IRSTEA – EABX
Aquatic Ecosystems and
Global Changes

Life+ Alosa alosa

Actions C1 – D7

Final Irstea report

2011 – 2015

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Abstract

This report presents works managed by Irstea, Aquarium La Rochelle and Borea Team concerning the setup of an ex situ rearing method for juveniles Allis shads *Alosa alosa*, and the monitoring of sexual maturation

It presents in a first part the rearing structures, the conditions of breeding and the feeding. The results in terms of survival, growth and the observed pathologies are then presented. The last part presents the results of the sexual maturation monitoring.

At the end of this 4 years-experiment the survival is 10 %. Very strong mortalities were recorded in the beginning of the rearing following the appearance of a cyst near the mouth. During the second year, a "nephrocalcinosis" was detected which also induced a strong mortality. Besides the direct mortalities, these pathologies caused delayed mortalities which were difficult to quantify.

The results of growth show a smaller mean size and weight of 4 years-old fish compared to wild fish.

A balanced sex ratio was observed within fish sampled. Five spermating males and one female with advanced maturation were recorded at 4 years-old. These results demonstrate for the first time, the ability to obtain matured allis shads in captivity, which represents a great interest in the framework of the reintroduction plan of this species.

Résumé

Ce rapport présente les travaux conduits par Irstea, Aquarium La Rochelle et l'équipe Borea dans le cadre de la mise au point d'une méthode d'élevage ex situ de juvéniles de grande alose *Alosa alosa* et du suivi de la maturation sexuelle. Il présente dans une première partie les structures utilisées, les conditions d'élevage et l'alimentation. Les résultats de l'élevage en termes de survie, de croissance et de pathologies observées sont ensuite détaillés. La dernière partie expose les résultats du suivi de la maturation sexuelle.

A la fin des 4 ans d'expérimentation la survie est de 10%. De très fortes mortalités ont été enregistrées en début d'élevage suite à l'apparition d'un kyste près de la bouche. Au cours de la deuxième année, une « néphrocalcinose » a été détectée qui a également engendré une forte mortalité. En plus des mortalités directes, ces pathologies ont induit des mortalités différées difficilement quantifiables.
Les résultats de croissance montrent que les poissons de 4 ans ont une taille et un poids moyens inférieurs à des poissons sauvages.

Un sexe ratio équilibré a été observé parmi les poisons échantillonnés. Cinq mâles spermiant et une femelle avec une maturation avancée ont été détectés chez les poissons âgés de 4 ans. Ces résultats montrent pour la première fois, qu'il est possible d'obtenir des grandes aloses matures en captivité, ce qui est un résultat majeur dans le cadre de la réintroduction de cette espèce dans le bassin du Rhin.

1. Objectives of this action (C1)

An ex situ stock of the American shad *Alosa sapidissima* for aquaculture purposes was established by American and Chinese biologists in China. Moreover, always in China, studies on Reeves shad *Tenualosa reevesii* have led to build up an ex situ stock which have allowed to obtain mature fish (Wang *et al.*, 1992; Wang *et al.*, 1995; Wang *et al.*, 2003). No experience with an ex situ stock of Allis shad exists in Europe so far.

The first objectives of this action are to test long term survival in captivity, to define main rearing conditions to obtain growth and normal development, and to monitor sexual maturation in these conditions.

The long term objective of this action is to obtain a captive broodstock to minimize the amount of adult fish caught in the wild for reproduction and larvae productions purposes within the framework of stocking activities.

2. Origin of the fish

At the end of the first Life project (LIFE06 NAT/D//000005), a batch of one month old larvae was transferred from St Seurin experimental station (Irstea) to Aquarium La Rochelle in June 2008. As the number of fish was too low to support samplings planed during this action, it was decided in January 2011 to transfer a new batch of larvae.

Unfortunately, a blackout occurred in November 2011, and all fish from the 2008 batch, and a part of those from the 2011 batch died.

The study was thus carried out on the 2011 batch only.

3. Monitoring of the 2011 batch

3.1 Origin of the batch

This batch arrived in Aquarium La Rochelle on 24 May and 25 October 2011, obtained from an artificial reproduction realized by Migado (Bruch). Hatching occurred on 14 May 2011. The first group was made up of approximately 2 500 ten day-old larvae. The second group of 400 juveniles was reared during 5 months in a pond at Bruch (**Figure 1**).

These 2 groups were put together in December 2011, with a total number of 539 juveniles.

This experimental rearing ended in June 2015.

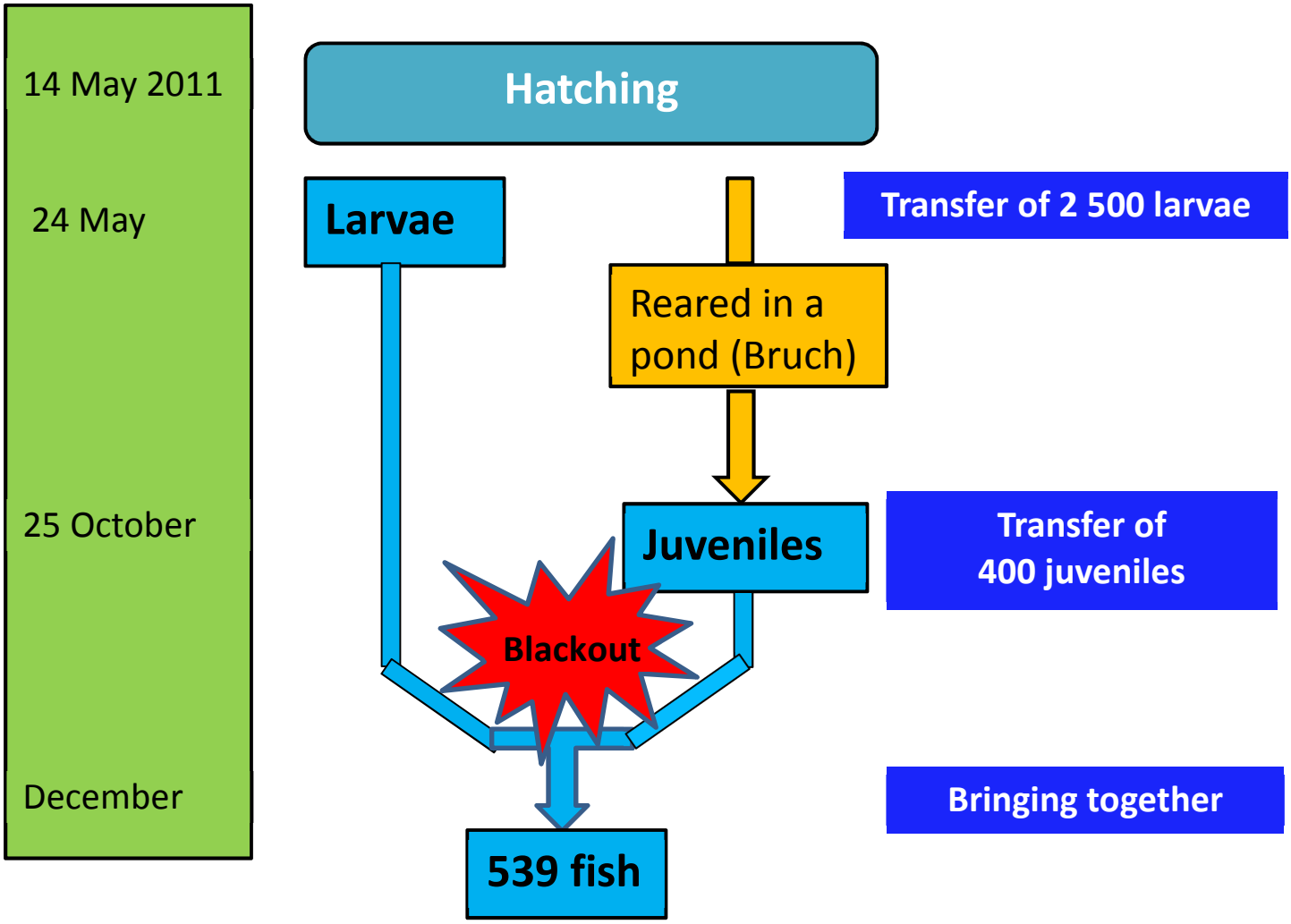


Figure 1 : Historic of the 2011 batch

3.2 Rearing structures

In December 2011, once the 2 groups brought together, fish were reared in a 2.6 m^3 tank, with a tangential water inlet to obtain circular water current (**Figure 2**). The tank diameter was 1.9 m and water height was 0.9 m. A net was simply installed on the tank to prevent fish escape. A lateral window allows observing fish while limiting fish disruption.

The increase of fish mass led to transfer fish to a 9.3 m^3 tank (**Figure 3**) with a diameter of 3.35 m and a water height of 1.05 m. The transfer occurred in September 2012. The tank was equipped with net to prevent fish escape. This first system was not efficient enough, and was modified in October 2013. This final system is a kind of "net wall" of 1 meter high. This height is necessary to be sure that adult fish could not jump outside (**Figure 4**).

Figure 2 : The first rearing tank (2.6 m^3)

Figure 3 : The 9.3 m^3 tank with the first system to prevent fish escape

Figure 4 : The 9.3 m^3 rearing tank with the final net system to prevent escapements.

Each tank was equipped with a recirculated system, with mechanical, biological filters and UV lamp for disinfection.

Because of the increase in biomass, and so feeding, the filtration capacity was not sufficient, leading to an increase of nitrite concentration in the rearing system. A second biological filter system has been set up at the end of April 2014, with a degassing procedure at the end of June 2014 (**Figure 5**).

Figure 5 : The biological filtration system installed in April 2014.

3.3 Rearing conditions

3.3.1 Salinity

Larvae arrived in May 2011 were rapidly acclimated to slightly salted water (8 – 10 ‰) in order to prevent diseases and to increase artemia survival (**Figure 6**).

Juveniles arrived in October 2011 were kept in freshwater until the 2 groups were put together, at 8 – 10 ‰.

Transfer to sea water occurred in December 2011, i.e. at 7 months-old. Fish were progressively acclimated to reach sea water salinity (30 ‰) in two weeks.

Mean salinity over the experiment was 28.4 ± 1.1‰ (**Figure 7**).

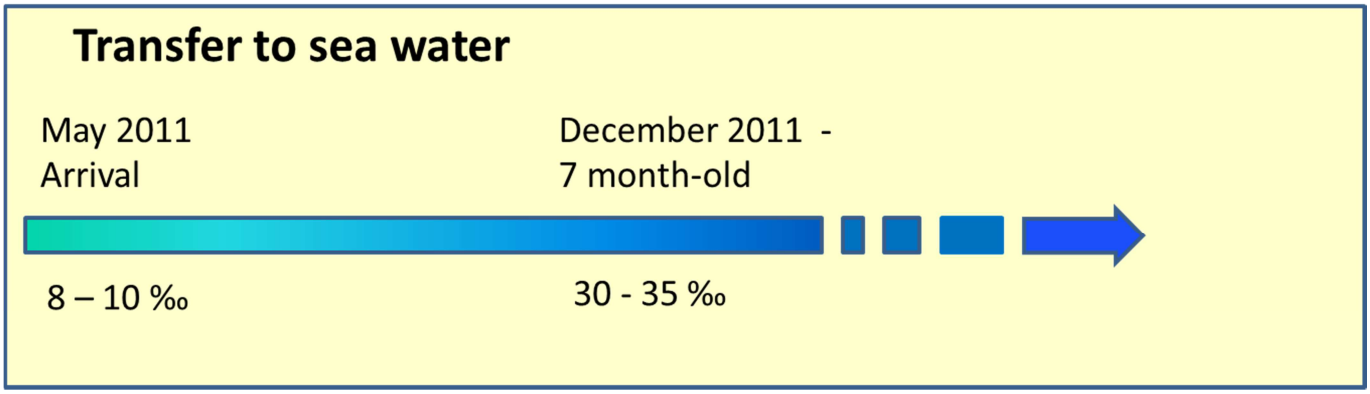


Figure 6 : Salinity change during the experiment

3.3.2 Temperature

Mean water temperature over the course of the experiment was 18.4 ± 0.2 °C with seasonal variations. Mean water temperature during winter was 17.4 ± 0.3 °C and 19.6 ± 0.2 °C during summer (**Figure 7**).

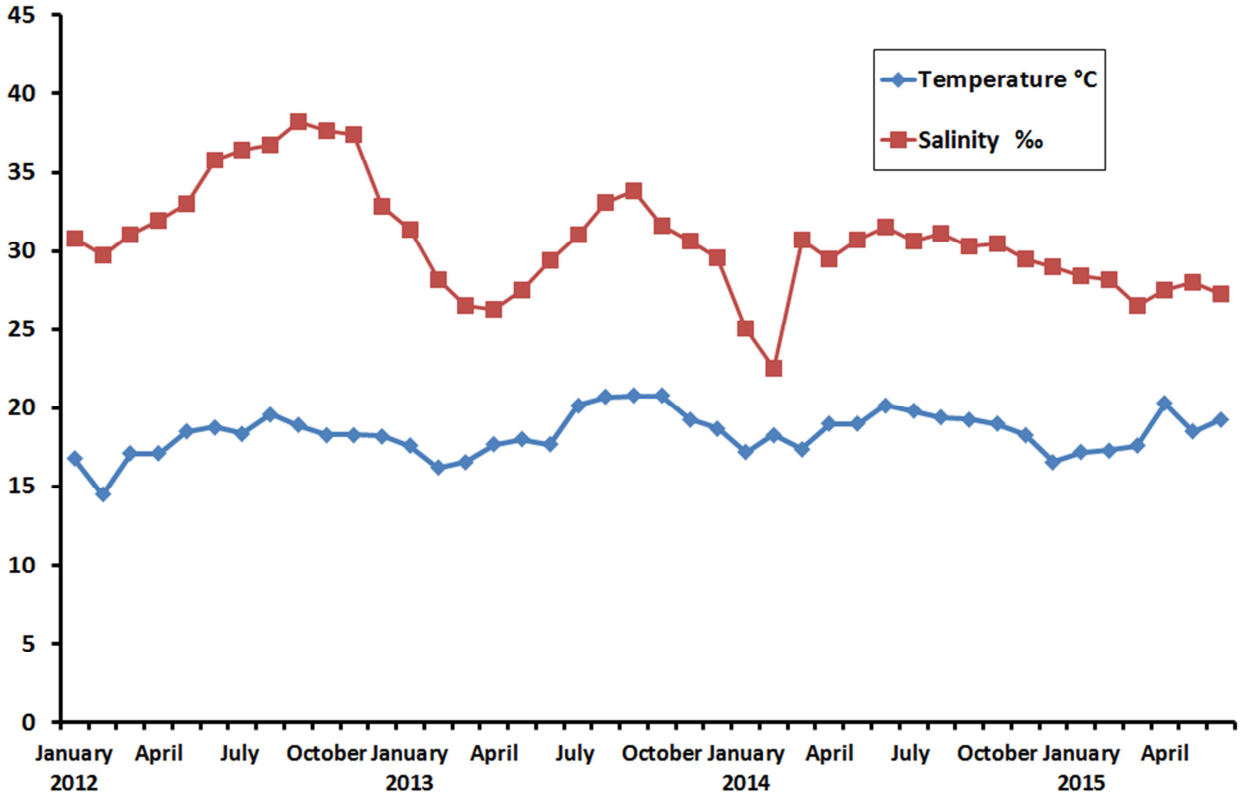


Figure 7 : Monitoring of temperature and salinity from January 2012 to June 2015 (end of the experiment)

3.3.3 Water current

The water current was first adjusted to have a correct compromise solution between self-cleaning of the rearing tank and suitable conditions for fish. From November 2012 until February 2014 the maximum water current measured in the middle of the water column, at 20 cm from the wall of the tank was 27± 0.6 $cm.s^{-1}$. This level of water current was satisfactory in terms of cleaning of the tank. But the current speed seemed too high when observing the fish behavior. Moreover results of growth were lower compared to wild fish (Taverny, 1991). This led us to think that a great part of energy could be allocated to swim against the current, and growth could be consequently impaired.

The current speed was decreased in March 2014 by modifying the direction of the water inlet. The water speed was then 13.4± 0.6 $cm.s^{-1}$ (measured in the same conditions).

3.3.4 Rearing density

Rearing density was assessed from results of samplings mainly carried out in June, from June 2012, and assessment of the number of fish remaining in the tank (**Figure 8**).

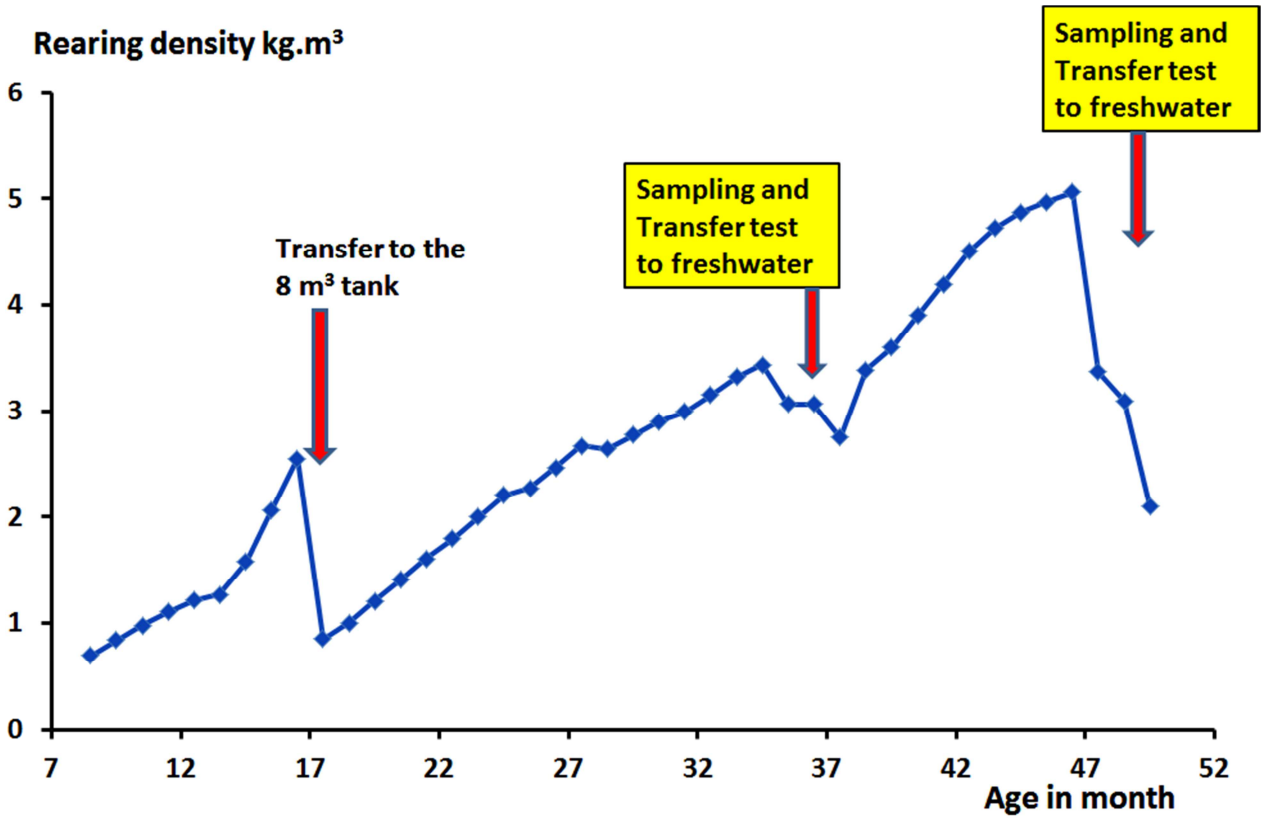


Figure 8 : Assessment of the rearing density as a function of age

Within the global trend of rearing density increase following fish growth, we can observe 3 periods of rapid decrease.

The first one is due to the change of rearing tank, which led to a logic reduction with the larger rearing volume. The second and the third ones correspond to removing of fish for annual samplings and to carry out test to simulate freshwater migration (see 2).

Rearing density reached 5.1kg.m^{-3} in March 2015. Following veterinarians' opinion, the rearing density for this kind of experimental rearing should not be higher than 2kg.m^{-3} to avoid pathologies and to obtain a correct growth.

3.3.5 Feeding

Feeding sequence

Before this first ex situ rearing experience, the only known feeding sequence for allis shad was for larvae and juveniles until 2 month-old. Various types of feed were thus tested during the first year, leading to a complex feeding sequence (**Figure 9**).

