www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 14: 929-938 (2014) DOI: 10.4194/1303-2712-v14_4_12

Effects of Artemia sp. Enrichment with Essential Fatty Acids on Functional and Morphological Aspects of the Digestive System in *Acipenser gueldenstaedtii* Larvae.

Maciej Kamaszewski[1,*], Teresa Ostaszewska[1], Maja Prusińska[2], Ryszard Kolman[2], Maciej Chojnacki[1], Juri Zabytyvskij[3], Barbara Jankowska[4], Robert Kasprzak[1]

[1] Warsaw University of Life Sciences (WULS-SGGW), Faculty of Animal Science, Department of Ichthyobiology and Fisheries, Poland.
[2] Stanislaw Sakowicz Inland Fisheries Institute, Olsztyn-Kortowo, Poland.
[3] National Academy of Agrarian Sciences, Institute of Fisheries, Lviv Research Station, Lviv, Ukraine.
[4] University of Warmia and Mazury, Department of Meat Technology and Chemistry, Olsztyn, Poland.

* Corresponding Author: Tel.: +48.22 5936645; Fax: +48.22 5936646;
E-mail: maciej_kamaszewski@sggw.pl

Received 5 August 2014
Accepted 19 December 2014

Abstract

The aim of this study was to compare the physiology and morphology of the digestive tract of Russian sturgeon (*Acipenser gueldenstaedtii*) larvae fed *Artemia* sp. nauplii enriched or non-enriched with essential fatty acids (EFA). Physiology was evaluated by digestive enzyme activity analysis, while morphology was assessed with histological methods. The larvae were divided into two groups, in which fish were fed either pure *Artemia* sp. nauplii or *Artemia* enriched in EFA. Both groups had similar survival rates, but fish fed EFA-enriched *Artemia* displayed higher, body weight and length. At the end of the experiment (22 dph): 1) the activities of lipase and leucine aminopeptidase were similar in both groups; 2) the activities of trypsin, alkaline phosphatase and γ-glutamyltransferase were insignificantly higher in fish fed pure *Artemia*; 3) the activity of α-amylase was significantly higher in the EFA-enriched feeding group; 4) lower hepatocyte lipid vacuole diameters and hepatocyte proliferation were measured in the EFA-enriched group. Lipid accumulation was observed in the anterior intestine of fish fed pure *Artemia* on the 15th and 22nd dph. The epithelial turnover was significantly lower in the EFA-enriched group, in the posterior intestine on the 15th dph, but no differences occurred between the groups on the 22nd dph, in either anterior or posterior intestine. In conclusion, the study revealed a positive effect of the EFA-enriched *Artemia*-based diet on the physiology and morphology of the digestive system of Russian sturgeon larvae.

Keywords: Sturgeon, feeding, enzymes, histology, liver, intestine.

Introduction

Generally, live preys play an important role in fish rearing, but optimal feeding conditions for many species are yet to be determined. In order to achieve higher survival, growth and stress-resistance numerous nutritional studies are conducted every year (Jalali *et al*., 2008; Ostaszewska *et al*., 2010; Ostaszewska *et al*., 2011; Noori *et al*., 2011).

Artemia sp. nauplii are live preys commonly used in aquaculture (Hanaee *et al*., 2005). Unfortunately, nutritional deficits were determined in their body composition, particularly of the two essential fatty acids (EFA): eicosapentaenoic (EPA) and docosahexaenoic (DHA) (Hanaee *et al*., 2005; Morais *et al*., 2007). This deficiency can be overcome by nutritional supplementation with highly unsaturated fatty acids (HUFA) (Hanaee *et al*., 2005).

In fish, the physiology of both the digestion and absorption of nutrients during early ontogenesis heavily depends on morphological and functional transformations that occur during that period (Izquierdo *et al*., 2000). The activity of digestive enzymes can be a very effective indicator of fish larvae development, allowing to predict larval mortality and to evaluate the digestive abilities of fish (Zambonino Infante and Cahu, 2001), as well as their overall nutritional condition (Kamaszewski *et al*., 2010).

The majority of wildly living sturgeons is threatened with extinction, but these fish are also highly desired in aquaculture. Enzymatic secretion during ontogenesis was studied on various *Acipenseridae* (Żółtowska *et al*., 1999; Napora-Rutkowski *et al*., 2009), but the physiological and morphological impact of different diets was not completely determined. However, free amino acids (FAA) and free fatty acids (FFA) are known to stimulate digestive processes and the assimilation of nutrients, and they also may have influence on digestive tract morphology, feeding behavior or food intake (Ostaszewska *et al*., 2008; Napora-Rutkowski *et al*., 2009; Naz and Türkmen, 2009; Ostaszewska *et al*., 2013).

The objective of this paper was to evidence how feeding Russian sturgeon (*Acipenser gueldenstaedtii*)

larvae with EFA-enriched *Artemia* sp. nauplii affects the activity of digestive enzymes (lipase, trypsin, α-amylase, alkaline phosphatase, γ-glutamyltransferase and leucine aminopeptidase), as well as the development and homeostasis of the alimentary tract.

Materials and Methods

Larvae (body weight = 19±5 mg; total length = 13.81±0.53 mm; n = 15) were placed on the hatching day in 200 L tanks with water recirculation (2 experimental groups, each in 3 replicates 1500 larvae; density: 7.5 larva L^{-1}). Exogenous feeding (50% of fish biomass day^{-1}) commenced 8 days post hatching (dph). Both groups were fed *Artemia* sp. nauplii, but in the EFA-enriched group the nauplii were supplied with a commercial preparation containing Polyunsaturated Fatty Acids (PUFA), according to the manufacturer's recommendations (Selco S. presso; Inve Aquaculture, Belgium). Water parameters were controlled, measured each 24h (n=3): 18±0.5°C, O_2 saturation >7 mg L^{-1} (Oxi 3205 SET3, WTW, Germany), NH_4^+<0.1 mg L^{-1} and NO_2^-<0.01 mg L^{-1} (Photometer LF205, Slandi, Poland). The tanks were cleaned twice a day.

Larvae were sampled 1, 8, 15, and 22 dph and anaesthetised with Propiscin (2 ml L^{-1}; Inland Fisheries Institute, Olsztyn, Poland). Every time, 12 fish from each tank were measured (electronic caliper: Z22855, Milomex Ltd., Pulloxhill, UK; ±0.1 mm) and weighed (scale: WPS 60/C/10, Radwag, Radom, Poland; ±1 mg). Samples for the enzymatic analysis were pooled, 0.5 g from each tank (whole larvae on 1 and 8 dph; dissected digestive tracts on 15 and 22 dph). Afterwards, they were frozen in liquid nitrogen and stored at -80°C. These larvae were excluded from survivability calculations. For histological and immunohistochemical analysis, 8 fish were taken from each tank. One half was fixed in Bouin's solution, while the other half was immediately frozen in liquid nitrogen and stored at -80ºC due to differences in the applied histological procedures.

