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THE IMPACT OF FEEDING ON THE RESULTS OF REARING LARVAL PIKEPERCH, *SANDER LUCIOPERCA* (L.), WITH REGARD TO THE DEVELOPMENT OF THE DIGESTIVE TRACT

Agata Kowalska[1], Zdzisław Zakęś[1], Krystyna Demska-Zakęś[2]
[1] *Department of Aquaculture, Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Poland*
[2] *Department of Ichthyology, University of Warmia and Mazury, Olsztyn, Poland*

ABSTRACT

The aim of this study was to determine the impact of the period mixed feed (formulated feed + *Artemia* sp.) is applied to the growth, survival, and digestive tract development of larval pikeperch (larva age – 6 days post-hatch (DPH)). The larvae were divided into three experimental groups and fed mixed feed from 6 to 12 DPH (group I), from 6 to 19 DPH (group II), and from 6 to 26 DPH (group III). After feeding with brine shrimp was discontinued (group I and II), the larvae were fed formulated feed exclusively. During rearing, the mean larval body weight increased from 0.7 to 20 mg (group I) and 25 mg (group III; $P < 0.05$). Larval survival in the tested groups was similar at approximately 60% ($P > 0.05$). The highest percentages of deformed larvae were noted in groups II and III (16 and 14%, respectively). Feeding was noted to have a significant impact on the development of the pikeperch digestive tract. The folds in the esophagus and posterior intestine of fish fed natural feed for the shortest period were the highest ($P < 0.05$). They also had the most intestinal pinocytotic vesicles and mucous cells. The smallest esophagus goblet cells and gastric glands were noted in group I ($P < 0.05$).

Key words: digestive tract histology, feeding, larvae, pikeperch (*Sander lucioperca*).

INTRODUCTION

Brine shrimp, *Artemia* sp., nauplii are used successfully to feed the larvae of most fish species. Although this feed is of unquestionable quality, it is fairly expensive. Currently, there is no commercial feed on the market adapted to the requirements of larval stages of most fish species. Only in a very few instances can commercial starters be used as an effective replacement for natural food. This refers to species that have functional stomachs when they hatch and whose digestive enzyme activity increases rapidly, as in salmonids [6]. Feeding the larvae of many fish species exclusively with formulated feed lowers survival and growth rates while contributing to the occurrence of body deformations [14, 26].

Pikeperch, *Sander lucioperca* (L.), belongs to the group of fish whose stomach (including the pyloric sphincter and pyloric caecae) forms fifteen to twenty days following hatch [7, 20]. The stomach plays an important role in hydrolyzing the ingredients of feed into simple components thus increasing their digestibility. Gastric glands, which contain one type of cell, secrete pepsin and hydrochloric acid. Their secretion is controlled by the nervous and hormonal systems. In the stomach mucosa (as well as in the intestines) there are also endocrine cells (that secrete gastrin and somatostatin) and mucous cells (responsible for secreting neutral and acidic mucins) [12]. The acidic digestive stomach acids are not only responsible for denaturing protein or emulsifying lipids, but also for activating pepsinogens (the pH in the stomach near the pyloric sphincter is 2.5). The time food is exposed to gastric juices is controlled by contractions of the pyloric sphincter muscle. Simultaneously, this muscle regulates the flow in the acidic stomach contents to the pyloric caecae and intestines. This is where further digestion initiated in the stomach, hydrolyzation, and nutrient absorption occurs. The epithelial cells of the intestines (enterocytes) play important digestive (through the excretion of, for example, aminopeptidases, carboxypeptidases, and lipases) and absorption roles. In fish, the digestive enzymes of the enterocytes might be contained in the supranuclear vacuoles or excreted into the lumen. This is also how intracellular and extracellular or lumenal digestion is differentiated (some of the enzymes are also associated with the cell membrane of the enterocytes) [15, 18].

The digestion of food in larvae with incomplete digestive tracts and an incomplete quantity of enzymes is retarded and reduces its digestibility [16]. This low digestion effectivity is somewhat compensated for by the pinocytosis process of protein macromolecules, which plays an important role in protein absorption in fish [11, 20]. At this time, peptides are assimilated and digested intracellularly in the posterior section of the intestines. Initially, the assimilation of proteins in larval fish occurs through intracellular digestion. During larval metamorphosis, the role of this process becomes less significant and is dominated by the extracellular or lumenal digestion of protein, whose significance increases along with the increase of acidity in the intestinal lumen [11].