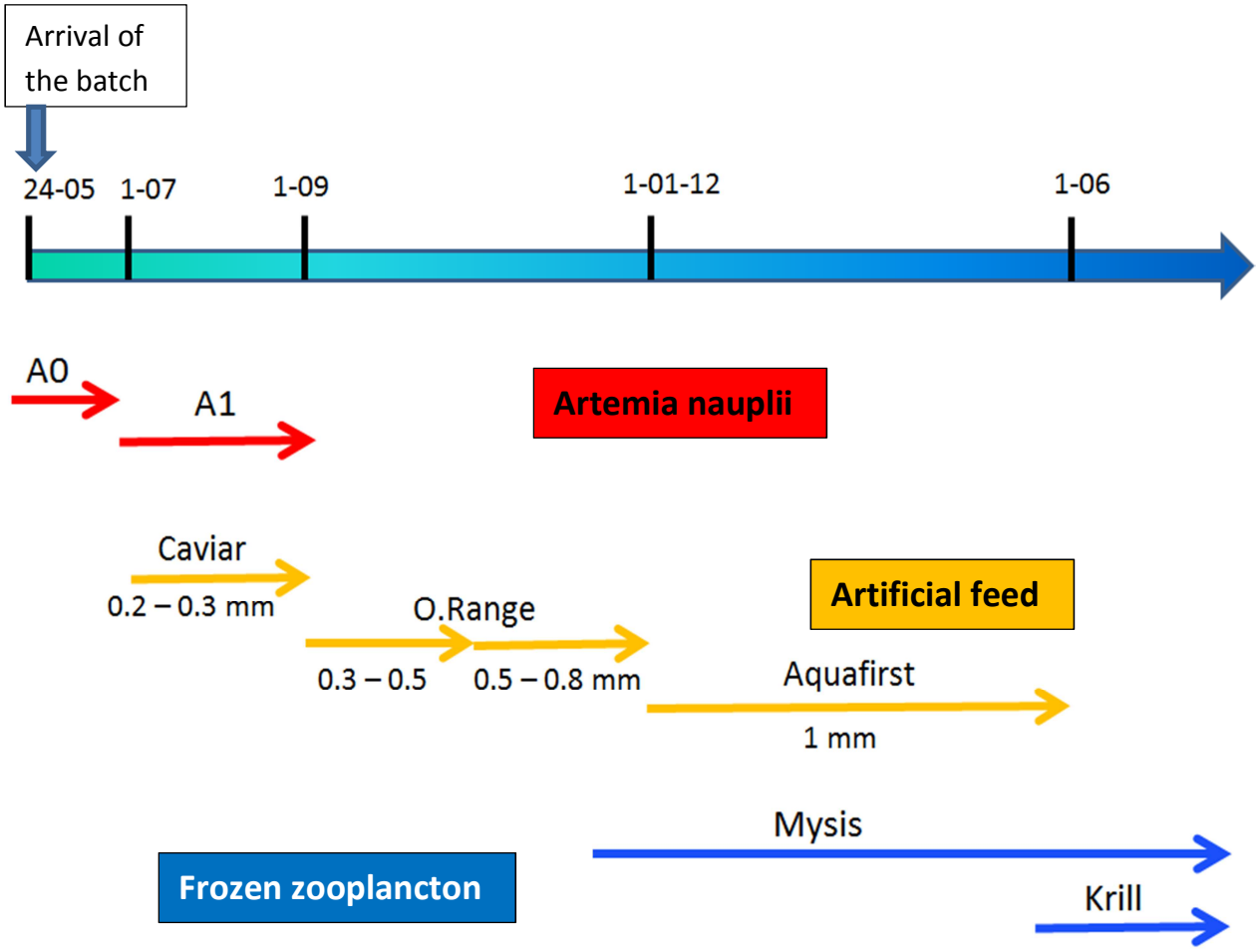


Figure 9 : Feeding sequence during the first year of rearing.

Larvae arrived in May were fed with artemia nauplii A0, in continuity of the feeding operated in Bruch. After this first phase which lasted around 1 month, Larvae were fed with A1 artemia nauplii (24 hours of enrichment – Selco) for 2 months.

At the same time, a progressive transition to artificial feed was carried out. This began with 0.2-0.3 mm Caviar pellets (Bernaqua) until September, following by O.Range pellets (INVE) 0.3-0.5mm and then 0.5-0.8mm until the end of December. In January 2012 the O.Range was replaced by 1mm Aquafirst N°3 (Tyca) which was delivered until the end of June 2012.

The feeding ration was supplemented with frozen zooplankton from December 2011 until the end of June 2012. Mysis was mainly distributed and Krill was added in June.

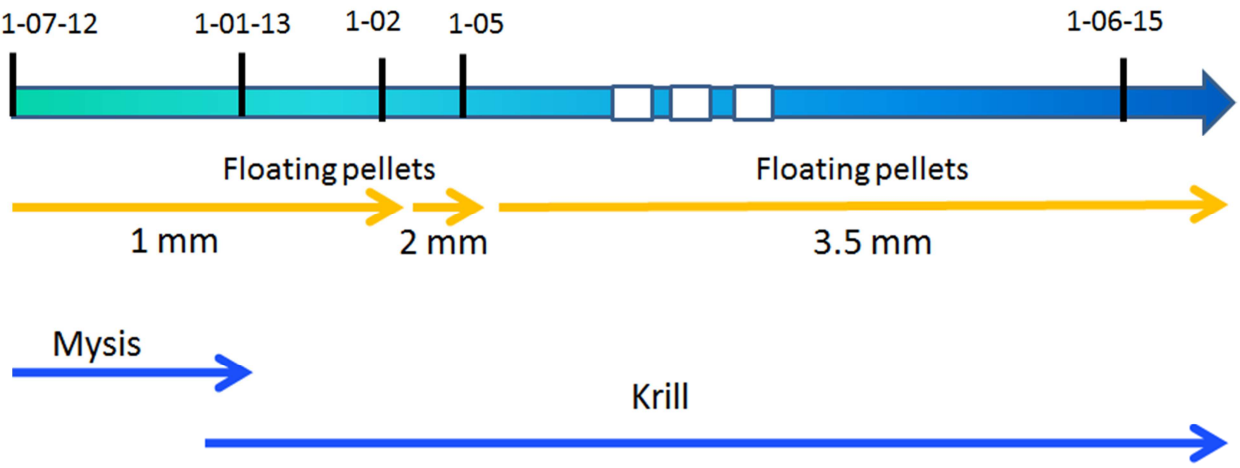


Figure 10 : Feeding sequence from July 2012 until June 2015

After this first year, juveniles were fed with both artificial feed and frozen zooplankton (**Figure 10**).

Allis shad chased and fed on preys in the water column. The Aquafirst pellets had a low floatability. They sink rapidly and the time spent by a pellet in the water column was not sufficient to allow a good feed intake. This feed appeared not well suitable for allis shad juveniles.

By July 2012, Aquafirst was replaced by Floating pellets (Tyca) with a size from 1mm until 3.5mm at the end of June 2015. Mysis was continued until the end of December 2012, and then was replaced by Krill until the end of the experiment.

Artificial feeds were distributed continuously during daylight by the mean of automatic belt feeder (**Figure 11**). Frozen zooplankton was manually distributed twice a day.

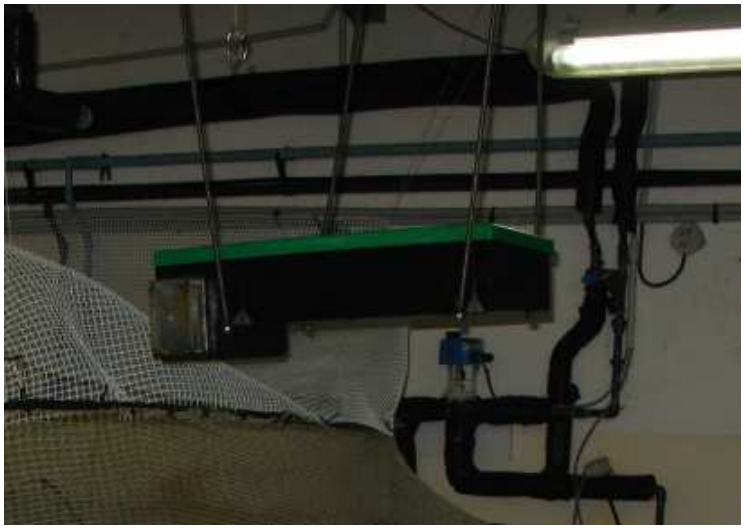


Figure 11 : Automatic belt feeder for artificial feed.

Feeding ratio

A total of 367.8kg of artificial feed and 128.6kg of frozen zooplankton was distributed from January 2012 until June 2015. A theoretical feeding ratio has been calculated according the growth rate calculated between 2 samplings, the number of live fish and food distributed. The mean feeding ratio was 1.35 and 0.79 for the artificial feed and zooplankton respectively.

3.4 Biological results

3.4.1 Survival

Monitoring of the survival is only based on natural mortality; fish collected for biological sampling or fish transferred to aquarium exhibition or in freshwater for test, were not counted.

An important mortality was recorded soon after the shipment to Aquarium La Rochelle (**Figure 12**). A part of this mortality can be attributed to the transfer: catching, shipment, modification of the rearing environment. The water temperature at the arrival of the larvae was 17.4°C. Increasing the temperature above 19°C seemed to stop mass mortality.

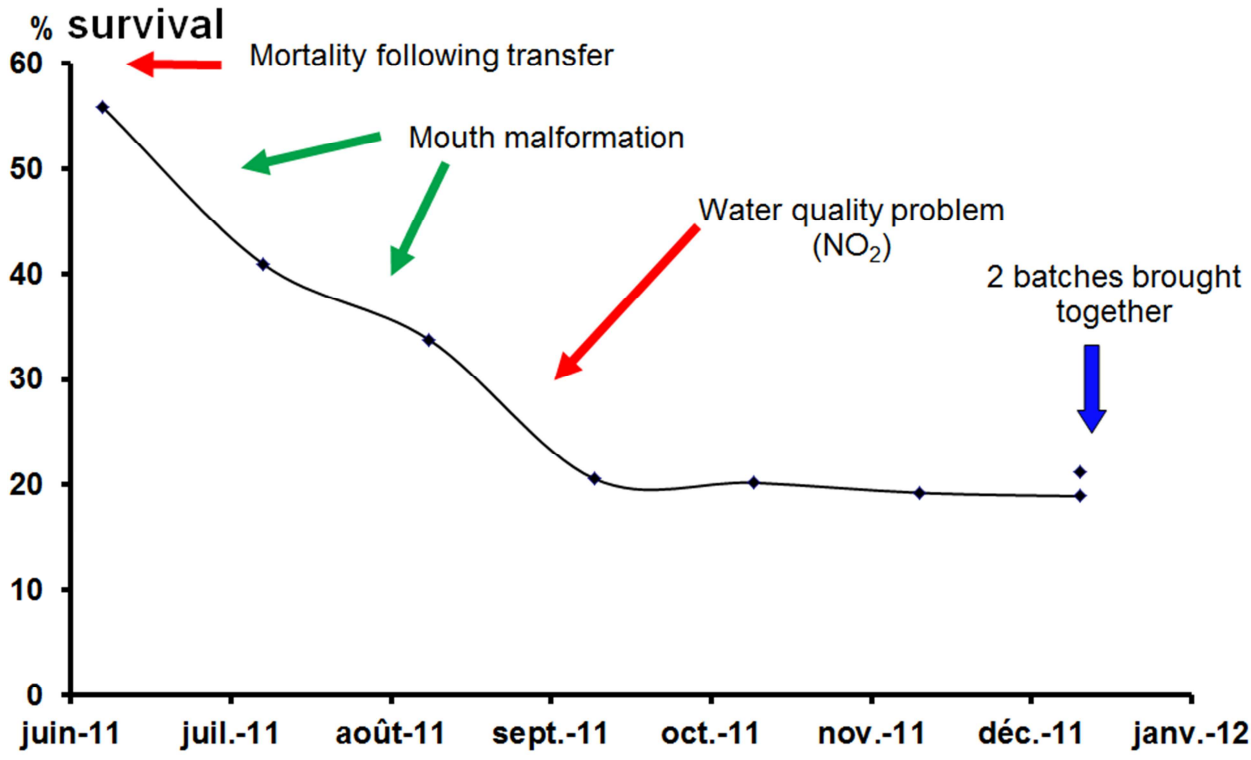


Figure 12 : Monitoring of the survival during the first 6 months of rearing.

The last potential cause of this mortality concerned the water salinity. Larvae were reared in freshwater until the transfer and placed in a 10‰ water salinity from their arrival onwards. DiMaggio *et al.* (2015) used a 5‰ water salinity from hatching as standard in an experimental rearing of *A. pseudoharengus* and *A. aestivalis*. Navarro *et al.* (2014) recorded at least a better survival in twaite shad larvae reared in 2.5, 5 or 10‰ from hatching until 10 days old. Moreover a rapid transfer from freshwater to 10‰ brackish water did not affect survival in 13 day-old allis shad larvae (Bardonnet and Jatteau, 2008). We can expect allis shad larvae can well withstand salinity soon after hatching.

But in our case, an acute salinity transfer combined with the stress caused by the shipment could have generated negative conditions and explained an important part of this mortality.

From the end of June to August a great number of larvae exhibited a nodular growth near the mouth (see 1.4.4.1). These nodules could likely have a negative effect on feeding even leading to death. The proportion of dead larvae affected by these nodules represented 87% in July and 44% in August and September.

With the increase of the feeding ratio, wastes increased too. Because the water current was not sufficient, waste settled in the bottom of the tank. This resulted in the degradation of the water quality in September with a high level of NO^2 (between 0.8 and 0.3 $ml.l^{-1}$). Mortality was stopped by an adjustment of the water flow circulating in the filter system.

During these first 4 months of rearing (until end of September), 2013 larvae died. The mortality is divided up as follows:

- 55% during the 2 first weeks following transfer
- 20% attributed to mouth deformation
- 17% attributed to peak of NO_2
- 8% of natural mortality

At the end of November, the overall survival of the batch arrived in May 2011 was 18.9%. In December the juvenile batch, arrived in October (n=59), was brought together with the first batch, and the number of individuals was 539, leading to a new value of survival of 21.3%.

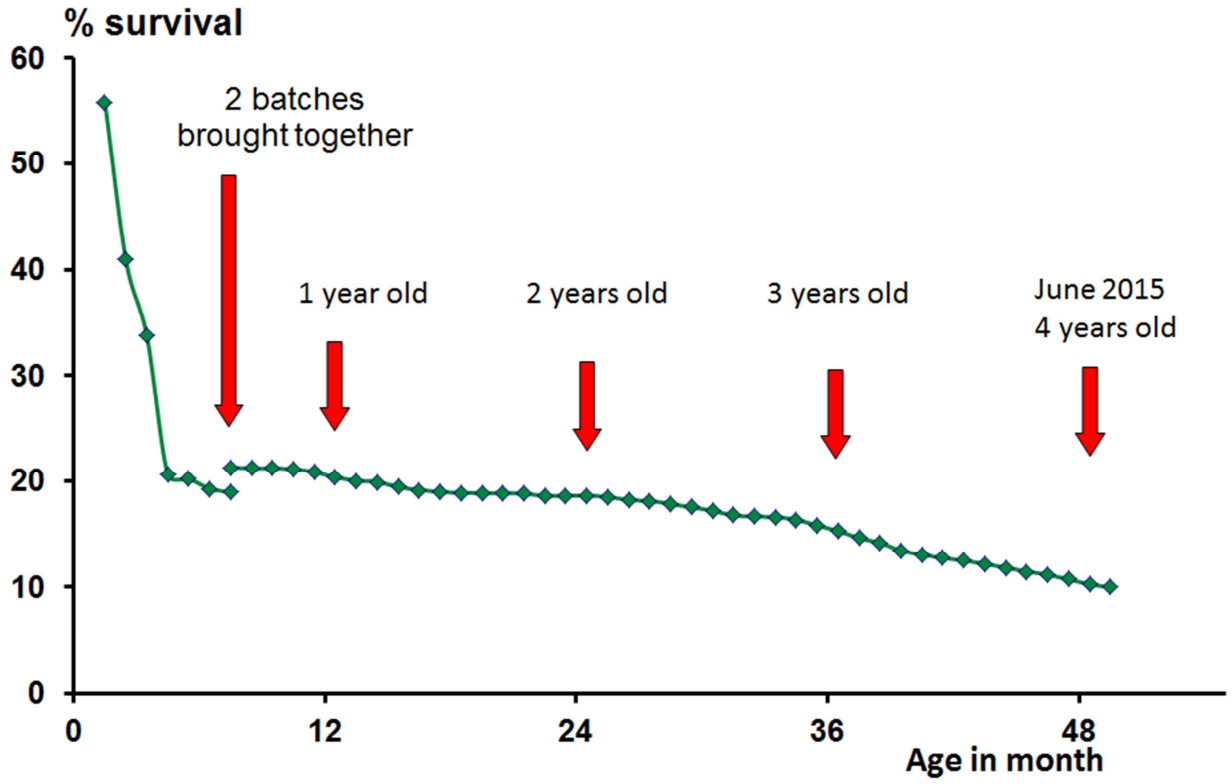


Figure 13 : Monitoring of the survival over the 4 years experiment.

Mortality was low during the following months (**Figure 13**). Survival was 18.5% in June 2013, i.e when fish were 2 year-old. The main cause of fish loss (63%) was the escapement outside the tank, which led us to modify the net around the tank (see 1.2).

Mortality was higher during the third and fourth year of rearing.

Ninety eight fish died during the third year. Two main mortality phases were observed. The first one during November – December 2013 corresponded to a parasitic and bacterial pathology which was stopped following a 6 days treatment. The second was observed from April. Dead fish were examined and presented calcium nodules in the kidney ("nephrocalcinosis") (See 1.4.4.2). Mortality was stopped thanks to the addition of the new biological filtration and degassing system. At the end of June 2014, survival was 14.6%.

Mortality rate increased during the last year of the experiment, with the loss of 118 fish, leading to a 10% survival rate.

No single cause could be highlighted considering water quality or feeding. A proportion of fish which were 4 year-old could be considered as maturing fish regarding results of sexual maturation monitoring (See Part 5). We can assume that environment rearing conditions for maturing fish could be unsuitable. When a level of sexual maturity is reached, fish will try to enter freshwater for reproduction when water temperature is 11°C and more, and stop feeding. In our rearing

conditions the temperature is above this threshold, and could trigger migration behaviour. The gap between fish requirements and actual environmental conditions could generate unsuitable conditions for these fish. More generally, older fish could be more sensitive to stress and contribute to increase mortality.

3.4.2 Growth

Length and weight were measured during annual samplings in June, and on two other occasions, in October 2011 and November 2012. Twenty fish were at least measured each time.

The result of linear (standard length) and weight growth are presented in **Figure 14**.

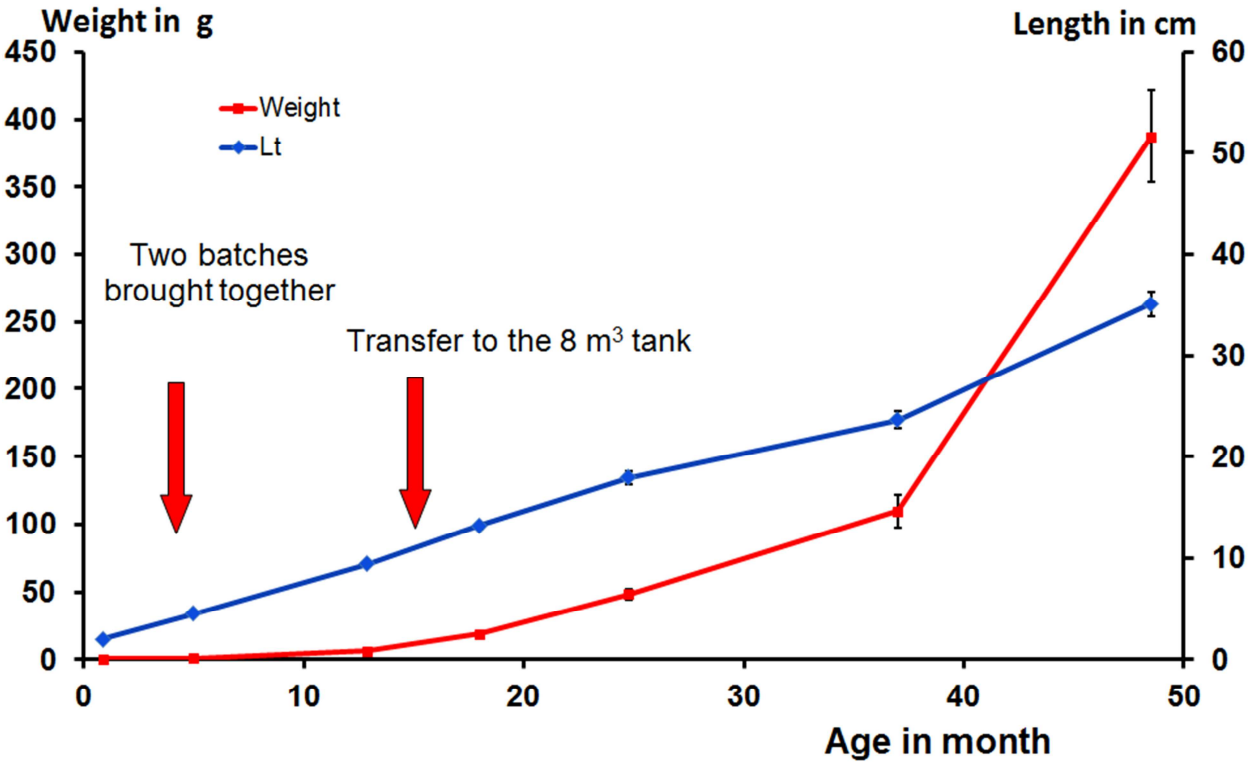


Figure 14 : Monitoring of the length (Total length) and weight until June 2015 - Standard error (SE) are mentioned.

We observe a rapid increase of weight, and of length to a lesser extent, during the last year of rearing.

The Specific Growth Rate (SGR) was calculated for each year as follows (same formulation for the weight):

$$SGR\% = [Ln(Lt2) - Ln(Lt1)]*100/t2-t1$$

The results are summarized in **Table 1**.

	Year 1	Year 2	Year 3	Year 4
Length	0.41	0.17	0.07	0.11
Weight	0.87	0.55	0.22	0.36

Table 1 : Linear and weight rate (SGR in %) calculated for the 4 years

The growth recorded in this experiment is low and fish are smaller either in terms of length or weight compared to wild fish. For example 4 years old wild females are around 45 cm and 1.6 kg (Martin Vandembulcke, 1999).

Different reasons could be proposed to explain this result:

- The level of salinity during the first months of rearing which could have impaired the growth
- An insufficient feeding ratio
- A too high density (food competition)
- The mouth malformation which prevents a good feed intake in some fish
- A too high water current until the beginning of 2014, which mobilized a part of energy for swimming

3.4.3 Length-weight relationships

The length-weight relationship was calculated from the beginning of the rearing. All measures realized during the time of the experiment are plotted in **Figure 15**. The relationship obtained from these data is:

$$W = 0.005 * Lt^{3.1626}$$

With Lt: Total length in cm
W: weight in g

We divide the results in 2 parts in order to have a more accurate analysis of the growth.

The first step corresponds to length until 15 cm, and the second one to the rest of the data.

