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Early life stages of Aquitanian pike *Esox aquitanicus* (Teleostei, Esocidae): first description of morphological development and trials of rearing

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Abstract – Aquitanian pike, *Esox aquitanicus*, is a new esocid species described in 2014 and endemic to Southwestern France. The four main goals of the present study were to (i) obtain the first morphological data on the early life stages, (ii) test the effects of temperature on their survival and development, (iii) compare the results with the Northern pike, and (iv) produce a few individuals to exhibit them at the Aquarium of Limoges. Eggs are yellowish, demersal, and sticky. The mean diameter was 2.73 ± 0.08 mm (n = 174). The larval size at hatching was 9.09 ± 0.24 mm (n = 14). The morphological development of 15 larvae was followed during 11 weeks as well as the snout growth in relation to eye size. It appeared that already after three weeks, slight differences between the two species were apparent, and after nine weeks, this snout/eye ratio was twice greater for Northern pike than Aquitanian pike. At the end of the yolk-feeding period, which lasted from 12 days at 18 °C to 36 days at 6 °C, the overall survival rate was over 85% and larvae were of similar size, except at 6 °C. After three months, the best results in term of survival were obtained at 12 °C (60%) and for growth at 15 °C (34.11 mm), followed at 12 °C (27.85 mm). Based on these results, we were able to rear Aquitanian pike for more than 2 years in the Aquarium of Limoges in order to promote the conservation of the species to the public.

Keywords: *Esox aquitanicus* / egg / larvae / temperature / endemic species

1 Introduction

Up to the early 2000s, it was supposed that the metropolitan French territory hosts about 80 freshwater and diadromous fish species, among which one-third had been introduced in the last century (Keith and Allardi, 1997). Yet, this number increased strongly in the recent years to reach more than 120 species, partly due to the introduction of new fish, such as gobies, but mostly due to the revision of the French fish species fauna based on integrative taxonomy (Keith *et al.*, 2020; Teletchea, 2020; Denys *et al.*, 2024). Hence, nine genera have been revisited so far, with the addition of 26 new species, most of them being endemic to mainland France (Denys *et al.*, 2024).

Among those revised genera are pikes *Esox*. For at least two centuries, the taxonomy of the Esocidae was considered well-known, including seven species occurring throughout North America and Eurasia (Raat, 1988; Crossman, 1996). Among these seven species, Northern pike *E. lucius* Linnaeus

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1758 is the most common esocid worldwide, with a circumpolar distribution covering both North America and Eurasia (Raat, 1988; Denys *et al.*, 2014). Besides, due to its high socio-economic interest for recreational and commercial fishing as well as aquaculture (Raat, 1988; Arlinghaus *et al.*, 2018), this species is one of the best studied fish species, particularly concerning its reproduction and early life stages (Teletchea *et al.*, 2007; Pospisilova *et al.*, 2019; Réalis-Doyelle *et al.*, 2022). This species is also regularly stocked in numerous water bodies including those where it is not native (*e.g.*, Cucherousset *et al.*, 2021). In France, Northern pike was considered to be the only esocid present, native to the Rhine, Seine, Loire (except in Brittany), and Rhône drainages (Denys *et al.*, 2014; Keith *et al.*, 2020). Yet, a taxonomic revision has allowed the discrimination of another pike species, the Aquitanian pike *E. aquitanicus* Denys, Dettai, Persat, Hautecoeur & Keith, 2014, an endemic species of Southwestern France from the Charente to the Adour drainages (Denys *et al.*, 2014). However, the ubiquist species *E. lucius* was also introduced in these catchments at least since the 50s with the development of aquaculture (Keith *et al.*, 2020).

The two French pike species differ according to morphological, mitochondrial and nuclear data (Denys *et al.*, 2014; Denys *et al.*, 2018; Jeanroy and Denys, 2019). Concerning morphology, the most obvious differences are that the Aquitanian pike possesses a marbled coat and a shorter snout than Northern pike (Denys *et al.*, 2014). Yet, even though it is supposed that Aquitanian pike should have similar biological traits than Northern pike, almost nothing is known about the biology of this new pike species, especially concerning its early life stages (Denys *et al.*, 2014). The breeding season is supposed to begin in February according to Chimits (1956), during which Aquitanian pike comes to spawn in the channels of marshy areas at the source of streams or in lagoons (Glize, 1993).

E. aquitanicus is currently listed as Vulnerable according to the French IUCN Red List of Threatened Species, particularly because of the degradation of its habitat – especially its breeding areas – and the introduction of *E. lucius* (Keith *et al.*, 2020). It is therefore important to better known the species biology and ecology to establish effective conservation and management policies. In this context, the four main goals of the present study were to (i) obtain the first morphological data on the early life stages of Aquitanian pike, and particularly the development of the snout length, (ii) test the effects of temperature on their survival and development, (iii) compare the results with the Northern pike, and (iii) produce a few individuals to exhibit them at the Aquarium of Limoges in order to promote the conservation of this species to the public.

2 Material and methods

2.1 Sampling of wild eggs

There is currently no commercial farming of Aquitanian pike, and no precise information were available about how and when to perform the artificial reproduction of this species. Therefore, we decided to sample eggs *in situ* in two sites located in the Landes department (Southwestern France), where it was demonstrated that only this species was present; thus, no risk of hybridization with common pike (Fig. 1). The first site is the marsh of Uza (Latitude: 44.025567, Longitude: −1.186314), a hunting reserve, located in a peat bog where the Vignac brook flows upstream of the pond, with water bodies on either side of the main stream that are connected to it during flood periods. The second site is the Plaine aux Pigeons in the township area of Lit-et-Mixe (Latitude: 44.052100, Longitude: −1.266470), a marshland managed by the Landes Hunting Federation and bordered by a brook called "Courant mort", which feeds tons of water for waterfowl hunting. Both sites are located within the Courant de Contis watershed (Fig. 1).