Unquestionably, fish larvae require specific diets that provide for appropriate growth rates and individual development. Live feed may also deliver exogenous enzymes requisite for digestion and/or that activate zymogenes (proenzymes) present in the primordial larval gut. Additionally, the movements of zooplankton nauplii in the esophagus irritate the epithelium, thus stimulating the release of enzymes [14, 16]. The physical properties of the feed (color, intensity, chemical properties determining flavor and taste) and environment factors (light intensity and reflection, degree of polarization and contrast) are significant in initiating exogenous feeding. It is known that the sense of sight and chemoreception (sense of smell) play key roles in the larval switch to exogenous feeding [11]. The failure of feeding exclusively formulated feed can thus be related to the inability of larvae to recognize feed granules as food or locate and ingest them. However, feeding larval stages exclusively with *Artemia* sp. is often unjustified economically. Additionally, this can, at a certain stage of ontogenic development, lead to lowered growth rates in comparison with those of specimens fed mixed feed (formulated feed + *Artemia* sp.) [27]. In many instances, mixed feeds insured optimal fish growth rates with minimal losses [27]. Optimizing (shortening) the period in which natural feed is delivered had a positive impact on the economic results and the labor intensity of rearing larvae. Not until fish have fully formed digestive tracts is it possible to apply commercial starters successfully without the necessity of supplementing the diet with natural feed. Most of the significant rearing indexes such as growth, survival, and larval body deformation are largely dependent on the length of time *Artemia* sp. is delivered; this is undoubtedly related to the ontogenesis of the digestive tract [14, 23].

The aim of the experiment was to determine what impact the period in which mixed feed (formulated feed + *Artemia* sp.) was applied had on the growth rates, survival, and the development of the digestive tract in larval pikeperch.

MATERIALS AND METHODS

Materials, rearing conditions, and experimental scheme

The experimental material was comprised of larval pikeperch 6 DPH of an initial body weight of 0.7 mg and a total body length of TL 5.9 mm. The material was obtained from artificial pikeperch spawning hormonally stimulated with human chorionic gonadotropin (hCG) [28]. The experiment tested the impact of the length of time the larval pikeperch were fed with live food, *Artemia* sp. The fish were fed with brine shrimp only in the first week of life (group I; larval age 6 – 12 DPH), for two weeks (group II; 6 – 19 DPH), and for three weeks (group III; 6 – 26 DPH). *Artemia* sp. (Neptune, U.S.A.) larvae were delivered three times daily (09.00, 14.00, 19.00) by hand (each feeding consisted of 2100 nauplii dm^{-3}). The pikeperch larvae were fed simultaneously with Perla 6.0 commercial starter (granule size 0.1 – 0.3 mm), and then Perla 5.0 (0.2 – 0.4 mm) and Perla 4.0 (0.3 – 0.5 mm) manufactured by Trouvit (Hendrix SpA, Italy). After the brine shrimp were discontinued, the fish in groups I and II were fed exclusively with formulated feed (group I – 13 – 26 DPH; group II – 20 – 26 DPH); the pikeperch in group III were fed with mixed feed until the conclusion of the experiment, i.e., from 6 – 26 DPH. Changes in granule size were preceded with a three-day transition period, during which a 50:50 granule mix was applied. The feed was delivered

around the clock ad libitum with a vibrating feeder with an electromagnetic mechanism (Sweeney, U.S.A.). The feed was delivered for 5 seconds at intervals of 18-minutes. According to manufacturer data, the feed contains 62% crude protein, 11% raw fat, 10% ash, 0.5% cellulose, 1.0% total phosphorus, and its digestible energy is 18.5 MJ kg^{-1}.

Rearing was conducted in rotating basins with a volume of 0.2 m^3 (at densities of 80 specimens dm^{-3}) which were part of a recirculating system (there were two replicates of each feeding group). The water temperature was maintained within the optimal range for larvae of this species at 20°C [25]. Permanent, twenty-four hour per day lighting was used and the light intensity above the water of the rearing basins was 40 lx. The oxygen concentration at the water inflow and outlet was 7.5 and 6.9 mg O_2 dm^{-3}, respectively. Nitrite contents in the outflowing water did not exceed 0.05 mg NO_2-N dm^{-3}, and the concentration of total ammonia nitrogen (TAN = NH_4^+-N + NH_3-N) did not exceed 0.25 mg TAN dm^{-3}. The basins were cleaned daily at 08.00, and fish losses were determined.

At weekly intervals (6, 13, 20, 27 DPH) during the experiment, 30 fish were randomly collected from each basin, sacrificed with a anesthetic solution of 4 cm^3 dm^{-3} (Propiscin, IRS Olsztyn [13]), and then preserved with 4% buffered formalin. During the experiment, the percentage of larvae with inflated swim bladders (13 DPH [7]) and the percentage of larvae with body deformations (27 DPH) were determined. The fish were weighed (BW ± 0.1 mg) and measured (Lt ± 0.01 mm), and the collected data were used to calculate the following zootechnical indices:

specific growth rate, SGR (% d^{-1}) = 100 (ln BW_2 – ln BW_1) t^{-1},
final coefficient of body weight variation, CV (%) = (SD BW $^{-1}$) 100,
daily growth rate, DGR (g d^{-1}) = (BW_2 – BW_1) t^{-1},
survival, S (%) = 100 (IN – DN) IN^{-1}.

where:

BW_1, BW_2 – initial and final body weight (g); t – rearing period (days); IN and DN – initial stocking density and losses (individuals).