Samples for the enzymatic activity analysis were homogenized in buffers according to the procedures described for: lipase (Winkler and Stuckman, 1979), trypsin (Erlanger *et al*., 1961), α-amylase (Foo and Bais, 1998), alkaline phosphatase (ALP) (Wenger *et al*., 1984), γ-glutamyltransferase (γ-GT) (Gendler, 1984) and leucine aminopeptidase (LAP) (Nagel *et al*., 1964) and centrifuged (4ºC, 15 min, 15000 G). The activity of all enzymes was measured at 25ºC (3 replicates each) and calculated for 1 mg of protein from the enzymatic extract (μmol of product 1 min^{-1}). Total protein content was determined by the method of Lowry *et al*. (1951). Absorbance was measured with a spectrophotometer (M501, Camspec Ltd., Sawston, UK).

Fixed samples were dehydrated in a graded series of ethanol, embedded in Paraplast and cut into thin (5 μm) longitudinal sections with a microtome (RM 2265, Leica Microsystems, Nussloch, Germany). Acidic and neutral carbohydrates were detected with a combined Alcian blue and Periodic acid-Schiff's stain (AB/PAS; pH = 2.5 and 1.0). PAS was also used as a control to stain glycogen with diastase (Gona, 1979). Antibodies directed against the proliferating cell nuclear antigen (PCNA) were applied to identify intestinal and hepatic cell proliferation (Ostaszewska *et al*., 2013). Apoptotic intestinal cells were detected immunohistochemically with a CPP-32 (caspase-3) rabbit polyclonal antibody (Ostaszewska *et al*., 2010).

Hepatocyte cell area and proliferation were measured in 4 fish per tank, each in 15 fields of view (35000 μm^2). The proliferative and apoptotic enterocyte indexes were calculated as the ratio of cells located either in the basal (PCNA-positive), or the apical (CPP-32-positive) part of folds, compared to all cells in the same area. Both indexes were estimated for 15 basal fold regions, for both the anterior and posterior (spiral) intestine, in 4 fish per tank. Epithelial turnover was determined as the ratio of these indexes.

Frozen material was sectioned into 10 μm slices with a cryostat (-20°C; CH 1900, Leica Microsystems, Nussloch, Germany) and stained with Oil Red O (70% isopropanol) for histochemical lipid examination. The diameter of lipid droplets was measured in the liver (15 fields of view, 35000 μm^2 each) of 24 fish (4 per tank).

Morphometric measurements were done at 400 magnification using a microscope (ECLIPSE 90i) equipped with a digital camera (DS5-U1) and connected to a PC with the NIS-Elements AR Image Analysis System (all elements: Nikon Corporation, Tokyo, Japan).

Fatty acid content of *Artemia* sp. nauplii and fish larvae (22 dph) was determined by total muscle lipid extraction (Folch *et al*., 1957) and was measured using gas chromatography (Hewlett-Packard 6890, Agilent Technologies Poland, Wroclaw, Poland). Methylation was conducted with a chloroform-methanol-sulfuric acid solution (100:100:1 by volume). Total fatty acid content was expressed as mg g^{-1} of dry weight (all measurements n=6).

The results were analyzed statistically using Statistica 10.0 and Statgraphics Plus 4.1. Survival, total length, weight of fish, fatty acid content, enzyme activity and morphometric parameters were expressed as mean ± standard deviation. Data were assessed for normality using a Shapiro-Wilk test and submitted to two-way ANOVA and Duncan's test.

Results

On the 22nd dph, the difference in survival between both groups was insignificant (non-enriched: 95.53±2.42%, EFA-enriched: 97.58±2.17%), but the means of body weight and length were significantly higher in the EFA-enriched feeding group (170±17 mg, 31.04±0.82 mm; compared to 138±15 mg,

28.95±1.08 mm; n=36; Figure 1A and 1B).

The differences in activity of lipase (Figure 2A), trypsin (Figure 2B), ALP (Figure 2D) and γ-GT (Figure 2E) were statistically insignificant between the two groups on both 15th and 22nd dph, however, the activities of trypsin, ALP and γ-GT were noticeably higher in fish fed non-enriched *Artemia*. The activity of α-amylase (Figure 2C) was

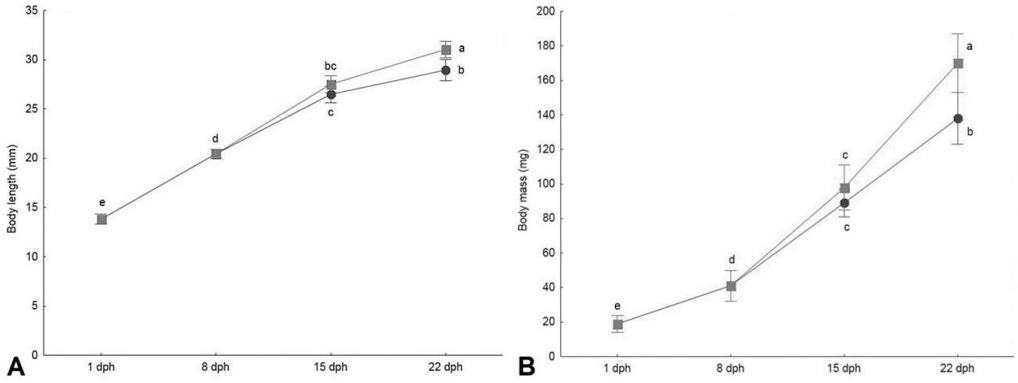

Figure 1. Growth of the Russian sturgeon larvae fed *Artemia* sp. nauplii (●) and *Artemia* sp. nauplii enriched in EFA (■), displayed as: A) total body length, B) wet body weight. Different letters indicate statistically significant differences (P≤0.05; n=36).