As pikes are phytophil, the harvesting method consisted of scraping the base of aquatic grass-like plants located on the inshore of the pond towards the water surface, using a landing or a kick net. Sampling was done three successive years: the first one from the 3rd to the 19th February 2021 ten times (from 9:00 a.m. until 4:30 p.m.), the second one from the 10th to the 13th of January 2022 as well as from the 31st January to the 1st February 2022, and the last one the 2nd February 2023. Once collected, the eggs were held and oxygenated in a bottle with water from the site until being sent by post to the University of Lorraine in Nancy (2021, 2022) and Aquarium of Limoges (2023), with an expected delivery time of less than 24 h. Upon their arrival, the water temperature was set at 12 °C, close to that observed at the sampling sites, and remained at this value until the end of the incubation period. Yet because we could not determine precisely when spawning had occurred and therefore how old the embryos were, we only start our experiments at hatching.

2.2 Morphological description of the early life stages

As eggs are not strictly spherical (Teletchea *et al.*, 2007), the diameter was calculated from the mean of two perpendicular diameters. Eggs were also observed to identify the stage of embryogenesis following Pospisilova *et al.*, 2019 to approximate the time of fecundation and hatching. At hatching, and then once a week, five larvae individually reared in a petri dish to avoid potential cannibalism frequently observed in Northern pike (Kucska *et al.*, 2007), were randomly sampled and photographed to follow the morphological development, and calculate the relative length of the snout (SnL) compared to the eye diameter (EyD) (Fig. 2).

2.3 Experimental conditions for testing the effect of temperature on the survival and development of early life stages

Eggs or recently hatched larvae were evenly distributed in three trays in each of the five incubators located at the Université of Lorraine in Nancy to test the effect of temperature (6, 9, 12, 15 and 18 °C), first on the endogenous feeding period of larvae (*i.e.*, from hatching up to the full resorption of the yolk sac) and then during 11 weeks post-hatching; for more information about the experimental design see Réalis-Doyelle *et al.* (2016, 2018, 2022). The temperature was applied as soon as all larvae hatched. The temperature was measured daily. Given the lack of any

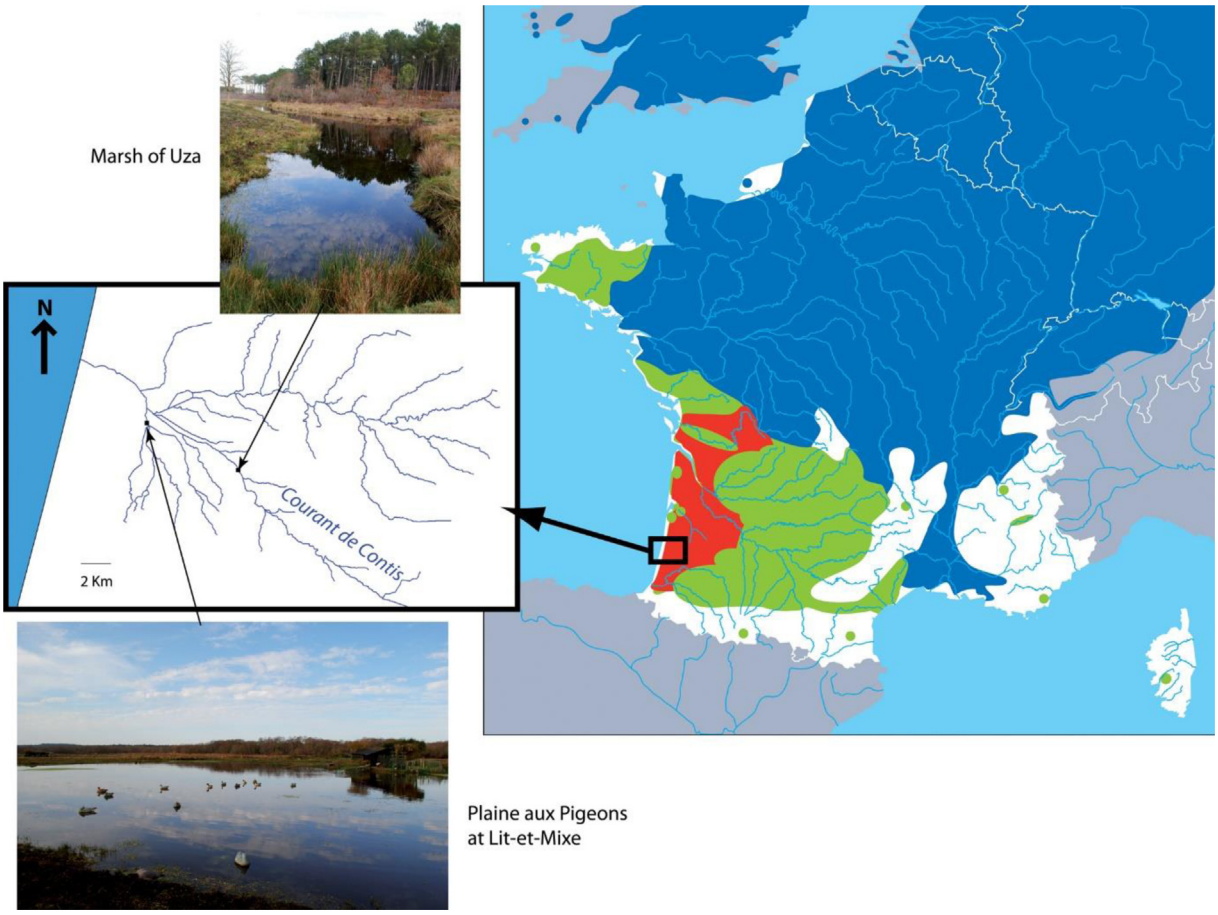
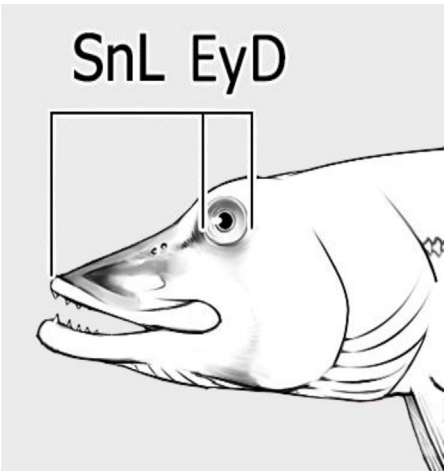