Histological analysis

On the last days of the subsequent weeks of rearing (age 13, 20, and 27 DPH), five fish were collected from each group and sacrificed with an anesthetic solution (Propiscin, 4 cm^3 dm^{-3}) for histological tests. The larvae were placed in a weight vessel and fixed with Bouin's solution. After several days, the fish were dehydrated with increasing concentrations of ethanol (from 70 to 100%), cleared in xylene, and embedded in paraffin blocks. This material was cut with a rotating microtome into slices 5 µm thick and then stained with hematoxylin and eosin [29]. These histological preparations were analyzed using a light microscope (Nikon, Japan). The computer program MultiScanBase v. 8.08 (Computer Scanning System Ltd., Warsaw, Poland) was used in observations and measurements of the ultrastructures of the digestive tract such as the height of the fold (± 0.01 µm), the size (ø ± 0.01 µm) and number of esophagus mucous goblet cells and gastric glands, the number of pinocytotic vesicles and intestinal mucous cells. The number of pinocytotic vesicles and mucous cells were counted in a field of vision of 1110 μm^2 (33 × 33 µm), while gastric glands were counted within a field of 1850 μm^2 (43 × 43 µm). The intestinal mucous cells were counted in longitudinal cross-sections of the intestines. The remaining quantitative and qualitative parameters were analyzed using cross-sections of the examined tissue.

Statistical analysis

The results were analyzed using one – way analysis of variance ANOVA. The differences between individual experimental groups were determined with the Tukey test ($P \leq 0.05$).

RESULTS

Growth, survival, and the biological quality of the material

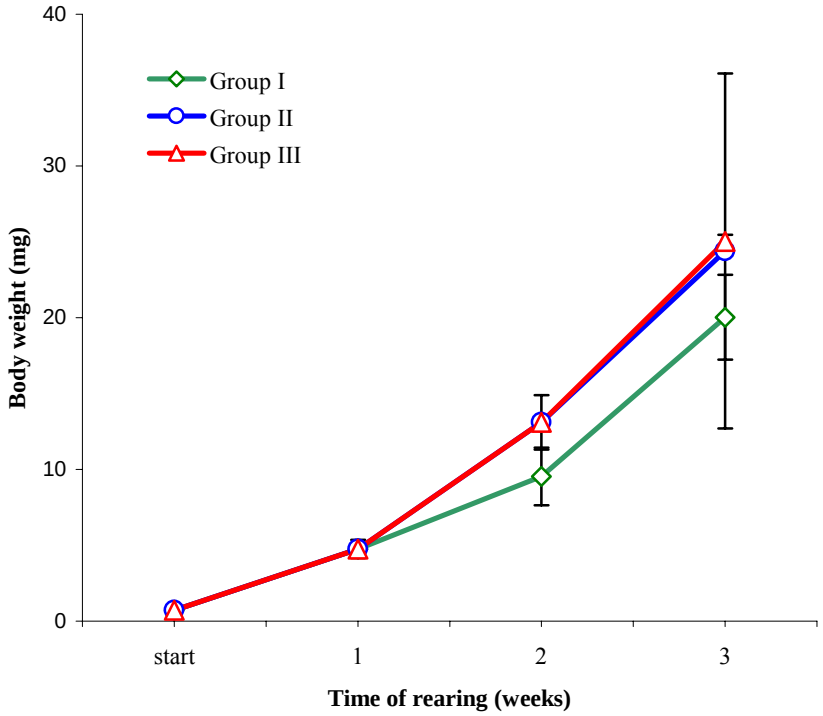
Feeding had a statistically significant impact on fish growth (Table 1; $P < 0.05$). On the final day of the experiment, the mean larval body weight ranged from 20 mg (group I) to 25 mg (group III). Larval body weight gain decreased as *Artemia* sp. was eliminated from the feeding of larvae. In the second week of rearing, the lowest body weight gain was noted in group I. This tendency was maintained throughout the experiment (Fig. 1). The relative SGR was the highest at 16.9% d^{-1} (±0.08) in group III. The final coefficient of body weight variation (CV, %) differed significantly statistically between groups I and III (Table 1; $P < 0.05$). No statistically significant differences were observed in the survival of larvae in the tested groups. The highest survival index (63.1%) was noted in group II (Table 1; $P > 0.05$).