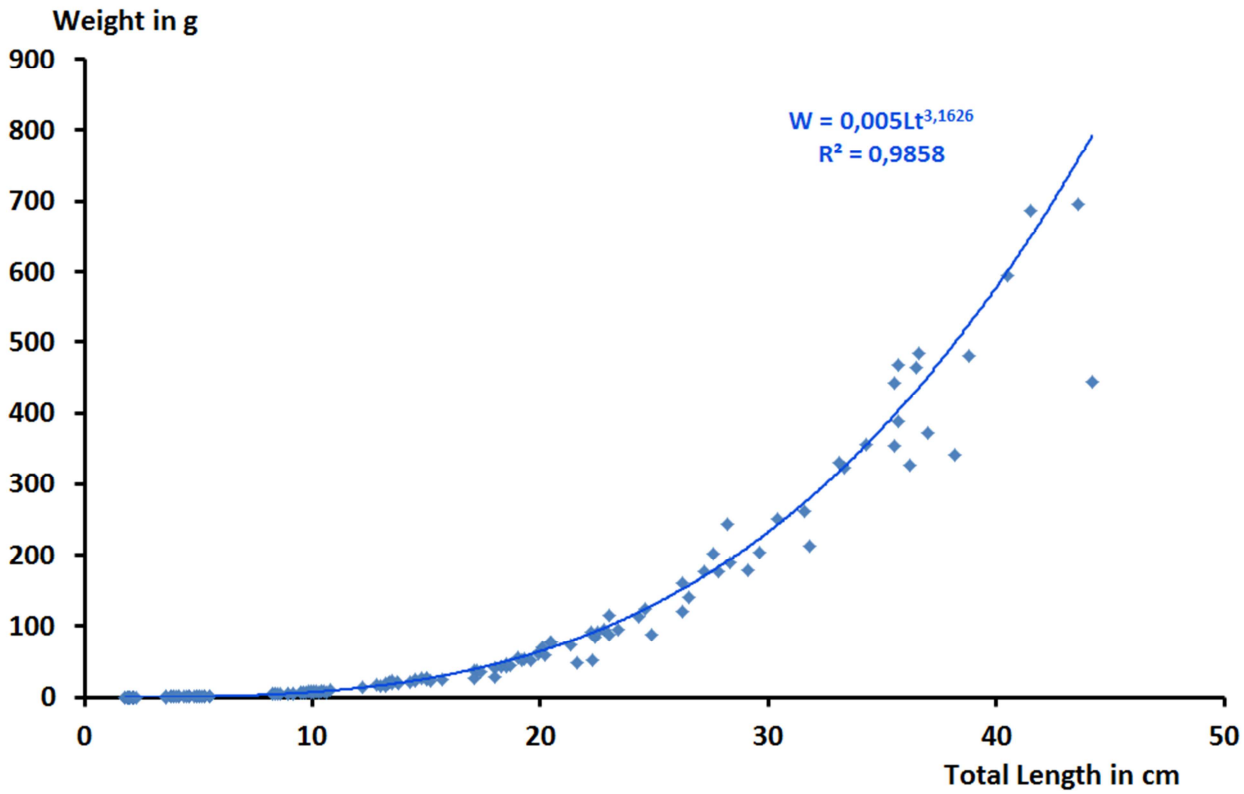


Figure 15 : Length-weight relationship calculated from all measures realized during the experiment

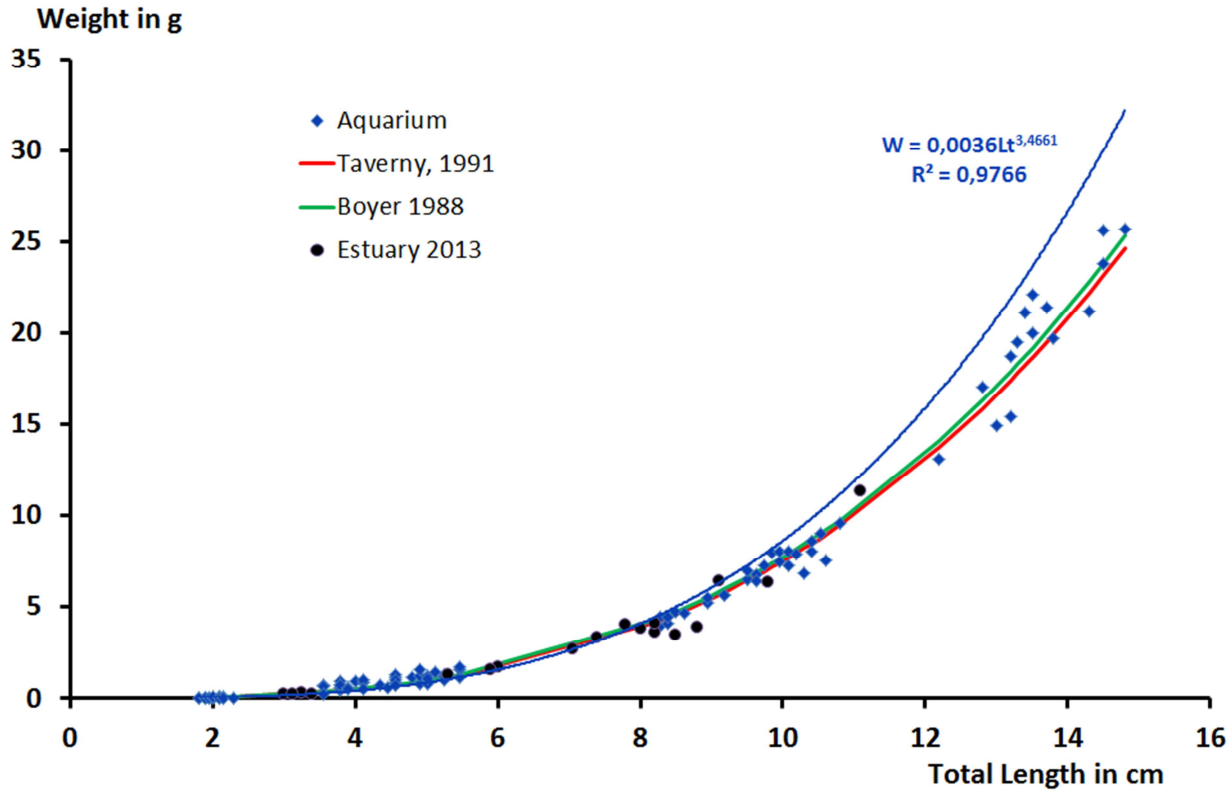


Figure 16 : Length-weight relationship for total length less than 15 cm.

During this first phase, the relationship obtains is:

$$W = 0.0036*Lt^{3.4661}$$

Three different sources of data were used and results were together plotted on this graph in order to compare results obtained in captivity and data collected in the wild.

All these data concerned juveniles, caught at the Golfech dam (Bernard and Larinier, 1988), or in the estuary, with data from 1988 (Taverny, 1991) or from 2013 (monthly samplings). We observe that results recorded during the ex situ experiment in Aquarium La Rochelle are consistent with data from wild fish.

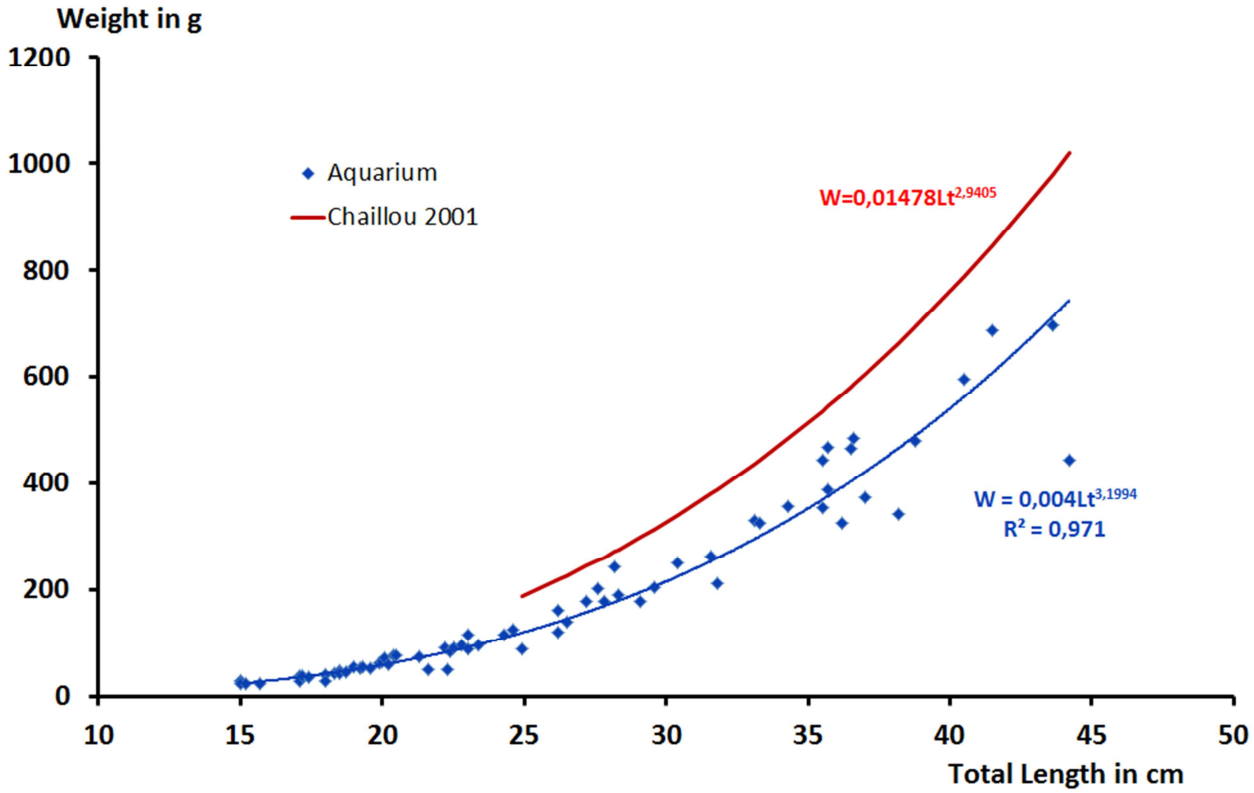


Figure 17: Length-weight relationship for total length upper than 15 cm.

The second step corresponds to juveniles with total length upper than 15 cm (**Figure 1**).

For this second phase, the relationship is:

$$W = 0.004*Lt^{3.1994}$$

The length-weight relationship calculated by Chaillou (2001) is also plotted in this figure. It was obtained from adults caught in the Dordogne River.

The lengths and weights measured during this ex situ experiment appeared lower than the wild fish relationship. That is, a fish of a given length is less heavy than wild fish.

3.4.4 Pathologies

Two main pathologies were observed during this rearing experiment. The first one concerned mouth malformations, and was detected during the first months of rearing. The second one was detected during the third year of rearing and concerned a kidney pathology.

3.4.4.1 Mouth cyst

The first indication of mouth malformations was recorded at the end of June 2011, when larvae were one month old. The first symptoms appear as a development of a little outgrowth located near the mouth (**Figure 18**) which grows with time (**Figure 19**).

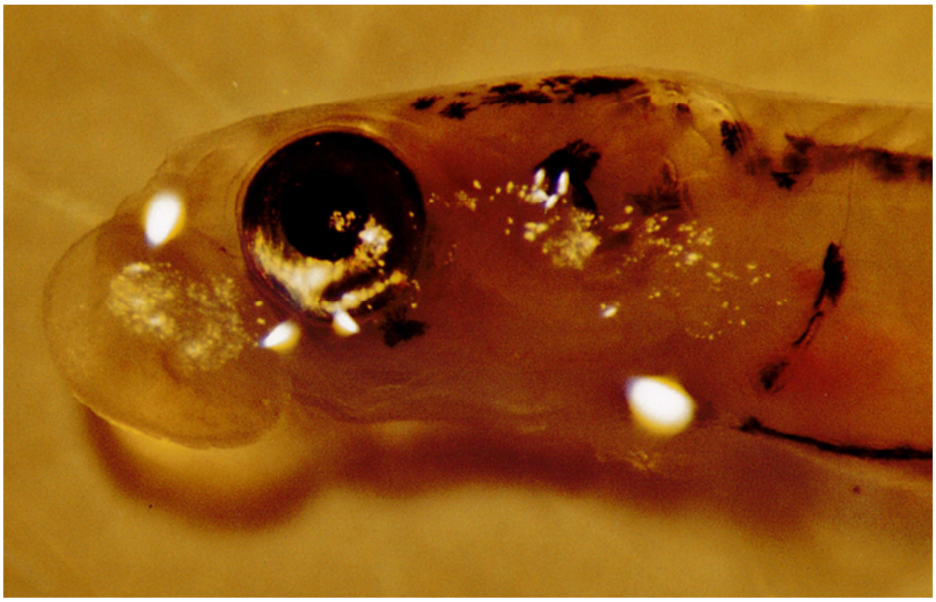


Figure 18: Mouth outgrowth of 1 mm (max diameter)

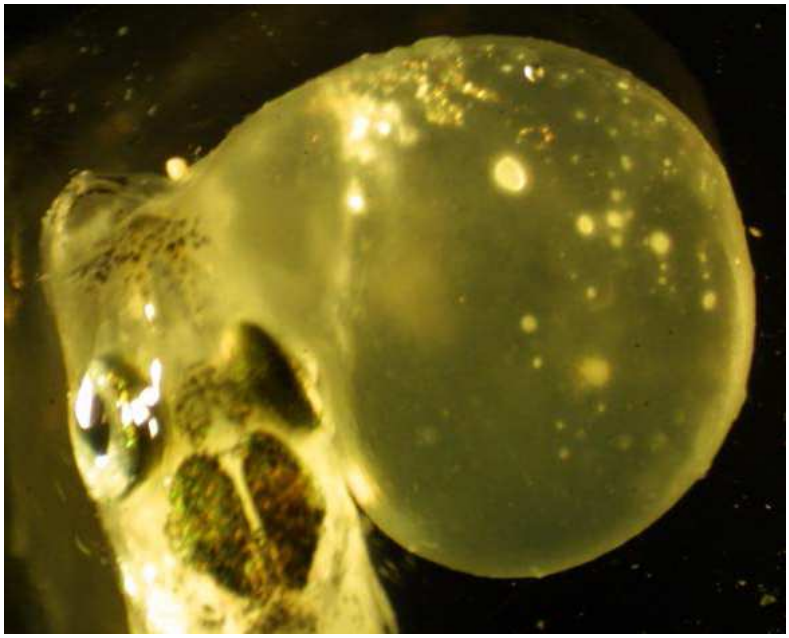


Figure 19: Mouth cyst on a dead larva

When the outgrowth becomes too large, feed intake is impaired, which leads to reduced growth or death.

The mouth malformation had a huge direct effect on mortality. In July, 87% of dead larvae presented this pathology and 44% in August. Resulting mortalities spread out over time but were difficult to quantify.

When larvae could survive, the size of the outgrowth decreased with time and finally disappeared.

Larvae presenting this pathology were recorded during annual samplings (**Table 2**). These figures and results of mortality during July and August 2011 show that a majority of fish were affected by this pathology.

	2 years-old	3 years-old	4 years-old
Fish affected	30	27	40

Table 2: Percentage of fish presenting signs of mouth malformation

Among fish which could survive despite this malformation, some of them were smaller showing that growth was impacted by this pathology. For example, the 3 years-old fish in **Figure 20** weighted 27g whereas mean weight at this age was 110g.

Figure 20: A 3 years-old larvae which survived to mouth malformation.

On the opposite, some fish grew normally and had even higher length and weight than means at age. In **Figure 21** this 4 years-old fish was 40.5 cm and 594g whereas means were 35cm and 387g.

Figure 21: A 4 years-old fish with a huge mouth malformation

Some surviving fish have kept a malformation of the jaws as shown in **Figure 21**. The after-effects of the outgrowth are visible on the lower jaw, with sometimes a huge malformation. But the upper jaw is also affected which is shorter as normally ("retrognathy").

The origin of this pathology is unknown. Analysis did not reveal the presence of infectious agents. It was previously observed in experimental rearing realized by Irstea from 2004 and in other shad species (DiMaggio *et al.*, 2015). But it was not observed in fish reared in ponds (natural feeding) and during experiments carried out by Irstea in 2000 and 2002 with a weaning realized with small size artemia.

From these observations 2 different causes can be proposed to explain this pathology.

- Feeding origin
- Mechanical origin by rubbing or collision against tank walls in relation with rearing density, prey avaibility

3.4.4.2 Kidney disease

Increasing mortality observed from April 2014 led to conduct analysis on dead fish. These autopsies revealed the presence of nodules located in the kidney (**Figure 22**). These nodules are made of urate and calcium carbonate. No infectious agent was detected.

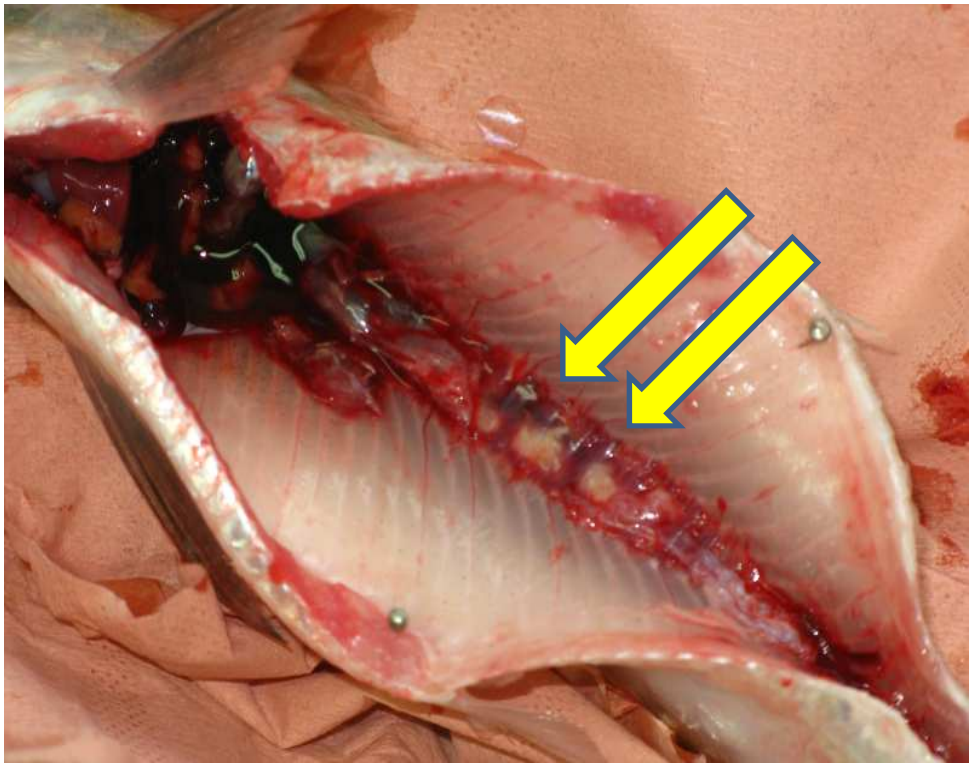


Figure 22: An example of fish presenting kidney nodules.

Histological examination of the kidney further revealed many intra-kidney nodules (**Figure 23**). This pathology is named “nephrocalcinosis”. This disease appears when pH is too low and concentration of CO_2 is too high (De Kinkelin *et al.*, 1985). The modification of the filtration system (see 1.2 and **Figure 5**) by adding of a second biological filter with injection of pressurized air allowed reducing the concentration of CO_2 and increasing pH and mortality was stopped few weeks later. A total loss of around 80 fish can be attributed to this pathology. During the last sampling in June 2015, around 52% of fish presented kidney nodules, demonstrating that the improvement of the water quality did not allow the resorption of these nodules.

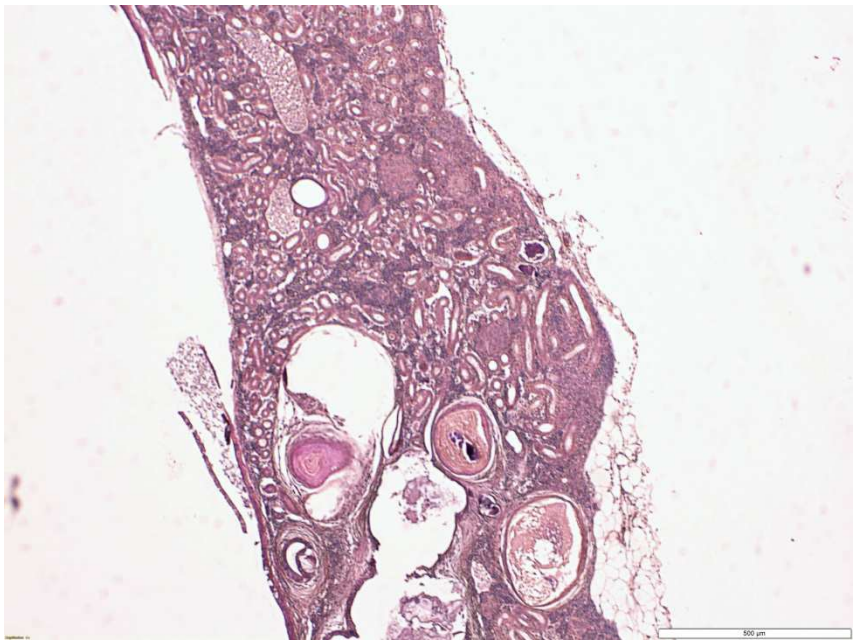


Figure 23 : Histological examination of the kidney tissue revealing many intra-kidney nodules.

4. Simulation of freshwater migration

4.1 Objective

Between 3 and 6 years, adult Shad leave the ocean to enter in the estuary and the river to reach the spawning grounds and spawn. During this migration, Shad switch therefore from a salted to a freshwater environment.

In the C1 'ex situ stock' action framework, the aim is to bring to maturity a batch of shad reared from the larval stage. In order to follow as much as possible the natural biological cycle of the species, it was therefore necessary to simulate the migration of subadults to the spawning grounds by transferring the fish in fresh water.

4.2 Protocol

Two transfer operations were realized in 2014 and 2015, when fish were 3 and 4 year-old.
The objective was to maintain the fish for 2 months in fresh water.
The transfers were managed in early April, and 30 fish were caught from sea water tank. They were transferred to the 2.6 m^3 tank. They were kept at sea water salinity for acclimation during 1 week in 2014 and 2 weeks in 2015. Feeding was maintained during this phase.