Fig. 1. Distribution area of the two pike species in France (*Esox aquitanicus* in red, *Esox lucius* in its native area in blue and introduced in green) (modified and adapted from Keith *et al.*, 2020; Royer *et al.*, 2024), with the sampling locations of Aquitanian pikes breeding areas in the Courant de Contis watershed (Landes department; southwestern France): the Plaine aux Pigeons at Lit-et-Mixe and the marsh of Uza; credits photos: G. Denys.

reference for the photoperiod and especially light intensity for this species, it was decided to leave the eggs, then the larvae, in the dark to limit stress. However, this decision was re-evaluated during the week 7, when the room's general lighting was used with a photoperiod of 8L/16D, *i.e.*, from 9 a.m. to 5 p.m. Once the endogenous feeding period was over, it was decided to feed pike. According to Kucska *et al.* (2007), pike larvae are capable of feeding on a floating pellet, whether fed continuously or twice a day, while Hubenova *et al.* (2010) recommended a feeding frequency of four times a day. Hubenova *et al.* (2010) and Pasquet *et al.* (2016) fed larvae first with live brine shrimp *Artemia salina* (Linnaeus, 1758) nauplii, followed by inert feed, with a transition period between feed types (co-feeding). Therefore, we decided to feed larvae four times a day, first with live *Artemia* and then pellets. Because of the low number of larvae obtained, three larvae were randomly anesthetized, sampled and measured per tray at both hatching and when the yolk sac was fully consumed, and then each week post-hatching. After being photographed, larvae were returned to their respective trays. Measurements of nitrite and ammonium concentrations (in mg/L) began on March 19 (T4), one to three times a week (Monday, Wednesday and/or Friday), from April 7 (T7) to May 5 (T11).



2. Snout length (SnL) versus horizontal eye diameter (EyD). Drawing by Guillaume Rech.

2.4 Observations and measurements

Total length (*TL*) and observations for both the individual rearing and the experiment on the effect of

temperatures were realized with a microscope (see Réalis-Doyelle *et al.*, 2022). Besides, for the individual rearing, the larvae were photographed individually each week, using a binocular magnifying glass, to measure the development of the snout. Measurements were taken using GIMP software. The survival rate (*SR*) was calculated as follows: $SR\ (\%) = n_f$ x 100 / n_i, where n_i and n_f are the initial and final number of larvae, respectively, measured both at the end of the endogenous feeding period and each week. The duration of the endogenous feeding period, corresponding to the duration between hatching and when all yolk sac is consumed, was calculated per temperature. Daily growth was calculated by considering the duration (d), and *TL* using the following formula: $DG\ (\text{mm / d}) = TL_f - TL_i$ / duration of the feeding period, with *TL* in mm and duration in days, *i* is hatching and *f* the end of endogenous feeding period. Then, larvae were measured each week.

2.5 Statistical analysis

Statistical analyses were performed using *R* (R Core Team, 2018). In this experiment, there is one quantitative dependent variable (total length or survival rate) for one quantitative independent variable (temperature, which has five modalities). We checked normality with the Shapiro Wilk test and variance homogeneity with a Levene test (R-package "car"; Fox and Weisberg (2019)), before choosing whether we could perform an ANOVA or a non-parametric Kruskall-Wallis test. Finally, the data were compared using a Tukey post hoc test.

2.6 Exhibiting the Aquitanian pike at the Aquarium of Limoges

Eggs sampled at the Plaine aux Pigeons in the township area of Lit-et-Mixe the 2nd February 2023 were sent to the aquarium by an express carrier. On their arrival, eggs were evenly distributed into six trays located in a specific aquarium (100 × 40 cm) with 20 cm of water depth. Based on our results of the first year of the project, the photoperiod was set as follows: 11L/13D; light was from 8 a.m. until 7 p.m. Larvae were fed, at least five times a day between 9:30 a.m. until 6:30 p.m., first *Artemi*a enriched with Selco (Ocean nutrition) and then pellets and various food items were gradually added.

3 Results

3.1 Egg samplings and their use

A total of 392 eggs were collected in shallow waters among sharp-flowered rush Juncus acutiflorus Ehrh. ex Hoffm. (1791), common rush J. effusus (Linnæus., 1753) and bog pondweed *Potamogeton polygonifolius* (Pourr., 1788) at a temperature of about 12 °C (see also Denys *et al.*, 2023), and sent by post in three batches. The first batch, comprising around 50 eggs, was collected from February 3rd to 4th 2021 and sent the last day, and arrived on the 6th in Nancy. The larvae hatched on the 7th and were only used to test an anesthetizing method (data not shown). The rare cases of cannibalism were observed on March 25, 46 days after hatching for this batch only (Fig. S1). The second batch contained 233 eggs, collected from February 4th to 8th 2021, before being shipped on the 8th and received at the laboratory the following day. They hatched from February 16th to 20th. Most larvae were evenly distributed into five incubators for the experiment on temperature, while 15 were put alone in modified petri dishes within a tray for the individual monitoring at 12 °C. The 2nd February 2023, about 100 eggs were collected in the bog pondweed and sent to the Aquarium of Limoges.

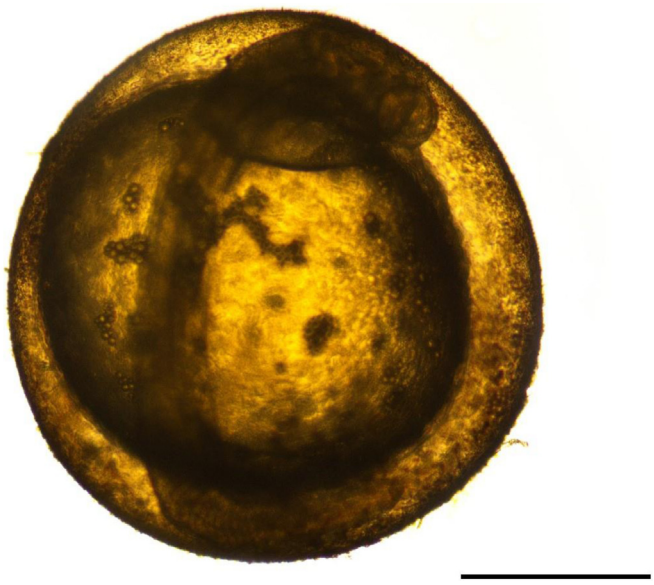


Fig. 3. Egg of Aquitanian pike (from batch 1, photographs taken on February 6th, 2021). Egg diameter = 2.83 mm. Scale = 1 mm.

