Effects of weaning age and diets on ontogeny of digestive activities and structures of pikeperch (*Sander lucioperca*) larvae

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Abstract Growth and ontogeny of digestive function were studied in pikeperch (*Sander lucioperca*) larvae weaned on artificial food at different ages. Three weaning treatments initiated respectively on day 9 (W9), day 15 (W15) or day 21 (W21) post-hatching (p.h.) were compared with a control group, fed *Artemia* nauplii from first feeding until the end of the rearing trial on day 36 p.h. The digestive enzyme activities and the ontogeny of digestive structures were investigated using enzymatic assays and histological methods. Growth of pikeperch larvae was significantly affected by precocious weaning. Pancreatic (trypsin and amylase) and intestinal (leucine-alanine peptidase, leucine aminopeptidase N and alkaline phosphatase) enzyme activities were detected from hatching onwards, increased at the moment of first feeding and then decreased. Pepsin secretion occurred at day 29 p. h. only, concurrently with the stomach development and differentiation of gastric glands. In the early weaning group (W9) the maturation process of intestinal enterocytes seems to be impaired and/or delayed and several signs of malnutrition were recorded. Except for alkaline phosphatase activity, no differences in enzyme activities and development of digestive structures were observed among the control, W21, and W15 groups. Moreover, at the end of the experiment, no differences in proteolytic activities were observed among larvae from the different treatments, indicating that, in surviving individuals, the digestive structures were properly developed and the larvae had acquired an adult mode of digestion. Based on the artificial diet used, our results suggested that pikeperch larvae can be weaned from day 15 p.h. without significant adverse effect on digestive capacities (except for alkaline phosphatase) or development of digestive tract, while earlier weaning impaired the onset of the maturation processes of the digestive system, both in terms of morphological structures and enzymatic activities.

Abbreviations
Amy	Amylase
AN	Leucine aminopeptidase N
AP	Alkaline phosphatase
Leu-ala	Leucine alanine peptidase
Try	Trypsin

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Introduction

Pikeperch (*Sander lucioperca*) is commercially valuable and is one of the main percid species that represents a great interest for aquaculture, restocking natural waters, and fishing (Kestemont and Mélard 2000). This species, which originated from Eastern Europe, was introduced into water reservoirs in North African countries and notably into Tunisia at the end of 1960s (Zaouali 1981). The species acclimated well to this geoclimatic context, showing satisfactory growth and an interesting production potential (Mhetli 2001). In the impounded reservoirs, it contributed to sustaining fishing activity thanks to its high commercial value. Nowadays, production of pikeperch fingerlings has become a priority because of increased fishing activity of this species and the objective of extending impoundment.

Several studies deal with the rearing and feeding of pikeperch at the juvenile stage (Zakes 1997, 1999; Zakes et al. 2001; Ljunggren et al. 2003), but the experience in the rearing of the North American species (*Stizostedion vitreum*; Moore 1996; Summerfelt 1996; Guthrie et al. 2000) is much more developed.

Most experiments of pikeperch rearing concerned the fingerlings and generally occurred in ponds (Klein Breteler 1989; Steffens et al. 1996; Ruuhijärvi and Hyvärinen 1996). Fish were generally fed on live prey (natural zooplankton, *Artemia* nauplii). Studies on the weaning of pikeperch larvae are rare (Ruuhijärvi et al. 1991; Schlumberger and Proteau 1991) and often lead to poor results in terms of survival and growth. In a review, Hilge and Steffens (1996) indicated the unsatisfactory quality of larval diets tested although they assumed that artificial diet can be used successfully from fingerling stage (4–5 cm). Yet, in a recent study, Ostaszewska et al. (2005) succeeded in rearing pikeperch larvae using formulated diets from first feeding.

In aquaculture, larval weaning is usually introduced as early as possible in order to reduce the constraints and costs of live prey production. Indeed, for European sea bass (*Dicentrarchus labrax*), Person-Le Ruyet et al. (1993) estimated the cost of this production at approximately 79% of the production cost to obtain a juvenile of

45 days of age. Difficulties of the larvae to accept and to digest artificial diets have been often attributed to their immature digestive system at hatching and low enzymatic capacities (Lauff and Hofer 1984; Person-Le Ruyet et al. 1989). Thus, knowledge of the larval ontogeny and the onset of functional digestive structures appeared necessary to define feeding strategies (nutritional requirements, formulation of feed, optimal weaning age).

A few authors studied pikeperch larval development (Mani-Ponset et al. 1994, 1996; Diaz et al. 1997, 2002) and their studies focused on the evolution of yolk reserves and digestive tract or lipid metabolism during the very early life stages. More recently, Ostaszewska et al. (2005) studied the changes in digestive tract of pikeperch larvae fed natural food or commercial diets.

Our work investigated the effects of different diets (live prey and dry diet) and weaning ages on growth and ontogeny of digestive function during the early development of pikeperch.

