped by adding 300 μ L stopping solution (alkaline solution) and absorbance was recorded at 405 nm.

The activity of disaccharidases (maltase, isomaltase, sucrose and trehalase) was determined according to the methods described by Dahlqvist [29], using maltose (Sigma no. M5885), isomaltase (Sigma no. I7253), sucrose (Sigma no. 84097) and trehalose (Sigma no. T9531) respectively as substrates. Incubation with 10 μ L homogenate and 10 μ L substrate buffer was performed at 37°C for 60 minutes and then stopped by adding 300 μ L Tris-glucose oxidase reagent. This was followed by another 60 minutes of incubation at 37°C for the colour to develop. Absorbance was recorded at 450 nm.

Lipase activity was determined according to Albro et al., [30] using p-nitrophenylmyristate (p-NPM; Sigma no. 70124) as substrate. In brief, the reaction mixture was prepared in a final volume of 1.0 mL and contained 100 mM Tris HCl (pH 8.0) 10 mM deoxycholate, 6 mM DL-dithiothreitol (DTT) and 0.5 mM p-NPM. Ethylene glycol monomethyl ether was used to dissolve p-NPM at 50°C. The reaction was started by adding 100 μ L homogenate and was monitored at 400 nm for 2 minutes at 30°C. The background (control without homogenate) change was subtracted from all values.

Trypsin and chymotrypsin activity was determined according to Rick [31] and Worthington [32] respectively. For trypsin, benzoyl-arginine-p-nitroanilide (BAPNA; Sigma no. B4875) was used as substrate and the reaction was followed at 390 nm for 2 minutes at 25°C. For chymotrypsin, N-benzoyl-L-tyrosine ethyl ester (BTEE; Sigma no. B6125) was used as substrate and the reaction was monitored at 256 nm for 2 minutes at 25°C.

Enzyme activity is expressed as specific activity in U mg⁻¹ protein or U mg⁻¹ sample (U= μ mol substrate hydrolysed min⁻¹). Protein was analysed using a Micro BCA Protein Assay kit, no. 23235 (Thermo Fisher Scientific Inc., IL, USA).

Statistical Analysis

Data was analysed with the statistical analysis system, version 9.3 (SAS Institute, Inc, Cary, NC, USA). The Mixed procedure was applied to calculate least square means (LSM) and standard errors (SE). Three different comparisons were made: the first analysing the effect of age and growth rate on carbohydrase and lipase activity in different regions of the GI tract of Eurasian perch, for which the statistical model included the fixed effects of groups (PYS, PYL, POS and POL) and tissues (LI, ST, PA, PC, MI and DI). In the second comparison, the digestive enzyme activity in different regions of the GI tract was analysed separately for Eurasian perch and Arctic charr with tissues as fixed effect. Finally, the effect of species on the enzyme activity in different regions of the GI tract of Eurasian perch (PYL was used) and Arctic charr of similar size was analysed. This model included the fixed effects of species and tissues. Two-way interactions between fixed factors (groups/species and tissues) were tested and excluded from the models if found to be non-significant (P>0.05). Fish within treatment was the experimental unit and treated as a random factor in all three models. The level of significance was set at P<0.05.

To compare the overall activity of individual carbohydrase and lipase in the GI tract, the activities in all tissues were summarised and in the remainder of this paper are referred to as 'total activity'. In addition, the total carbohydrase activity (sum of total sucrase, maltase, isomaltase, trehalase and α -amylase activity) was compared with the total lipase activity within and between species. The PDIF option was used to calculate P values when testing differences between LSMs. The

most relevant comparisons—differences between tissues and differences between species within the same tissue—were selected. To adjust for multiple comparisons using the Bonferroni method, the P values obtained were multiplied by the number of comparisons (indicated in the tables and figures).

Results

Enzyme activities in the GI tract of Eurasian perch

In terms of enzyme activity in different regions of the GI tract of Eurasian perch, the activity of carbohydrases and lipase was unaffected by growth rate and age (P>0.05). However, significant differences were found in the enzyme activity between different tissues (Figure 1A). The highest sucrase activity was found in MI, followed by LI, which was not significantly higher than the activity in PC (P=0.125). The lowest sucrase activity was found in ST, PA and DI. The maltase activity was higher in PC than in MI (P=0.008), whereas the isomaltase activity was higher in MI than PC (P=0.029) and also higher in these two tissues than in the other tissues (P<0.001). The activity of trehalase was higher in PA (P<0.001) than in all the other tissues. The activity of α -amylase was higher in PA than in all the other tissues (P<0.001), followed by the activity in PC, which was higher than the activity in LI, ST and DI. Lipase activity was highest in PA (P<0.001) and similar in all other tissues except for LI, where the activity was lower than in DI (P=0.049). The trypsin and the chymotrypsin activities were higher in PC than in MI and DI (P<0.05 for all).

The total lipase activity (142 U mg⁻¹ protein) in the GI tract was about 23 times higher than the total carbohydrase activity (6.2 U mg⁻¹ protein) (P>0.001). Among the carbohydrases, maltase had the highest total activity (4.8 U mg⁻¹ protein), followed by isomaltase (0.95 U mg⁻¹ protein), and then trehalase, α -amylase and sucrase (0.96, 0.17 and 0.13 U mg⁻¹ protein respectively) (P<0.001 for all). Total chymotrypsin activity (110 U mg⁻¹ sample) was significantly higher than total trypsin activity (0.27 U mg⁻¹ sample).

