Life+ Alosa alosa Irstea report 2: Actions C1 - D7 July 2012-June 2013

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IRSTEA – EPBX
Estuarine Ecosystems and
Diadromous Fish

Life+ Alosa alosa
Irstea report 2
Actions C1 – D7
July 2012 – June 2013

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Pour mieux affirmer ses missions, le Cemagref devient Irstea

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Table of contents

Abstract – Résumé **1**

1. Monitoring of the 2011 batch **2**

1.1 Origin of the batch 2

1.2 Rearing conditions 2

1.3 Feeding 3

1.4 Mortality 3

1.5 Growth 4

2. Monitoring of the sexual maturation **6**

2.1 Histological analysis of the gonads of one year old shads 6

2.1.1. Histological preparations

2.1.2. Histological determination of gonadal sex

2.2 Histological determination of gonadal stage 7

2.2.1 Females 7

2.2.2 Males 8

2.3 Samplings carried out in 2013 9

3. Aquarium exhibition (Action D7) **11**

4. Technical and management meetings **11**

Annex **13**

Abstract

This report presents works managed by Irstea, Aquarium La Rochelle and Borea Team concerning the set up of an ex situ rearing method for juveniles Allis shads *Alosa alosa*, and the monitoring of sexual maturation. Fish were moved to a larger tank in September 2012, which probably improve growth. Mortality has been stabilized. Histological analysis of samples collected in June 2012 showed a normal gonadal development in 50% of the samples. A second set of samplings for the monitoring of sexual maturation was carried out in June 2013.

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. Les poissons ont été transférés dans plus grand bac en septembre 2012, ce qui a probablement permis d'améliorer la croissance. La mortalité a été stabilisée. Les analyses histologiques des prélèvements effectués en juin 2012 ont montré un développement normal des gonades dans 50 % des échantillons. Une seconde série de prélèvements a été réalisée en Juin 2013.

1. Monitoring of the 2011 batch

1.1 Origine of the batch

This batch is the only one left after the death of individuals from the 2008 batch in November 2011.

This batch arrived in Aquarium La Rochelle on 24 May and 25 October 2011. These 2 groups were put together in December 2011. Salinity was increased from 10 to 30 ‰ during the course of December 2011 (a one week transfer).

1.2 Rearing conditions

Because of their growth, fish were transferred from a 3 m^3 to a 8 m^3 tank in September 2012 (Figure 1). The tank is covered by a net to prevent fish to jump outside. Rearing conditions were not modified, but fluctuated during the year (Figure 2). Mean water temperature was 17.6 ± 0.3 °C and mean salinity was 32 ± 0.9 ‰. Photoperiod was constant at 12/12.