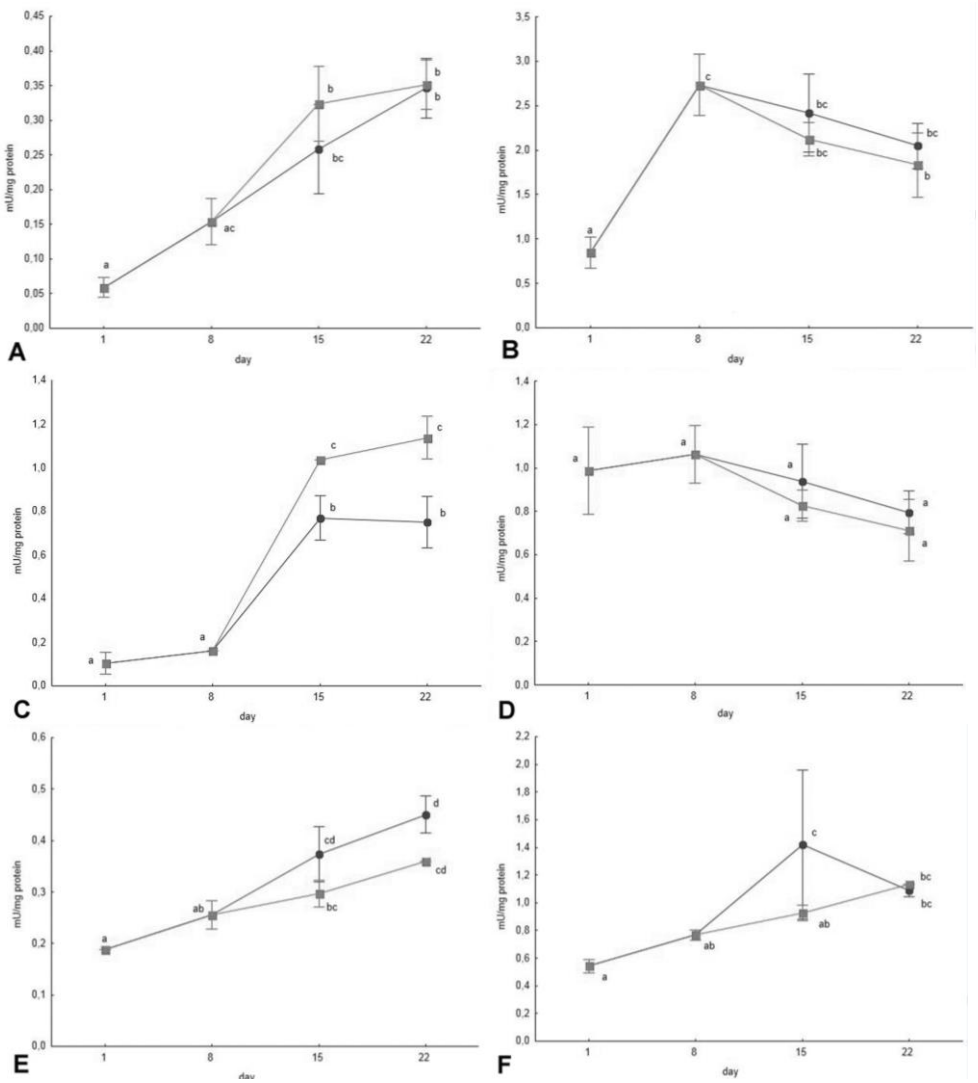

Figure 2. Enzyme activity in Russian sturgeon fed *Artemia* sp. nauplii (●) and *Artemia* sp. nauplii enriched in EFA (■): A) lipase, B) trypsin, C) α-amylase, D) ALP, E) γ-GT, F) LAP; different superscripts indicate significant differences at P<0.05 (n=9).

significantly higher on both 15th and 22nd dph in the EFA-enriched feeding group, while the activity of LAP (Figure 2F) was significantly higher in the non-enriched group only on the 15th dph.

The differences in hepatocyte area between the two groups were statistically insignificant (Figure 3A). Histological analysis revealed lesser amounts of accumulated glycogen grains (PAS-positive areas) and higher numbers of lipid vacuoles in hepatocytes of fish fed non-enriched *Artemia* (Figure 4A and 4B). The average lipid vacuole diameters on the 22nd dph were statistically significantly smaller in fish fed EFA-enriched *Artemia* (Figure 3B, 4C and 4D). On the 22nd dph, cell proliferation was statistically significantly lower in the liver parenchyma of fish from the EFA-enriched feeding group (Figure 3C; 4E and 4F).

Small lipid vacuoles were visible in the supranuclear region of enterocytes in the anterior intestine of fish fed non-enriched *Artemia* (15 and 22 dph) and fish fed EFA-enriched *Artemia* (only 22 dph; Figure 5A and 5B; 5C and 5D). PAS-positive granulation was observed in enterocytes of the posterior (spiral) intestine of fish from both groups (15 and 22 dph; Figure 5E and 5F). PCNA-positive cell nuclei were found mainly in the basal part of the folds, in both the anterior and posterior intestine (Figure 6A), while CPP-32-positive cells were observed mostly in the apical part of the folds (Figure 6B). Fish fed EFA-enriched *Artemia* were characterized by lower epithelial turnover values in both sections of the intestine, but the difference was statistically significant only in the posterior intestine on the 15th dph (Figure 6C and 6D). Proliferation prevailed over apoptosis in both groups during the entire experiment (epithelial turnover >1).

Fatty acid content of EFA-enriched *Artemia* was over two times higher (5509.29±197.52 mg g^{-1}) when compared to the non-enriched *Artemia* (2184.83±119.21 mg g^{-1}). Also, on the 22nd dph, fish from the EFA-enriched feeding group were characterized by higher fatty acid content (5799.11±245.08 mg g^{-1}) than fish from the non-enriched group (3809.12±164.86 mg g^{-1}).

Discussion

The study revealed a favorable effect of the EFA-enriched *Artemia* sp. nauplii live preys on growth and fatty acid content of Russian sturgeon larvae, similarly as in the Persian sturgeon (*Acipenser persicus*; Hafezieh *et al*., 2009) and the beluga (*Huso huso*; Jalali *et al*., 2008). Moreover, simultaneous HUFA and vitamin C addition decreases the frequency of opercula deformations and results in increased tolerance for abiotic conditions (Noori *et al*., 2011). However, in a study on the walleye (*Stizostedion vitreum*) increased dietary HUFA content did not affect the growth of fish (Kolkovski *et al*., 2000).

Although lipids are essential for the growth and development of fish, the dietary demand of various