Enzyme activities in the GI tract of Arctic charr

Differences in disaccharidases and lipase activity in different regions of the GI tract are presented in Figure 1B. Sucrase activity was higher in LI (P<0.001) and maltase activity was higher in PC and MI (P<0.001) than in the other tissues. The activity of isomaltase was highest in PC, followed by MI, whereas the activity in the other sampled tissues did not differ. Trehalase activity was highest in PC and MI and lowest in ST. The highest α -amylase activity was observed in PA (P<0.001). The lipase activity was higher in PC and MI than in all other tissues (P<0.001). The trypsin and the chymotrypsin activities were higher in MI than in PC and DI (P<0.05 for all).

Total lipase activity (111 U mg⁻¹ protein) in the gut was 46 times higher than total carbohydrase activity (2.4 U mg⁻¹ protein) (P>0.001). Among the carbohydrases, maltase had the highest total activity (1.5 U mg⁻¹ protein), followed by isomaltase (0.70 U mg⁻¹ protein) and then trehalase, sucrase and α -amylase (0.17, 0.04 and 0.03 U mg⁻¹ protein respectively) (P<0.001 for all). Total chymotrypsin activity (193 U mg⁻¹ sample) was significantly higher than total trypsin activity (0.24 U mg⁻¹ sample).

Comparison of enzyme activities in the GI tract of Eurasian perch and Arctic charr

The activity of sucrase was significantly higher in the LI, PC and MI of Eurasian perch than in Arctic charr (Figure 2A). Eurasian perch

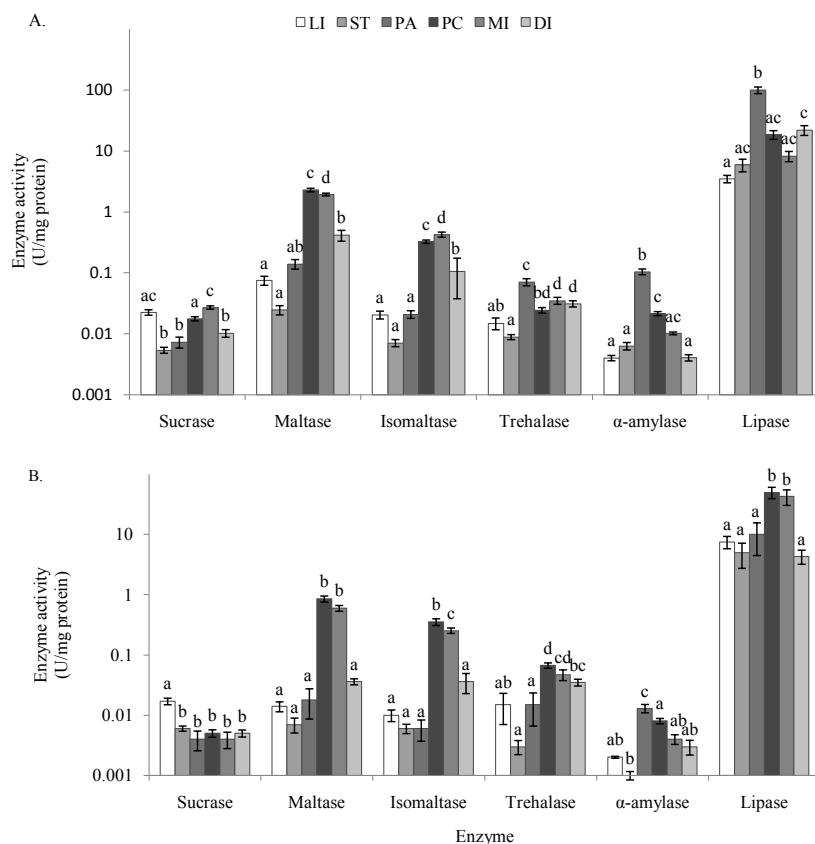


Figure 1: Enzyme activities ($U\ mg^{-1}\ protein$, logarithmic y axis) in sections of the gastrointestinal tract of A) Eurasian perch and B) Arctic charr. Disaccharidases (sucrase, maltase, isomaltase and trehalase), α -amylase and lipase in the liver (LI), stomach (ST), pancreas (PA), pyloric caeca (PC), mid-intestine (MI) and distal intestine (DI) of A) Eurasian perch ($n=24$) and B) Arctic charr ($n=6$). Bars within enzymes marked with different letters are significantly different ($P<0.05$). Results presented are least squares means and standard error.

had significantly higher maltase activity in PC, MI and DI than Arctic charr (Figure 2B), whereas the activity of isomaltase (Figure 2C) only was higher in DI of perch ($P<0.001$). Trehalase and lipase activities were significantly higher in PA of Eurasian perch than of Arctic charr (Figure 2D and 3B), but the opposite relationship was found in PC and MI ($P<0.001$). In Eurasian perch, the activity of α -amylase was higher in PA and PC than in charr (Figure 3A).