Table 1. Zootechnical indicators of the rearing of pikeperch larvae fed mixed feed (formulated feed + *Artemia* sp.) for a period of one (group I), two (group II) and three (group III) weeks (mean ± SD; each variant was conducted in two replicates; description of groups in Materials and Methods)

Specification	Experimental groups		
	group I	group II	group III
Initial body weight BW (mg)	0.7 ± 0.00	0.7 ± 0.00	0.7 ± 0.00
Final body weight BW (mg)	20^{a} ± 2.8	24^{b} ± 11.7	25^{b} ± 0.45
Daily growth rate (g d^{-1})	0.92^{a} ± 0.13	1.12^{b} ± 0.56	1.15^{b} ± 0.00
Specific growth rate SGR (% d^{-1})	15.8^{a} ± 0.68	16.5^{ab} ± 2.39	16.9^{b} ± 0.08
Final coefficient of body weight variation CV (%)	33.3^{a} ± 2.98	41.2^{ab} ± 2.82	41.5^{b} ± 16.21
Final body lenght TL (mm)	13.3^{a} ± 0.83	13.5^{a} ± 1.83	14.0^{a} ± 0.23
Share of larvae with body deformity (%)	9.5^{a} ± 9.19	16.5^{b} ± 4.94	14.5^{b} ± 2.12
Survival (%)	57.3^{a} ± 16.43	63.1^{a} ± 3.14	59.2^{a} ± 5.61

Data in the same row with the same superscript do not differ significantly statistically (P > 0.05).

Fig. 1. Growth of larval pikeperch in three feeding groups (mean ± SD; * – statistically significant intergroup differences (P < 0.05); description of groups in Materials and Methods)



The share of fish with inflated swim bladders (larva age 13 DPH) was 52% (± 4.95). Although body deformations were observed in all of the experimental groups, there were noted primarily in fish uninflated swim bladders. Spine anomalies also occurred. Kyphosis (curvature of the spine towards the back) and lordosis (curvature of the spine towards the stomach) were observed most frequently (Photo 1), while scoliosis was noted rarely. On the final day of the experiment (27 DPH) the percentage of deformed larvae in the particular groups differed statistically significantly at 9.5% (group I) to 16.5% (group II) (Table 1; P < 0.05).

Phot. 1. Pikeperch larvae with inflated (upper) and uninflated swim bladders with visible skeletal deformations (lower)

Histology of the digestive tract

At the conclusion of the first week of rearing (13 DPH), in which mixed feeding was applied in all tested experimental groups, the anterior, central, and posterior segments of the digestive tract were differentiated. The well-formed folds of the anterior intestine reached an average height of 64 µm (Table 2). The mucous goblet cells occurred numerously between the membrane cells of this part (Photo 2A). The number of them in the cross-section under a field of vision of 1110 µm^2 was 1.5 (Fig. 2). The diameter of the goblet cells was 10 µm (Table 2). No gastric glands were noted on this day. In the central and posterior intestines, which were separated by the intestinal valve, the folds were well-built, and their heights averaged 42 and 46 µm (Table 2). Pinocytotic vesicles were visible in the mucous membranes of the central intestine and small light vacuoles were noted in the enterocyte cytoplasm (Photo 2B). The posterior part of the intestine was characterized by the presence of large light vacuoles Photo 2C). There was an average of 2.5 pinocytotic vesicles in a given field of vision of the cross-sections (Fig. 2). No mucous cells were confirmed in the central and posterior intestines. Brush borders were, however, present on the surface of the enterocytes.

Table 2. Size of intestinal folds and excretion cells of the digestive tract in pikeperch larvae in the first (13 DPH, fed mixed feed), second (20 DPH, Group I – fed formulated feed exclusively, groups II and III – feeding continued with mixed feed) and third weeks of rearing (27 DPH, Group I and II – fed only formulated feed, Group III – still fed mixed feed) (mean ± SD, each variant was conducted in two replicates)

Specification	The first week of rearing	The second week of rearing		The third week of rearing		
	groups I, II, III	group I	groups II, III	group I	group II	group III
Height of folds (µm)						
Oesophagus	64.5 ± 2.76	79.7^{a} ± 2.00	71.8^{b} ± 3.14	81.0^{a} ± 1.00	74.9^{b} ± 2 .41	75.0^{b} ± 2.12
Stomach	absent	85.5^{a} ± 3.82	87.5^{a} ± 0.76	95.8^{a} ± 8.20	96.3^{a} ± 5.23	109.2^{b} ± 4.49
Central intestine	42.5 ± 5.86	60.6^{a} ± 2.28	62.1^{a} ± 0.38	88.5^{a} ± 0.23	70.8^{b} ± 1.65	93.4^{c} ± 0.35
Posterior intestine	46.8 ± 7.77	77.8^{a} ± 6.01	69.9^{b} ± 4.66	71.7^{a} ± 6.95	65.2^{b} ± 6.91	67.7^{ab} ± 2.22
Size of cells (µm)						
Goblet cells	10.0 ± 0.38	9.8^{a} ± 0.67	9.9^{a} ± 1.00	9.4^{a} ± 0.27	10.5^{b} ± 0.80	10.1^{b} ± 0.21
Gastric glands	absent	22.2^{a} ± 2.16	22.6^{a} ± 1.08	31.4^{a} ± 0.52	33.3^{ab} ± 3.90	33.9^{b} ± 0.60

Data in the same row with the same superscript do not differ significantly statistically ($P > 0.05$).