The decrease of salinity was gradual and lasted 2 weeks in 2014 and 1 week in 2015, by adding freshwater during the day, to obtain a decrease of approximately 1-5‰ per day.
The temperature was increased until 20°C.
Feeding was maintained until the beginning of the salinity decrease, and then progressively reduced and finally stopped with decrease and stop of fish appetite.
The current speed was maintained around 15cm.sec^{-1}.
The duration of the light phase was also increased according to the following scheme:
In April: photoperiod 12N / 12L (from 7 h to 19 h)
In May: photoperiod 11N / 13L (from 7: 00 to 8: 00 pm)
In June: photoperiod 10N / 14L (from 07: 00 to 21: 00)

4.3 Results

In 2014, during the first trial 4 juvenile shads died soon after handling and were renewed.

Only 4 fish died during the salinity decrease, showing that 3 year-old fish well withstood transfer to freshwater.
Majority of shads seemed to rapidly stop feeding.
By the end of April, level of NO_2 increased because of the death of bacterial biomass in the biological filter due to passage to freshwater, which unfortunately induced the death of all fish.

In 2015, prior the transfer, a filter system adapted to freshwater was prepared and was connected to the 2.6 m^3 tank as soon as the salinity 3-5‰. This allowed to prevent the rise of NO_2 concentration, and the water quality was thus maintained to a good level.

Two fish died soon after handling and were renewed.
Three fish died during the salinity decrease. A chronic mortality arose after 1 week in freshwater, and continued despite antibiotic treatment. At the beginning of June, 6 fish were alive and were sampled.

4.4 Conclusion

These first trials demonstrated that 3 and 4 years-old shads can tolerate transfer from seawater to freshwater within 1 or 2 weeks, without significant mortality.

The objective of these transfers was to simulate the upstream migration of adults for spawning. In the wild, and all the more, in captivity where environment conditions were not optimal, not all fish reach maturity at 4 years-old. The starting of the upstream migration likely depends on the level of the sexual maturation and the level of reserves necessary to undertake the migration. In our case, fish were randomly caught, and this limits the relevance of this operation.

5. Monitoring of the sexual maturation (Borea Research Unit)

5.1 Materials and methods

Yearly samplings were performed in June during 4 following years, 2012, 2013, 2014 and 2015, and fish were 1 to 4 years-old. Fish were sampled after euthanasia in a concentrated bath of eugenol.

Following parameters and samplings have been recorded and made:

- Length, weight, height, weight of the digestive tract, gonad and liver
- Blood, gonad, digestive tract and liver

In addition, samples of gill, liver, brain and pituitary have been realized to open the possibility for future analyses, considering that these individuals from this pioneer experiment are very unique.

Blood samples have been taken directly in the heart. After centrifugation, plasma was kept frozen at -20°C.

Gonad samples were fixed in Bouin solution for histological studies.

After Bouin's fixation, tissue samples were dehydrated by successive washes in ethanol 70% (6 baths), 95 % (2 baths), 100 % (2 baths) and finally in butanol (2 baths). Each sample was then included in paraffin, and cut with a microtome. After paraffin removal and rehydration, gonad sections were coloured with Hemalun-eosine, and embedded in Eukitt. Observations and numerical pictures were made using an Olympus BX 63 microscope.

The following indexes were calculated:

The gonadosomatic index (GSI: gonad weight x100/ body weight)

The hepatosomatic index (HIS: liver weight x100/ body weight)

The digestive tract–somatic index (DTSI: digestive tract weight x100/ body weight)

5.2 Results

5.2.1 Histological determination of gonadal sex

5.2.1.2 Sampling of 1 year-old fish (2012)

Twenty shads had been sampled in June 2012.

For 8 fish, gonads could be recognized by eye during the body dissection. They appeared as small strings along the body walls. They were dissected out and quickly placed in freshly prepared Bouin's fixative solution, for histological analyses. For the other fish, when gonads were too small to be identified and dissected, a transversal body section was fixed in Bouin's solution.

Histology indicated that macroscopically observable gonads (that could have been dissected out, as indicated above) corresponded to ovaries (4 fish) or to testis (4 fish).

For body sections, gonads could be identified by histology for 3 individuals, and corresponded either to ovaries (2 fish), or to testis (1 fish). In the other cases we could not observe the gonads on the histology of the body slices. The reasons could be either that the gonads did not remained attached to the body walls during histological processing, or that the body section was made anterior to the site of fixation of the gonads to the body walls.

Altogether, histological analysis of the gonads could be successfully performed for 11 one-year old shads, and revealed that they corresponded to 6 females and 5 males.

This first result shows a balanced sex ratio, which allows to hypothesize that controlled reproduction and rearing have no effect on sex ratio.

5.2.1.2 Sampling of 2 year-old fish (2013)

A total of 20 shads were sampled in June 2013.

Gonads could be recognized by eye along the body walls, during the body dissection. Ovaries were larger than the testes and with a light orange colour, while testes had a white colour (**Figure 24**). Gonads were dissected out and quickly placed in freshly prepared Bouin's fixative solution, for histological analyses.

Histology confirmed the visual sex determination, except for two fish (n° 38 and 42), for which the histological samples collected did not include the gonads. These two fish were removed from the analyses.

Altogether, histological analysis of the gonads could be successfully performed for 18 two-year old shads, and revealed that they corresponded to 9 females and 9 males. This further assessed that the ex-situ rearing conditions did not affect sex differentiation and sex-ratio.

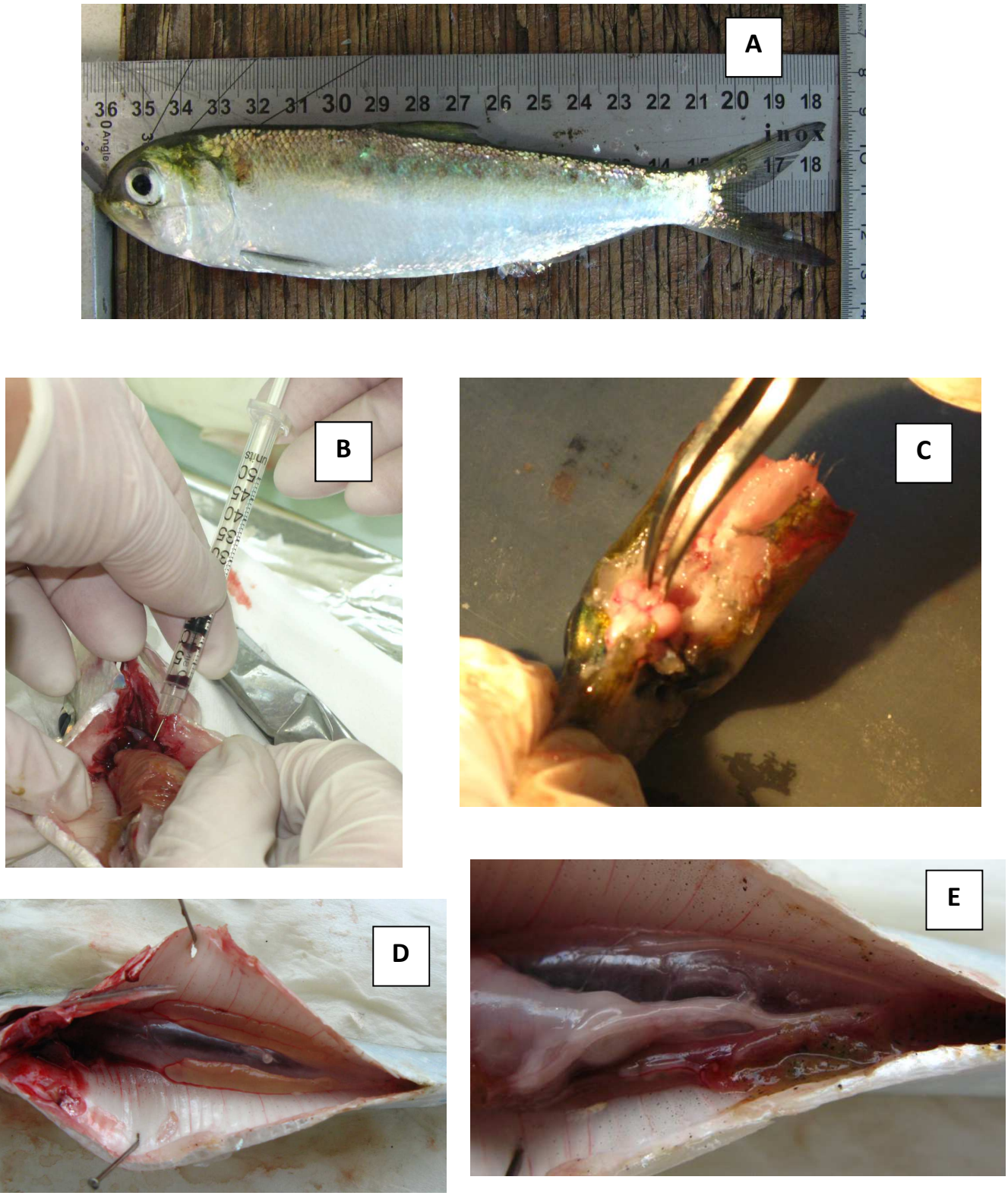


Figure 24 : Length measurement, A – blood sample, B – brain sample, C – ovary, D – testis, E.

5.2.1.3 Sampling of 3 year-old fish (2014)

Twenty two fish were sampled in June 2014. One fish (n°47) was one of the few survivors of the transfer from seawater to freshwater, performed in April 2014. This male fish had the smallest size, possibly reflecting stress and arrest of feeding. All the other fish were from the batch maintained in seawater.

During the dissection, ovaries (large size and orange colour) could be distinguished from testis (smaller size and white colour) in most three-year-old fishes.

Gonad histology confirmed visual sex determination, except for fish n° 57, for which the gonad could not be recovered from the histological sample. This fish was removed from the following analyses.

Among the 21 fish analyzed, 13 were females and 8 males.

5.2.1.4 Sampling of 4 year-old fish (2015)

Twenty five fish were sampled. Among them, five had been transferred from seawater (SW) to freshwater (FW) during the spring (3 females: n°69, 70, 71; and 2 males: n°72 and 73). The others had been maintained in seawater.

The liver and digestive tract were also dissected and weighed for the determination of the hepatosomatic index (HSI) and digestive tract-somatic index (DTSI), respectively.

During the dissection, ovaries and testis could be easily visually distinguished in most four-year-old shads. Some male and females had even very well developed gonads (**Figure 25** and **Figure 26**). When handled after anesthesia, some male fish were spermiating.

Gonad histology confirmed visual sex determination, except for fish n° 73, for which the gonads could not be recovered from the histological sample. This fish was removed from the following analyses.

Among the 24 fish analyzed, 6 were females, and 18 males.

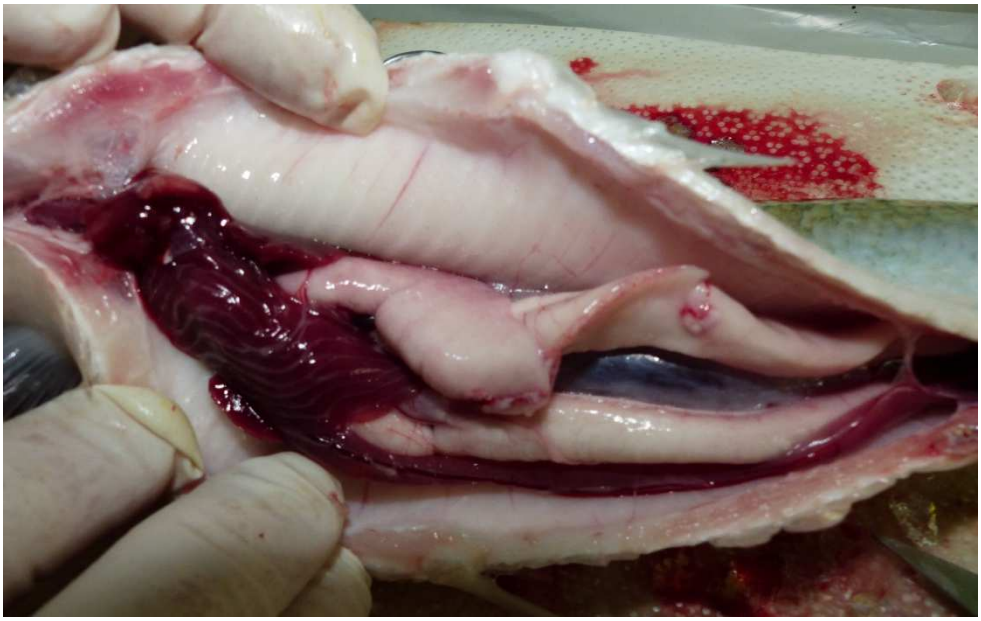


Figure 25 : Dissection of a 4 year-old male (male N°76 ; GSI=3.42)

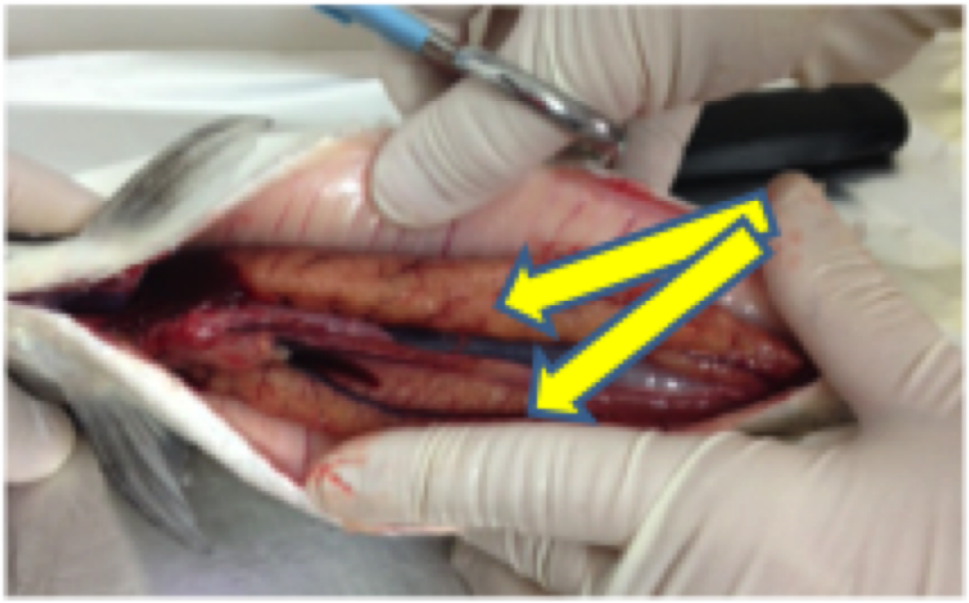
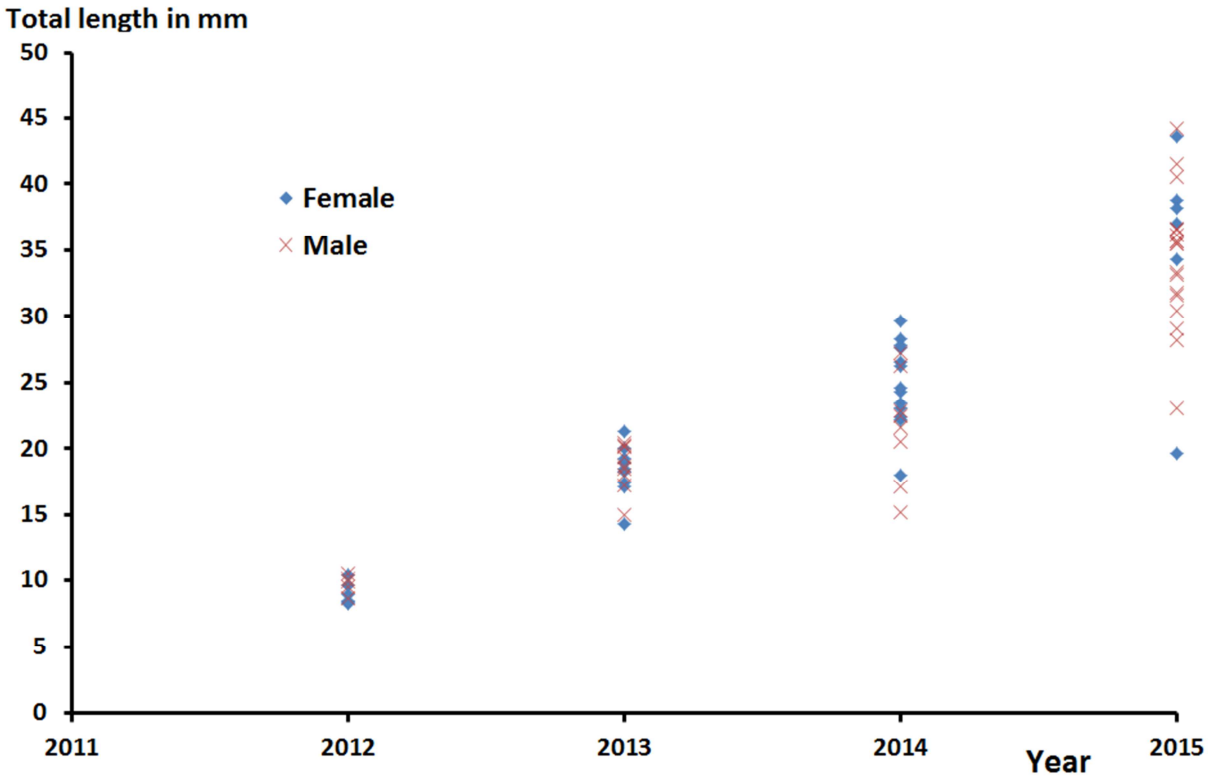


Figure 26 : Dissection of a 4 year-old female (female N°69 ; GSI=6.75)

5.2.2 Biometric parameters

There was no significant difference between females and males, whatever the age, neither for the total length nor for the weight (**Figure 27**). This similar growth between sexes is not observed in the wild, and could be likely due to our rearing conditions.



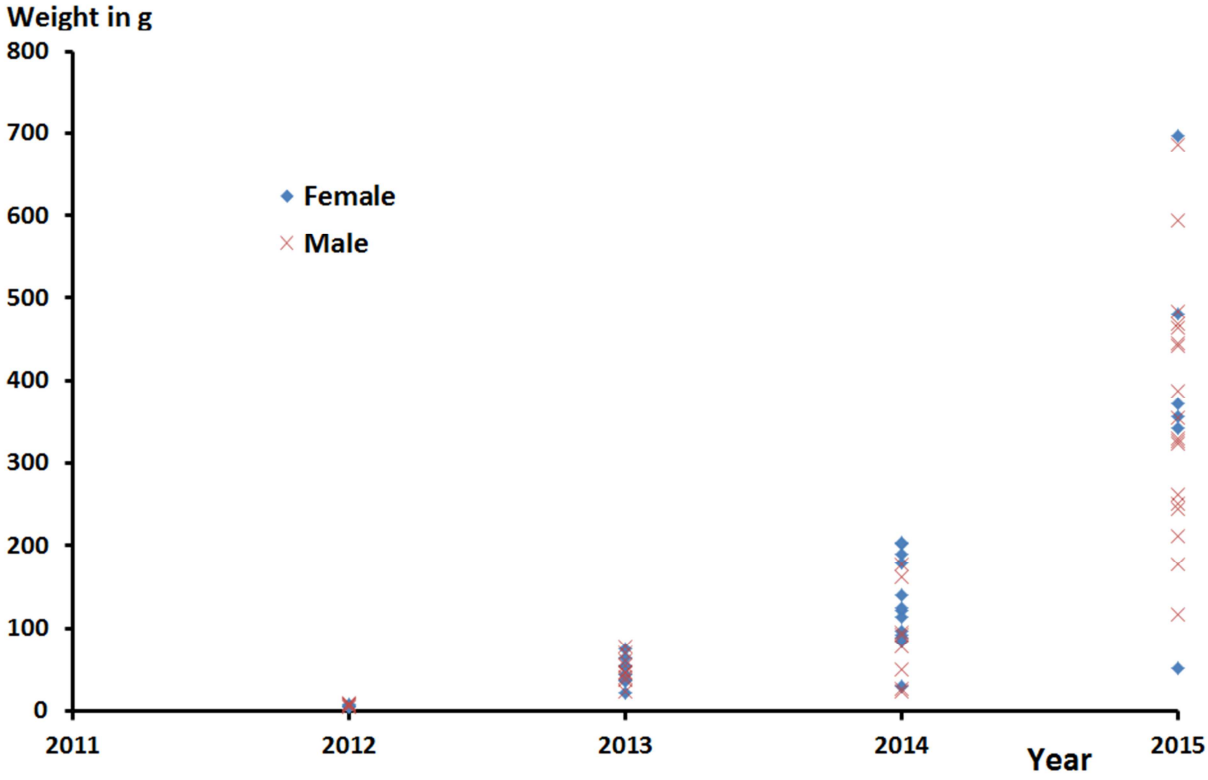


Figure 27 : Monitoring of the body length (total length – above) and the body weight (below) for females and males

The Gonado Somatic Index was higher in females, but nearly constant between 2 year-old and 3 year-old fish (**Figure 28**).