There were interactions between species and tissues in trypsin and chymotrypsin activities ($P<0.001$). Both enzymatic activities were higher in PC of Eurasian perch than of Arctic charr, whereas charr had higher activities in MI (Figure 4A and 4B). In DI, no significant differences between the two species could be observed. In Arctic charr, the activity of lipase, trypsin and chymotrypsin was higher in MI than in DI ($P<0.001$ for all), whereas no such difference was observed for Eurasian perch ($P>0.05$ for all).

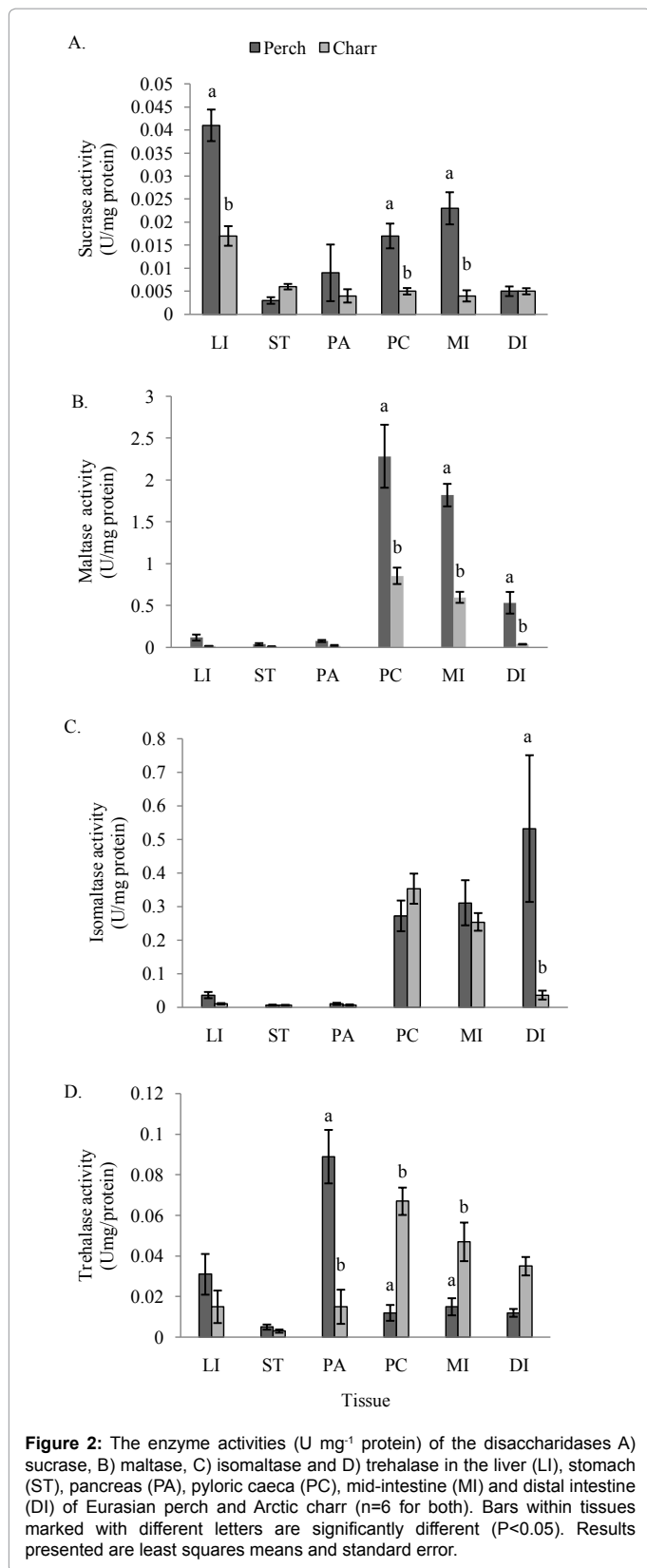
Total lipase activity did not differ between the species ($P=0.420$). Eurasian perch had a higher total carbohydrase activity than Arctic charr ($P<0.001$). The total activity of the individual carbohydrases (maltase, sucrase and α -amylase) was also significantly higher ($P<0.001$) in Eurasian perch than in Arctic charr, and isomaltase activity tended to be higher ($P=0.061$), whereas trehalase activity was higher in Arctic charr than in Eurasian perch ($P=0.022$). The total activity of chymotrypsin was higher for Arctic charr than for Eurasian perch ($P<0.001$). For total trypsin activity, no difference could be found ($P=0.469$).

Discussion

Enzyme activities in perch in relation to age and size

No effect of age or size on enzyme activity in Eurasian perch was observed in the present study. In contrast, Kuz'mina [2] noted a decrease in sucrase and α -amylase activity in adult Eurasian perch compared with fry. However, in this study, the comparison was between farmed fish fed a commercial diet, and the age range covered was narrower than in the study by Kuz'mina [2]. Moreover, the body size of the small fish differed from the fry examined by Kuz'mina [2]. In the wild, Eurasian perch changes its feeding habits as it grows, from initially eating predominantly plankton as fry to gradually eating more benthos as it continues to grow in size. According to [16], Eurasian perch starts its adaptation to become piscivorous at an approximate body length of 14-18 cm, above which the diet is dominated by fish. Thus it appears reasonable to assume that the perch examined in the present study were physiologically already more or less adapted to eating fish, which explains the lack of impact of age and size on carbohydrase activity.