Materials and methods

Facilities and fish

Pikeperch larvae were obtained from a private hatchery (Viskweekcentrum Valkenswaard, The Netherlands). On the day of mouth opening, day 4 p.h., 12,000 larvae were transferred to the rearing facilities of the laboratory (URBO). Larvae were maintained in a 200-l tank (19°C) for acclimation for 4 days with a low water supply and air flow. They were fed on small size *Artemia* nauplii (AF, INVE, Dendermonde, Belgium) ad libitum each hour from 8 a.m. to 8 p.m. At day 9 post-hatching, all larvae were transferred to the experimental unit in a recirculating rearing system of 12 rectangular grey tanks of 20 l. Temperature and dissolved O_2, controlled daily, were maintained at 19–20°C and over 7 mg l^{-1} respectively. A 12:12 h light regime was provided by fluorescent tubes (40 W) giving a moderate light intensity at the water surface. The recirculating water was purified by a bio-filter system.

Each tank was stocked with 1,000 larvae (50 larvae/l). Four treatments in triplicate were

randomly assigned to the tanks. The larval feeding scheme is summarized in Table 1. Newly hatched AF *Artemia* nauplii (INVE) were used as first live preys from day 4 to day 10 p.h.. They were replaced by newly hatched EG *Artemia* nauplii (INVE), from day 11 to 15 p.h. and then, by metanauplii enriched for 24 h with Super Selco (INVE) from day 16. The artificial diet Lansy CW 2/3 (200–300 µm) was used for weaning and was introduced from days 9, 15, and 21 p.h. (treatments W9, W15, and W21). After day 29 (p.h.), it was replaced by the larger brand Lansy CW 3/5 (300–500 µm). Its proximate composition is described in Table 2. The control group (A) was fed exclusively on *Artemia* nauplii. Food was distributed manually (live prey every 90 min and dry diet every hour) from 8 a.m. to 8 p.m. The feeding levels were fixed on a dry weight basis at 25, 20, 15, and 10% of larval wet weight during the first, second, third and fourth week respectively, corresponding to 0.25–0.5; 1–2; 2–3; 3–5 g tank^{-1} day^{-1} (dry weight). A period of 6–7 days of co-feeding was applied to habituate the larvae to the dry diet.

Table 1 Feeding regimes followed during the larval rearing of *Sander lucioperca*

Days	A (control)	W9	W15	W21
4–8	A_0	A_0	A_0	A_0
9–14	A_0	$A_0 + L_1$	A_0	A_0
15–20	A_1S	L_1	$A_1S + L_1$	A_1S
21–28	A_1S	L_1	L_1	$A_1S + L_1$
29–36	A_1S	L_2	L_2	L_2

A_0, *Artemia* nauplii (AF from day 5 to 10 and EG from day 11 to 15); A_1S, metanauplii enriched for 24 h with Super Selco (INVE, Dendermonde, Belgium); L_1, Lansy 1 (200–300 µm); L_2, Lansy 2 (300–500 µm)

Table 2 Proximate composition of the dry diet Lansy CW

Ingredients	Percentage dry matter
Crude protein	58
Crude lipids	15
Crude ash	12
Crude fiber	1
Vitamin A	30,000 IU kg^{-1}
Vitamin D3	2,500 IU kg^{-1}
Vitamin E	700 mg kg^{-1}
Vitamin C	2,000 mg kg^{-1}

Sampling

Growth rate was monitored by sampling 30 larvae from each tank at days 5, 9, 15, 21, and 29. The larvae were weighed immediately. About 400 larvae were collected on days 0 and 5 for histology and enzymatic assays; 80, 60, 40, 30, and 20 larvae per tank on days 9, 15, 21, 29, and 36 respectively in order to determine the pattern of enzymatic activity. Ten to 15 larvae per treatment were also collected for histological study. Samples were taken before food distribution and immediately stored at –80°C for biochemical analysis or fixed in Bouin's fluid for histology. Larvae were weighed per group from day 0 to day 29. On day 36, all surviving larvae were weighed individually. The coefficient of variation (CV, %) was calculated as 100 SD/mean and the specific growth rate (SGR, % day^{-1}) as $100(\text{LnWf} - \text{LnWi})\Delta T^{-1}$ where Wf, Wi = final and initial weight of larvae (mg), T = time (days).

Enzymatic assays

Larvae younger than 15 days were relieved of head and tail to isolate their digestive segment. Older larvae were cut into four parts as described by Cahu and Zambonino Infante (1994), on a glass maintained on ice (0°C) under binoculars, to separate their pancreatic and their intestinal segments.

Samples were homogenized in five volumes (v/w) of ice-cold distilled water. Pancreatic enzymes trypsin (Try) and amylase (Amy) were assayed according to Holm et al. (1988) and Metais and Bieth (1968) respectively. BAPNA ($N\alpha$-Benzoyl-DL-Arginine-p-Nitroanilide) and starch were respectively used as substrates for these two enzymes. When larvae were dissected assays were conducted on the pancreatic segment. Intestinal enzymes, leucine alanine peptidase (Leu-ala), alkaline phosphatase (AP), and leucine aminopeptidase N (AN) were assayed respectively, according to Nicholson and Kim (1975), Bessey et al. (1946), and Maroux et al. (1973) using respectively Leucine-alanine, p-nitrophenyl phosphate and L-leucine p-nitroanilide as substrates. When larvae were dissected assays were conducted on the intestinal segment. Pepsin was

assayed according to Worthington (1982). Enzyme activities are expressed as specific activities (U mg protein^{-1}). Protein was determined by Bradford's (1976) procedure.