Phot. 2. Histopathological picture of the digestive tract: A – esophagus; B – central intestine; C – posterior intestine (13 DPH); D – stomach (20 DPH); E – central intestine (27 DPH); LU – intestinal lumen, mc – mucous cells, pv – pinocytotic vesicles, lv – lipid vacuoles, gg – gastric glands

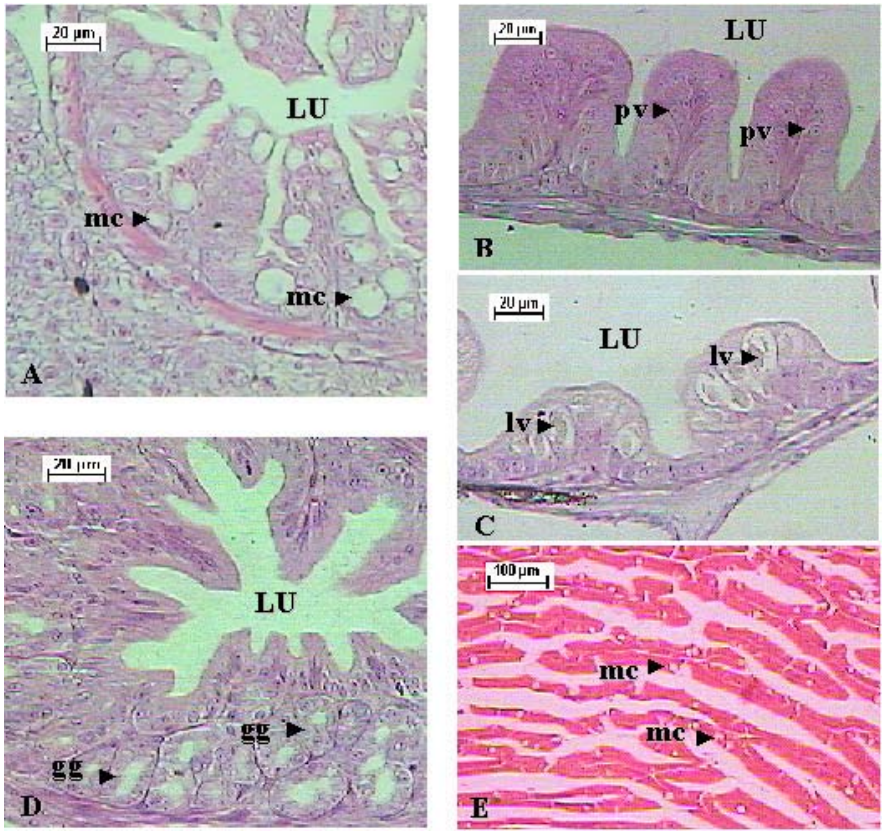
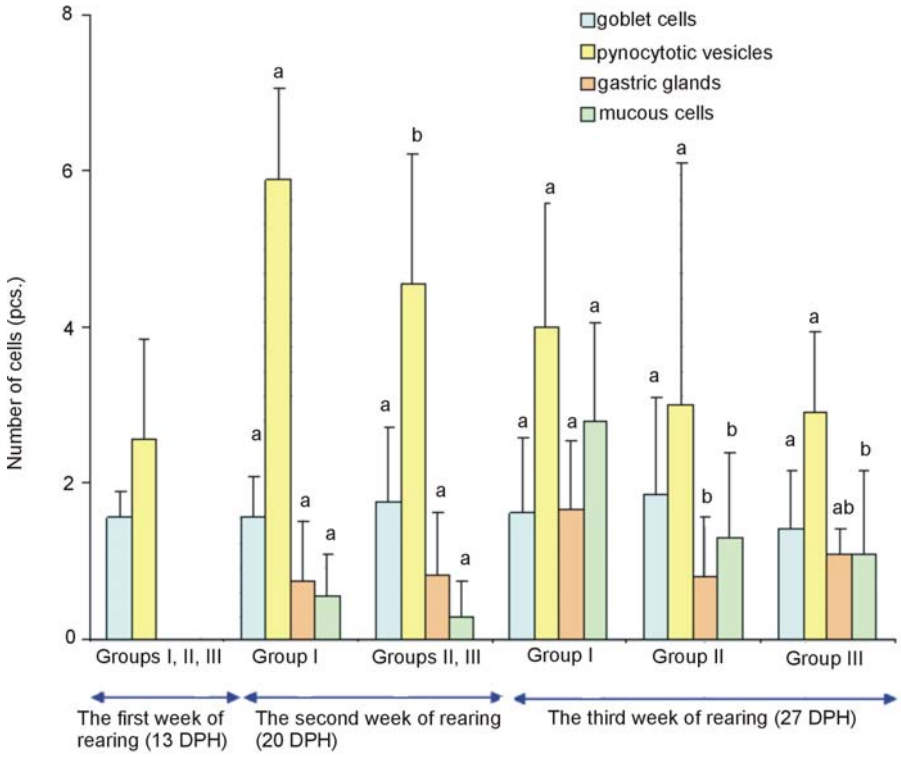