The hypothesis here of slow-growing Eurasian perch having a lower digestive enzyme activity was not supported by the results. Similarly, Blier et al., [33] studied the activity of several different enzymes, including trypsin, chymotrypsin and alkaline phosphatase, in transgenic Coho salmon and found little or no difference between fast-growing transgenic and non-transgenic fish. Kuz'mina [2] did not find any difference in proteolytic activity in large and small fry of perch or



pike, but in bream (*Abramis abramis*) and roach larger fry had higher proteolytic activity. However, Ditlecadet et al., [34] found higher trypsin activity in fast-growing Arctic charr than in slow-growing Arctic charr.

In the present study, no distinction was made between endogenous and exogenous digestive enzymes. Askarian et al., [35] found amylase-, lipase- and protease-producing bacteria in Atlantic cod. However, although it is possible that the gut microbiota may contribute to fish nutrition, the role of enzyme-producing bacteria isolated from the fish gut remains unclear, mainly due to the different utilisation of substrates *in vitro* and *in vivo* [36].

Carbohydrases

The α -amylase activity in both Eurasian perch and Arctic charr was found to be highest in diffuse pancreatic tissues, as also reported by Fish [7]. For omnivorous/herbivorous species such as the Mozambique tilapia (*Oreochromis mossambicus*), goldfish (*Carassius auratus*) and river garfish (*Hyporhamphus regularis ardelio*), the amylase activity shows a more widespread presence in the gut [7,37,38]. In contrast, Sarbahi [37] did not find any α -amylase activity in the gut of the carnivorous largemouth bass (*Micropterus salmoides*). Reimer [39] found very low α -amylase activity in Arctic charr, and suggested that it is of exogenic origin. However, α -amylase activity has been found in the gut of some carnivorous species such as yellowfin tuna (*Thunnus albacares*) [40], rainbow trout [41] and pike (*Esox lucius*) [2].

Total α -amylase activity in the different tissues was higher in Eurasian perch than in Arctic charr (0.13 vs. 0.03 U mg⁻¹ protein, respectively), including higher α -amylase activity in the pancreas and pyloric caeca. This could be a species specific effect and indicates that Eurasian perch may have a higher capacity to digest starch than Arctic charr. There is a variation in α -amylase activity between species and it appears to be higher in herbivorous and omnivorous species than in carnivorous ones [2,4,41,42]. Thus, this suggests that feeding habits and long-term

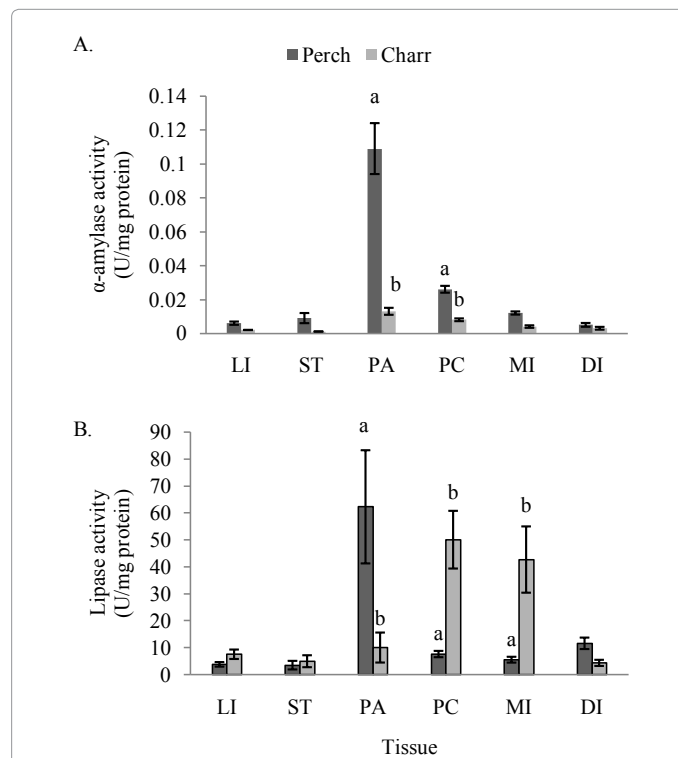
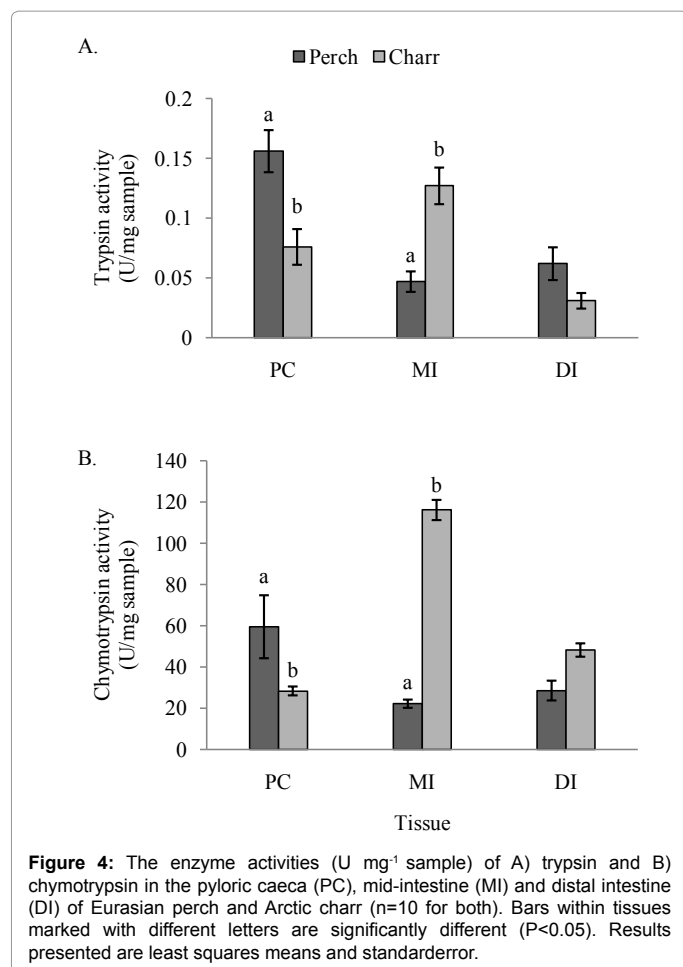