Histological method

Fixed larvae were embedded in paraffin, and 6-μm longitudinal sections were stained with Masson trichrome (Gabe 1968). Ten larvae per treatment were observed with light microscope.

Statistical analyses

Results are given as mean ± SD (n = 3). Values of weights and enzymatic activities were log10 transformed, and percentages (SGR and CV) were arcsin transformed. Weights, SGR, CV and enzymatic activities were compared using one-way and two-way (only enzymatic activities) analysis of variance followed by LSD multiple range test when significant differences were found with a level of significance of $P < 0.05$. Homogeneity of variances was first verified using Levene's test.

Results

Growth

On days 15 and 21, there were no differences in the larval weights among the four experimental groups. On day 29, larvae fed on live prey (A) or weaned on day 21 (W21) and day 15 (W15) displayed significantly higher body weights than larvae weaned on day 9 (W9). At the end of the experiment, the difference was not significant between larvae fed the different diets due to the high variability between replicates (Table 3).

Cannibalism, observed from day 16, is an important cause of mortality. It reached 40–50% whatever the diet. Because of the important mortality of W9 larvae (and sampling on day 29), only one tank remained in the W9 treatment by day 36. SGR varied between 5.3 and 12.8% day^{-1} and were similar among the A (control), W21, and W15 groups. Coefficient of variation of weights varied between 44 and 55% and did not differ significantly among treatments.

Enzymatic activities

Pancreatic and intestinal enzyme activities were detected as early as hatching. For all enzymes except Try and AP, activities increased at first feeding (especially Amy, Leu-ala, and AN) and then sharply decreased after day 5. Trypsin-specific activity remained almost constant during the first days of development (until day 9). It increased on day 15, significantly in control and W15 larvae, but not in the W21 and W9 groups. On days 21 and 29, it significantly increased in the control group and was significantly higher than in the weaned groups (Fig. 1a). As for the other proteolytic enzymes, there were no differences among treatments by the end of the experiment.

Amylase-specific activity was 0.81 ± 0.15 mU mg protein^{-1} at hatching and reached 5.28 ± 1.67 mU mg protein^{-1} at mouth opening. Then it decreased to about 1 mU mg protein^{-1} and remained almost constant until day 36. There were no differences between treatments except on day 29 when Amy activity in W9 larvae was significantly higher than in other treatments (Fig. 1b).

**Table 3** Growth of pikeperch larvae under different treatments

	A	W9	W15	W21
Weight (mg) D29	32.7 ± 26.0[a]	7.1 ± 0.9[b]	25.0 ± 11.5[a]	41.2 ± 21.8[a]
Final weight (mg) D36	79.4 ± 70.3[a]	9.05*	26.2 ± 15.7[a]	98.7 ± 100.1[a]
SGR (% day^{-1})	12.5 ± 3.0[a]	5.9*	8.8 ± 2.4[a]	12.8 ± 4.7[a]
CV (%) at D36	54.9 ± 19.7[a]	51.7*	46.4 ± 3.1[a]	43.9 ± 18.1[a]

Means ± SD (n = 3). Values with different superscript letters in the same line are significantly different ($P < 0.05$)

SGR: specific growth rate; CV: coefficient of variation

* Only one measurement (two tanks were collected on day 29 for enzymatic assays)

Fig. 1 Specific activities of pancreatic enzymes trypsin (**a**) and amylase (**b**) in pikeperch larvae weaned at different times. Means ± SD
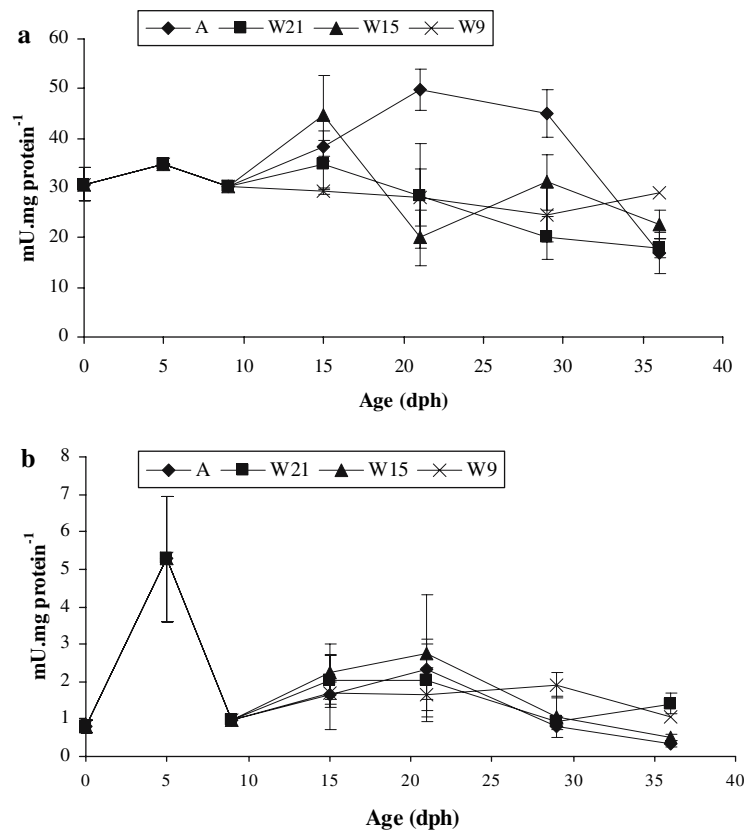
Amy activity in the W21 group was also significantly higher than in the other groups, but this seems to be an artifact. The two-way ANOVA showed that treatment effect was much more significant ($P < 0.001$) than time (''age'') effect ($P = 0.008$) on Try activity. On the other hand, Amy activity was significantly affected by time ($P = 0.0018$), but not by treatment ($P = 0.793$).