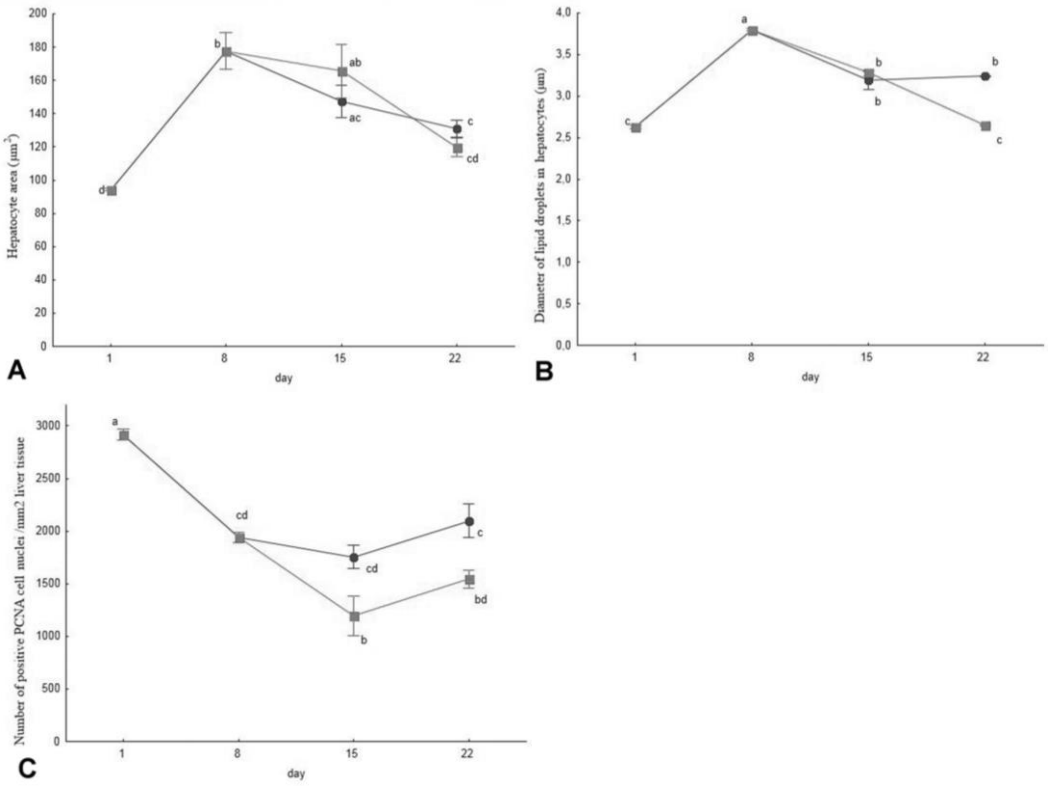

Figure 3. Morphometric liver parameters of Russian sturgeon fed *Artemia* sp. nauplii (●) and *Artemia* sp. nauplii enriched in EFA (■): A) hepatocyte area, B) diameter of lipid droplets in hepatocytes. C) number of positive PCNA cell nuclei in 1mm² liver tissue; different superscripts indicate significant differences at P<0.05 (n=12).

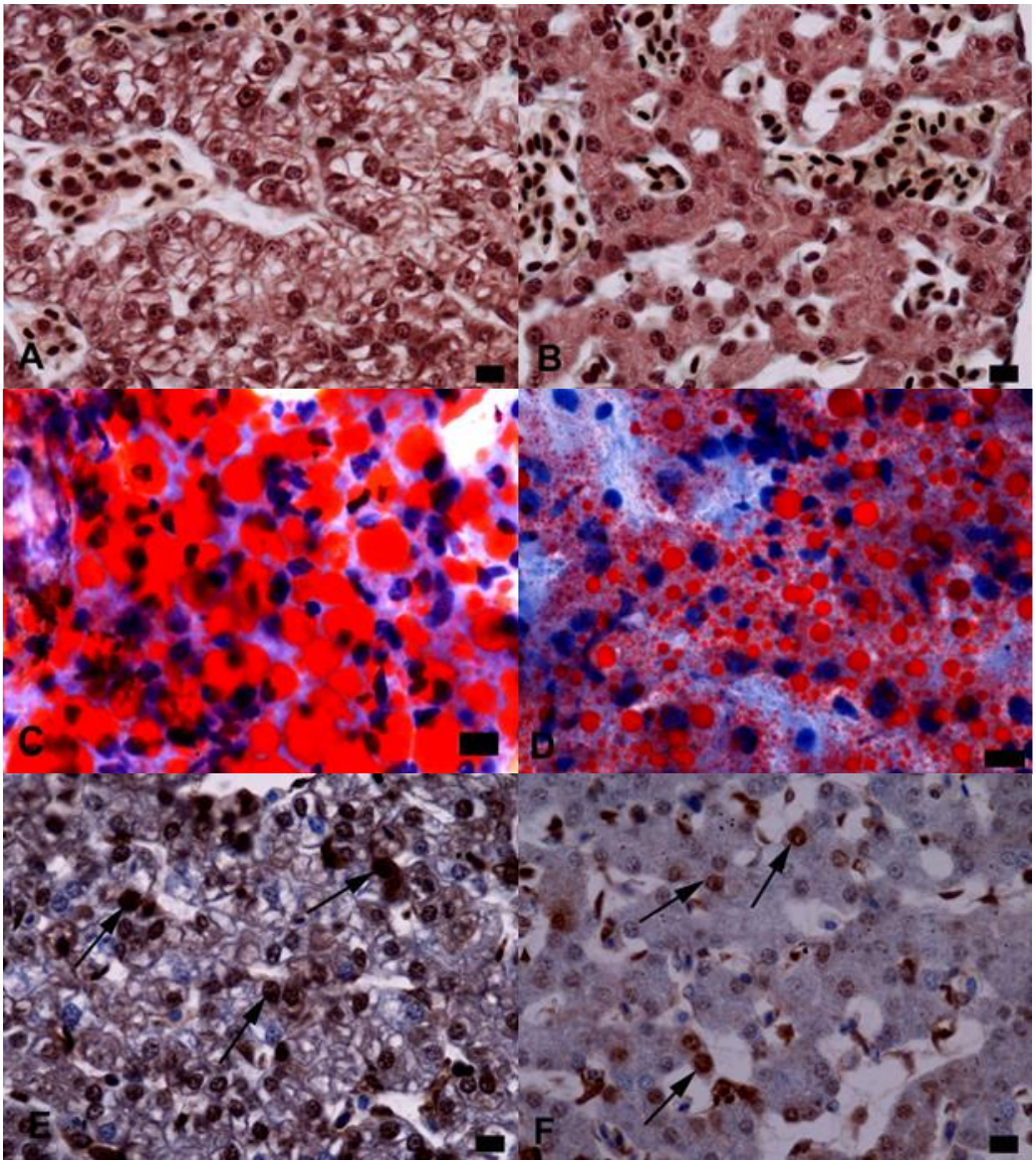

Figure 4. Histological image of liver (22 dph) of Russian sturgeon fed *Artemia* sp. nauplii (A, C, E) and *Artemia* sp. nauplii enriched in EFA (B, D, F); AB/PAS staining (A, B), Oil Red O staining (C, D), Immunohistochemical detection of PCNA-positive nuclei (arrows) (E, F); Scale bars=10 µm.

fish species for this component has been studied insufficiently (Hanaee *et al*., 2005). According to Izquierdo *et al*. (2000), the activity of lipase is affected by the fatty acid composition of the dietary lipids. In fish, the longer and less saturated the carbon chains are, the lower fatty acid digestibility values are recorded (Morais *et al*., 2005). Fish lipases prefer PUFA as substrate, more than MUFA and saturated fatty acids (Olsen *et al*., 1998). In Russian sturgeon larvae fed EFA-enriched *Artemia*, higher lipase activity 15 days post hatching could be the result of the higher dietary PUFA content, similarly as in the gilt-head seabream (*Sparus aurata*) fed PUFA-rich fish oil diet (Izquierdo *et al*., 2000). However, the statistically insignificant differences between the two experimental groups imply that lipase activity is not so heavily influenced by nutritional factors (Żółtowska *et al*., 1999).