Figure 3: The enzyme activities (U mg⁻¹ protein) of A) α -amylase and B) lipase in the liver (LI), stomach (ST), pancreas (PA), pyloric caeca (PC), mid-intestine (MI) and distal intestine (DI) of Eurasian perch and Arctic charr (n=6 for both). Bars within tissues marked with different letters are significantly different (P<0.05). Results presented are least squares means and standard error.



selection and adaptation could explain species related differences. In some studies, no relationship between α -amylase activity and feeding habits has been found [1,8]. The difference in α -amylase activity between species might also be related to the higher NFE content in the diet fed to Eurasian perch. However, recent experiments conducted by this research group (Abro, Lundh and Lindberg, unpublished data) confirmed a higher α -amylase activity in Eurasian perch than in Arctic charr when feeding fish diets that have different inclusion levels of NFE. Furthermore, no significant correlation between α -amylase activity and dietary NFE levels could be found in that study.

The relatively high activity of some digestive enzymes in the liver of both Eurasian perch and Arctic charr was unexpected. This may be due to an infiltration of endocrine or exocrine pancreatic tissue into the liver [5,9,17] or due to intracellular liver enzymes. The liver contains amylo-1,6-glucosidase, acid α -glucosidase and neutral α -glucosidase [43], which might have cleaved glucose units off the disaccharides. However, digestive enzyme activities have been found in the liver of rabbit fish (*Siganus canaliculatus*) [5] and in that of animals such as rats [44].

The highest sucrase activity in Arctic charr was found in the liver, while in the liver of Eurasian perch it was as high as in the mid-intestine (Figures 1A and 1B). Moreover, the activity was twice as high in the liver of Eurasian perch as in that of Arctic charr (Figure 2A). The disaccharidase with the highest activity in both the Eurasian perch and the Arctic charr was maltase, followed by isomaltase, trehalase and

sucrase in that order. Higher maltase activity than trehalase and sucrase activity has also been reported for hybrid tilapia (*O. niloticus* × *O. aureus*), hybrid striped bass (*Morone saxatilis* × *M. chrysops*), rainbow trout and Atlantic salmon [3,9,45]. In the present study, sucrase, maltase and isomaltase activity was higher in Eurasian perch than in Arctic charr. Consequently, this indicates that the capacity to digest carbohydrate-rich feed is higher in Eurasian perch than in Arctic charr.

Lipase

Carnivorous fish usually consume a fat-rich diet [1], which might explain the much higher activity of lipase than of carbohydrases in Eurasian perch and Arctic charr in this study, as well as in other carnivorous fish species [7,12]. The highest lipase activity was found in the pyloric caeca and mid-intestine of the Arctic charr, and in the diffuse pancreatic tissue of the Eurasian perch. The lipase activity in the pancreas of the perch was around six times higher than in charr, while the lipase activity in charr pyloric caeca and mid-intestine was about seven and eight times higher respectively than in perch. Noailles-Depyre and Gas [46] reported that lipids are absorbed in the proximal segment of the intestine in Eurasian perch, which is in line with observations in several other fish species [47]. Thus it could be expected that the highest lipase activity should be found in the same region of the GI tract in both Eurasian perch and Arctic charr. The unexpected discrepancy in enzyme activity in different gut segments between the two species might be explained by complications with sampling. In comparison to the Arctic charr, the Eurasian perch had more loose connective tissue along the GI tract, of which some was sampled as diffuse pancreatic tissue. Much of this loose connective tissue consists of visceral adipose tissue which is known to express hormone-sensitive lipase [48] and lipoprotein lipase activity [49]. The method used in this study did not distinguish between the activities of different types of lipases. Consequently, the exocrine pancreas in Eurasian perch synthesises lipase, which is secreted into the lumen, but not to the same extent as in Arctic charr. In the present study it cannot definitely be shown that the higher lipase activity in PC and MI of Arctic charr is a species-related effect, or whether the slightly higher dietary lipid intake in Arctic charr has influenced the secretion and expression of the lipase.