Leucine alanine peptidase (Leu-ala) activity reached a maximum (810.0 ± 94.6 U mg protein^{-1}) at first feeding (5 days p.h.) and then decreased in all treatments until day 36 (Fig. 2a). In the W9 group, Leu-ala activity remained significantly higher than in other treatments (day 29). The specific activity of the alkaline phosphatase (AP) increased progressively from hatching up to day 36 p.h., but peaked at maximal values (115.5 ± 16.8 and 108.6 ± 24.1 mU mg protein^{-1}) immediately after weaning in the W9 and W15 groups respectively (Fig. 2b). The AP increase was concurrent with the progressive decrease in

Leu-ala. The leucine aminopeptidase (AN)-specific activity also increased between days 15 and 29, except in the W9 group in which activity remained stable between days 21 and 29. On day 29, the AN activity in W9 larvae was significantly lower than in W21 larvae (Fig. 2c). Leu-ala/AP and Leu-ala/AN ratios sharply decreased between days 5 and 29, except in the W9 larvae in which they remained significantly higher (on day 29) than in the other groups (Table 4).

For the three intestinal enzymes, ANOVA 2 showed a highly significant effect of time on their activities ($P < 0.001$ for AP, AN, and Leu-ala) while treatment effect was not significant for AP and AN ($P = 0.546$ and $P = 0.540$ respectively), although it was significant for Leu-ala ($P = 0.014$).

Pepsin activity was detected for the first time on day 29. It varied between 55 and 112 mU mg protein^{-1}, but was not significantly different between treatments (Table 4). Pepsin

Fig. 2 Specific activities
of intestinal enzymes
leucine alanine peptidase
(**a**), alkaline phosphatase
(**b**), and aminopeptidase
N (**c**) in pikeperch larvae
weaned at different times.
Means ± SD
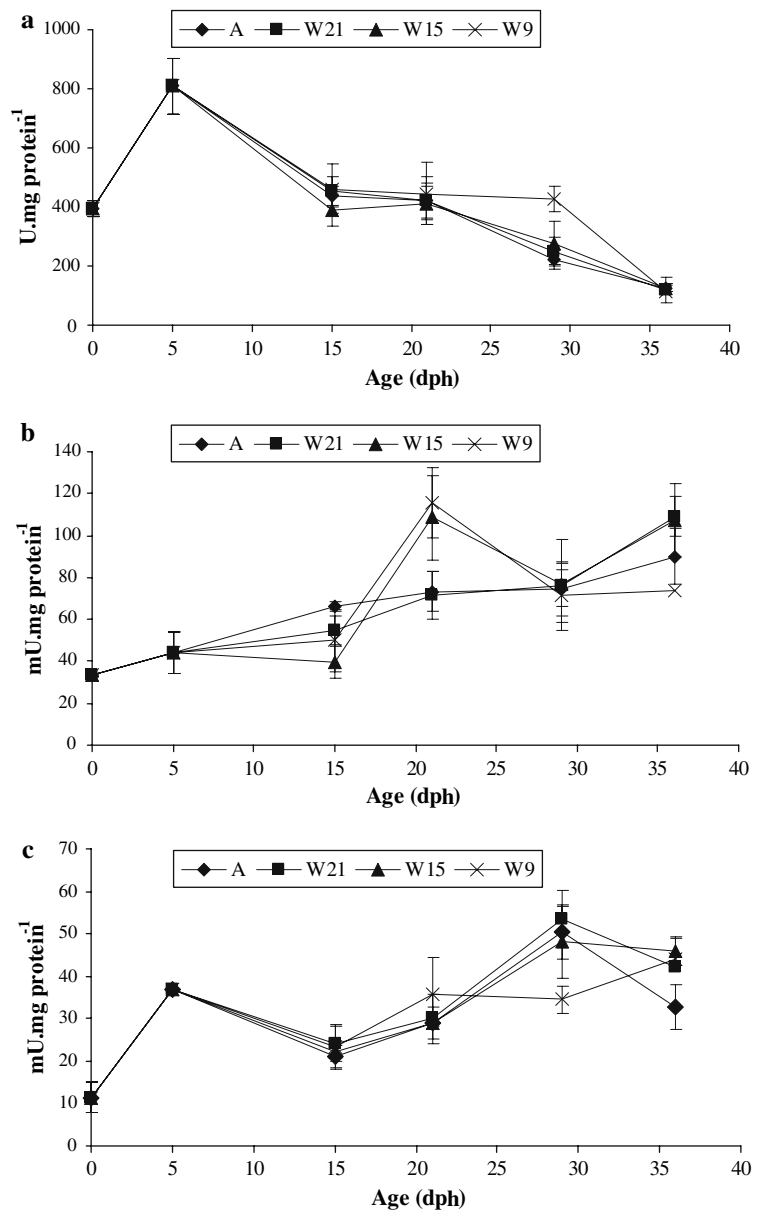
activity was significantly higher in large fish (it
reached 253 ± 51 mU mg protein^{-1}).