Trypsin activity in fish depends not only on the diet, but also on a variety of conditions in the digestive tract, like temperature or pH (Napora-Rutkowski *et al*., 2009; Kamaszewski *et al*., 2010). Proteolytic activity can be a useful indication of the larvae's ability to digest different types of meals (Okan Kamaci *et al*., 2010). The studies of Cahu *et al*. (1999) revealed that the activity of trypsin depends on the protein content passing through the intestinal lumen, but Naz and Türkmen (2009) evidenced that feeding *Artemia salina* enriched in lysine does not affect trypsin activity in *S. aurata* larvae until 40 dph. In this research, trypsin activity was insignificantly lower in the EFA-enriched feeding group, suggesting that the activity of trypsin in Russian sturgeon larvae is not heavily influenced by the changes in dietary fatty acid content, similarly as in the Atlantic sturgeon, *Acipenser oxyrinchus* (Kamaszewski *et al*., 2014).

The dietary composition affects the activity of α-

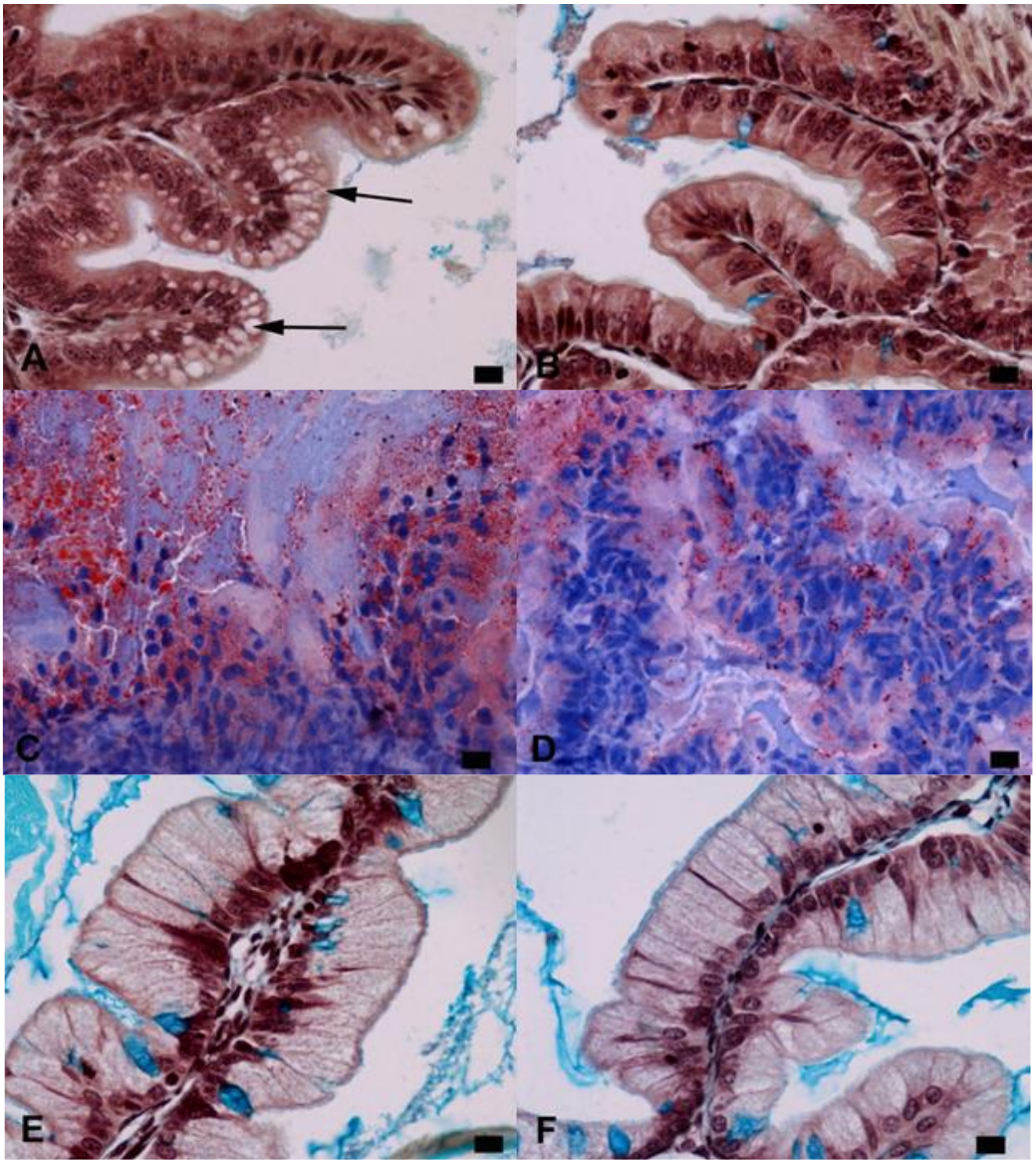

Figure 5. Histological image of anterior (A, B, C, D) and posterior (E, F) intestine (22 dph) of Russian sturgeon fed *Artemia* sp. nauplii (A, C, E) and *Artemia* sp. nauplii enriched in EFA (B, D, F); AB/PAS staining (A, B, E, F), Oil Red O staining (C, D); Lipid vacuoles in the supranuclear regions of enterocytes (arrows); Scale bars=10µm.

amylase (Zambonino-Infante and Cahu, 1994; Naz and Türkmen, 2009). Lipid-rich feeds were the cause for increased total α-amylase activity in the pike-perch, *Sander lucioperca* (Kamaszewski *et al*., 2010) and resulted in increased pancreatic secretion in the red drum, *Sciaenops ocellatus* (Buchet *et al*., 2000). Additionally, both of these phenomena occurred in the common carp, *Cyprinus carpio* (Manjappa *et al*., 2002). In this study, the significantly higher activity of this enzyme in the EFA-enriched feeding group can be explained as the result of increased levels of cholecystokinin (CCK), the primary regulator of pancreatic secretion. High levels of dietary lipids may stimulate the release of CCK in rats (Liddle, 1995) and a similar model has been proposed for fish (Zambonino-Infante and Cahu, 1999; Buchet *et al*., 2000).

ALP (Gisbert *et al*., 1999), γ-GT (Zambonino-Infante and Cahu, 1994) and LAP (Kvale *et al*., 2007) are markers of enterocyte maturation in fish. Increased activity of ALP in the intestine of young Siberian sturgeons (*Acipenser baerii*) indicates the presence of functionally developed enterocytes (Gisbert *et al*., 1999), while several studies conducted on various species proved that γ-GT activity is higher in larvae fed artificial diets when compared to live preys (Zambonino-Infante and Cahu, 1994; Tibaldi *et al*., 2006). However, in this study ALP and γ-GT activity remained similar in the two groups, while LAP activity was significantly higher in the non-enriched group only on the 15th dph. All that implies, that the dietary HUFA addition has only a minor impact on the development of the intestinal epithelium.