Trypsin and chymotrypsin

Torrissen [50] found higher trypsin activity in the second half of the small intestine and the first part of the large intestine than in the pyloric caeca and second part of the large intestine in Atlantic salmon and rainbow trout. This is in accordance with our results for Arctic charr, but not for Eurasian perch, which had highest activity in the pyloric caeca. In Atlantic salmon, trypsin and chymotrypsin enter the gut through the pyloric caeca [51], but are stored in and secreted from the pancreas [52]. Probably trypsin and chymotrypsin are stored and secreted in the same manner in Arctic charr and Eurasian perch as in Atlantic salmon. Therefore, it is unexpected that the highest proteolytic activities were found in the pyloric caeca in Eurasian perch and not in the mid-intestine as in Arctic charr. The proteolytic activity declined in the distal intestine in Arctic charr, but was unchanged in Eurasian perch. Similar results were reported by Fish [7], who found a higher activity of alkaline proteases in the lower intestine than in the upper intestine and pyloric caeca in Eurasian perch.

Chymotrypsin activity was higher than trypsin activity in both Eurasian perch and Arctic charr, in agreement with findings in several other species: Atlantic salmon [51,52], silver carp (*Hypophthalmichthys molitrix*) and common carp [53], *Limia vittata* and *Gambusia punctata* [4] and Spotted sorubim (*Pseudoplatystoma corruscans*). In contrast,

some authors found the opposite relationship between trypsin and chymotrypsin activities in Eurasian perch larvae [19], Coho salmon (*O. kisutch*) [33], wels catfish (*Silurus glanis*) [53] and Atlantic salmon [54].

Total chymotrypsin activity was higher in Arctic charr than in Eurasian perch, while no difference in total trypsin activity was found between the two species. Previous studies have reported higher trypsin and chymotrypsin activities or total proteolytic activity in carnivorous fish than in omnivorous, herbivorous and detritivorous fish [4,12]. However, in a study by Hidalgo et al., [41] no correlation was found between total proteolytic activity and feeding habits.

In conclusion, the high lipase and protease activity and low carbohydrase activity in both species can be linked to their carnivorous feeding habits. Total carbohydrase activity was higher in Eurasian perch than in Arctic charr, which had a higher total chymotrypsin activity and lipase activity in the mid-intestine. The data presented suggests that feed formulation should be different for Eurasian perch and Arctic charr in order to match their inherent digestive enzyme activities. This applies in particular to the carbohydrate content and composition of the feed. Based on the recorded α -amylase and carbohydrase activities, the carbohydrate content, in particular starch, can be higher in feed for Eurasian perch than in feed for Arctic charr.

Acknowledgments

This research was supported by the Faculty of Veterinary Medicine and Animal Science at the Swedish University of Agricultural Sciences and by Formas (Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning). Special thanks to Kristina Andersson at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, for her helpful assistance with the statistical analyses and for reading the manuscript.