Histological development

At hatching, the mouth and anus were closed.
The yolk vesicle occupied a large volume and
was disposed posteriorly to the oil globule
(Fig. 3a). The digestive tract appeared as a
simple straight tube composed of the buccoph-
aryngeal cavity, esophagus, anterior and poster-
ior intestine separated by the intestinal valvula.
The enterocytes were well differentiated and the
brush border membrane was visible. The liver
was a visible mass between the heart and the
intestine, but was not yet differentiated from the
pancreas.

On the first feeding (day 5), the mouth and
anus opened. Yolk reserves were in the process of
being resorbed, but the oil globule volume was
still substantial. The pancreas and the liver were
functional and developed several mitotic cells

Table 4 Pepsin-specific activity and Leu-ala/AN and Leu-ala/AP ratios on day 29 in different treatments

	A	W9	W15	W21
Pepsin-specific activity (mU mg protein^{-1})	90.2 ± 44.1[a]	54.6 ± 11.0[a]	112.5 ± 47.3[a]	92.6 ± 30.1[a]
Leu-ala/AN (10^3)	4.42 ± 0.75[a]	12.61 ± 2.78[b]	6.04 ± 2.64[a]	4.82 ± 1.42[a]
Leu-ala/AP (10^3)	2.96 ± 0.11[a]	6.02 ± 0.52[b]	3.66 ± 0.78[a]	3.37 ± 0.99[a]

Means ± SD ($n = 3$). Values with different superscript letters in the same line are significantly different ($P < 0.05$)

(Fig. 3b). The intestinal epithelium presented a well-organized brush border membrane.

At 9 days p.h., reserves were almost totally resorbed. Pharyngeal teeth were visible in the buccal cavity and the goblet cells secreting mucous were numerous in the esophagus. The gut was convoluted. The intestine became wider with well-developed enterocytes (Fig. 3c). Numerous lipid inclusions were present in the liver indicating lipid absorption and/or storage.

At 15 days p.h., a ''rough shape'' of stomach (gastric area) was observed (Fig. 4a). The enterocytes were reduced in height and less developed in W9 larvae (Fig. 4b) compared with the larvae fed live prey. No effect was observed in the liver. At 21 days p.h. (5 mg individual weight), the stomach was differentiated, but gastric glands were not visible (Fig. 5a). The effects of dietary treatments on intestinal (enterocytes) development did not appear clearly in W9 larvae, which presented well-developed enterocytes (Fig. 5b). The enterocytes of W15 larvae were not affected by weaning (Fig. 5c).
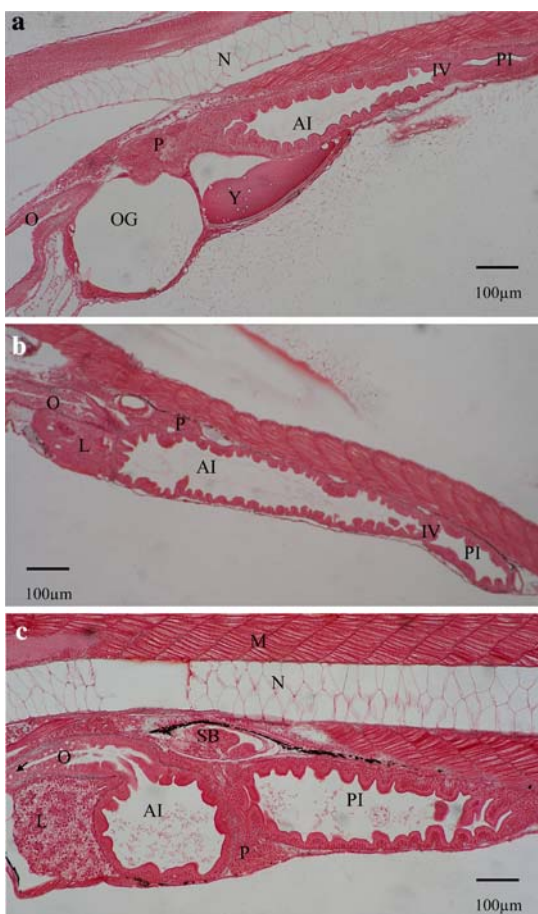