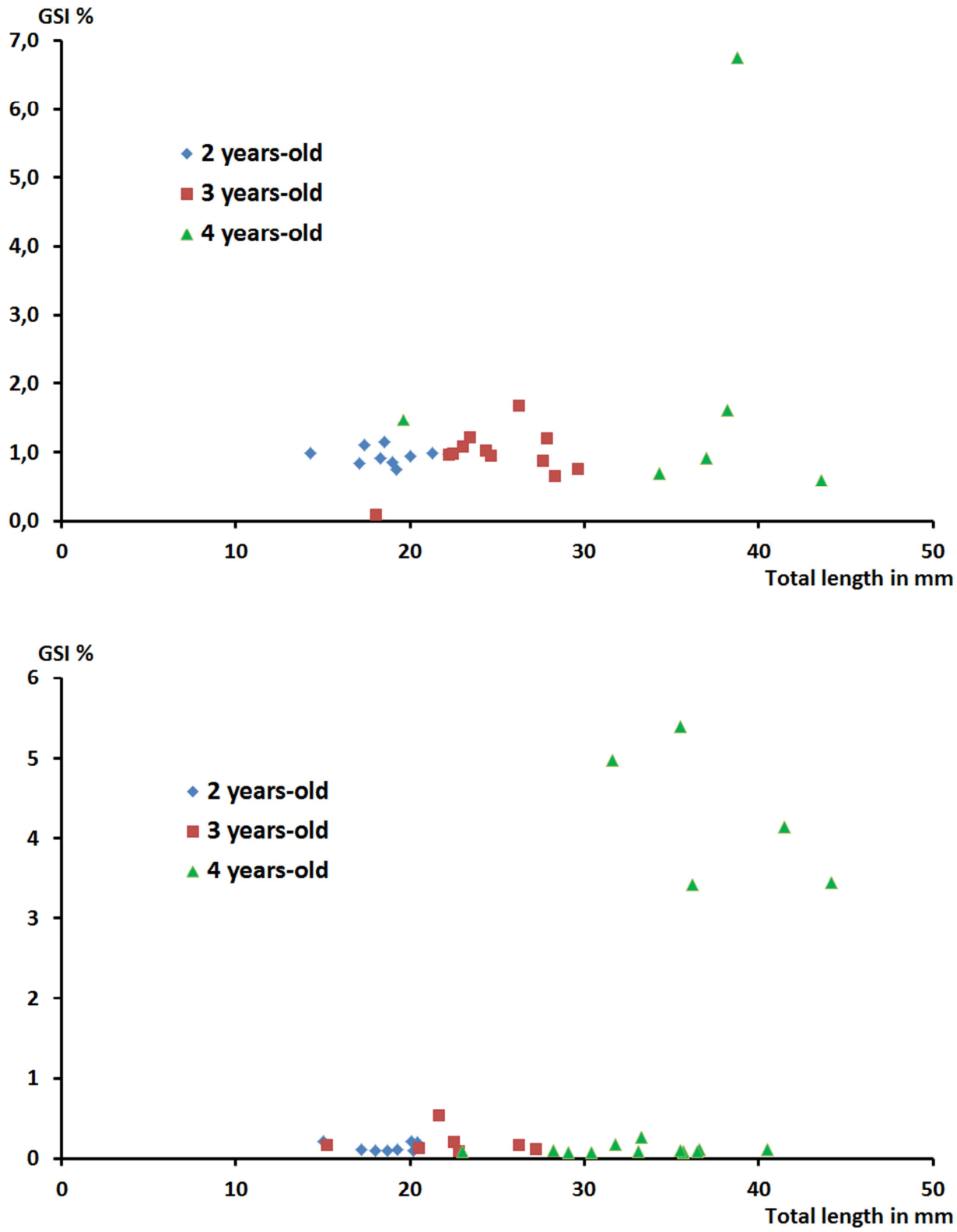


Figure 28: Monitoring of the GSI in females (above) and males

The gonadosomatic index calculated in 4 year-old fish, (June 2015), ranged between 0.60 and 6.75 (mean 2.0 ± 0.9) in females and between 0.07 and 5.4 (mean 1.21 ± 0.47) in males. This was the first year that a high GSI was observed in a female (in one over six sampled females: n° 69, in freshwater) and in some males (in five over eighteen sampled males, n° 74, 75, 78, 91, 92, in seawater).

Hepatosomatic Index and the Digestive-Tract Somatic Index were also similar between sex in 4 year-old fish. The absence of mean HIS difference between females and males, could be explained by the very low number of female with advanced vitellogenesis.

In the other hand, fish having a high GSI also presented a low DTSI (**Figure 29** and **Figure 30**). During the final maturation phase and migration to freshwater, fish stop feeding. Our results could reflect this physiological trait, showing that some fish would have been in final maturation.

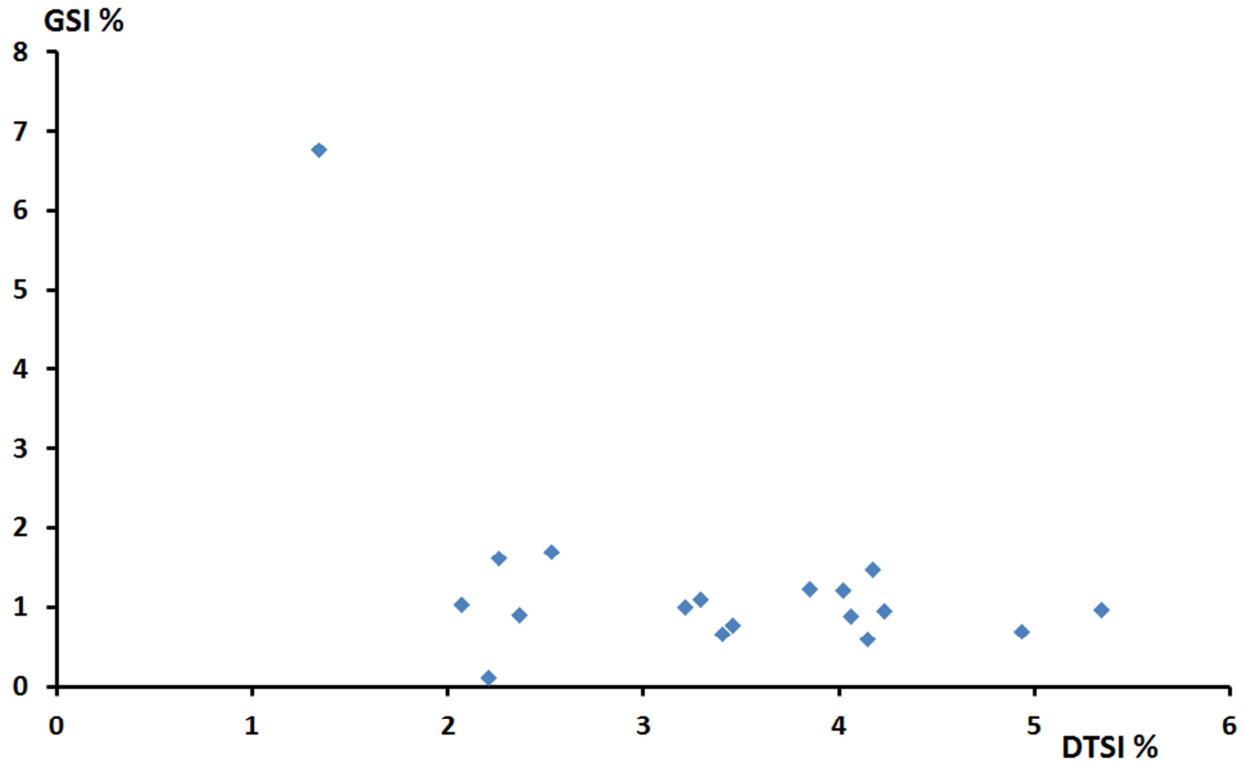


Figure 29 : Relationship between GSI and DTSI in females (3 and 4 years-old)

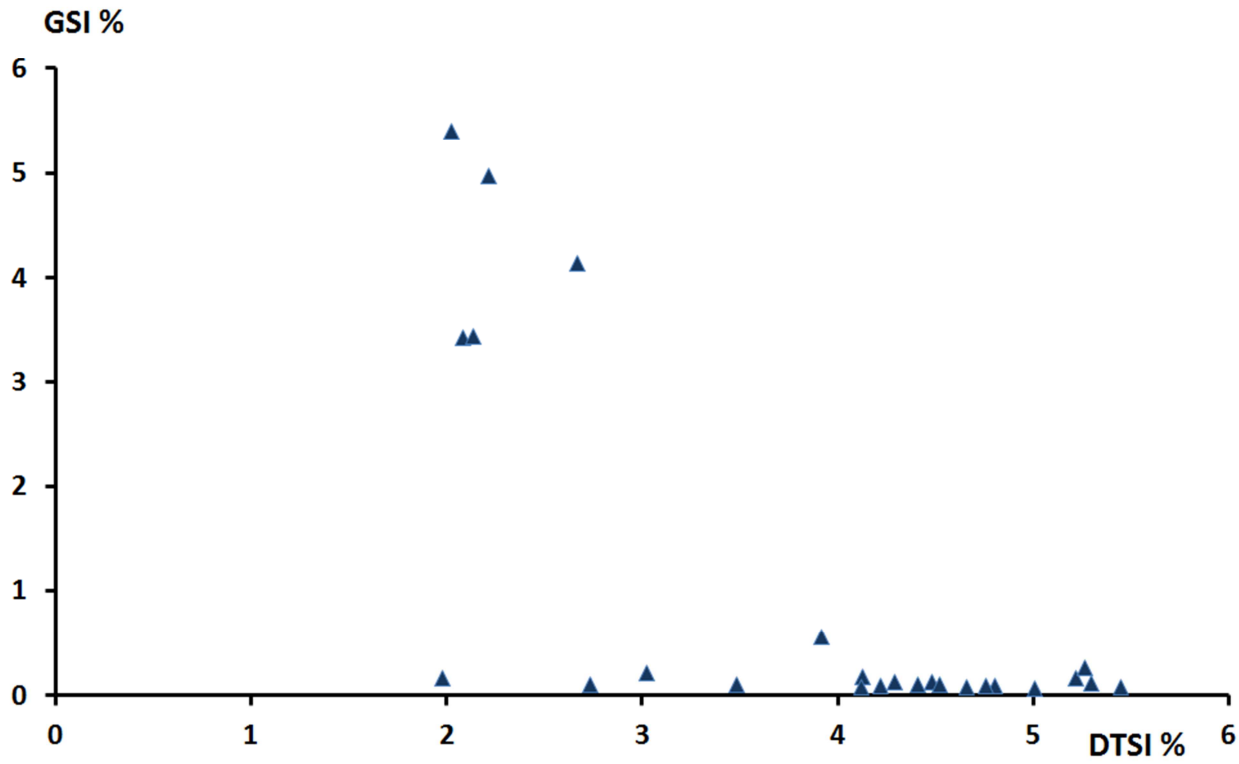


Figure 30 : Relationship between GSI and DTSI in males (3 and 4 years-old)

5.2.3 Histological analysis of the gonads

For each female, the diameter of the 10 larger oocytes was measured to monitor the oocyte development.

5.2.3.1 One year-old fish (sampling June 2012)

In female ovaries, histology showed the presence of oogonia, figures of mitosis, as well as primary oocytes with a large nucleus and dense ooplasma (**Figure 31**). This indicates that one year-old females were at the gonial and primary oocyte stage. Oocytes of various diameters were observed. Mean diameter for all females ± SE is 36.7 ± 4.62 µm. We can notice difference in mean diameter between females, ranging between 20 ± 1 and 49.8 ± 1.8 µm.

Histological observation of the testis showed spermatogonia, and many figures of mitosis. This indicates that one-year old males were at a spermatogonial stage with active gonial division, pointing out a normal development (**Figure 32**).

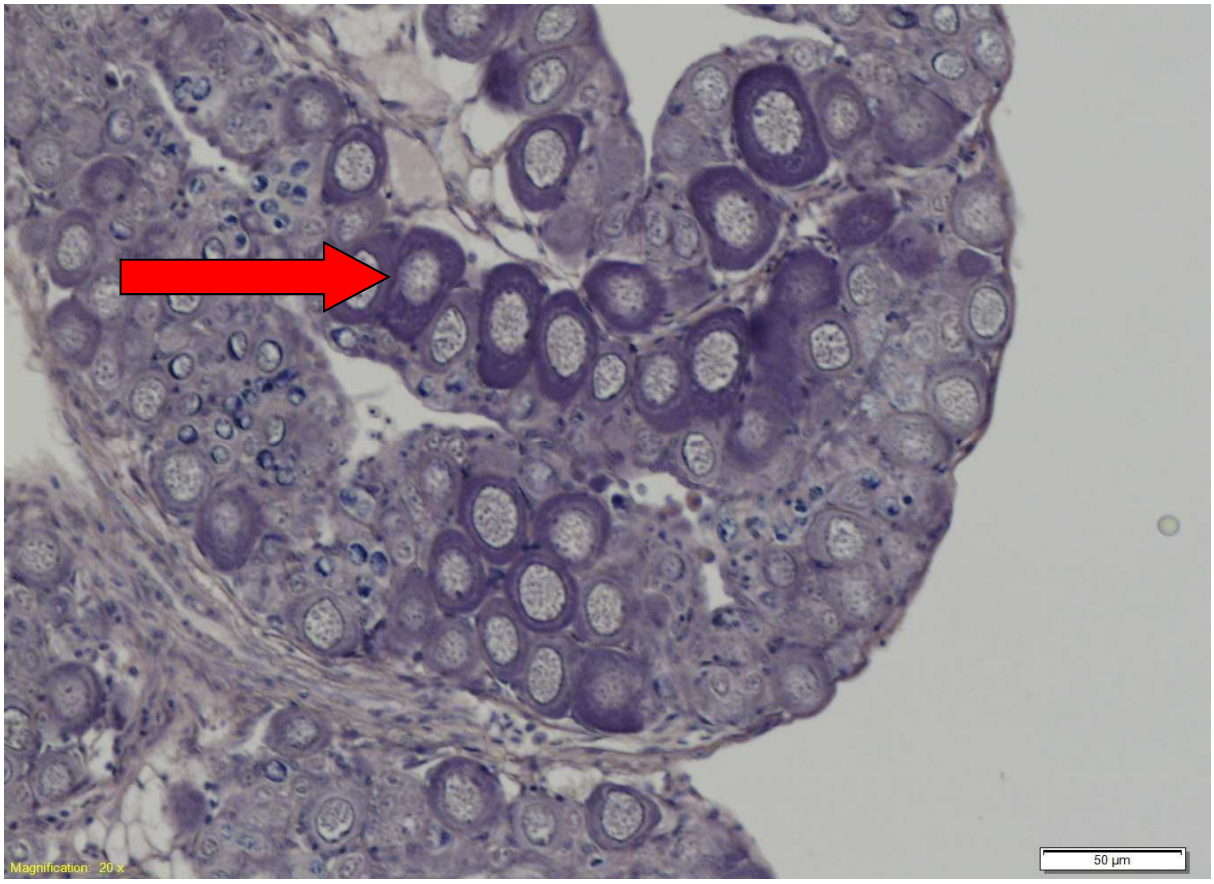


Figure 31 : Histological section of female gonad showing oocytes stage 1 (red arrow).

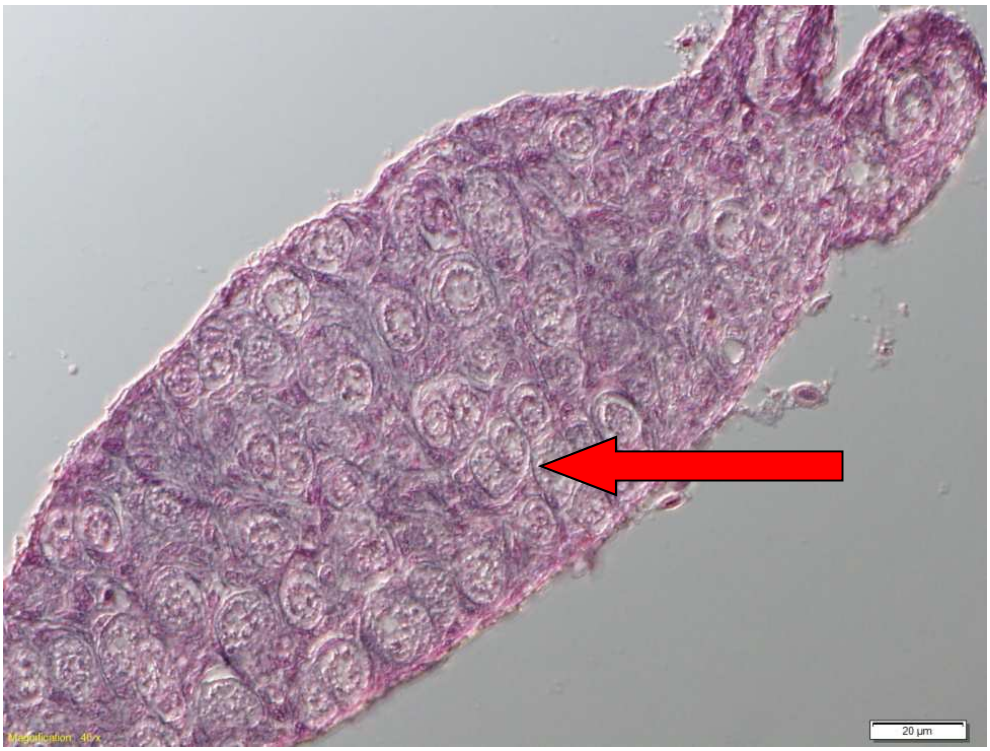


Figure 32 : Male gonad with numerous spermatogonia, and figure of mitosis (red arrow).

5.2.3.2 Two year-old fish (sampling June 2013)

Histology of the ovaries showed the presence of numerous primary oocytes, characterized by a dense ooplasma, and a large nucleus with many perinuclear nucleoli (**Figure 33** and **Figure 34**). This indicates that two year-old females are at the primary oocyte stage. As compared to one-year-old females, the oocytes of two-year old females are enlarged, but still at the primary oocyte stage.

The presence in the ooplasma of a few lipid vesicles, indicating the initiation of the lipid vesicle stage, could be observed only in one fish (**Figure 35**).

The mean oocyte diameter ranged between 93 ± 3.4 (SEM) µm to 137.7 ± 4.9 µm according to fish. The mean diameter of the largest oocytes of two-year old shads was 109.8 ± 4.3 (SEM) µm.

As compared to one-year old fish (36.7 ± 4.6 µm), these results indicate a 3 fold increase ($P<$ 0.001) in the primary oocyte diameters in two-year old fish, which reflects the progress of primary oocyte growth.

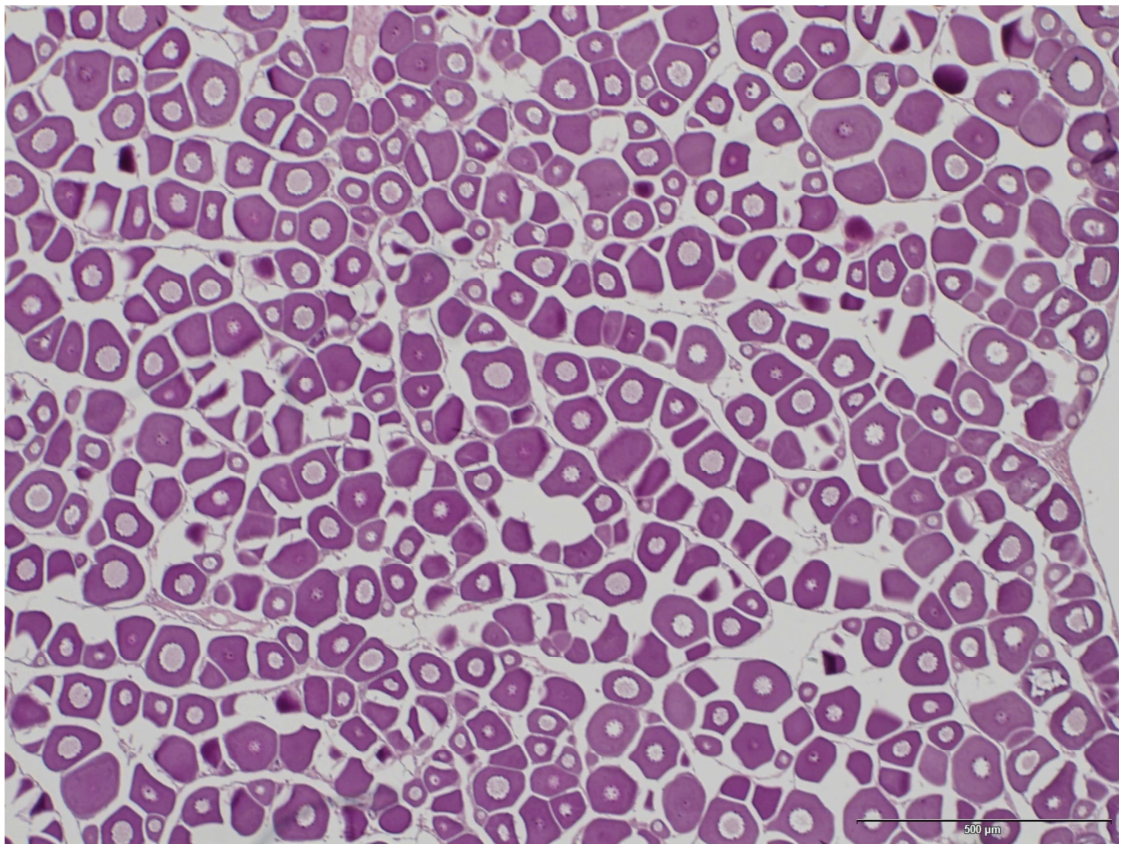


Figure 33 : Histological section of ovary from two-year old female *Alosa alosa* (Female n°40; x 4) - Numerous primary oocytes are observed.

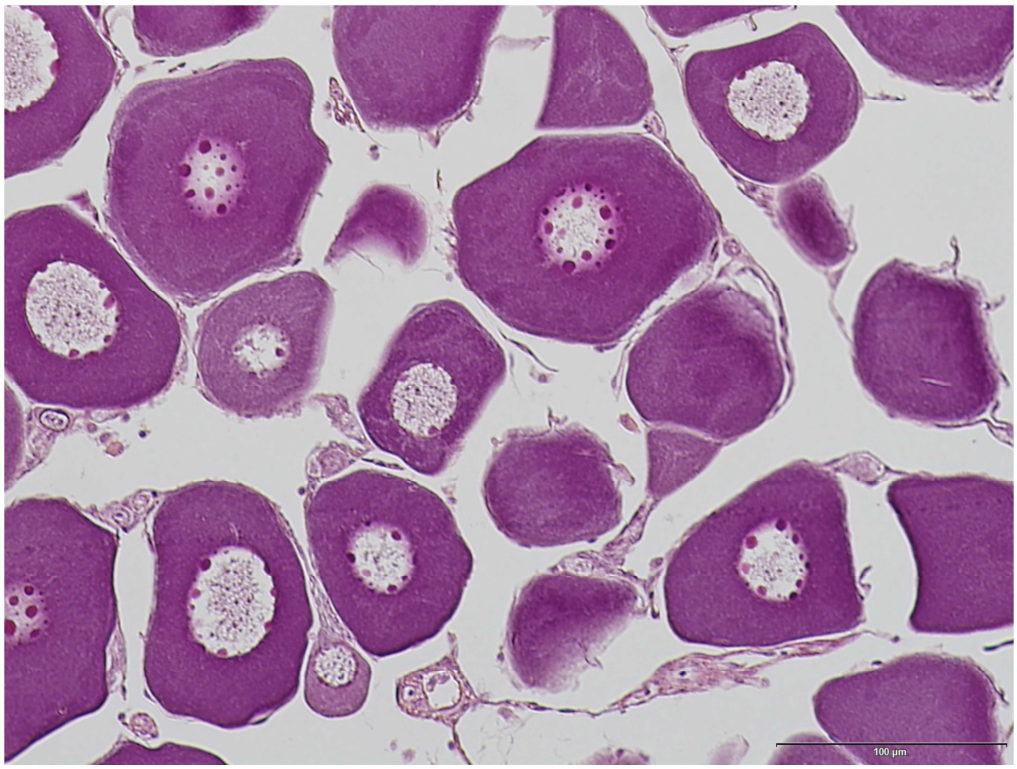


Figure 34 : Histological section of ovary from two-year old female *Alosa alosa* (Female n°40; x 20) Primary oocytes with dense ooplasma. and perinuclear nucleoli are observed.