Apart from the enzymatic procedures, histological analysis provided further data about the morphology of the digestive tract. Hepatocytes of fish fed EFA-enriched *Artemia* were smaller and

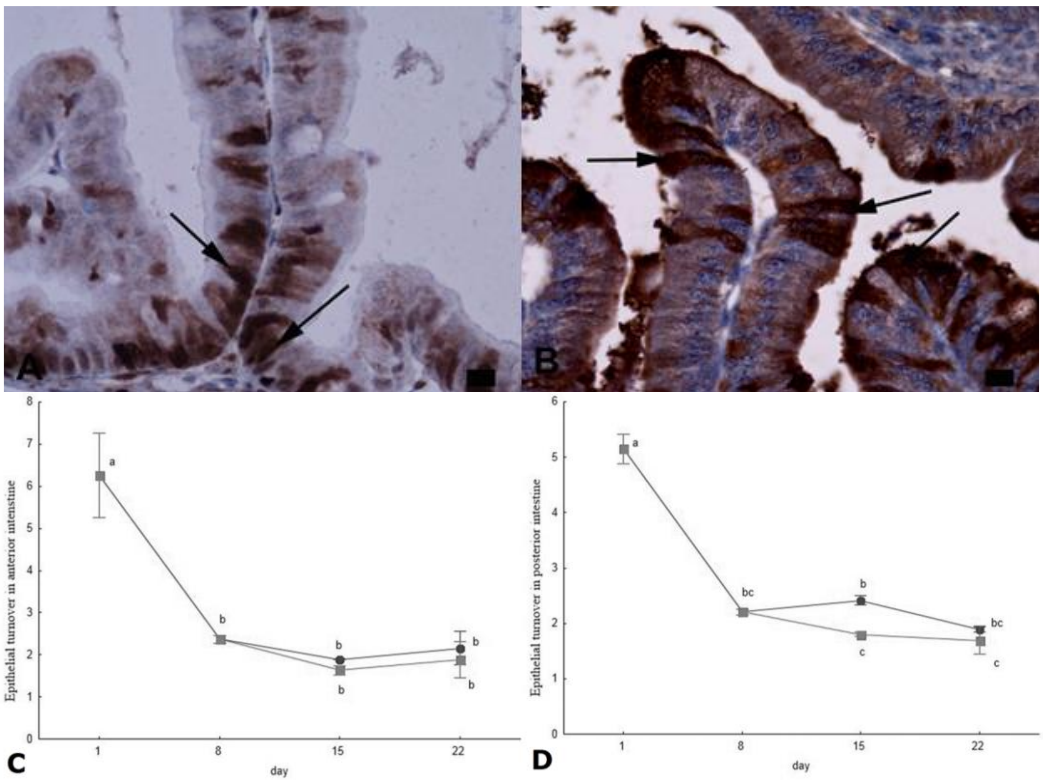

Figure 6. Histological image of anterior intestine (22 dph) of Russian sturgeon: detection of PCNA-positive nuclei (arrows) (A) and caspase-3-positive nuclei (arrows) (B); Scale bars=10µm; Morphometric intestine parameters of Russian sturgeon fed *Artemia* sp. nauplii (●) and *Artemia* sp. nauplii enriched in EFA (■): C) epithelial turnover in anterior intestine, D) epithelial turnover in posterior intestine; different superscripts indicate significant differences at P<0.05 (n=12).

contained smaller lipid droplets than hepatocytes of fish from the non-enriched group, but no pathological changes that would suggest *steatosis* were determined in either group. The difference in lipid accumulation resulted most likely from the variety of fatty acid profiles of the provided live preys (Ostaszewska and Boruta, 2006). Watanabe *et al*. (1989) determined that diets covering nutritional requirements of fish contribute to efficient lipid utilization, while food inadequate to these demands can cause lipid accumulation in the liver.

Increased hepatocyte proliferation may indicate pathogenesis resulting from toxicological factors (Dabrowska *et al*., 2012) or improper dietary composition (Ostaszewska *et al*., 2010). Lower numbers of PCNA-positive nuclei were observed in fish fed EFA-enriched *Artemia*, suggesting that the fatty acid profile of the provided live food was adequate to the feeding requirements of the larvae and thus did not lead to unnecessary, reparative hepatocyte proliferation (Ostaszewska *et al*., 2013).

Lipid vacuoles were detected in the supranuclear region of enterocytes in the anterior intestine of fish in both experimental groups. The presence of these vacuoles can be interpreted as a temporary accumulation form of esterified fatty acids (Fontagné *et al*., 1998) and may occur due to various disturbances in lipid transport from enterocytes to the circulatory system. Smaller lipid droplets were observed in the enterocytes of fish fed EFA-enriched *Artemia*, implying positive influence of the EFA. However, Luizi *et al*. (1999) discovered that fat-rich diets cause the growth of these lipid vacuoles, but Caballero *et al*. (2002) concluded that lipid accumulation may result from inadequate fatty acid ratio in fish feeds.

PAS-positive vacuoles in the supranuclear region of enterocytes in the posterior (spiral) intestine were described in a number of fish species (Ostaszewska *et al*., 2005) and were also found in both feeding groups. These vacuoles are the result of pinocytotic protein absorption from the intestinal lumen and therefore indicate proper digestion and nutrient intake (Ostaszewska *et al*., 2005).

Cell proliferation and apoptosis are two basic mechanisms sustaining the integrity of the intestine (Kamaszewski and Ostaszewska, 2014). New intestinal epithelial cells develop in basal parts of the folds and then migrate to the apical regions, where they last until being removed via apoptosis (Olsvik *et al*., 2007). In fish however, epithelial cell proliferation occurs over the entire length of the folds (Sanden *et al*., 2005). In this study, high numbers of PCNA-positive cells were observed in basal parts of the folds in the anterior and posterior intestine, but single proliferating cells were found even at half of the folds' height. Increased proliferation might appear due to cell maturation or feeding stress (Kamaszewski and Ostaszewska, 2014). Meanwhile, apoptotic cells were located mainly on apical parts of the intestinal

folds, similarly as in other fish species (Ostaszewska *et al*., 2010 and 2011; Kamaszewski and Ostaszewska, 2014).

No statistically significant differences in epithelial turnover were observed between the experimental groups in the anterior intestine. However, significantly higher epithelial turnover values were calculated in the posterior intestine of fish fed non-enriched *Artemia* on the 15th dph. This implies intensive epithelial regeneration, similarly as in fish fed diets including soybean meal (Sanden *et al*., 2005).

In conclusion, the study revealed that enriching *Artemia* live preys in essential fatty acids has a positive effect on growth of Russian sturgeon larvae and causes lower lipid deposition in the liver and lower hepatocyte proliferation. The analysis of digestive enzyme activity and morphology of the intestine showed no significant differences (apart from increased α-amylase activity). All of these remarks suggest that *Artemia* nauplii supplied with PUFA can be recommended for use in *A. gueldenstaedtii* larvae rearing.

Acknowledgements

The Project was co-financed by the Polish development cooperation programme 2011 of the Ministry of Foreign Affairs of the Republic of Poland. The publication expresses exclusively the views of the author, and cannot be identified with the official stance of the Ministry. Authors declare that all experiments presented in this study comply with the current laws of Poland.

References

Buchet, V., Zambonino Infante, J.L. and Cahu, C.L. 2000. Effect of lipid level in a compound diet on the development of red drum (*Sciaenops ocellatus*) larvae. Aquaculture, 184: 339-347. doi:10.1016/S0044-8486(99)00325-7.