Fig. 2. Number of goblet cells in the esophagus, pinocytotic vesicles, and mucous cells in the intestine in a field of vision of 1110 µm^2, gastric glands in a field of vision of 1850 µm^2 in the digestive tracts of pikeperch larvae tested in different feeding groups (mean ± SD; data with the same superscript do not differ significantly statistically (P > 0.05); description of groups in Materials and Methods)



In the next week (20 DPH), in addition to significant intergroup differences in larval growth, differences were observed in the ultrastructures of the digestive tract of larvae from various feeding groups. The height of the folds of the various sections of the intestines increased and was significantly different in the esophagus and the posterior intestine. In the larvae fed formulated feed exclusively in the second week of rearing (group I) and in those that were still fed mixed feed (groups II and III), the mean height of the folds in the esophagus was 79 and 71 µm, in the central intestine – 60 and 62 µm, and in the posterior intestine – 77 and 69 µm, respectively (Table 2). The pyloric caecae formed, and the gastric glands in the connective tissue already occurred numerously (Photo 2D). The diameter of the gastric glands (cross-section of the glandular duct) in all of the groups was an average of 22 µm (Table 2). The number of pinocytotic vesicles in the central intestine increased. In group I, the quantity of vesicles in a given field of vision of the cross-sections was 5.8 and was significantly higher than in group II and III, were it was 4.5 (Fig. 2). Few mucous cells were visible in the intestine, and the quantity of them in group I (in a field of vision of 1110 μm^2 on longitudinal cross-sections) was 0.5, while in groups II and III it was 0.28 (Fig. 2). These differences were not statistically significant ($P > 0.05$). The quantity and size of the goblet cells in the esophagus did not change (Table 2), and the quantity of goblet cells and gastric glands was similar in the studied groups (Fig. 2; $P > 0.05$).

On the final day of the experiment (27 DPH), the mean height of the esophagus folds ranged from 81 l'm (group I) to 75 µm (groups II and III). These differences were statistically different (Table 2; $P < 0.05$). In group I, the highest were in the posterior intestine; their mean height was 71 µm and was significantly higher than in group II (65 µm) (group III – 67 µm). The mean height of the folds in the central intestine was significantly different in all feeding groups with the highest in group III (93 µm) and the lowest in group II (70 µm). The mean height of stomach folds, which was similar in groups I and II (95 and 96 µm, respectively), was significantly lower than in group III (109 µm) (Table 2; $P < 0.05$). Differences in the size of the esophagus goblet cells and gastric glands also appeared. The average size of these cells in group I was significantly the smallest (Table 2). There were similar quantities of goblet cells in all the groups. The number of gastric glands grew nearly twofold in the larvae from group I (up to 1.66 in a field of vision of 1850 μm^2), while in group II there were no changes (0.80). In group III, this value was in between at 1.10 (Fig. 2; $P > 0.05$). Mucous cells occurred numerously in the central intestine (Photo 2E). In the larvae from group I, the number of mucous cells was significantly statistically higher at 2.81. The quantity of these cells in the other groups ranged from 1.3 (group II) to 1.1 (group III) (Fig. 2).

DISCUSSION

Supplementing the diet with live feed provides conditions for the fast growth of many fish species [8]. Any formulated feed whatsoever can be acceptable in initiating exogenous feeding, although feeding formulated feed exclusively often results in low larval growth and survival [23]. It can be concluded from the comparison of larval growth in three feeding groups that a longer period of feeding mixed feed (for two to three weeks) results in better larval growth in comparison to that of fish fed such feed for only one week of rearing. Kestemont et al. [14] reported that supplementing the diet with *Artemia* sp. has a beneficial impact on the growth of larval European perch, *Perca fluviatilis* L. In the case of this species, the poorest rearing results were obtained when formulated feed was applied exclusively. There was no confirmation, however, of any difference in growth or survival between groups fed natural feed (*Artemia* sp.) exclusively or mixed feed (formulated feed supplemented with *Artemia* sp.). Xu et al. [27] observed in European pikeperch larvae fed *Artemia* sp. exclusively, that on day 12 post-hatch, the body weight gain was better than in larvae fed formulated feed supplemented with natural feed. However, it was determined that in the subsequent week of rearing better results were obtained with mixed feed since the larvae achieved better body growth. Additionally, the share of deformed specimens was the lowest in this feed group. In the current study which tested three feed groups, no differences were noted among larval survival, and the share of deformed fish was the highest in groups II and III, which were fed mixed feed for two (6 – 19 DPH) and three (6 – 26 DPH) weeks, respectively. Ostaszewska et al. [21] maintained that pikeperch larvae fed commercial starter exclusively (fish age 5 – 35 DPH) demonstrated similar survival (and growth) as the larvae fed *Artemia* sp. exclusively. Perhaps the quicker growth of larvae from groups II and III caused the occurrence of a greater number of deformed specimens. It is postulated that body deformation in percoid fish can stem from a deficit of highly unsaturated fatty acids (HUFA), which is effectively eliminated by supplementing the larval diet with *Artemia* sp. [14, 27]. Additionally, according to Xu et al. [27], differences in growth and deformations must be explained by the ability of the larvae to digest and absorb nutritional substances in the feed at a given stage during ontogenic development.