References

- Chakrabarti I, Gani MA, Chaki KK, Sur R, Misra KK (1995) Digestive Enzymes in 11 Freshwater Teleost Fish Species in Relation to Food Habit and Niche Segregation. *Comparative Biochemistry and Physiology Part A: Physiology* 112: 167-177.
- Kuzmina VV (1996) Influence of Age on Digestive Enzyme Activity in Some Freshwater Teleosts. *Aquaculture* 148: 25-37.
- Harpaz S, Uni Z (1999) Activity of Intestinal Mucosal Brush Border Membrane Enzymes in Relation to the Feeding Habits of Three Aquaculture Fish Species. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 124: 155-160.
- Falcon-Hidalgo B, Forrellat-Barrios A, Farnes OC, Hernandez KU (2011) Digestive Enzymes of Two Freshwater Fishes (*Limia vittata* And *Gambusia punctata*) with Different Dietary Preferences at Three Developmental Stages. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 158: 136-141.
- Sabapathy U, Teo LH (1993) A Quantitative Study of Some Digestive Enzymes in the Rabbit fish, *Siganus canaliculatus* and The Sea Bass, *Lateolabrax japonicus*. *J Fish Biol* 42: 595-602.
- Kapoor BG, Smit H, Verighina IA (1976) *The Alimentary Canal and Digestion in Teleosts*, Academic Press.
- Fish GR (1960) The Comparative Activity of Some Digestive Enzymes in the Alimentary Canal of Tilapia and Perch. *Hydrobiologia* 15: 161-178.
- Chan AS, Horn MH, Dickson KA And Gawlicka A (2004) Digestive Enzyme Activities in Carnivores and Herbivores: Comparisons among Four Closely Related Prickleback Fishes (Teleostei : Stichaeidae) From A California Rocky Intertidal Habitat. *J Fish Biol* 65: 848-858.
- Krogdahl Å, Hemre GI, Mommsen TP (2005) Carbohydrates in Fish Nutrition: Digestion and Absorption in Postlarval Stages. *Aquacult Nutr* 11: 103-122.
- Frøystad MK, Lilleeng E, Sundby A, Krogdahl Å (2006) Cloning and Characterization of α -Amylase from Atlantic Salmon (*Salmo salar* L.). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 145: 479-492.
- Kolkovski S (2001) Digestive Enzymes in Fish Larvae and Juveniles— Implications and Applications to Formulated Diets. *Aquaculture* 200: 181-201.
- Furné M, Hidalgo MC, López A, García-Gallego M, Morales AE, et al. (2005) Digestive Enzyme Activities in Adriatic Sturgeon *Acipenser naccarii* And Rainbow Trout *Oncorhynchus mykiss*. A Comparative Study. *Aquaculture* 250: 391-398.
- Twining SS, Alexander PA, Huibregtse K, Glick DM (1983) A Pepsinogen from Rainbow Trout. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* 75: 109-112.
- Spannhof L and Plantikow H (1983) Studies on Carbohydrate Digestion in Rainbow Trout. *Aquaculture* 30: 95-108.
- Labeelund JH, Langeland A, Saegrov H (1992) Piscivory By Brown Trout *Salmo trutta* L and Arctic Charr *Salvelinus alpinus* (L) In Norwegian Lakes. *J Fish Biol* 41: 91-101.
- Allen KR (1935) The Food and Migration of the Perch (*Perca fluviatilis*) in Windermere. *J Anim Ecol* 4: 264-273.
- NRC (2011) *Nutrient Requirements of Fish and Shrimp*, The National Academies Press, Washington DC, USA.
- Dabrowski K (1993) Ecophysiological Adaptations Exist In Nutrient Requirements of Fish: True Or False? *Comparative Biochemistry and Physiology Part A: Physiology* 104: 579-584.
- Cuvier-Peres A, Kestemont P (2001) Development of Some Digestive Enzymes in Eurasian Perch Larvae *Perca fluviatilis*. *Fish Physiol Biochem* 24: 279-285.
- Lemieux H, Le Francois NR, Blier PU (2003) The Early Ontogeny of Digestive And Metabolic Enzyme Activities In Two Commercial Strains Of Arctic Charr (*Salvelinus alpinus* L.). *J Exp Zool Part A* 299A: 151-160.
- Dabrowski K, Krumschnabel G, Paukku M, Labanowski J (1992) Cyclic Growth and Activity of Pancreatic Enzymes In Alevins of Arctic Charr (*Salvelinus alpinus* L.). *J Fish Biol* 40: 511-521.
- Blier PU, Pelletier D, Dutil JD (1997) Does Aerobic Capacity Set A Limit on Fish Growth Rate? *Reviews in Fisheries Science* 5: 323-340.
- Lemieux H, Blier P, Dutil JD (1999) Do Digestive Enzymes Set A Physiological Limit on Growth Rate and Food Conversion Efficiency in The Atlantic Cod (*Gadus morhua*)? *Fish Physiol Biochem* 20: 293-303.
- Torrissen KR, Shearer KD (1992) Protein Digestion, Growth and Food Conversion In Atlantic Salmon and Arctic Charr With Different Trypsin-Like Isozyme Patterns. *J Fish Biol* 41: 409-415.
- Kawai S, Ikeda S (1973) Studies on Digestive Enzymes of Fishes. Iii. Development of the Digestive Enzymes of Rainbow Trout after Hatching and the Effect of Dietary Change on the Activities of Digestive Enzymes in the Juvenile Stage. *Bull Jap Soc Sci Fish* 39: 819-823.
- Melard C, Kestemont P, Grignard JC (1996) Intensive Culture of Juvenile and Adult Eurasian Perch (*P. fluviatilis*): Effect of Major Biotic and Abiotic Factors on Growth. *J Appl Ichthyol* 12: 175-180.
- Kwasek K, Dabrowski K, Ware K, Reddish JM, Wick M (2012) The Effect Of Lysine-Supplemented Wheat Gluten-Based Diet On Yellow Perch *Perca flavescens* (Mitchill) Performance. *Aquac Res* 43: 1384-1391.
- Nilsson J, Brännäs E, Eriksson LO (2010) The Swedish Arctic Charr Breeding Programme. *Hydrobiologia* 650: 275-282.
- Dahlqvist A (1968) Assay of Intestinal Disaccharidases. *Anal Biochem* 22: 99-107.
- Albro PW, Hall RD, Corbett JT, Schroeder J (1985) Activation of Nonspecific Lipase (Ec 3.1.1.-) By Bile Salts. *Biochimica et Biophysica Acta* 835: 477-90.
- Rick W (1974) *Methods of Enzymatic Analysis*, Academic Press, New York.
- Worthington TM (1982) *Enzymes and Related Bio chemicals*. Biochemical Products Division. Worthington Diagnostic System Inc., Freehold, New Jersey.
- Blier PU, Lemieux H, Devlin RH (2002) Is the Growth Rate of Fish Set By Digestive Enzymes or Metabolic Capacity Of The Tissues? Insight from Transgenic Coho Salmon. *Aquaculture* 209: 379-384.
- Ditlecadet D, Blier PU, Le François NR, Dufresne F (2009) Digestive Capacities, Inbreeding and Growth Capacities in Juvenile Arctic Charr *Salvelinus alpinus*. *J Fish Biol* 75: 2695-2708.