3.2 Morphological description of the early life stages

Eggs are yellowish, demersal, and sticky (Fig. 3); some particles were often stuck on them. For the first batch collected in February, 2021 the mean egg diameter was 2.81 ± 0.04 mm (n = 25). Among the 25 eggs observed on February 6th, heartbeats were visible for only one, while muscle contractions were observed for three embryos. The diameter of the second batch measured on February 15th 2021, was 2.71 ± 0.08 mm (n = 134). Some embryos were at the pre-hatching stage according to the Northern pike development table (Pospisilova *et al.*, 2019). Finally, on February 18, 2021, the eggs of the second batch used for the individual monitoring had a diameter of 2.72 ± 0.06 mm (n = 15). In conclusion, all eggs combined measured 2.73 ± 0.08 mm (n = 174).

Larvae at hatching are characterized by a curved, transparent body with a light-brown coloration (Figs. 4, and 5). The curved trunk rests on a voluminous, ovoid yolk reserve. The head, bearing large eyes, is bent downwards at the anterior part of the yolk. With the exception of the pectoral fins, the other fins are not yet differentiated from the protopterygia. Melanophores are found on the dorsal side (and the upper part of the yolk bladder) but not on the ventral part, while pigments accumulate along the lateral line. The larval size at hatching, measured on February 18th, 2021 was 9.09 ± 0.24 mm (n = 14).

The morphological development of 15 larvae, reared individually at 12 °C, was followed during 11 weeks (Tab. 1,

Fig. 4. Larvae at hatching of the Aquitanian pike larvae (batch 2, from February 19, 2021). TL = 9.24 mm. Scale = 1 mm.

Fig. 5). Besides, we also calculated the snout growth in relation to eye size on the same 15 larvae. The snout was about half the size of the eye 15 days after hatching, and became larger than the eye at T9, just over two months after hatching (Fig. 6).

3.3 Effects of five temperatures on the survival and development of the early life stages

Overall, 114 larvae were tested, ranging from 19 to 25 per temperature and between 3 and 10 per tray (Tab. 2). The overall survival rate was over 85% during the entire larval endogenous feeding period for all tested temperatures, with a maximum of 96% at 12 °C (Fig. 7A). No significant difference was found. Except at 6 °C, the larvae show a similar size at the end of the endogenous period (Fig. 7B). The endogenous feeding period lasted from 12 days at 18 °C to 36 days at 6 °C (Fig. 7C). Thus, daily growth increased fourfold between 6 and 18 °C (Fig. 7D).

During the first week (T1), most larvae were immobile, remaining either at the bottom of the tray or attached to the walls of the trays. This behaviour changed during the second week (T2) when the yolk sac resorbed and the larvae began to swim. However, larvae at 6 and 9 °C remained inactive throughout the experiment (T11). They swam only when a current was generated by sucking up and expelling water with a dropper pipette. Even the presence of food (nauplii or pellets) did not seem to make them reacted. After 11 weeks, the best survival rate was obtained at 12 °C with 61% (Fig. 8). For the other temperatures tested, values at 6, 9 and 15 °C were below 5%. At 18 °C, all larvae were dead, the last one died during week T6 (Fig. 8).

As for the end of the endogenous period, three larvae were randomly taken and photographed, for each of the 3 trays in the 5 incubators, bringing the total number of samples per week to 45. The total larval length at 12 °C had doubled in 8 weeks (Fig. 9). At the same time, the size of larvae at 15 °C tripled in 10 weeks, while at 6 and 9 °C, size changed little over the course of the experiment.

3.4 Rearing Aquitanian pike at the aquarium of Limoges

Based on the results obtained in 2021, we decided to feed the larvae daily from 9.30 a.m. to 6.30 p.m., with *Artemia* nauplii only, for over a month from February 16 to March 19, 2023, with an average of 7.4 meals between March 4 to 19. Pellets were then provided alternately with *Artemia* from March 20 to May 27, at a rate of almost 9 meals a day. For 3