Figure 1: The 8 m^3 rearing tank

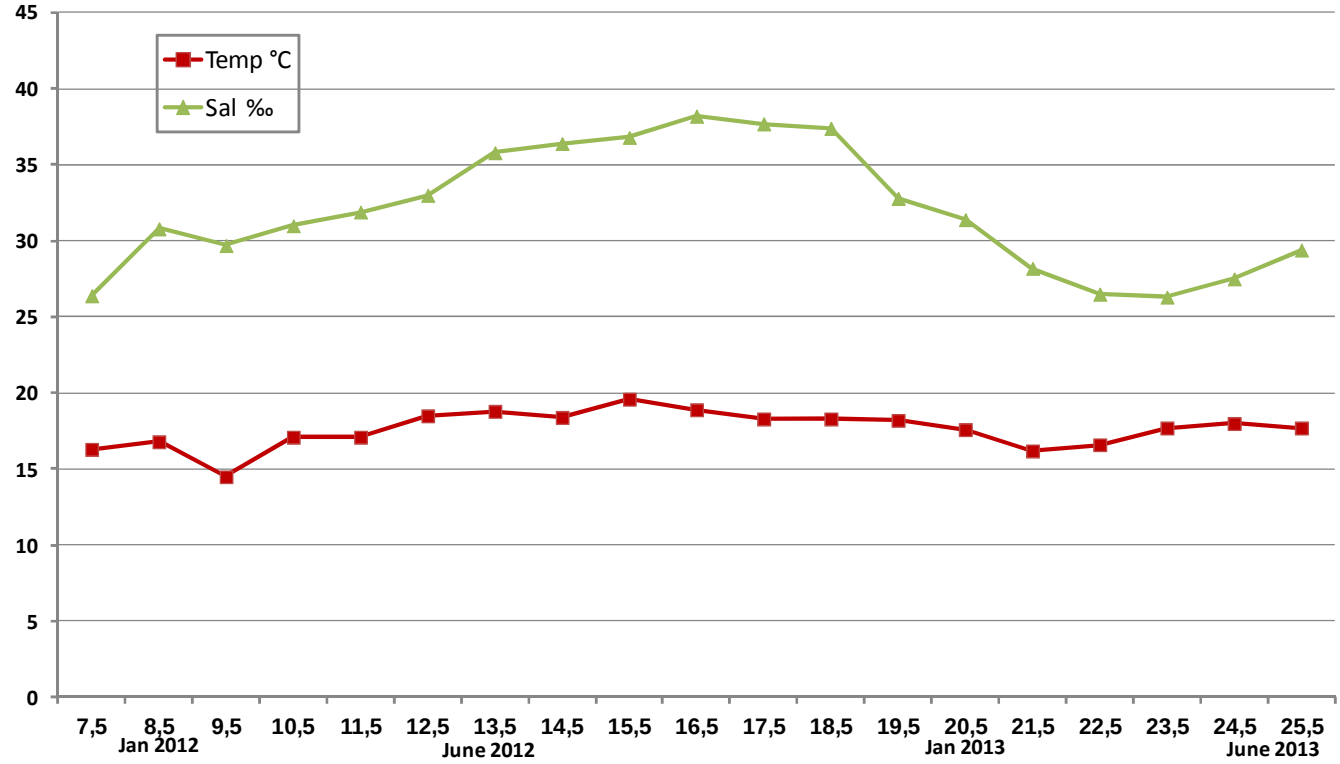


Figure 2: Monitoring of the temperature and the salinity from December 2011 to June 2013

1.3 Feeding

Fish were fed with artificial feed and frozen zooplankton.

From July until January 1 mm half-floating pellets were distributed, following by 2 mm floating pellets until April. From May to June they fed 3.5 mm floating pellets.

Frozen Mysis were first distributed until December, following by Krill pacifica which corresponds to larger preys.

1.4 Mortality

Mortality was low during this second year of rearing (Figure 3). Monitoring of the survival is only based on natural mortality; dead fish following samplings or anesthetic test were not counted. Natural mortality corresponded to 38 fish, among which 24 were due to jump outside which leads to a modification of the net covering the tank. At the end of June 2013, 473 fish remained in the tank.