Initial segmentation of the digestive tract in larval pikeperch is connected to the resorption of the yolk sac and the beginning of exogenous feeding [17, 20]. Three segments (anterior, central, posterior) are already visible in the digestive tract of larvae 4 – 7 DPH. According to Govoni et al. [9], the end of yolk sac absorption and related exogenous feeding is the period when the larval primordial gut becomes segmented into three. During this period, mucous cells appear in the anterior and supranuclear inclusions in the posterior segments of the intestine, which are indicators of the functioning of the digestive tract. Results obtained by other authors investigating different fish species confirm this [1, 2, 22]. The observed changes in the larval digestive tract during this period attest to the

advent of digestive processes. The characteristic intestinal valve, which separated the last two segments of the digestive tract, retains enzymes in the intestine of larvae in early developmental stages. The secretions of goblet cells in the anterior segment play a protective role in the digestive tract. The mucous cells of the posterior segment secrete digestive enzymes and substances that permit absorption processes different from those of mucous [11, 24], while the light vacuoles in the cytoplasm of the enterocytes attest to the presence of lipids. It is precisely in the anterior intestine that lipids are hydrolyzed by lipases and absorbed in the form of micells by the enterocyte brush borders. From here they diffuse into the interior of the cell where they are resynthesized and grouped with proteins (in chylomicrons and lipoproteins) and then either stored or transported to the circulatory system. As reported by Ostaszewska et al. [21], excess enterocyte vacuolization in this segment can result in halted intracellular or lumenal digestion and the transport of products to the circulatory system. Further, the absence of light vacuoles might be the result of starvation or the proper absorption, synthesis, and transport of lipoproteins to the circulatory system. Based on histological observations conducted within the scope of the current study, it can be concluded that pikeperch larvae at 13 DPH are capable of digesting lipids. The large light vacuoles in the supranuclear regions of the cytoplasm of mucous membrane cells of the posterior segment attest to intestinal absorption. The presence in these vacuoles of acid-absorbing granules was the result of pinocytosis of protein macromolecules from the intestinal lumen, which resulted from the low concentration of digestive enzymes and the lack of gastric glands that produce proteolytic enzymes. This is the effect of intracellular digestion that plays a key role in the assimilation of protein prior to the formation of the stomach [11]. Longer and more differentiated mucous membrane folds in the posterior segment of the intestine of pikeperch larvae in comparison with the central segment indicates that at this stage in development (13 DPH) it was more active in the absorption of nutrients. The large and numerous supranuclear vacuoles in the cytoplasm of the enterocytes attest to this. The higher folds in the posterior segment of the larval pikeperch digestive tract as compared to those in the anterior segment were maintained in the second week of rearing. On the final day of the experiment (27 DPH), the intestinal folds of the central segment were already higher than those in the posterior segment. In species such as flounder, *Paralichthys dentatus* (L.), the central intestinal folds are higher than those of the posterior segment as early as 9 DPH [1]. Furthermore, in pike eel, *Muraenesox cinereus* (Forsskål), the central and posterior segments were still of similar height at 6 DPH [22]. Higher intestinal folds in the larval pikeperch from group I, which was fed mixed feed for only a week (6 – 12 DPH), increased the surface area of the intestinal mucous membrane responsible for secretion and absorption during the digestion process. An increase in the size of the intestinal folds caused an increase in the size of the digestive surface (including the striated border of the enterocytes), and, in consequence, the exposition and absorption of assimilable nutrients. Depending on the species, protein and lipid absorption in the intestines can begin at the moment feeding begins or can pertain initially to one of the components. The microvilli present along the entire length of the central and posterior intestine indicate that there is active transport in the intestinal epithelial [1]. Cousin et al. [5] reported that the hydrolysis of nutritional substances into assimilable monomers occurs with the participation of the enzymes of the striated border of the enterocytes.