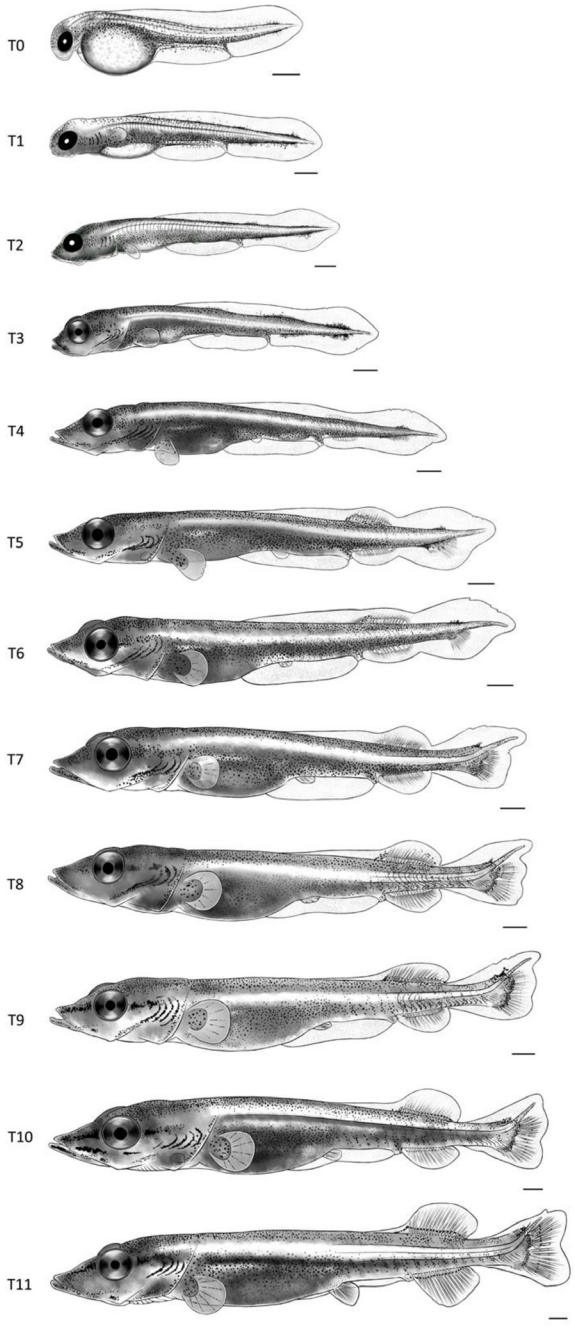


Fig. 5. Morphological development of the Aquitanian pike during the first 11 weeks post-hatching (n = 15 larvae; reared individually in a petri dish) at 12 °C, see Table 1. Drawing by Guillaume Rech. Scale = 1 mm.

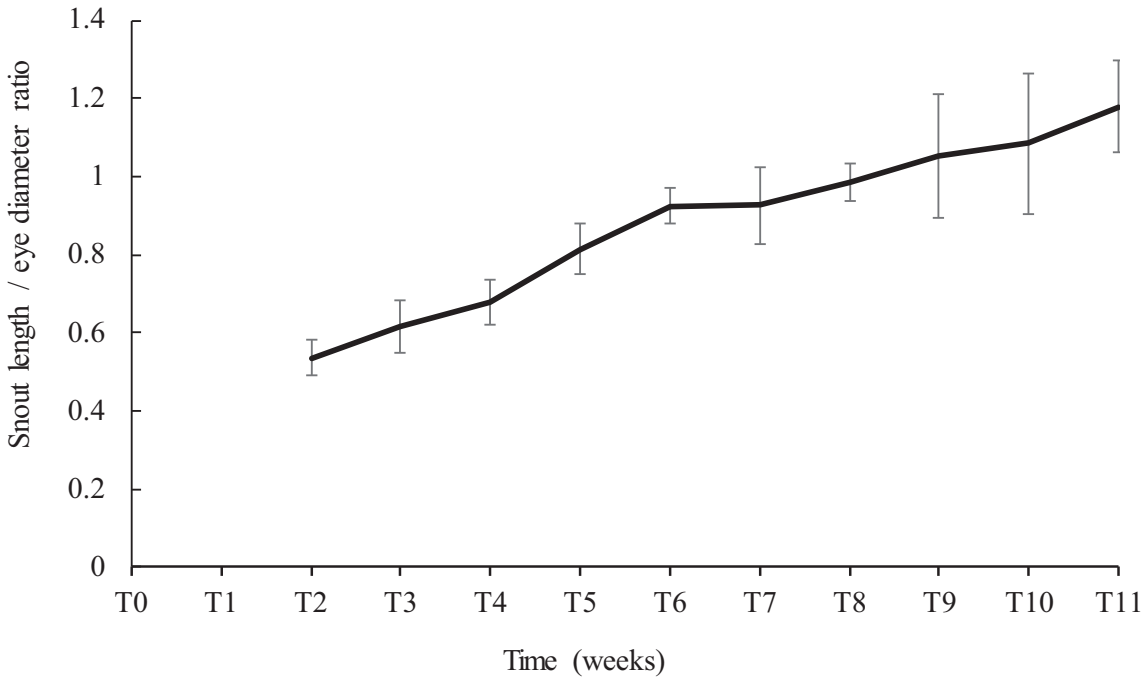


Fig. 6. Evolution of the ratio of snout length compared to the eye diameter during 11 weeks at 12 °C (mean ± SD).

days (03/31, 04/1, 04/2), feeding pellets only was tested, but the results were inconclusive (see Denys *et al.*, 2023). Therefore, it was decided to maintain co-feeding throughout the rearing period, which showed that it was very difficult to feed wild animals with pellets, and so the production of live prey has proved necessary to date. The trial lasted about 3.5 months, from February 16th to May 27th 2023. Larvae died during the first three weeks; no larvae died after April 24th. It should be noted that no cases of cannibalism were observed, probably due to the low density of pike per tray. Overall, 40% of the larvae (26 out of 63) survived (see Denys *et al.*, 2023). Almost 2 years after, two fish are still present in the Aquarium.

4 Discussion

This study represents the first attempt to obtain wild eggs of Aquitanian pike directly from the field and rear them in controlled conditions. Despite the relatively low number of eggs sampled (ca. 400), we were nevertheless able to get new morphological data on both egg and larvae (Tab. 3) and to test the effects of five temperatures on the survival and growth for the first 3 months of life of this species.

Because there are neither Aquitanian pike farm nor data on the reproductive season of this species available, we had to rely on the information published on Northern pike to determine our sampling field period. According to most studies, Northern pike spawns from February to May depending on latitude (Teletchea and Teletchea, 2020). In France, Northern pike spawns mostly from February in the South to April in the North (Keith *et al.*, 2020; Teletchea, 2020). Yet, according to our observation in the field, the first two weeks of February might be the end of the breeding period for the Aquitanian pike. Moreover, spawners in these locations were relatively small: 20–30 cm total length so with weights around 70–100 g (40 cm TL and 680 g for the longest female observed à Lit-et-Mixe in 2021) (Royer *et al.*, 2024). So, if we estimate that the fecundity of the Aquitanian pike is the same as Northern pike (30,000 eggs/kg; Souchon, 1983), a single female isolated in a pond of both locations may do multiple spawning laying a maximum of 3 000 eggs during the breeding period. All these may explain why we were only able to collect so few eggs. However, more investigations are necessary with a specific survey in order to better characterize the breeding period of the Aquitanian pike which may be earlier than Northern pike.