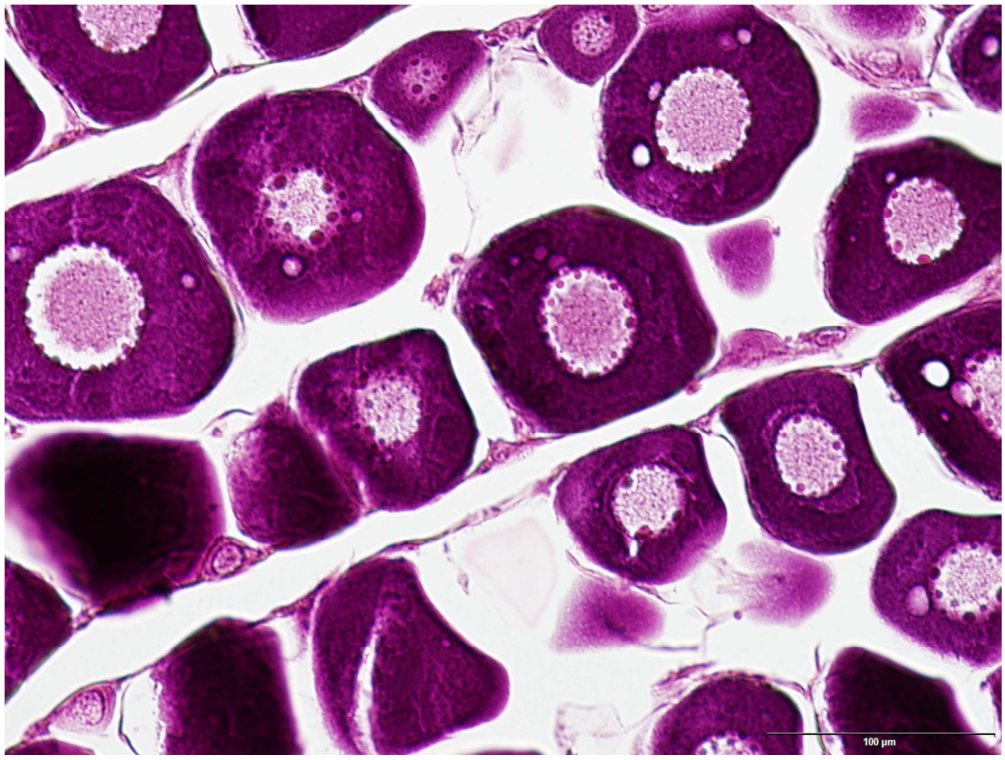


Figure 35 : Histological section of ovary from two-year old female *Alosa alosa* (Female n°31; x 20) Primary oocytes with dense ooplasma and a few lipid vesicles, and perinuclear nucleoli are observed.

Histological observation of the testes showed numerous spermatogonia, with many figures of mitosis. This indicates that the two-year old males are at a spermatogonial stage with active gonial division. The most advanced stages showed formation of cysts (**Figure 36**). As compared to one-year old males, the testes of two-year old males are larger, but still at the spermatogonia proliferation stage.

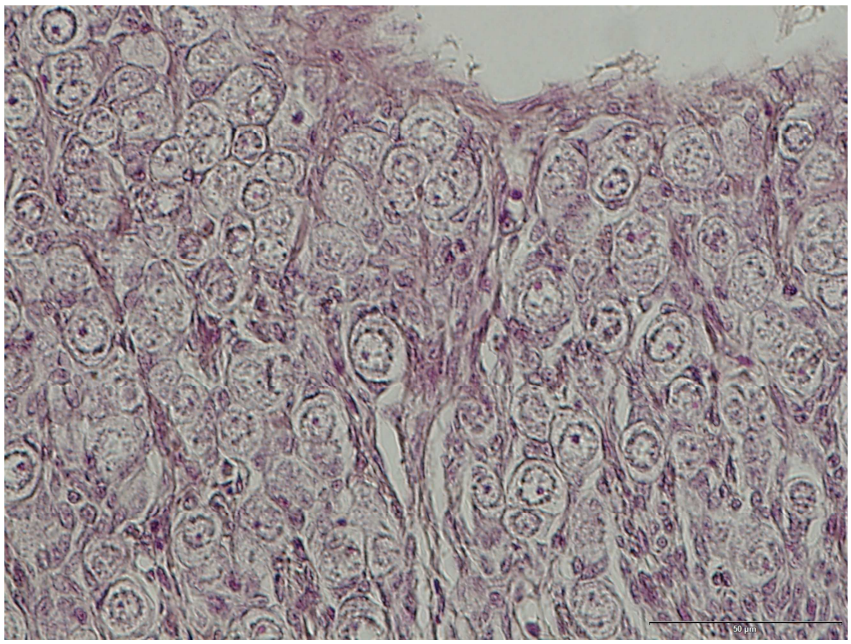


Figure 36 : Histological section of testis from two-year old male *Alosa alosa* (Male n° 25; x 40) - Numerous spermatogonia organized in cysts, with many mitoses are observed.

5.2.3.3 Three year-old fish (sampling June 2014)

The ovaries of most three-year-old females were still at the primary oocyte stage (**Figure 37**). In one case, more advanced vitellogenic oocytes were observed (**Figure 38**).

The mean oocyte diameter ranged between 61.7 ± 12.8 to 189.8 ± 10.4 µm, according to fish. Altogether, the mean diameter of the largest oocytes of three-year-old shads was 140.8 ± 9.2 µm. As compared to two-year-old females (109.8 ± 4.3) this indicates an average 1.3 fold increase.

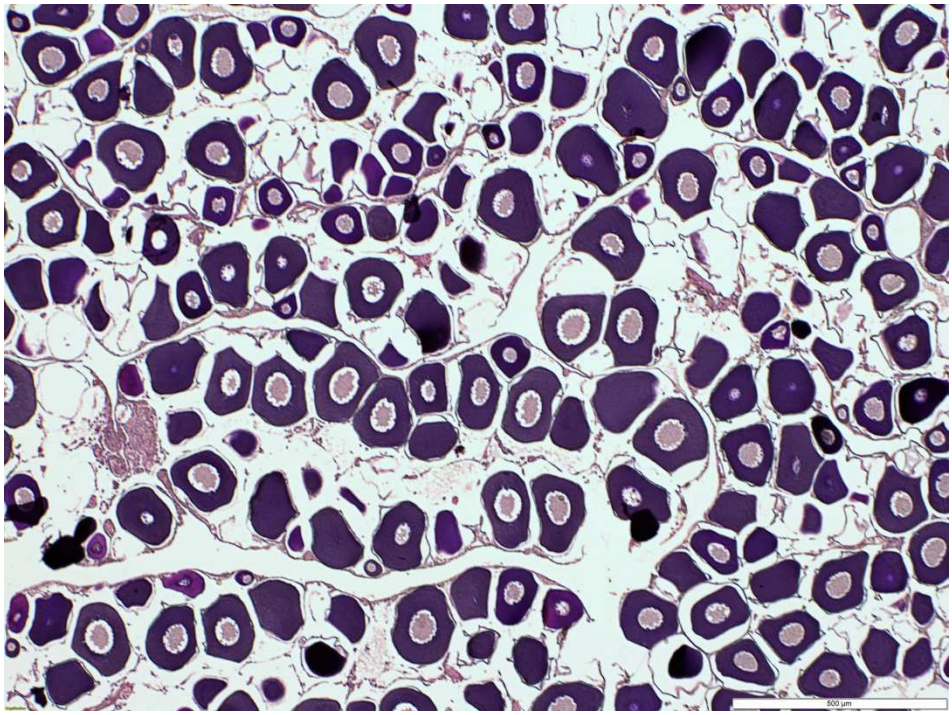


Figure 37 : Histological section of ovary from three-year-old female *Alosa alosa* (Female n° 51; GSI: 0.88) x 4. Primary oocytes, with dense ooplasma and perinuclear nucleoli are observed.

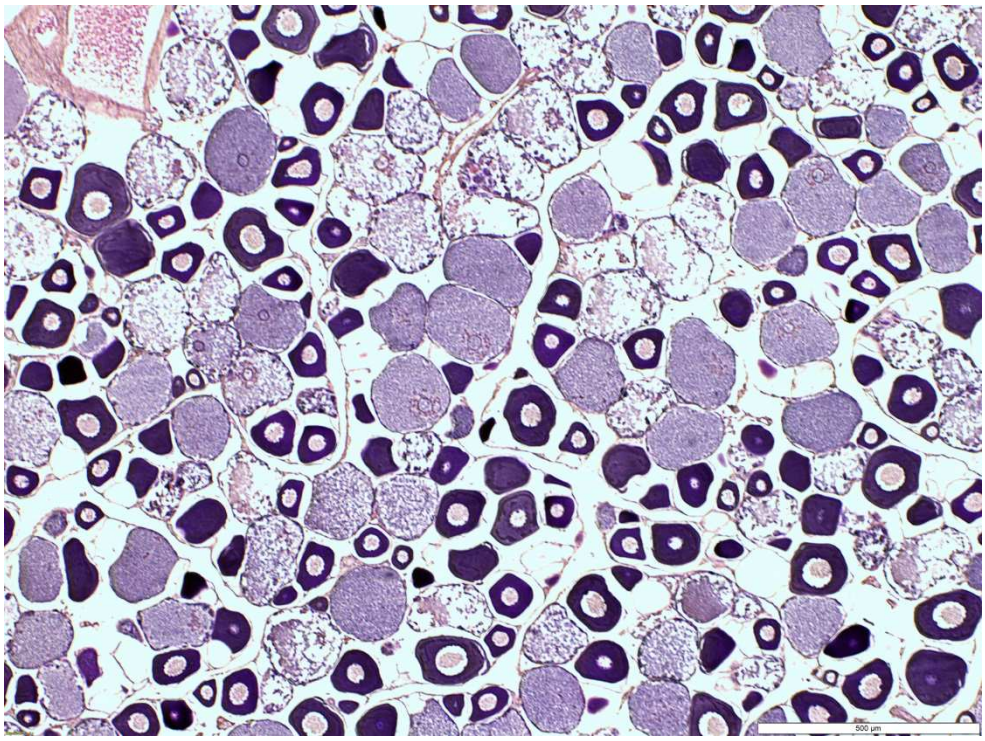


Figure 38 : Histological section of ovary from three-year-old female *Alosa alosa* (Female n°55; GSI: 1.69) x 4. Primary oocytes with dense ooplasma, as well as larger vitellogenic oocytes are observed.

In some three-year-old males, testes were still at the spermatogonial proliferation stage, as in previous years (**Figure 39**). The most advanced stage was found in one fish, with numerous spermatids (**Figure 40**).

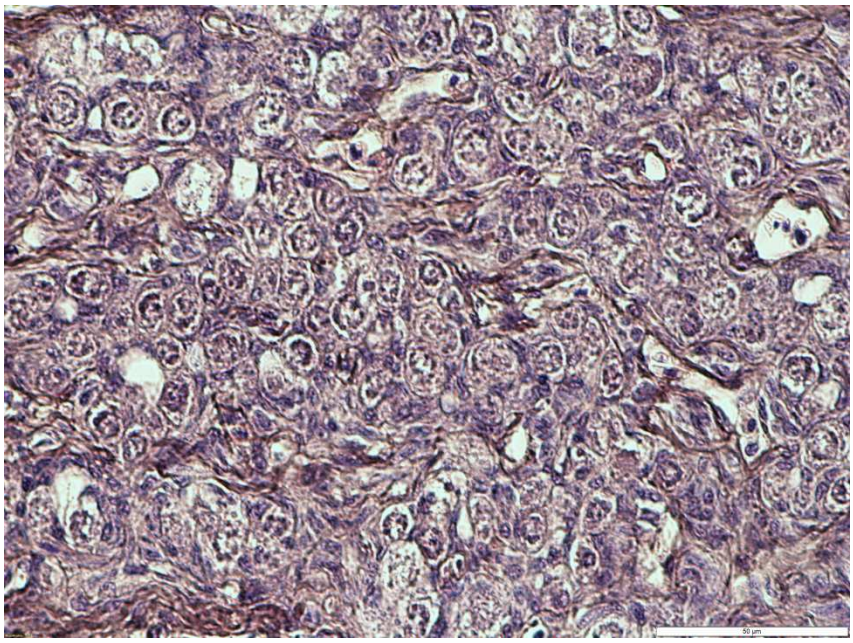


Figure 39 : Histological section of testis from three-year-old male *Alosa alosa* (Male n° 67; GSI: 0.09) x 40. Spermatogonial mitoses are observed.

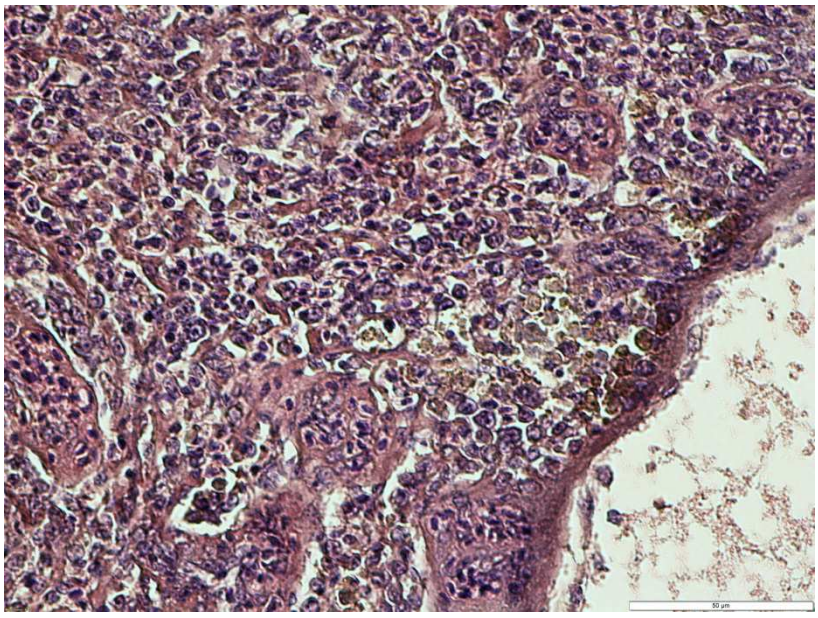


Figure 40 : Histological section of testis from three-year-old males *Alosa alosa* (Male n° 53; GSI: 0.7) x 40. Advanced stage of spermatogenesis, with numerous post-meiotic small cells (spermatids).

5.2.3.4 Four year-old fish (sampling June 2015)

Some four-year-old females were still at an early vitellogenic stage (**Figure 41**). In contrast, in one female, highly developed oocytes could be observed for the first time (**Figure 42** and**Figure** 43).

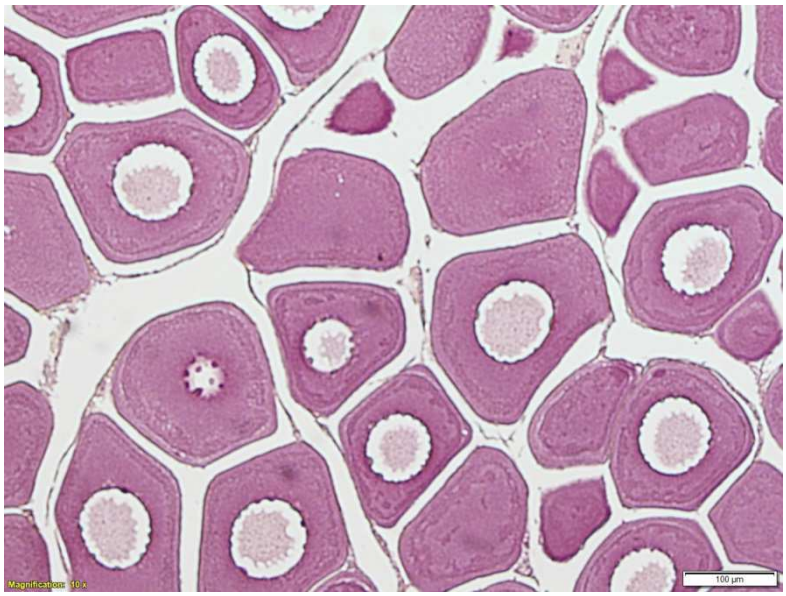


Figure 41 : Histological section of ovary from four-year-old female (Female n° 71 (FW); GSI= 0.91) x10. Early vitellogenic oocytes are observed.

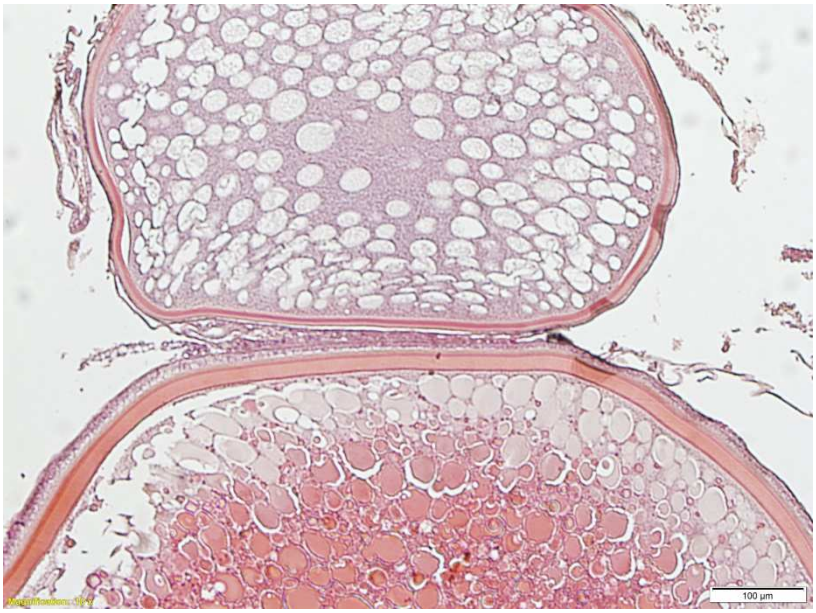


Figure 42 : Histological section of ovary from four-year-old female (Female n° 69 (FW); GSI: 6.75) x10. Large full vitellogenic oocyte are observed.

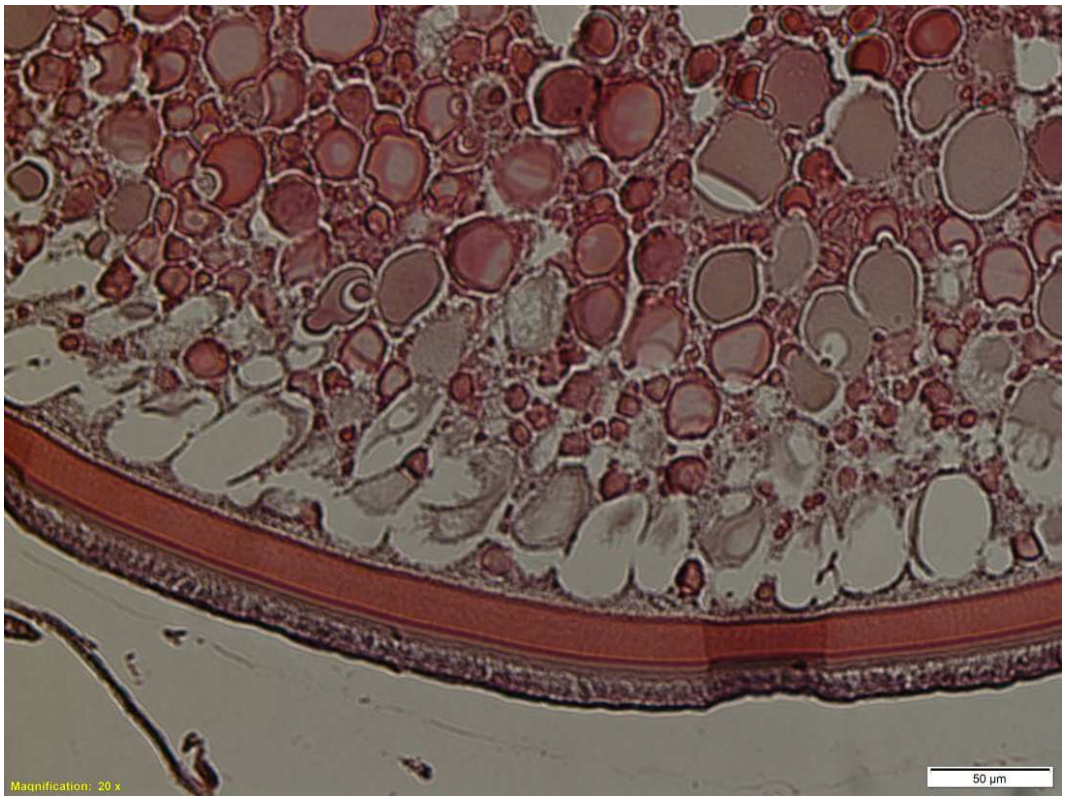


Figure 43 : Histological section of ovary from four-year-old female (Female n° 69 (FW); GSI: 6.75) x20. Higher magnification providing details on the oocyte envelope (zona radiata) and ooplasma inclusions.