Caballero, M.J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. and Izquierdo, M.S. 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 214: 253-271. doi: 10.1016/S0044-8486(01)00852-3

Cahu, C.L., Zambonino Infante, J.L., Quazuguel, P. and Le Gall, M.M. 1999. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass (*Dicentrarchus labrax*) larvae. Aquaculture, 171, 109-119. doi: 10.1016/S0044-8486(98)00428-1

Dabrowska, H., Ostaszewska, T., Kamaszewski, M., Antoniak, A., Napora-Rutkowski, Ł., Kopko, O., Lang, T., Fricke, N.F. and Lehtonen, K.K. 2012. Histopathological, histomorphometrical, and immunohistochemical biomarkers in flounder (*Platichthys flesus*) from the southern Baltic Sea. Ecotoxicology and Environmental Safety, 78: 14-21. doi: 10.1016/j.ecoenv.2011.10.025

Erlanger, B., Kokowsky, N. and Cohen, W. 1961. The preparation and properties of two new chromogenic substrates of trypsin. Archives of Biochemistry and Biophysics, 95: 271-278. doi:10.1016/0003-9861(61)90145-X

Folch, H., Less, M. and Stanley, H.A. 1957. A simple method for isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry, 226: 497-499.

Fontagné, S., Geuden, I., Escaffre, A.M. and Bergot, P. 1998. Histological changes induced by dietary phospholipids in intestine and liver of common carp (*Cyprinus carpio* L.) larvae. Aquaculture, 161: 213-223. doi: 10.1016/S0044-8486(97)00271-8

Foo, Y.A. and Bais, R. 1998. Amylase measurement with 2-chloro-4-nitrophenyl maltotrioside as substrate. Clinica Chimica Acta, 272: 137-147. doi: 10.1016/S0009-8981(98)00009-6

Gendler, S. 1984. γ-GT. In: L.A. Kaplan and A.J. Pesce (Eds.), Clinical Chemistry: Theory, Methods and Practice, C.V. Mosby Co, St. Louis, USA: 1120-1123.

Gisbert, E., Sarasquete, M.C., Williot, P. and Castelló-Orvay, F. 1999. Histochemistry of the development of the digestive system of Siberian sturgeon during early ontogeny. Journal of Fish Biology, 54: 596–616. doi: 10.1111/j.1095-8649.1999.tb00702.x

Gona, O. 1979. Mucous glycoproteins of teleostean fish: a comparative histochemical study. The Histochemical Journal, 11: 709-718. doi: 10.1007/BF01004734

Hafezieh, M., Kamarudin, M.S., Bin Saad, C.R., Abd Sattar, M.K., Agh, N. and Hosseinpoar, H. 2009. Effect of enriched *Artemia urmiana* on growth, survival and composition of larval Persian sturgeon. Turkish Journal of Fisheries and Aquatic Sciences, 9: 201-207. doi:10.4194/trjfas.2009.0212

Hanaee, J., Agh, N., Hanaee, M., Delazar, A. and Sarker S.D. 2005. Studies on enrichment of *Artemia urmiana* cysts for improving fish food value. Animal Feed Science and Technology, 120: 107-112. doi: 10.1016/j.anifeedsci.2005.01.010

Izquierdo, M.S., Socorro, J., Arantzamendi, L. and Hernández-Cruz, C.M. 2000. Recent advances in lipid nutrition in fish larvae. Fish Physiology and Biochemistry, 22: 97-107. doi:10.1023/A:1007810506259

Jalali, M.A., Hosseini, S.A. and Imanpour, M.R. 2008. Effect of vitamin E and highly unsaturated fatty acid-enriched *Artemia urmiana* on growth performance, survival and stress resistance of Beluga (*Huso huso*) larvae. Aquaculture Research, 39: 1286-1291. doi: 10.1111/j.1365-2109.2008.01992.x

Kamaszewski, M., Napora-Rutkowski, Ł. and Ostaszewska, T. 2010. The effect of feeding on activity of digestive enzymes and morphological changes in pike-perch (*Sander lucioperca*) liver and pancreas. The Israeli Journal of Aquaculture-Bamidgeh, 62: 225-236.

Kamaszewski, M. and Ostaszewska, T. 2014. The effect of feeding on morphological changes in intestine of pike-perch (*Sander lucioperca L.*). Aquaculture International, 22: 245-258. doi:10.1007/s10499-013-9693-y

Kamaszewski, M., Wójcik, M., Ostaszewska, T., Kasprzak, R., Kolman, R. and Prusińka, M. 2014. The effect of essential fatty acid (EFA) enrichment of *Artemia* sp. nauplii on the enzymatic activity of Atlantic sturgeon (*Acipenser oxyrinchus* Mitchill, 1815) larvae–preliminary study. Journal of Applied Ichthyology, 30, 1256-1258.doi:10.1111/jai.12561

Kolkovski, S., Czesny, S., Yackey, C., Moreau, R., Cihla, F., Mahan, D. and Dabrowski, K. 2000. The effect of vitamins C and E in (n-3) highly unsaturated fatty acid enriched *Artemia* nauplii on growth, survival and stress resistance of freshwater walley *Stizostedion vitreum* larvae. Aquaculture Nutrition, 6: 199-206. doi: 10.1046/j.1365-2095.2000.00112.x

Kvale, A., Mangor-Jensen, A., Moren, M., Espe, M. and Hamre, K. 2007. Development and characterisation of some intestinal enzymes in Atlantic cod (*Gadus morhua* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. Aquaculture, 264: 457-468. doi:10.1016/j.aquaculture.2006.12.024

Liddle, R.A. 1995. Regulation of cholecystokinin secretion by intraluminal releasing factors. American Journal of Physiology, 269: 319-327.

Luizi, F.S., Gara, B., Shields, R.J. and Bromage, N.R. 1999. Further description of the development of the digestive organs in Atlantic halibut (*Hippoglossus hippoglossus*) larvae, with notes on differential absorption of copepod and *Artemia* prey. Aquaculture, 176: 101-116. doi:10.1016/S0044-8486(99)00054-X

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry, 193: 265–275.

Manjappa, K., Keshavanath, P. and Gangadhara B. 2002. Growth performance of common carp, *Cyprinus carpio* fed varying lipid levels through low protein diet, with a note on carcass composition and digestive enzyme activity. Acta Ichthyologica et Piscatoria, 32: 145-155.

Morais, S., Koven, W., Rønnestad, I., Dinis, M.T. and Conceição, L.E.C. 2005. Dietary protein/lipid ratio and lipid nature affects fatty acid absorption and metabolism in teleost larva. British Journal of Nutrition, 93: 813-820. doi: 10.1079/BJN20051378

Morais, S., Conceição, L.E.C., Rønnestad, I., Koven, W., Cahu, C., Zambonino Infante, J.L. and Dinis, M.T. 2007. Dietary neutral lipid level and source in marine fish larvae: Effects on digestive physiology and food intake. Aquaculture, 268: 106-122. doi: 10.1016/j.aquaculture.2007.04.033

Nagel, W., Willing, F. and Schmidt, F.H. 1964. On amino acid arylamidase (so-called leucine aminopeptidase) activity in the human serum Klinische Wochenschrift. 42: 447-449. doi: 10.1007/BF01486 025 (in German).