Aquitanian and Northern pike possess quite similar egg and larvae (Tab. 3). The eggs are demersal, adhesive and large (Fig. 3). The egg diameter obtained here are similar to the values (2.5 to 2.9 mm) described for Northern pike females ranging from 0.3 to 4.4 kg (Bonislawska *et al.*, 2000). Similarly, Aquitanian pike larvae resemble Northern pike larvae at hatching (see drawings in Dorier, 1938; Pospisilova *et al.*, 2019). At hatching, larvae of Aquitanian pike measured 9.09 ± 0.24 mm (n = 14) at 12 °C, which is comprised in the range of sizes described between early hatched larvae (8.17 mm) and late hatched larvae (9.50 mm) coming from Northern pike eggs incubated as the same temperature (Trabelsi *et al.*, 2013). The development (Tab. 1, Fig. 5) is similar to the one described for Northern pike by Dorier (1938) and for the first four weeks by Pospisilova et (al., 2019).

First feeding Northern pike larvae are usually feed either brine shrimp (Hokanson *et al.*, 1973; Hubenova *et al.*, 2010) or sieved zooplankton collected in ponds (Dorier, 1938; Salonen and Engström-Öst, 2013) as artificial diet is unsuitable for them (Wurtz, 1944; Hubenova *et al.*, 2010; Imentai *et al.*, 2024). Yet, fish farmers are interested in introducing inert items as early as possible to avoid producing live preys, which is particularly time-consuming and costly (Imentai *et al.*, 2024). Here, we also

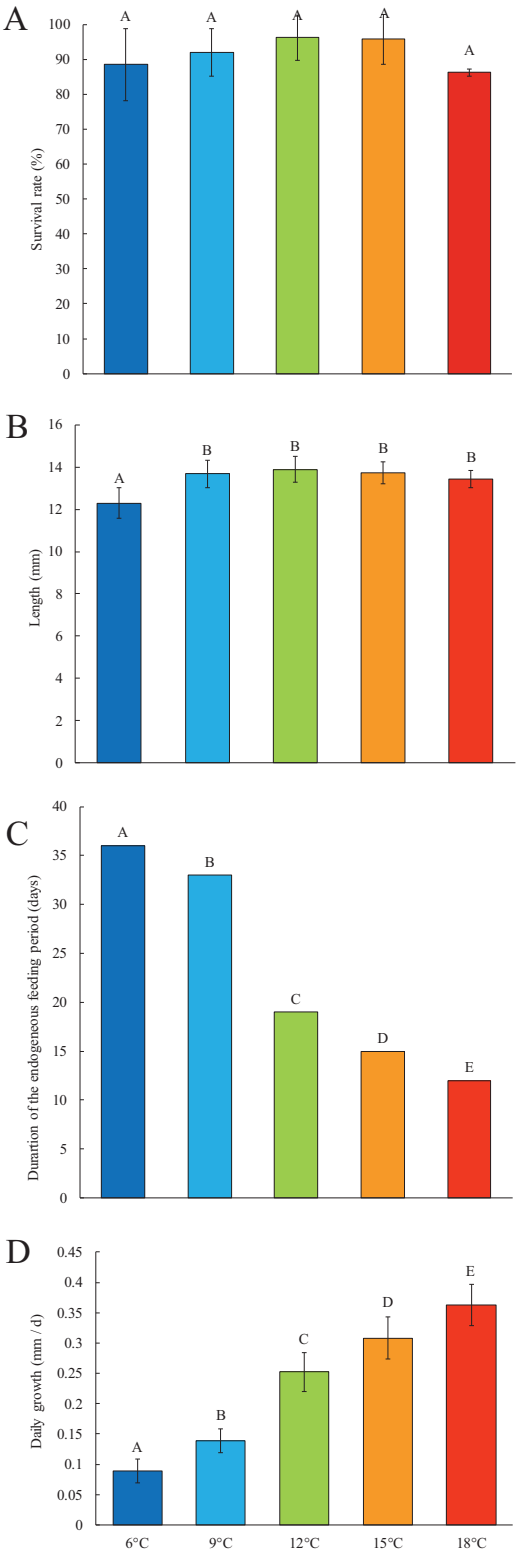


Fig. 7. Effect of temperature on the survival rate (A), larval size (B), duration (C) and daily growth (D) during the endogenous feeding period, i.e, from hatching until the full yolk sac resorption (T0 to T3 at 12 °C) (mean ± SD).

found that Aquitanian pike were reluctant to eat inert food and fed almost exclusively on live preys during the first weeks of rearing (Figs. 6, 8 and 9). This result might also be partly due to the fact that we chose to rear the larvae in the dark during the first 7 weeks to limit stress as much as possible. Indeed, it has been shown that Northern pike larvae could feed on living active prey, even in the dark, whereas inert food was taken only in the light (Billard, 1996). Imentai *et al.* (2024) found that pike larvae and early juveniles grew better when fed life prey than dry diet, and that growth and survival increased with increasing light period; all larvae reared in complete darkness died during their experiment. This might also explain why the growth obtained here at 12 °C (Fig. 9) was slower during the first weeks compared to the results obtained for Northern pike (data not shown, see Denys *et al.*, 2023). In conclusion, Aquitanian larvae should first be fed live preys for at least one week (but probably more) before starting co-feeding with inert food with at least 16 h of light (Imentai *et al.*, 2024). At last, we observed only one case of cannibalism, which is probably due to the low density used here (Imentai *et al.*, 2024). To limit cannibalism, Imentai *et al.* (2024) recommended a stocking density of 20 ind L^{-1} for Northern pike between 14- and 27-days post-hatching at a temperature of 20 ± 0.5 °C.

To our best knowledge, there are no snout/eye ratio values published at the larval stage comparable to those determined in juveniles and adults (Fig. 2 in Denys *et al.*, 2014). Nevertheless, a comparison with other references was made (Dorier, 1938; Wurtz, 1944); Raat, 1988; Lancioni *et al.*, 2005; Pospisilova *et al.*, 2019), based on photos or scientific drawings, to calculate this ratio in Northern pike (data not shown, see Denys *et al.*, 2023). Even though this preliminary comparison should be taken cautiously because data were obtained in different rearing conditions, it seems that already after three weeks, slight difference between the two species are apparent, and at T9, this ratio is twice greater for Northern pike than Aquitanian pike (Dorier, 1938; Lancioni *et al.*, 2005). According to Shamardina (1957) who did the ratio between the snout length and the total length among the Northern pike, this ratio stabilizes at the T3 stage. Thus, if our result is confirmed, this could imply that these two species could be identified morphologically based on this ratio after only a few weeks post-hatching.