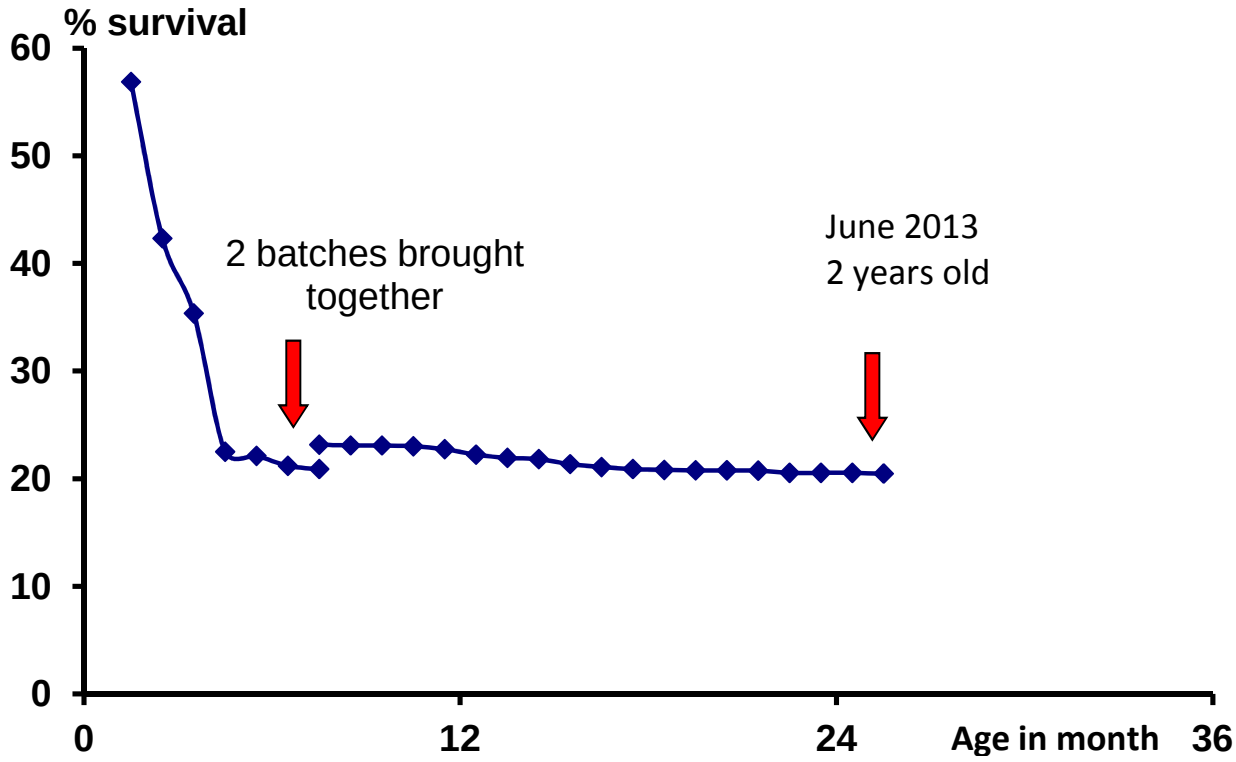


Figure 3: Survival after 25 months of rearing

1.5 Growth

A sampling was organized in November 2012 to have an intermediary statement for growth. Nineteen fish were caught and anesthetized. Ten fish were found died the day after. It was decided that no further sampling for growth measurements will be undertaken due to the extreme sensitivity of shad to handling.

The growth until June 2013 is represented in Figure 4. Specific growth rate (SGR, % body length day^{-1}) is 0.17 for length and 0.53 for weight between June 2012 and June 2013.

Food efficiency (or food conversion ratio) is basically assessed from biomass gain and dry food distributed

FCR = Food distributed / biomass gain

In our case, around 40% of food is frozen food, ie with a high level humidity, which doesn't allow comparison with aquaculture data. Food conversion ratio calculated during the second year of rearing is 4.97, so 4.97 kg of food (dry and moist food) is necessary to product one kg of fish.

Length – weight relationship was calculated from the beginning of the rearing. The relationship is plotted on Figure 5.