The first gastric glands appear in pikeperch larvae at 15 – 20 DPH along with the recesses of the central intestine [17, 20]. In some species they occur much later regardless of the formation of the pyloric caecae [1, 2, 5]. Stomach development in the larval pikeperch in the current study was similar in the three feeding groups. Not until the final phase of the experiment (27 DPH) did differences appear in the size and number of gastric glands. It is plausible that fish fed formulated feed, in order to achieve better assimilation, developed a larger number of gastric glands. It is known that *Artemia* sp. is an exogenous source of enzymes that play a simultaneous role in autolysis. This is why the physiological effort expended by the larvae during brine shrimp digestion is lower than it is with formulated feed. Ostaszewska et al. [21] did not note a difference in stomach development and the presence of gastric glands in larval pikeperch fed *Artemia* sp. and two commercial starters. According to these authors, fully formed gastric glands were noted at the conclusion of the experiment (larva age 35 DPH) in these three tested groups. It should be emphasized that the presence of gastric glands is not always indicative of stomach function, and the activity of pepsin and an acidic pH in the stomach lumen appear somewhat later [19]. However, the occurrence of pepsinogen in the gastric glands and neutral mucins in the stomach lumen, which protect the mucous membranes from hydrochloric acid and enzymes, were confirmed in pikeperch at this stage of development in other studies [20].

The number of pinocytotic vesicles decreased in all pikeperch feeding groups beginning in the second week of rearing and, at the same time, was the highest in group I until the conclusion of the experiment. Perhaps the increased number of pinocytotic cells in the intestines in group I attests to the domination of this process in the absorption of nutrients. Supposing, as did Cahu and Zambonino Infante [4], that the increased secretion of pepsinogen and hydrochloric acid causes rapid protein digestion in the stomach and simultaneously reduces the activity of pinocytotic and intracellular digestion in the enterocytes, it can be concluded that pinocytosis was less important in protein digestion in groups II and III than it was in group I. These studies also confirm previous reports by Cahu and Zambonino Infante [3], who also maintained that the activity of digestive enzymes in larvae is connected with live food.

In the current experiment, intestinal mucous cells were noted in pikeperch larvae at the end of the second week of rearing (20 DPH). The first intestinal mucous cells in the central and posterior intestine appeared in pikeperch larvae at 16 DPH. These cells synthesize neutral and acidic mucins [20]. Grau et al. [10] reported that the mucous substances in the intestines participate in digestive and absorption processes. The abundance of mucous cells in the anterior as compared with the posterior intestine attests to the functionality of the intestine in the digestive tract. These differences refer simultaneously to the ability to absorb and secrete mucous in this segment [11]. In the concluding phase of the experiment it was precisely in group I that the highest number of these cells was confirmed, which may attest to intensified excretion processes. This reaction may stem from the fish adjusting to assimilate formulated feed effectively, as was the case with gastric glands. Shortening the feeding period with *Artemia* sp., a carrier of exogenous enzymes, results in the formation of a larger number of structures responsible for digestion.

The results of many studies indicate that feeding live feed contributes to better growth and the assimilation of formulated feed [16]. It can be concluded that the enzymes in live feed support digestive processes in the incomplete digestive tract [2]. It has been demonstrated that diet might be a decisive factor in the activity of digestive enzymes. The activity of pepsin (as well as of trypsin and chymotrypsin) in larval perch changes in response to changes in diet, e.g., it decreased during periods of body growth in larvae taught to consume formulated feed.

Feeding live feed did not affect the activity of these enzymes during larval and postlarval development [14]. It is assumed that larvae require external feed enzymes and hormones provided by natural feed. However, supplementing the feed of yellow perch, *Perca flavescens* (Mitchill), with neurohormones did not improve growth rates or survival. Additionally, the secretion of the studied enzymes was similar to that of fish fed formulated feed [16]. Similar results were obtained in cyprinids and bass, *Dicentrachus labrax* (L.) [16]. Further, in gilthead bream, *Sparus aurata* L., enzyme supplementation to the diet had a positive impact on growth [16]. These authors reported that a diet supplemented with these components did not produce the anticipated effect when there was a sufficient amount of enzyme in the developed larval digestive tract.

CONCLUSIONS

The physiological effort exerted by pikeperch larvae to digest formulated feed is undoubtedly higher than that required for natural feed. The higher energy consumption required for digesting formulated feed may have resulted in, among other things, the creation of a greater number of cell structures and lower larval growth rates. A statistically significant intergroup difference was noted in the number of gastric glands and intestinal mucous cells. The current experiment indicates that the second week of rearing (13 – 19 DPH, water temperature – 20°C) is a crucial period as significant changes occur in the larvae that determine their growth and development. In older larvae (20 – 26 DPH) fed exclusively formulated feed (third week of rearing), no differences were noted in body weight increases in comparison with larvae fed mixed feed. Thus, it can be concluded that eliminating *Artemia* sp. from the larval diet at this age does not have a negative impact on individual growth, development, or the results of larval pikeperch rearing.

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Agata Kowalska
Department of Aquaculture,
Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Poland

Zdzisław Zakęś
Department of Aquaculture,
Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Poland

Krystyna Demska-Zakęś
Department of Ichthyology,
University of Warmia and Mazury, Olsztyn, Poland
Oczapowskiego 10, 10-719 Olsztyn, Poland