The mean diameter ranged between 87 ± 9.27 and > 600 µm, according to fish. In the case of female n°69, as the current histological sections did not cross through nuclei, the size was estimated as > 600 µm. Altogether, the mean diameter of the largest oocytes of four-year-old shads was 217 ± 68 µm.

In some four-year-old males, testes were still at the spermatogonial proliferation stage, as in previous years (**Figure 44**). In contrast, fully mature testes, with spermatozoa, could be observed for the first time in five males (**Figure 45**).

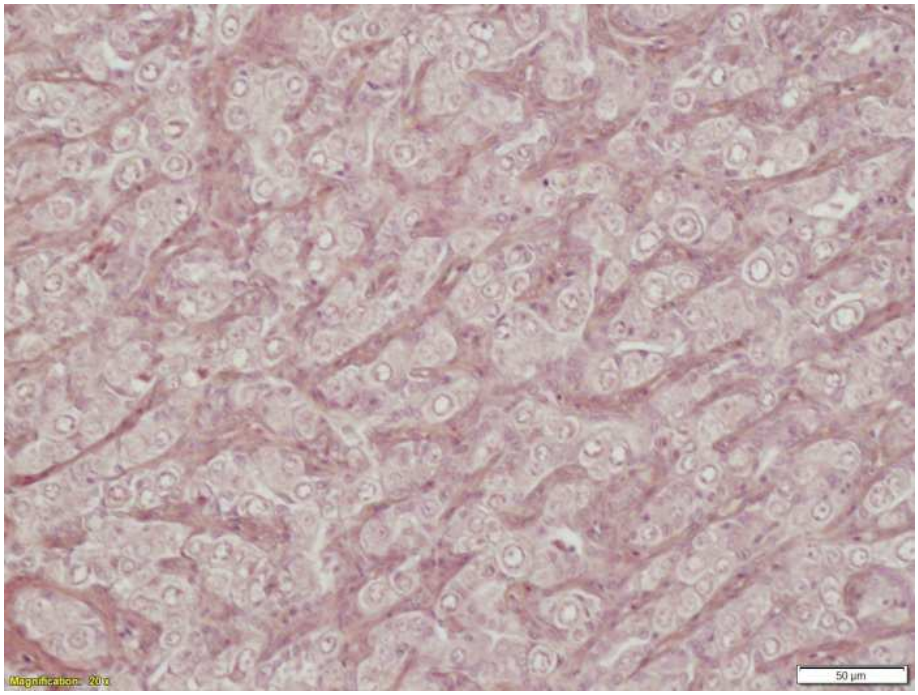


Figure 44 : Histological section of testis from four-year-old male (Male n° 72 (FW); GSI: 0.17) x20. The testis is still immature, at the spermatogonial stage.

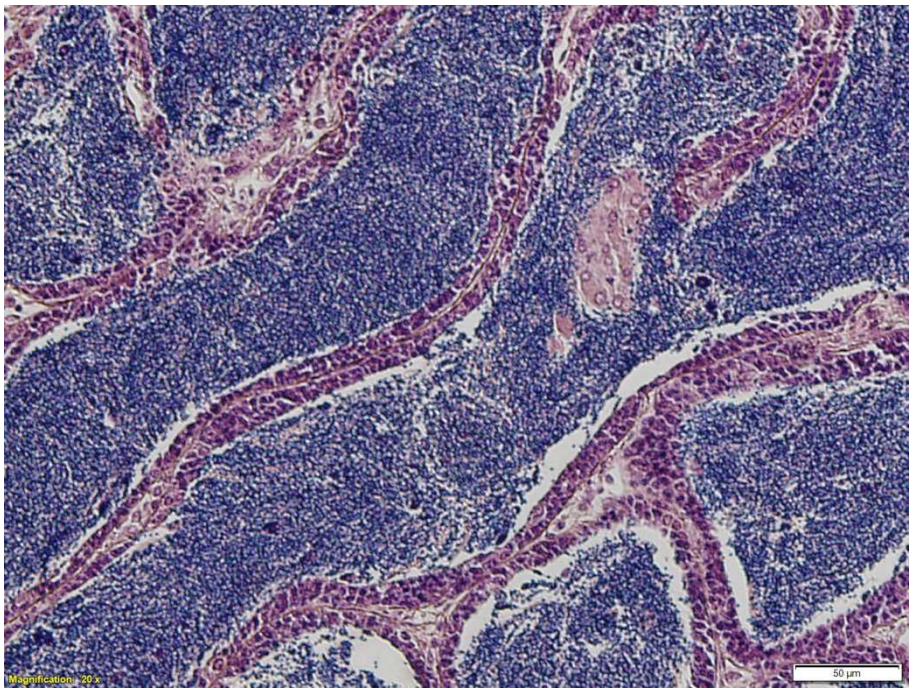


Figure 45 : Histological section of testis from four-year-old male (Male n° 78 (SW); GSI: 4.14) x20. The testis is fully mature with numerous spermatozoa (blue color).

5.2.4 Role of transfer to freshwater on sexual maturation

Interestingly, the five mature (spermiating) four-year-old males had been maintained in seawater. This indicates that transfer to freshwater would not be necessary for male full sexual maturation. Further studies should still aim at investigating the quality and viability of sperm in seawater versus freshwater.

In contrast in females, the only mature four-year-old female, was an individual that had been transferred to freshwater (**Figure 42 andFigure** 43). This suggests that transfer to freshwater may be necessary for inducing advanced vitellogenesis.

However, in the case of another female that has been also transfered to freshwater (**Figure 46** and**Figure** 47), histological analysis revealed both small primary oocytes and numerous unusual features that could be interpreted as resorption of larger follicles. This may suggest that transfer to FW would may have induced resorption of early vitellogenic oocytes.

However, since these observations are relying on a too few number of fish, further studies are strongly required in order to address these hypothesis.

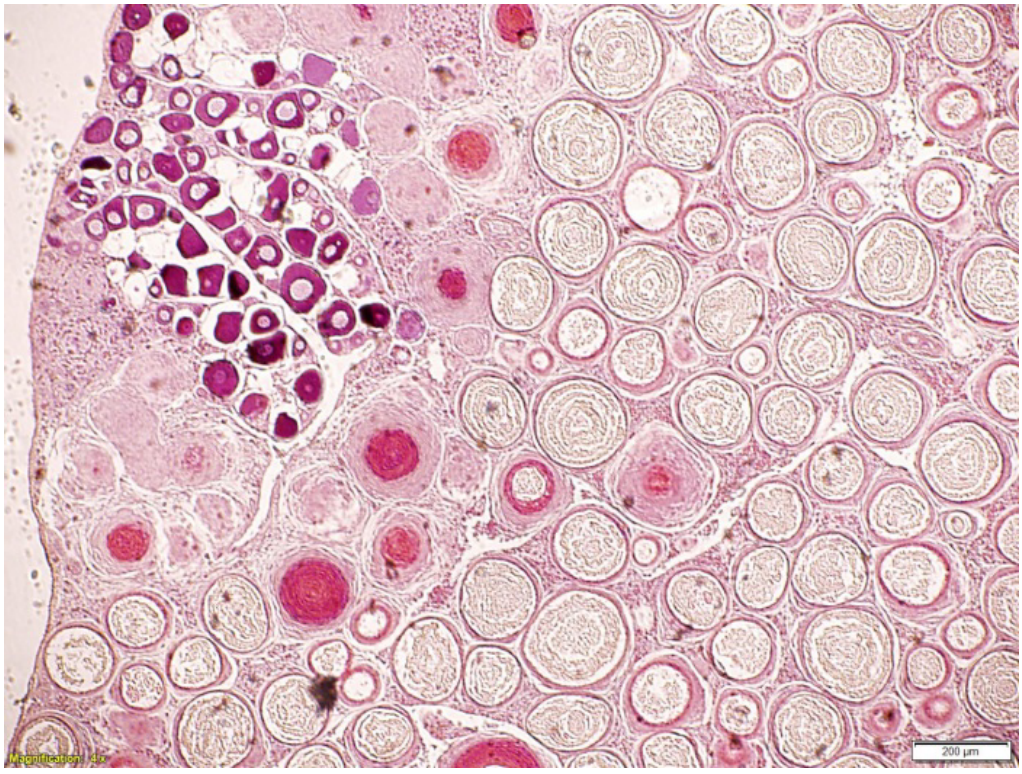


Figure 46 : Histological section of ovary from four-year-old female (Female n° 70 (FW); GSI: 1.46) x4. Some small primary oocytes (upper left) are observed beside numerous larger oocytes undergoing resorption.

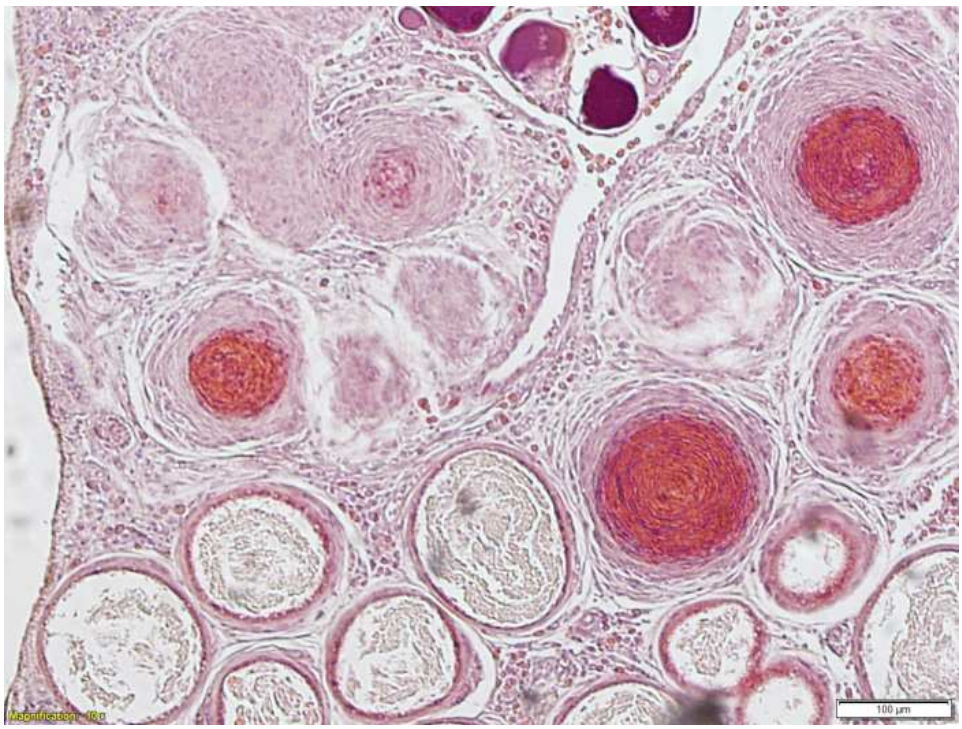


Figure 47 : Histological section of ovary from four-year-old female (Female n° 70 (FW); GSI: 1.46) x10. Higher magnification showing oocytes under resorption.

5.2.5 Synthesis of the gonadal development in allis shad raised in captivity

Testes were at spermatogonial stage in two-year-old males (sampled in 2013), as well as in most three-year-old males (sampled in 2014), and still in some four-year-old males (sampled in 2015). One case of advanced spermatogenesis (with spermatids) could be observed in one three-year old male.

Importantly, five (over eighteen) four-year-old male were fully matured, with many spermatozoa in the testis. Spermiation could be noticed, when handling the anesthetized fishes. This suggests that a relevant proportion (> 25%) of four-year-old males may reach sexual maturation in captivity. The smallest of these mature males had a body length of 31 cm and body weight of 225 g, which together with the age of 4 years, could be considered, in our rearing conditions, as minimal thresholds for male maturation.

Ovaries were at the primary oocyte stage in two-year-old females. Some significant growth in primary oocyte size, as well as some cases of early vitellogenic oocytes were observed in three-year-old females.

Mean oocyte diameters for all females sampled are plotted against total body length (**Figure 48**), or GSI **(Figure 49**). These figures highlight the only female showing an advanced gonadal maturation.

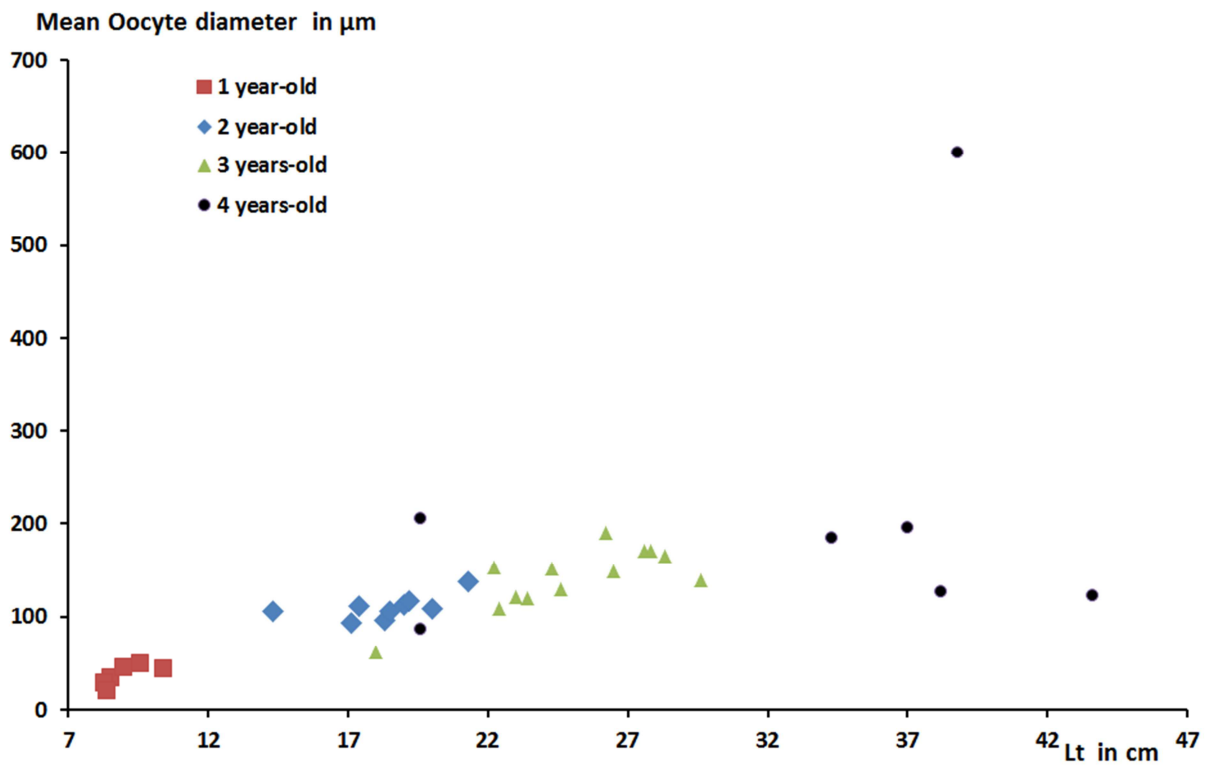


Figure 48 : Mean oocyte diameter plotted against total length for all females sampled.

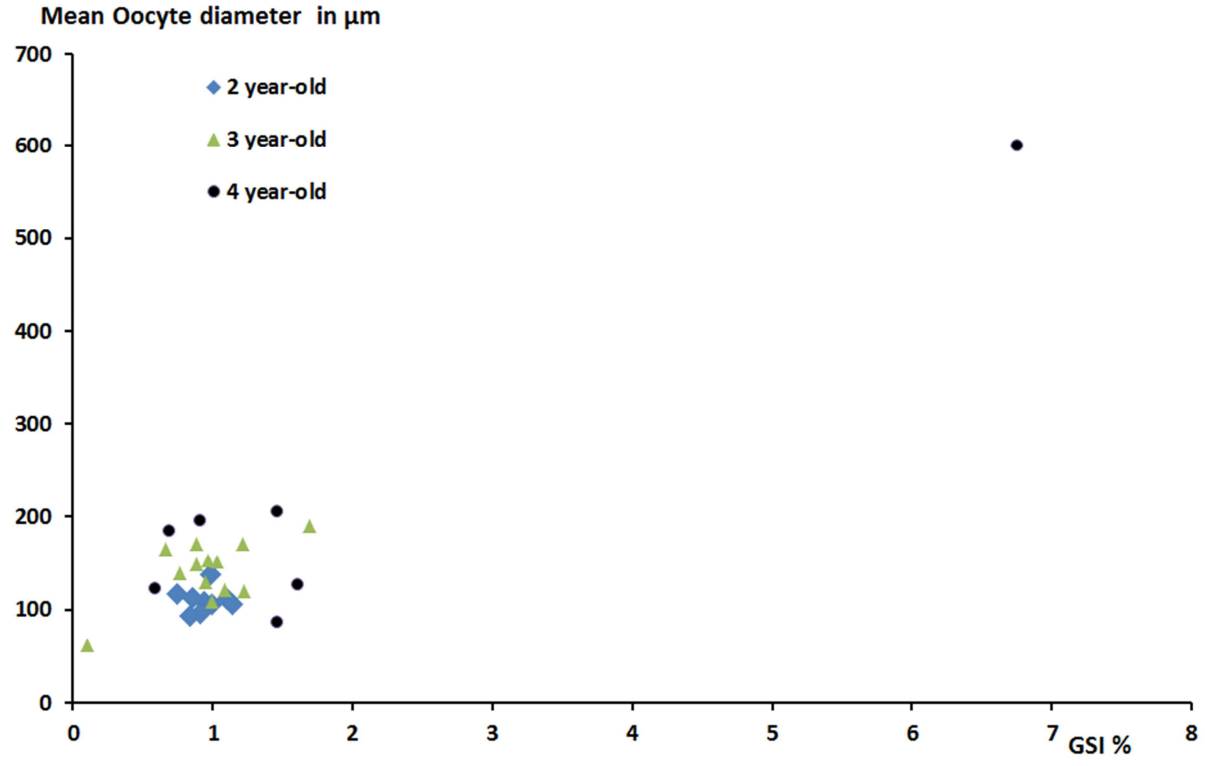


Figure 49 : Mean oocyte diameter plotted against GSI for all females sampled.

Remarkably, a single case of advanced vitellogenesis was observed in a four-year-old female (female n°69: GSI: 6.75). This female had a body length of 38.8 cm and a body weight of 480 g, which together with age of 4 years, could be considered as minimal thresholds for female sexual maturation, in our rearing conditions. However this unique case suggests that this may still be an exception in four-year-old females.

Comparison between males and females suggest that females may reach maturity at an older age and larger size than males. This represents a common feature in this species and among many other teleost species.

5.2.6 Immunoenzymatic assays of plasma steroid levels

The study of gonadal development was completed by immunoenzymatic assays (ELISA) of sex steroid plasma levels.

5.2.6.1. Estradiol plasma levels in females

After various tests previously performed on unextracted and extracted plasma samples from shads and eels (see previous report), we had discarded various kits such as the kit from Labor Diagnostica Nord Gmbf, Ref FR E-2000, which produced artefactual cross-reactions with fish plasma samples, and selected the kit Nova Tec, Ref DNOV003. We used this kit for the following assays in female shads.

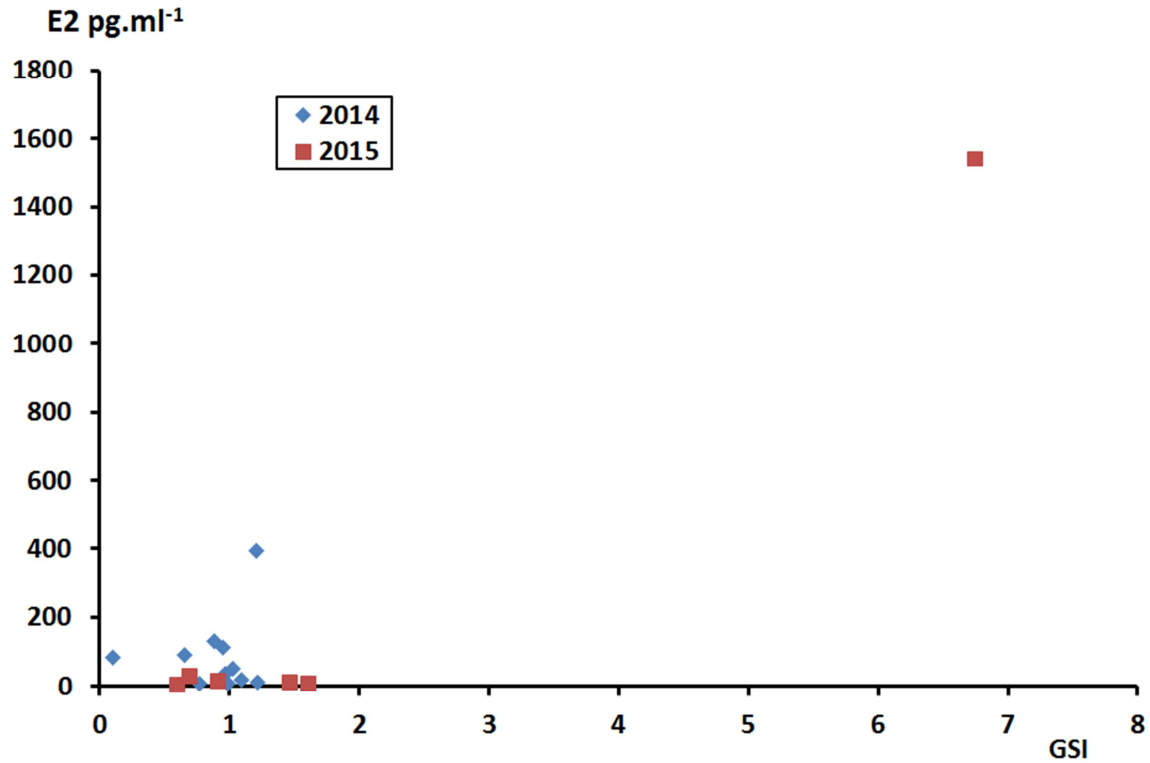


Figure 50: Relationship between gonadosomatic index and estradiol plasma levels in three- and four-year-old females.