Fig. 3 a Sagittal section of pikeperch larva at hatching (day 0; GX100), **b** at first feeding (day 5; GX100), and **c** pikeperch larva fed Artemia (day 9; GX100). *AI* anterior intestine, *OG* oil globule, *O* oesophagus, *arrow* goblet cell, *IV* intestinal valvula, *K* kidney, *L* liver, *M* muscle, *N* notochord, *P* pancreas, *PI* posterior intestine, *SB* swimbladder, *Y* yolk
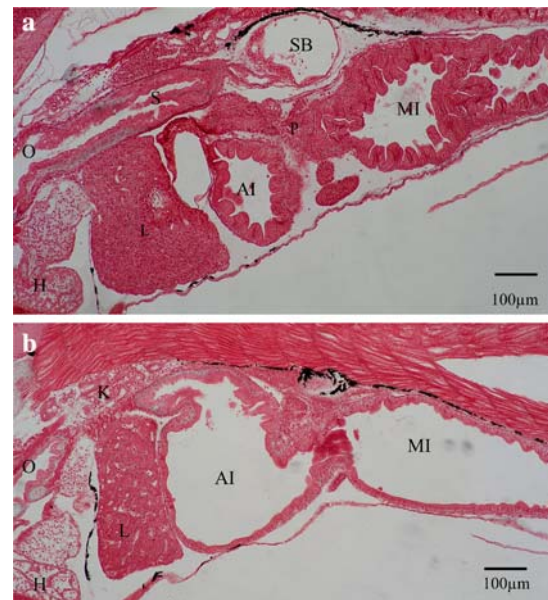

Fig. 4 Day 15. **a** Sagittal section of pikeperch larva fed Artemia (GX100). **b** Weaned on day 9 (GX100). *AI* anterior intestine, *H* heart, *L* liver, *MI* median intestine, *O* oesophagus, *P* pancreas, *S* stomach (here gastric area), *SB* swimbladder
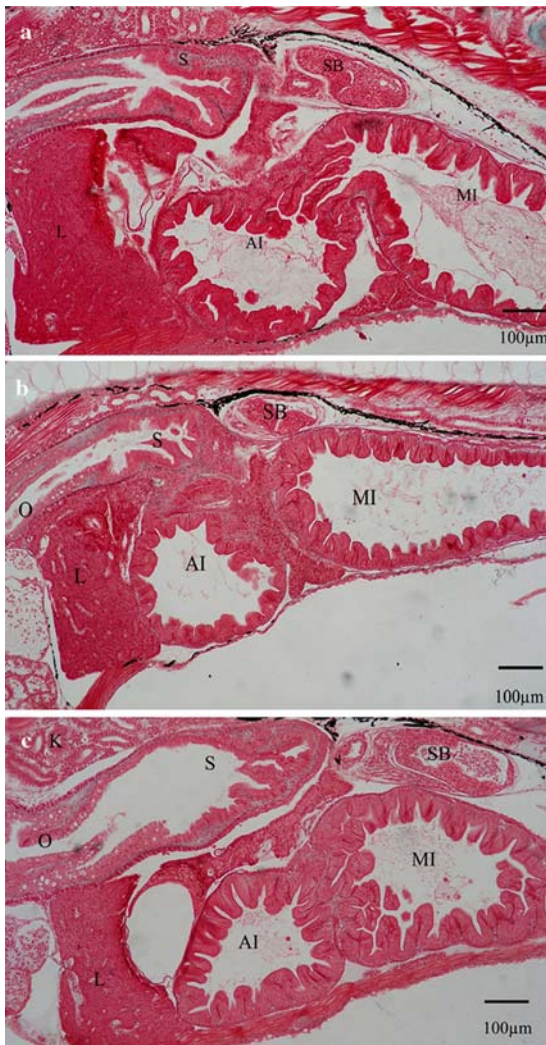
Fig. 5 Day 21. **a** Sagittal section of pikeperch larva fed live prey (GX100). Stomach is developed without gastric glands (GX100). **b** Weaned on day 9 (GX100) and **c** weaned on day 15 (GX100). *AI* anterior intestine, *K* kidney, *L* liver, *MI* median intestine, *O* oesophagus, *S* stomach, *SB* swimbladder

Gastric glands appeared only by day 29 (Fig. 6) for an individual weight of 20–30 mg. On the same day, three pyloric caeca were visible. At 36 days p.h., the stomach appeared similar to that of an adult fish and the pyloric caeca were present in all dissected fish. The stomach appeared better developed with more numerous gastric glands in fish exclusively fed with *Artemia* nauplii or weaned on day 21 than in fish weaned on day 15 (Fig. 7), but the stomach structure and size were largely dependent on the fish size.
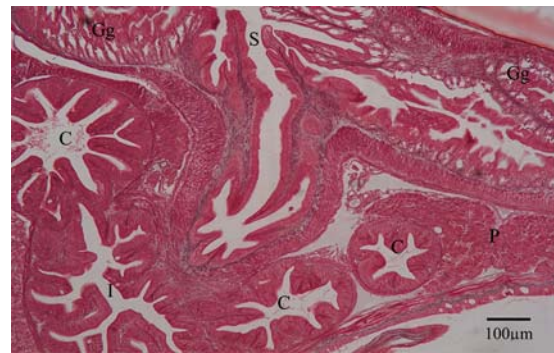

Fig. 6 Day 29. Sagittal section of pike perch larva fed live prey. Detail of the stomach (gastric glands) and pyloric caeca (GX100). *C* pyloric caeca, *Gg* gastric glands, *I* intestine, *P* pancreas, *S* stomach
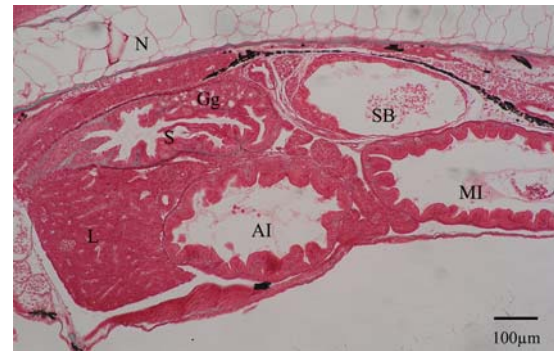