It is well-known that temperature strongly impacts the survival and development of early life stages of fish (Réalis-Doyelle *et al.*, 2016, 2018, 2022). For Northern pike, several studies have tested the effects of temperature from fertilization up to hatching, known as the incubation period, and concluded that the optimal range for survival and development of embryo is probably comprised between 10 and 14 °C (Teletchea and Teletchea, 2020; Réalis-Doyelle *et al.*, 2022), and explain why we selected 12 °C here for incubating the Aquitanian pike eggs; this was also the temperature measured on the field in 2021. As for incubation, increasing temperature strongly decreased the period from hatching until the full resorption of the yolk-sac (Fig. 7C). Besides, the survival rate was over 85% (Fig. 7A), which would confirm that Aquitanian pike larvae are as tolerant as Northern pike (Réalis-Doyelle *et al.*, 2022). At last, larvae at the end of the yolk feeding period were similar (about 13.5 mm), except at 6 °C, where they were significantly smaller

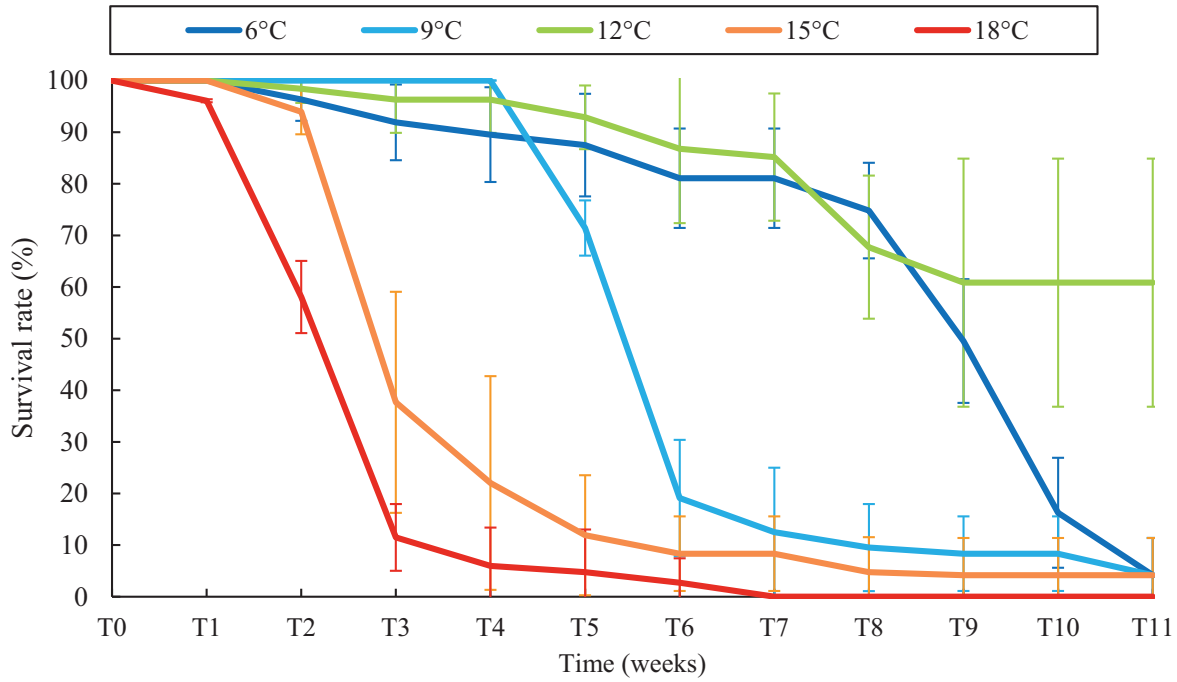


Fig. 8. Effect of temperature on the survival rate of Aquitanian pike during the first 11 weeks of life (mean ± SD).

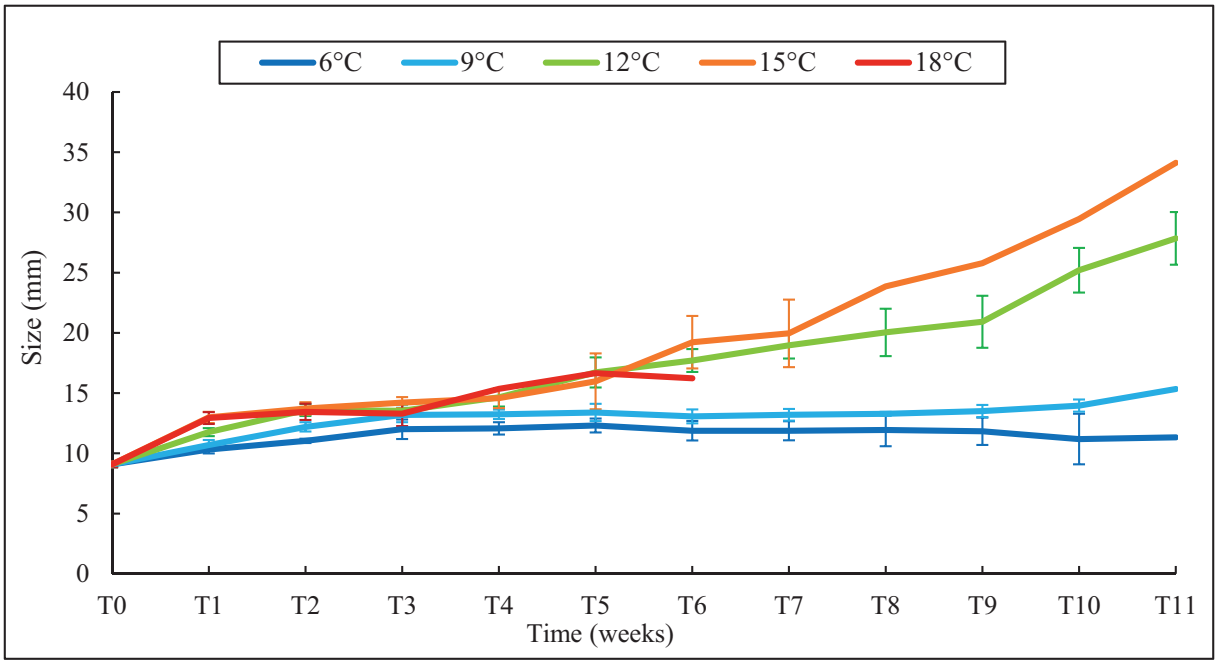


Fig. 9. Effect of temperature on the growth of Aquitanian pike during the first 11 weeks of life (mean ± SD).