Napora-Rutkowski, Ł., Kamaszewski, M., Bielawski, W., Ostaszewska, T. and Wegner, A. 2009. Effects of starter diets on pancreatic enzyme activity in juvenile sterlet (*Acipenser ruthenus*). The Israeli Journal of Aquaculture–Bamidgeh, 61: 143-150.

Naz, M. and Türkmen, M. 2009. Changes in the digestive enzymes and hormones of gilthead seabream larvae (*Sparus aurata*, L. 1758) fed on *Artemia* nauplii enriched with free lysine. Aquaculture International, 17: 523-535. doi: 10.1007/s10499-008-9221-7

Noori, F., Takami, G.A., Van Speybroeck, M., Van Stappen, G. and Sorgeloos P. 2011. Feeding *Acipenser persicus* and *Huso huso* (*Acipenseriformes*) larvae with *Artemia urmiana* nauplii enriched with HUFA and vitamin C: II. Effect on tolerance to shock exposure of environmental factors. Journal of Applied Ichthyology, 27: 787-795. doi: 10.1111/j.1439-0426.2011.01700.x

Okan Kamaci, H., Suzer, C., Çoban, D., Saka, Ş. and Firat, K. 2010. Organogenesis of exocrine pancreas in sharpsnout sea bream (*Diplodus puntazzo*) larvae: characterization of trypsin expression. Fish Physiology and Biochemistry, 36: 993-1000. doi: 10.1007/s10695-009-9377-8

Olsen, R.E., Henderson, R.J. and Ringø, E. 1998. The digestion and selective absorption of dietary fatty acids in Arctic Charr, *Salvelinus alpinus*. Aquaculture Nutrition, 4: 13-21. doi:10.1046/j.1365-2095.1998.00099.x

Olsvik, P.A., Torstensen, B.E. and Berntssen, M.H.G. 2007. Effects of complete replacement of fish oil with plant oil on gastrointestinal cell death, proliferation and transcription of eight genes encoding proteins responding to cellular stress in Atlantic salmon *Salmo salar* L. Journal of Fish Biology, 71: 550–568. doi: 10.1111/j.1095-8649.2007.01521.x

Ostaszewska, T., Dabrowski, K., Czumińska, K., Olech, W. and Olejniczak, M. 2005. Rearing of pike-perch larvae using formulated diets–first success with starter feeds. Aquaculture Research, 36: 1167-1176. doi: 10.1111/j.1365-2109.2005.01332.x.

Ostaszewska, T. and Boruta, A. 2006. The effect of diet on the fatty acid composition and liver histology of pike perch (*Sander lucioperca* L.) larvae. Archives of Polish Fisheries, 14: 53–66.

Ostaszewska, T., Dabrowski, K., Hliwa, P., Gomółka, P. and Kwasek, K. 2008. Nutritional regulation of intestine morphology in larval cyprinid fish, silver bream (*Vimba vimba*). Aquaculture Research, 39: 1268-1278. doi: 10.1111/j.1365-2109.2008.01989.x

Ostaszewska, T., Dąbrowski, K., Kamaszewski, M., Grochowski, P., Verri, T., Rzepkowska, M. and Wolnicki, J. 2010. The effect of plant protein-based diet supplemented with dipeptide or free amino acids on digestive tract morphology and PepT1 and PepT2 expressions in common carp (*Cyprinus carpio* L.). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 157: 158–169. doi:10.1016/j.cbpa.2010.06.162

Ostaszewska, T., Dabrowski, K., Verri, T., Kamaszewski, M., Napora-Rutkowski, Ł. and Śliwiński, J. 2011. The effect of various diet formulation (experimental and commercial) on rainbow trout (*Oncorhynchus mykiss*) intestine and liver morphology. Aquaculture Research, 42: 1796–1806. doi: 10.1111/j.1365-2109.2010.02779.x

Ostaszewska, T., Dabrowski, K., Kamaszewski, M., Kwasek, K., Grodzik, M. and Bierla, J. 2013. The effect of dipeptide, Lys-Gly, supplemented diets on digestive tract histology in juvenile yellow perch (*Perca flavescens*). Aquaculture Nutrition, 19: 100–109. doi:10.1111/j.1365-2095.2012.00948.x

Sanden, M., Berntssen, M.H.G., Krogdahl, Å., Hemre, G.I. and Bakke-McKellep, A.M. 2005. An examination of the intestinal tract of Atlantic salmon, *Salmo salar* L., parr fed different varieties of soy and maize. Journal of Fish Diseases, 28: 317-330. doi:10.1111/j.1365-2761.2005.00618.x

Tibaldi, E., Hakim, Y., Uni, Z., Tulli, F., de Francesco, M., Luzzana, U. and Harpaz, S. 2006. Effects of the partial substitiution of dietary fish meal by differently processed soybean meals on growth performance, nutrient digestibility and activity of intestinal brush border enzymes in the European sea bass (*Dicentrarchus labrax*). Aquaculture, 261: 182-193. doi:10.1016/j.aquaculture.2006.06.026

Watanabe, T., Thongrod, S., Takeuchi, T., Satoh, S.,

Kubota, S.S., Fujimaki, Y. and Cho, C.Y. 1989. Effect of dietary n-6 and n-3 fatty acids on growth, fatty acid composition and histological changes of white fish *Coregonus lavaretus maraena*. Bulletin of the Japanese Society of Scientific Fisheries, 55: 1977–1982. doi: 10.2331/suisan.55.1977

Wenger, C. 1984. Alkaline phosphatase. In: L.A. Kaplan and A.J. Pesce (Eds.), Clinical Chemistry: Theory, Methods and Practice, C.V. Mosby Co, St. Louis, USA: 1094-1098.

Winkler, U.K. and Stuckmann, M. 1979. Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. Journal of Bacteriology, 138: 663-670. ISSN: 0021-9193.

Zambonino Infante, J.L. and Cahu, C.L. 1994. Development and response to a diet change of some digestive enzymes in sea bass (*Dicentrarchus labrax*) larvae. Fish Physiology and Biochemistry, 12: 399-408. doi: 10.1007/BF00004304

Zambonino Infante, J.L. and Cahu, C.L. 1999. High dietary lipid levels enhance digestive tract maturation and improve *Dicentrarchus labrax* larval development. The Journal of Nutrition, 129: 1195-2000.

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

Żółtowska, K., Kolman, R., Łopieńska, E. and Kolman, H. 1999. Activity of digestive enzymes in Siberian sturgeon juveniles (*Acipenser baeri* Brandt) – A preliminary study. Archives of Polish Fisheries, 7: 201-211. ISSN:2083-6120.