Fig. 7 Day 36. Sagittal section of pikeperch larva weaned on day 15. Note the less developed stomach and the much less numerous gastric glands compared with the control on day 29 (GX100). *AI* anterior intestine, *Gg* gastric glands, *L* liver, *MI* median intestine, *N* notochord, *S* stomach, *SB* swimbladder

Discussion

The growth of pikeperch larvae was similar in the control, W21, and W15 groups, but it was significantly affected by precocious weaning, particularly at day 9 (W9 group). Similar results were obtained for weaned larvae of European sea bass (Person-Le Ruyet et al. 1993; Cahu and Zambonino Infante 1994). In a recently published study, satisfactory growth was reported by Ostaszewska et al. (2005) when pikeperch larvae were fed exclusively on dry diets from mouth opening. These results could be

explained by more adequate dry diets and/or by rearing conditions. In our study, intra-treatment variability was reflected by the coefficient of variation, which reached more than 50% by day 36. Cannibalism, well-known in this species, was the main cause of this growth heterogeneity, as reported in many other species during the larval stage (Baras 1998).

Ontogeny of the digestive system

Few studies have been dedicated to the digestive system ontogeny of pikeperch larvae (Mani-Ponset et al. 1994; Ostaszewska 2002; Ostaszewska et al. 2005) using histological methods. In this study, we studied both structural and enzymatic development. The histological development of the digestive system observed in the control group (fed *Artemia*) of this study is comparable to the description of the previously cited studies. Digestive enzyme activities were detected in the pikeperch larvae since hatching as was observed in several other species like cod *Gadus morhua* (Hjelmeland et al. 1984) and herring *Clupea harengus* (Pedersen et al. 1990). At first feeding, histological study revealed the onset of all of the digestive structures of pikeperch larvae except the stomach. Liver and pancreas were functional and the intestine contained enterocytes with a well-developed brush border. It was also reported by Mani-Ponset et al. (1994), who considered that lipid absorption from initiation of exogenous feeding implies a capacity to digest food in pikeperch larvae. The enhancement of pancreatic (particularly Amy) and intestinal (Leu-ala and AN) enzymes at first feeding reflects the development of pancreatic exocrine function and the intestinal enzyme activities respectively. On day 9, we observed the yolk resorption and the convolution of the gut with fully developed intestinal enterocytes.

Between days 15 and 21, folds of intestinal mucosa developed concurrently with the increase in intestinal enzyme activities. This period was concomitant with a non-glandular stomach differentiation. The decrease in cytosolic enzyme (Leu-ala) activity concurrent with the increase in brush border enzyme activities (AN and AP) has been presented as a normal evolution reflecting

the maturation of intestinal enterocytes (Cahu and Zambonino Infante 1994). This indicated that brush border enzymes relayed cytosolic enzyme for digestion. In the larvae of the control, we observed the same evolution pattern even if we did not isolate the brush border membrane as indicated by these authors. The same evolution was also shown in Eurasian perch larvae (Cuvier-Péres and Kestemont 2002).

On day 29, we observed the appearance of gastric glands concurrent with pepsin secretion. On the same day, pyloric caeca were present. They allow enhancement of nutrient digestion and absorption, according to Hossain and Dutta (1998). For pikeperch, Ostaszewska (2002) reported the appearance of gastric glands and pyloric caeca on day 25 p.h.

According to our results, pikeperch larvae acquired an adult mode of digestion around day 29. Indeed, the development of the stomach and functionality of the gastric glands with pepsin secretion indicated the end of the larval stage (Kolkovski 2001).

Weaning effect on digestive capacities

Previously, several studies used enzymatic criteria (Lauff and Hofer 1984; Hjelmeland et al. 1984; Cahu and Zambonino Infante 1994; Cuvier-Péres and Kestemont 2002) or histological methods (Deplano et al. 1991; Segner et al. 1993; Rodriguez Souza et al. 1996; Ostaszewska et al. 2005) to study the effects of different diets on the digestive structures of the larvae. Among them, few studies related these two approaches (Kestemont et al. 1996) to correlate information about larval digestive capacities.

In the present work, higher tryptic activities were observed at days 21 and 29 in the larvae fed live prey compared with the weaned larvae. This has also been observed in European sea bass larvae by Nolting et al. (1999), who attributed it to the fact that live prey stimulates the enzymatic secretion in the larvae more. In our results, this difference cannot be strictly explained by diet. In fact, on day 21 larvae of the control group and W21 group were both fed on *Artemia* nauplii. Moreover, the variability of Try activity did not allow any clear conclusions.

Amylase activity reached a peak at first feeding and then sharply decreased. It did not vary among treatments except at day 29 for the W9 group. In European sea bass, amylase activity of weaned larvae was significantly higher than in larvae fed *Artemia* (Zambonino Infante and Cahu 1994). According to these authors, this may be due to the adaptation of the larvae to the level of starch in the food. Such a statement cannot be made in our study, since Amy activity modification appeared to be a long time (15–20 days) after weaning. Our results showed that the evolution of enzymatic activities was more determined by the larval age or development stage than by the dietary treatment, as shown by Zambonino Infante and Cahu (2001).