35. Askarian F, Sperstad S, Merrifield DL, Ray AK, Ringø E (2012) The Effect of Different Feeding Regimes on Enzyme Activities of Gut Microbiota in Atlantic Cod (*Gadus Morhua* L.). Aquac Res 44: 841-846.
36. Ray AK, Ghosh K Ringø E (2012) Enzyme-Producing Bacteria Isolated from Fish Gut: A Review. Aquacult Nutr 18: 465-492.
37. Sarbahi DS (1951) Studies of the Digestive Tracts and the Digestive Enzymes of the Goldfish, *Carassius Auratus* (Linnaeus) and the Largemouth Black Bass, *Micropterus Salmoides* (Lacépède). Biological Bulletin 100: 244-257.
38. Day RD, German DP, Tibbetts IR (2011) Why Can't Young Fish Eat Plants? Neither Digestive Enzymes Nor Gut Development Preclude Herbivory In The Young of a Stomachless Marine Herbivorous Fish. Comparative Biochemistry and Physiology Part B, Biochemistry & Molecular Biology 158: 23-9.
39. Reimer G (1986) The Relationship between the Digestive Enzymes in Arctic Char, *Salvelinus-Alpinus* (Salmonidae, Osteichthyes) and Its Ability To Survive in Extreme Environments. Hydrobiologia 133: 65-72.
40. Buentello JA, Pohlenz C, Margulies D, Scholey VP, Wexler JB, et al. (2011) A Preliminary Study of Digestive Enzyme Activities and Amino Acid Composition of Early Juvenile Yellow Fin Tuna (*Thunnus Albacares*). Aquaculture 312: 205-211.
41. Hidalgo MC, Urea E, Sanz A (1999) Comparative Study of Digestive Enzymes in Fish with Different Nutritional Habits. Proteolytic and Amylase Activities. Aquaculture 170: 267-283.
42. Al-Hussaini AH (1949) On the Functional Morphology of the Alimentary Tract of Some Fish in Relation to Differences in their Feeding Habits: Cytology and Physiology. Quarterly Journal of Microscopical Science S3-90: 323-354.
43. Mehrani H, Storey KB (1993) Characterization of A-Glucosidases from Rainbow Trout Liver. Archives of Biochemistry And Biophysics 306: 188-194.
44. Horvat A (1973) The Occurrence and Intracellular Localization of Sucrase Activity in Rat Liver. Biochimica ET Biophysica Acta (BBA) - General Subjects 297: 413-422.
45. Krogdahl Å, Sundby A, Olli JJ (2004) Atlantic Salmon (*Salmo Salar*) and Rainbow Trout (*Oncorhynchus Mykiss*) Digest and Metabolize Nutrients Differently. Effects of Water Salinity and Dietary Starch Level. Aquaculture 229: 335-360.
46. Noaillac-Depeyre J, Gas N (1979) Structure and Function of The Intestinal Epithelial Cells in the Perch (*Perca Fluviatilis* L.). Anat Rec 195: 621-639.
47. Bakke AM, Glover C, Krogdahl Å (2010) 2 - Feeding, Digestion and Absorption of Nutrients, Academic Press.
48. Hazel JR, Sidell BD (2004) The Substrate Specificity of Hormone-Sensitive Lipase from Adipose Tissue of The Antarctic Fish *Trematomus Newnesi*. J Exp Biol 207: 897-903.
49. Albalat A, Sánchez-Gurmaches J, Gutiérrez J, Navarro I (2006) Regulation of Lipoprotein Lipase Activity in Rainbow Trout (*Oncorhynchus Mykiss*) Tissues. General and Comparative Endocrinology 146: 226-235.
50. Torrissen KR (1984) Characterization of Proteases in the Digestive Tract of Atlantic Salmon (*Salmo Salar*) In Comparison With Rainbow Trout (*Salmo Gairdneri*). Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 77: 669-674.
51. Einarsson S, Davies PS, Talbot C (1996) The Effect of Feeding on The Secretion of Pepsin, Trypsin and Chymotrypsin in The Atlantic Salmon, *Salmo Salar* L. Fish Physiol Biochem 15: 439-446.
52. Einarsson S, Davies PS (1996) On the Localisation and Ultrastructure of Pepsinogen, Trypsinogen and Chymotrypsinogen Secreting Cells in The Atlantic Salmon, *Salmo Salar* L. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 114: 295-301.
53. Jonas E, Ragyanszki M, Olah J, Boross L (1983) Proteolytic Digestive Enzymes of Carnivorous (*Silurus Glanis* L), Herbivorous (*Hypophthalmichthys Molitrix* Val) and Omnivorous (*Cyprinus Carpio* L) Fishes. Aquaculture 30: 145-154.
54. Torrissen KR, Moss R, Andresen LH, Berg A, Waagbo R (2006) Different Expressions of Trypsin and Chymotrypsin in Relation To Growth in Atlantic Salmon (*Salmo Salar* L.). Fish Physiol Biochem 32: 7-23.

Citation: Langeland M, Lindberg JE, Lundh T (2013) Digestive Enzyme Activity in Eurasian Perch (*Perca Fluviatilis*) and Arctic Charr (*Salvelinus Alpinus*). J Aquac Res Development 5: 208 doi:10.4172/2155-9546.1000208

Submit your next manuscript and get advantages of OMICS Group submissions

Unique features:

- User friendly/feasible website-translation of your paper to 50 world's leading languages
- Audio Version of published paper
- Digital articles to share and explore

Special features:

- 300 Open Access Journals
- 25,000 editorial team
- 21 days rapid review process
- Quality and quick editorial, review and publication processing
- Indexing at PubMed (partial), Scopus, EBSCO, Index Copernicus and Google Scholar etc
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at: <http://www.omicsonline.org/submit>