(12.3 mm) (Fig. 7B), which are comparable to the length (13–13.5 mm) obtained for Northern pike (Réalis-Doyelle *et al.*, 2022). Then, we applied the same five temperatures up to three months after hatching to evaluate the possible effects for such a long period, which has never been tested on Northern pike, *i.e.*, only up to four weeks (Hokanson *et al.*, 1973). After 28 days post-hatching (T4), Aquitania pike were much smaller than Northern pike for the three tested temperatures in common between the two studies: 14 *vs* 25 mm (at 12 °C), 14 *vs* 34 mm (at 15 °C) and 15 *vs* 42 mm (at 18 °C) (Hokanson *et al.*, 1973). This slower growth might be due to the fact that we reared larvae in total obscurity up to T7 and that we had to anesthetize them regularly (ca. once every two weeks) to follow growth. Besides, for Northern pike, larvae longer than 30 mm were fed an excess ration of yolk-sac larvae to minimize cannibalism and insure good growth or larger individuals (Hokanson *et al.*, 1973). At the end of the three months (T11), the better results in term of survival (Fig. 8) were obtained at 12 °C (60%) and for growth

Table 1. Morphological development of the Aquitanian pike during the first 11 weeks post-hatching when reared at 12 °C (n = 15 larvae; reared individually in a petri dish), see Figure 5.

Week	Mean total length (mm)	Description
T0	9.09	Curved body (inclined head) resting on a voluminous, ovoid yolk sac, only pectoral fins differentiated from the protopterygia; diphycercal caudal: symmetrical protopterygia in relation to the terminal part of the notochord, pigmentation localized on dorsal surface and lateral line
		Mouth still closed
T1	11.76	Mouth open, in terminal position
		Resorption of the yolk sac
		Development of the snout
		Yolk sac reabsorbed, but lipids (energy reserves) still present
T2	13.59	Propterygia starts to atrophy at the ventral and dorsal surfaces (between anus and tip of notochord)
		Development of pelvic fin
T3	13.54	All energy reserves consumed: end of the endogenous feeding period
T4	14.65	Snout is formed, but its length is less than the eye diameter
T5	16.72	Development of dorsal, anal and caudal fin rays (lower lobe)
T6	17.71	Beginning of pigmentation at the base of dorsal, anal and caudal fin rays
T7	18.97	Heterocercal caudal fin
		Upper caudal fin lobe: acute (notochord)
		Lower caudal fin lobe: rounded (rays)
T8	20.04	The caudal fin has a three-lobed shape as a result of the progressive regression of the protopterygia.
		Upper lobe: begins to merge with upper rays
		Lower lobe: begins to separate into 2 parts
T9	20.93	Snout length greater than eye diameter
T10	25.20	Caudal fin separated from dorsal and anal fins (atrophied proto pterygia)
T11	27.85	Homocercal caudal fin: upper lobe (raised notochord + rays) and lower lobe (rays)
		Anal and dorsal fins shaped and individualized
		Pectoral and pelvic fins formed

Table 2. Initial number of larvae per temperature tested, and per tray.

Temperature	6 °C	9 °C	12 °C	15 °C	18 °C
Tray 1	7	9	3	7	7
Tray 2	10	8	7	8	8
Tray 3	8	8	9	8	7
Total	25	25	19	23	22

Table 3. Comparison of early life stages of Aquitanian and Northern pike. Data for Northern pike were taken from Teletchea *et al.* (2007), completed with those on-line here https://www.storefish.org/species/esox-lucius). Definitions of traits can be found here https://storefish.org/description.

Trait #	Trait description	Aquitanian pike	Northern pike
Egg			
1	Oocyte diameter (mm)		2.31
2	Egg size after water-hardening (mm)	2.73 ± 0.08	2.64
3	Egg Buoyancy	Demersal	Demersal
4	Egg adhesiveness	Adhesive	Adhesive
5	Incubation time (days)		11
6	Temperature for incubation (°C)		9.5
7	Degree-days for incubation (°D)		100
Larvae			
8	Initial larval size (mm)	9.09 ± 0.24	7.43
9	Larvae behaviour	Demersal	Demersal
10	Reaction to light		Photopositive
11	Temperature during larval development (°C)	12	12

Table 3. (continued).

Trait #	Trait description	Aquitanian pike	Northern pike
12	Sibling intracohort cannibalism	Present	Present
13	Full yolk-sac resorption (°D)	19 days at 12 °C	15 days at 12 °C
14	Onset of exogeneous feeding (°D)		7 days at 12 °C

(Fig. 9) at 15 °C (34.11 mm), followed at 12 °C (27.85 mm). Given the low number of larvae tested per temperature (Tab. 2), those results should be considered preliminary, but is seems that the optimal temperature of Aquitanian pike larvae is comprised between 12 and 15 °C.

Based on these results, we were able to rear Aquitanian pike in the Aquarium of Limoges in 2023 and then present two individuals for more than 2 years in order to promote the conservation of this species to the public, illustrating the potential of collaborations between angling federations, public aquariums and academic research for both research and conservation of fish (Teletchea *et al.*, 2023).

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Supplementary material

Fig. S1. Cannibalism observed among larvae at 12 °C on March 25, 2021 (46 days after hatching). Predator = 17.18 mm; Prey = 13.25 mm. The Fig. S1 is available at https://doi.org/10.1051/kmae/2025030/olm

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2025030/olm.

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