The effect of weaning was more evident on the intestinal enzymatic activities. From first feeding, Leu-ala activity decreased whatever the treatment, but remained significantly higher for W9 larvae (day 29). This may reflect an impairment and/or delay in maturation process of intestinal enterocytes. Indeed, according to Cahu and Zambonino Infante (2001) artificial feed can delay the maturation process and inadequate food can even prevent it, leading to the death of larvae. The higher activity of AP after weaning could be explained by the high phosphate level of dry diet compared with live prey (Watanabe et al. 1983) or by the fact that larvae have to secrete more enzymes due to the low digestibility of food. Cahu and Zambonino Infante (1994) observed a similar effect of the weaning (days 10 and 15) on the specific AP activity in sea bass larvae. The immediate increase in AP activity after weaning may reveal a perturbation in the secretion process and/ or be a sign of malnutrition. Furthermore, the increase in AN between day 15 and day 29 in all groups except for W9 larvae might also reflect some perturbation or delay in the maturation of the intestine in this last group. Segner et al. (1989) also observed higher activity of aminopeptidase in the gut of whitefish larvae *Coregonus lavaretus* fed zooplankton than in the gut of larvae fed on dry diets. These results were confirmed by Leu-ala/AN and Leu-ala/AP ratios, which remained significantly higher for W9 larvae compared with the other groups by day 29. It is therefore expected that the intestinal enzymes were not produced at a

sufficient level to relay efficiently the cytosolic enzyme to ensure good digestion.

At the end of the experiment, no differences in intestinal proteolytic activities were observed among larvae from different treatments. It is probably due to the fact that digestive structures were properly developed and that larvae had acquired an adult mode of digestion at that stage.

Weaning effect on histogenesis

The histological study clearly showed the effect of dry diet on the intestinal epithelium of the larvae precociously weaned, especially the W9 group. Indeed, at day 15, number and height of the enterocytes were strongly reduced and the epithelium appeared atrophied compared with the control larvae. It can be a mechanical effect of the artificial diet, which erodes the intestinal epithelium. Ostaszewska (2005) observed the same effects in pikeperch larvae fed prepared diet containing casein or casein hydrolysate, not with the commercial diets. On this basis, the dry diet used in our study may not be convenient for pikeperch larvae at this stage. In the same way, Deplano et al. (1991) examined the disappearance of the intestinal folds in 18- to 19-day-old sea bass larvae fed artificial diet. The height of the enterocytes, particularly in the midgut, was considered to be one of the nutritional indices (Segner et al. 1993).

Gastric glands were less numerous and more poorly developed in W9 and W15 larvae compared with larvae fed *Artemia*. This was related to the growth of the larvae, as well as with pepsin secretion. For the W15 group, the effect of the weaning was not noticeable on epithelium development.

On days 21 and 29, the effects of weaning on the intestinal epithelium of the larvae appeared less clearly according to histological observations. Nevertheless, enzymatic analysis during the same period revealed perturbations of the intestinal enzyme activities. For the larvae weaned on day 21, we did not observe any differences from the control in terms of both enzymatic activities and histogenesis. On days 29 and 36, digestive structures of W21 larvae were similar to those of the control group.

Some authors associated the adequate timing of weaning with stomach differentiation and pepsin secretion (Walford and Lam 1993; Person-Le Ruyet et al. 1993; Segner et al. 1993), which enhanced considerably the digestion of artificial feed. However, the results of Cahu et al. (2003) showed that sea bass larvae can be weaned from day 9, although their stomach did not develop until around day 25. Similar results were observed in this study with relatively successful weaning even in the W15 group while the stomach was not completely developed. More precocious weaning (day 9) provoked malnutrition effects, a delay in the maturation of digestive structures, and perturbation of enzymatic secretion processes.

We can assume that weaning at day 9 could be feasible with a more convenient diet. Curnow et al. (2006) compared the effect of two commercial microdiets on the growth of *Lates calcarifer* larvae. These authors attributed the lower growth performance of the larvae fed with Proton (compared with Gemma microfed larvae) to a lower lipid and free amino acid content in this diet. Indeed, Nyina-Wamwiza et al. (2005) and Molnar et al. (2006) suggested 10–16 g kg^{-1} and 18 g kg^{-1} lipid content respectively in the diet for pikeperch fingerlings. We know that fish larvae generally need a higher lipid level in the diet than juveniles, so we can suppose that this diet containing 15 g kg^{-1} lipids was not adequate for their lipid requirement.

In conclusion, based on the artificial diet used in this study, pikeperch larvae can be weaned from day 15 (around 3 mg) p.h. without any significant adverse effect on digestive capacities (except for AP activity) or digestive tract development. Earlier weaning impaired the onset of maturation processes of the digestive system, both in terms of morphological structures and enzymatic activities. The effect of diet and precocious weaning were notable on the development of digestive structures as well as on the enzymatic capacities. The enzymatic approach gave supplementary information on enzymatic secretion processes and appeared a useful indicator of the nutritional status of the larvae and their digestive capacity.

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