Estradiol (E2) plasma levels were measured in plasma samples of three-year-old and four-year-old females. High level was only found in the case of the four-year-old female with high GSI (n° 69; GSI: 6.75) (**Figure 50**)

5.2.6.2. Testosterone plasma levels in males

For testosterone we had previously validated the kit Eurobio Ref EUDNOV002, based on eel plasma samples. We used this kit for the following assays in male shads.

High testosterone plasma levels were found in the five mature males with GSI > 3.4 (**Figure 51**). A significant correlation was observed between GSI and testosterone plasma levels (r^2= 0.848).

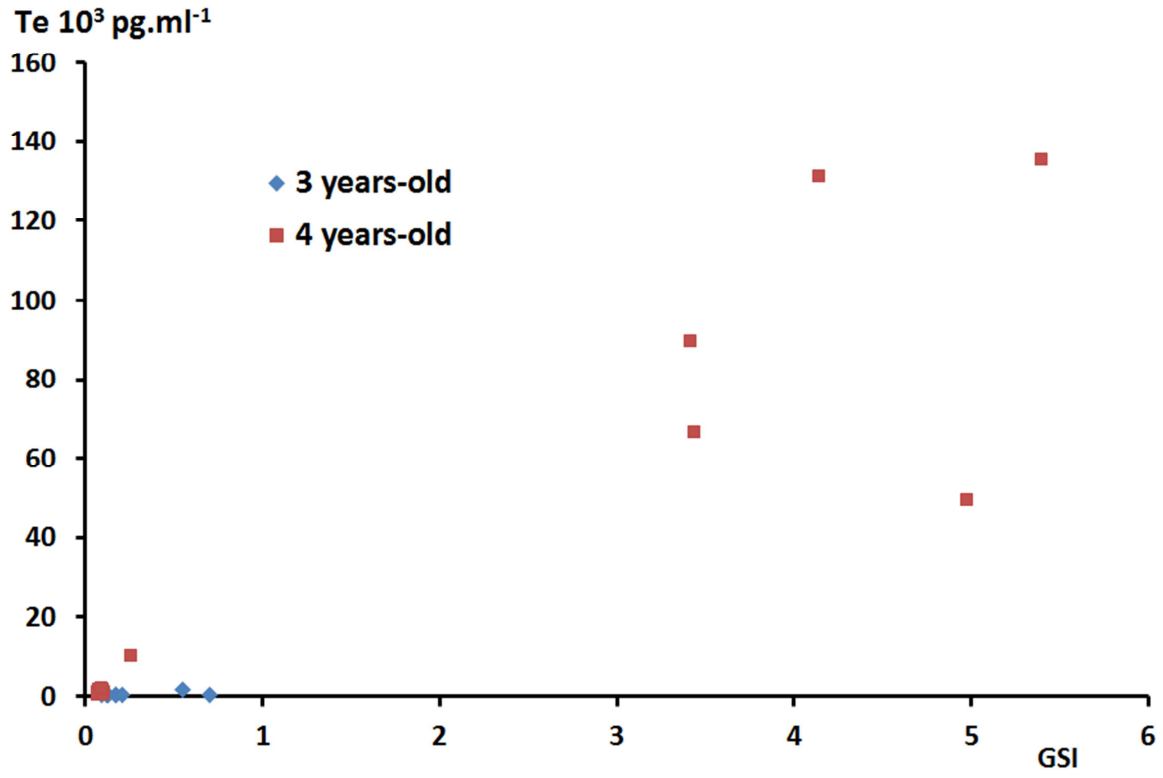


Figure 51: Relationship between gonadosomatic index and testosterone plasma levels in three- and four-year-old males.

5.2.6.3. 11-Keto-testosterone plasma levels in males

11-Keto-testosterone (11-KT) is an androgen characterized in teleosts. 11-KT was measured using the Kit Cayman Chemical Company, Ref 58271, that we had previously validated in shad (see previous report).

High 11-KT plasma levels were found in the five mature males (**Figure 52**). A significant correlation was observed between GSI and 11-KT plasma levels (r^2= 0.808).

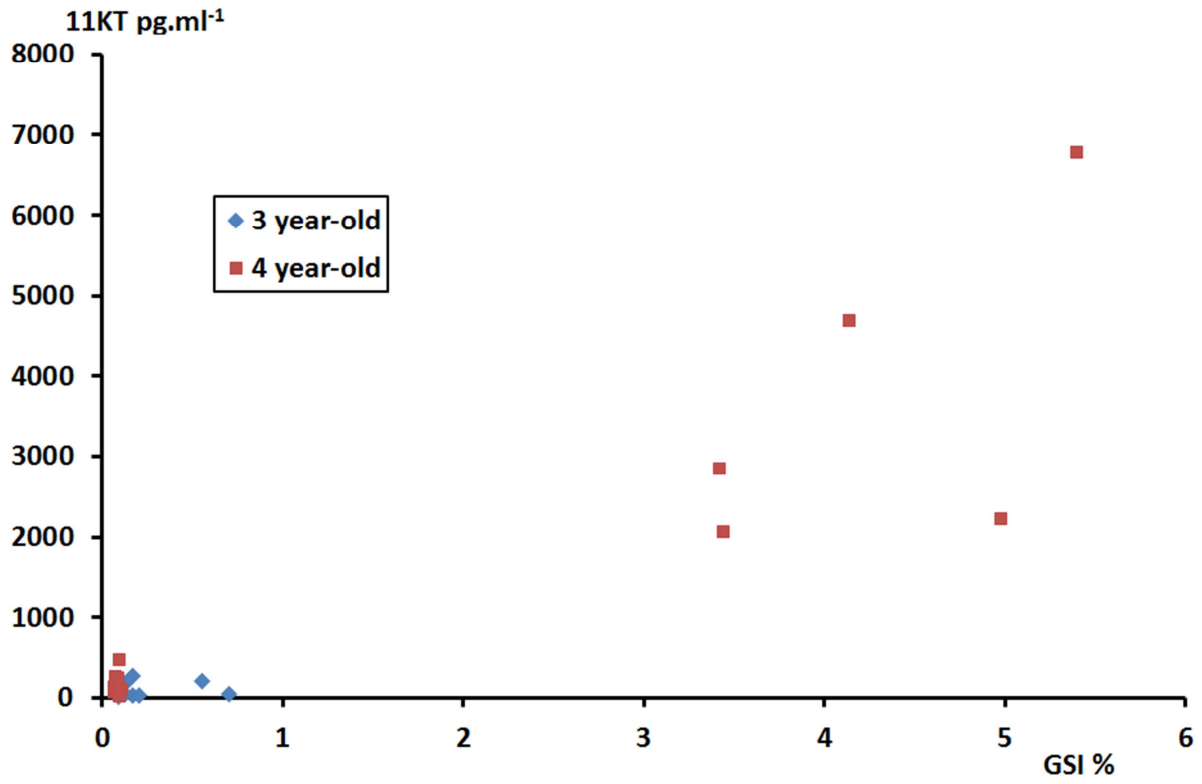


Figure 52: Relationship between gonadosomatic index and 11-Keto-testosterone plasma levels in three- and four-year-old male**.**

Finally, a high correlation was observed between both androgen (testosterone and 11-KT) plasma levels in male shads (r^2=0.936) (**Figure 53**).

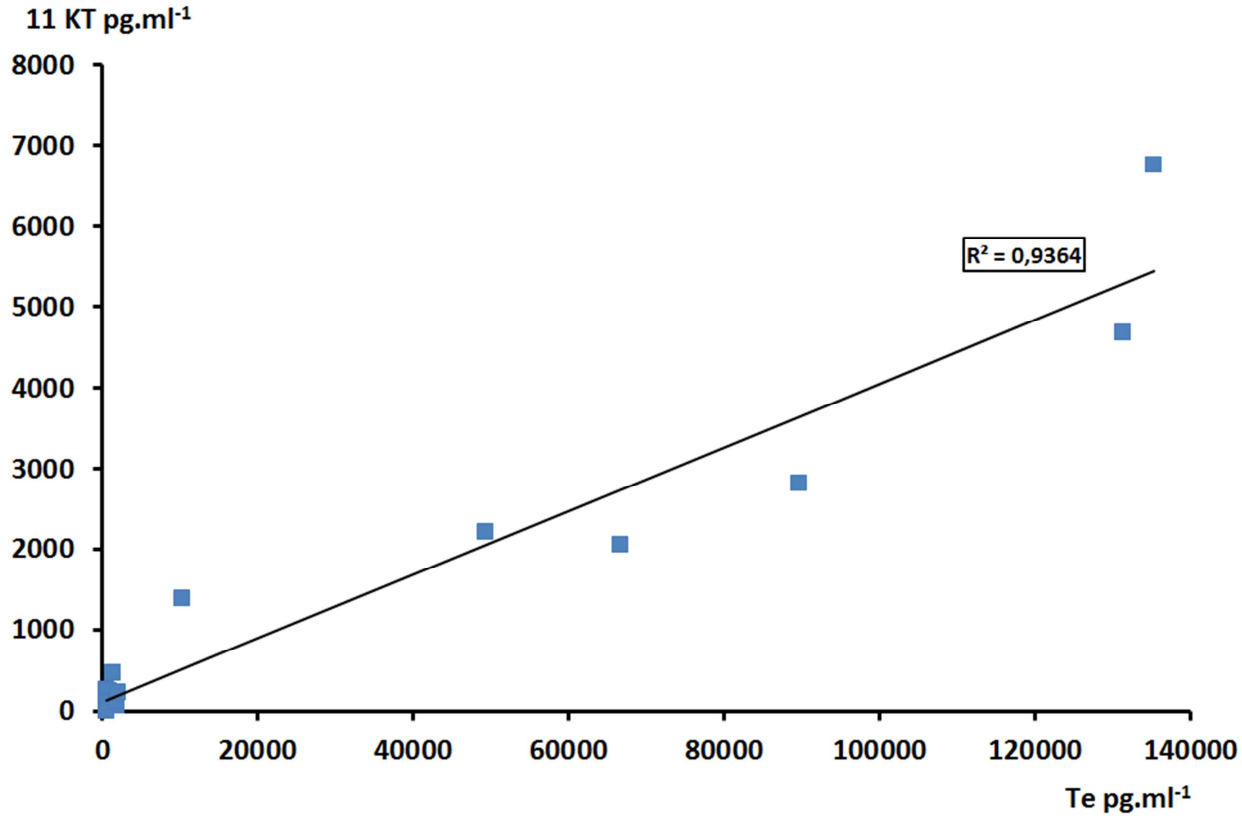


Figure 53: Correlation between testosterone and 11-Keto-testosterone plasma levels in three- and four-year-old male.

6. Conclusion and learnings of this action

This experiment showed that it was possible to realize the breeding of shads from the larval stage until the adult phase, and in the same time, to monitor the sexual maturation of fish between 1 and 4 year-old. Even if the conditions of breeding were not optimal, some fish from the age of 4 years reached an advanced stage of sexual maturation. The initial objective of this action is thus reached.

This first experiment allowed to highlight numerous advances in terms of rearing. The main recommendations are the following ones:

During the first weeks, the rearing density must be sufficient to elicit an effective feeding behaviour. It seems that the density of 50 larvae per liter is a minimum (Howey, 1985).

The appearance of the oral cysts generated an important direct or indirect mortality and probably had a negative incidence on the growth. Thus it is essential being in control of this disease to assure the viability of future operations. He seems necessary before any future operations of breeding to realize experiments to verify the positive effect of a weaning on small size artemia.

Larvae can very early withstand a 10‰ brackish water (Bardonnet and Jatteau, 2008). Slightly salted water allows to limit the risks of diseases development. Nevertheless it is possible that such a level of salinity can have a negative effect on the growth. It is thus recommended to limit the level of salinity to 5‰ (DiMaggio *et al.*, 2015). The passage to sea water can be made from the age of 3-4 months over a period of one week.

The speed of the current has to allow a good self-cleaning of the tank. Nevertheless it must not be too high to increase energy expenditure necessitated by swimming countercurrent. A too high current speed will penalize later growth. This speed must be adapted according to the size of the tank and the size of the individuals.

It is necessary to have a long enough lit phase during which the feeding will take place, because larvae have a positive phototropism, and they feed during the lit phase (Wiggins *et al.*, 1985; Hendricks, 2003; Jatteau and Bardonnet, 2008).

From the juvenile stage, the capacities of jump increase, and the risks of escape by jumps are important. It is thus necessary to install a system preventing the jumps outside the tank. We installed a safety net with a height of 1 m which prevents the risk of escape even for adult fish.

The density of breeding for subadults and adults must be limited. For this type of breeding realized with not domesticated fishes, without sorting allowing to limit the food competition, the recommended density is from 2 to 5 kg m^3 (Lambert and Dutil, 2001).

A special attention must be focused on the follow-up of the pH. To prevent the risks of nephrocalcinosis, it is imperative to maintain the pH around 7.8. The addition of a degassing system contributes to maintain the pH by limiting the CO_2 concentration.

The monitoring of the gonadal development allowed us to show that we obtained fish with a balanced sex ratio. This demonstrates that our rearing conditions are good enough to prevent appearance of unbalanced sex ratio which is observed for instance in eels in high density aquaculture conditions.

Fish transferred to freshwater were randomly chosen, ie independently of their level of maturity. The absence of direct mortality due to the shift in salinity, demonstrates that fish are able to sustain seawater at 3 years-old, and there is probably no threshold in relation with sexual maturity. As well as for the ability of allis shad larvae to withstand the transfer from freshwater to seawater, the ability of juveniles and subadults to adapt from seawater to freshwater reveals a remarkable euryhalinity of the allis shad.

However, since the transfer from seawater to freshwater induces an arrest of feeding behavior, an early transfer of immature fish to freshwater is deleterious for fish further development and survival.

First sexually matured or advanced fish were recorded at 4 years-old. One female with an intermediate GSI, compared to wild matured female, was observed in freshwater, which is in accordance with the life cycle. The five more advanced males were fully matured, and sperm was obtained by manual stripping. These males were more advanced in the maturation process compared to females, which is also in accordance with the biology of the species, where males reach maturity earlier than females (Mennesson-Boisneau *et al.*, 2000). On the other hand these males were recorded in seawater. This result is important in rearing point of view, because it shows that the transfer to freshwater is not necessary to reach sexual maturity in males. Nevertheless, it would be necessary to verify the quality of the gametes.

Finally, this study allowed us to set up the method to analyze steroid hormones in allis shad. High steroid plasma levels, estradiol in females, androgens (testosterone and 11-Keto testosterone) in males, were measured in mature shads. We can propose to use plasma steroid levels as markers for selecting future genitors to be transferred to freshwater. This selection is necessary to avoid early transfer of immature fish, leading to arrest of feeding and development.

7. Action D7

A new batch of 27 shads has been transferred in the aquaria exposition in September 2013.

A new informative panel was installed in 2015. This panel groups together information and a video concerning three migratory fish presented in this area, allis shad (*Alosa alosa*), European sturgeon (*Acipenser sturio*) and eel (*Anguilla anguilla*). An illustration of the panel is presented in **Figure 54** andFigure 55.

Figure 54 : The information panel in Aquarium La Rochelle.

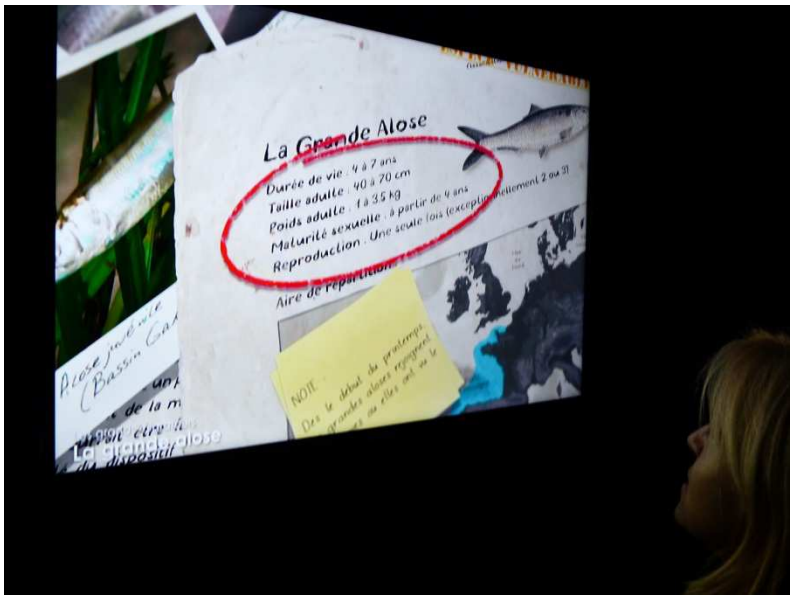


Figure 55 : A picture extracted from the video.

8. Technical and management meetings

The kick off meeting of action C1 took place on 18 January 2011 at the Aquarium La Rochelle. This meeting allowed to present the project and to establish the sampling program.

On the occasion of this meeting, the Life + Alosa alosa project was presented to the media, including France 3 (TV) and Sud-Ouest (newspaper) (**Figure 56**).

Andreas Scharbert, project coordinator, has visited the rearing installations on 31 May 2011 and detailed studies planned during action C1 were presented

Other technical meetings were held in Aquarium La Rochelle :

- On 29 November 2011
- On 11 May 2012
- On 11 July 2012
- On 15 November 2012
- On 13 March 2014
- On 24 September 2014
- On 4 June 2015

Moreover a technical and management meeting with members of Aquarium La Rochelle, Borea Unit was held at the end of each yearly sampling.

Three meetings were held at Conseil Regional Aquitaine:

- On 22 February 2011
- On 28 March 2012
- On 17 April 2013

The 2 first were management meetings.

Mots : 551

SUD OUEST

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Des aloses captives

LE SAUVETAGE DU POISSON MIGRATEUR S'ORGANISE

Une Charente-Maritime **Des aloses captives** La grande alose. Qu'est-ce que les scientifiques savent sur ce poisson migrateur emblématique des grands fleuves, qu'il est interdit de pêcher depuis 2008 dans la Garonne et la Dordogne, où a été observé l'effondrement vertigineux de ses stocks ? « Peu d'études lui ont été consacrées, déplore Philippe Jatteau. On connaît sa vie adulte, lorsqu'elle arrive dans les estuaires. Mais on connaît mal sa vie en mer, et sa phase juvénile en eau douce. » L'ingénieur de recherche au Cemagref (institut scientifique girondin) était à l'Aquarium de La Rochelle, hier, pour coucher de nouveaux chapitres dans la littérature scientifique consacrée à ce cousin de la sardine et de l'anchois, un beau poisson argenté de la famille des clupéidés, dont la pêche artisanale ne peut plus se pratiquer dans nos fleuves. Le Cemagref, l'Aquarium de La Rochelle, et le Muséum d'histoire naturelle de Paris ont signé la convention avec laquelle ils se donnent trois ans pour analyser l'évolution sexuelle des aloses en captivité. Avec ce que la démarche sous-tend comme prolongements éventuels pour repeupler des secteurs où l'espèce est menacée, voire éteinte. Les biologistes ont ainsi défini les étapes de ce programme de recherche pour la conservation et la restauration de l'alose, qui a reçu le soutien de l'Union européenne, à travers le fonds Life nature. Ces travaux s'inscrivent dans la continuité d'une première phase de recherche au cours de laquelle les chercheurs s'intéressèrent à la disparition de l'alose dans le Rhin. Entre 2007 et 2010. Le Cemagref fut l'un des piliers de ces travaux qu'avait consacrés en 2008 le prix Best European Maritime Project du Comité des Régions. 5 millions d'alevins lâchés En lien avec l'association Migado (pour la restauration et la gestion des poissons migrateurs), 5 millions d'alosons avaient alors été lâchés dans le fleuve frontalier. La phase de recherche qui débute poursuit un double objectif. D'une part, transférer en Allemagne les techniques utiles à l'effort de réintroduction de l'espèce. D'autre part, améliorer la connaissance sur le franchissement des obstacles, les zones de frayères, la descente des juvéniles entre la frayère et l'estuaire. Mais, cette fois, le champ des recherches est élargi aux bassins de la Garonne et de la Dordogne, fleuves où le lâcher d'alevins n'est pas encore d'actualité. Dans ce dispositif, l'Aquarium de La Rochelle propose sa connaissance sur la vie de l'alose en milieu artificiel. 150 spécimens, que lui avait fournis le Cemagref en 2009, s'y développent pour partie devant les visiteurs et, pour le reste, dans les locaux techniques de l'entreprise touristique. « Elles mesuraient 3 centimètres à leur arrivée, évoque le biologiste de l'Aquarium, Pierre Morinière. Il se tient devant le bassin où des poissons de 20 centimètres s'excitent aujourd'hui à l'approche des intrus qui les observent. « Adulte, la grande alose peut atteindre 40 à 50 centimètres. » Mais, qu'une seule écaille de son bel habit de lumière soit abîmée, et sa vie peut être menacée. Un poisson fragile, et difficile à élever dans 8 mètres cubes d'une eau dont la température est comprise entre 16 et 18°, dans laquelle il est nourri au plancton congelé et au granulé d'aquaculture. « 150 aloses sont élevées à l'Aquarium depuis deux ans. Elles sont nourries avec du plancton congelé »

PHILIPPE BAROUX

Figure 56 : Newspaper article published in Sud-Ouest on 19 January 2011.

9. Valorization

A presentation of the Life+ Alosa alosa project was realized on 17 April 2013 at the Conseil Régional Aquitaine on the occasion of the visit of a Delegation of Hessian Members of Parliament.

During the Life+ Alosa alosa conference held in October 2015 in Bergerac, the main results of this action were presented (**Figure 57**).

Figure 57 : Title of the presentation realized during the Life+ Alosa alosa Conference

Two other presentations are planned in 2016:

- Poster presentation at the XXIXth Congress of the French Association of Histotechnology, Paris, June 2016.
  Provisional title: Monitoring of the gonadal development of Allis shad Alosa alosa, reared for the first time in captivity. Gonnet F., Baloche S., Campo A., Jatteau Ph., Morinière P., Dufour S.
- Oral presentation at the International Aquarium Congress, Vancouver, September 2016
  Provisional title: First rearing attempt of the diadromous Allis shad *Alosa alosa:* from larvae to adult. Morinière P., Jatteau Ph., Dufour S., Baloche S. and Gonnet F.

A scientific publication dealing with rearing results and sexual maturation monitoring is in preparation.

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