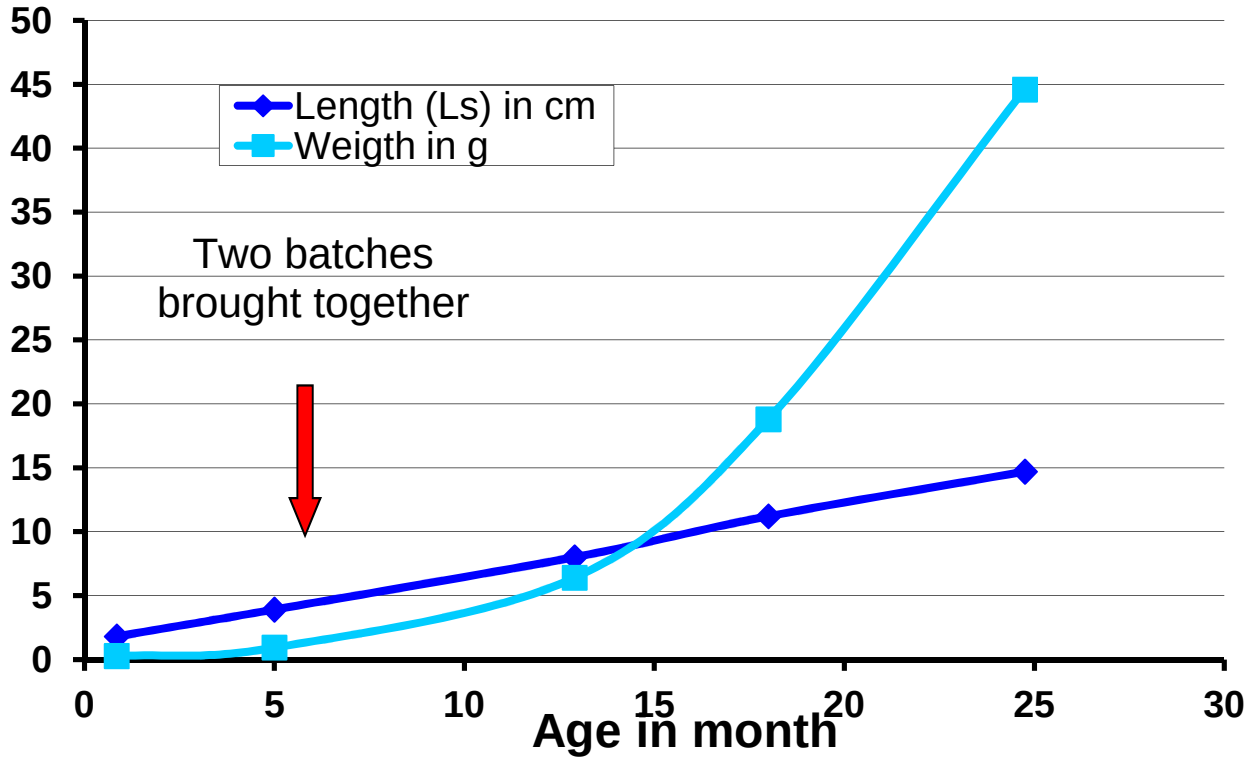


Figure 4: Linear and weight growth until June 2013

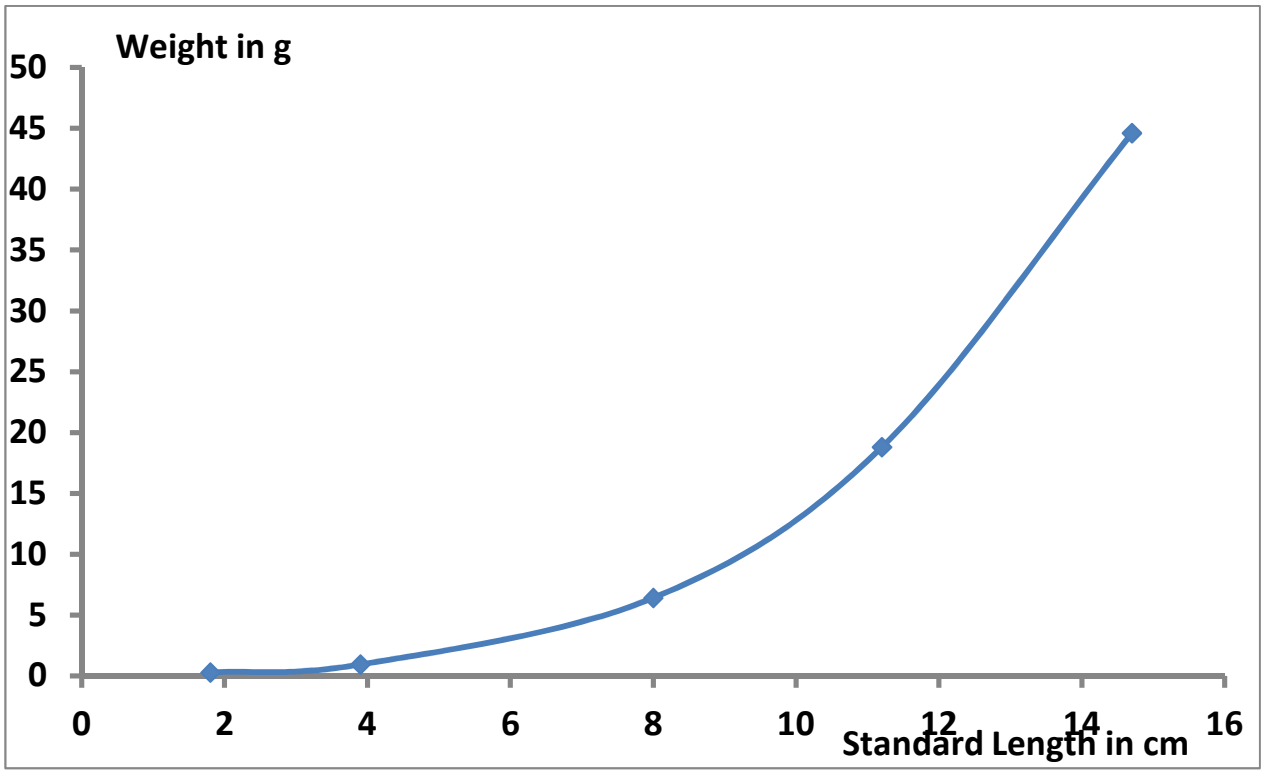


Figure 5: Length-weight relationship

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

2.1 Histological analysis of the gonads of one year old shads.

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.

2.1.1 Histological preparations

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.

2.1.2 Histological determination of gonadal sex

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 individuals, or to testis. 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.

All together, 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. There was no significant difference between females and males for the body length (females: 8.2 $\pm$ 0.30 cm (SE); males: 8.8 $\pm$ 0.29 cm; P=0.79) nor for the body weight (females: 5.46 $\pm$ 0.66 g; males: 7.42 $\pm$ 0.76 g; P=0.89).

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

2.2 Histological determination of gonadal stage

2.2.1 Females

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 6). This indicates that one year-old females were at the primary oocyte stage. Oocyte of various diameter were observed. On each preparation, the diameter of the 10 larger ovocytes was measured (Table I). Mean diameter ± 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. Mean oocyte diameter plotted against body length shows a tendency to a positive but weak relationship (Figure 7).

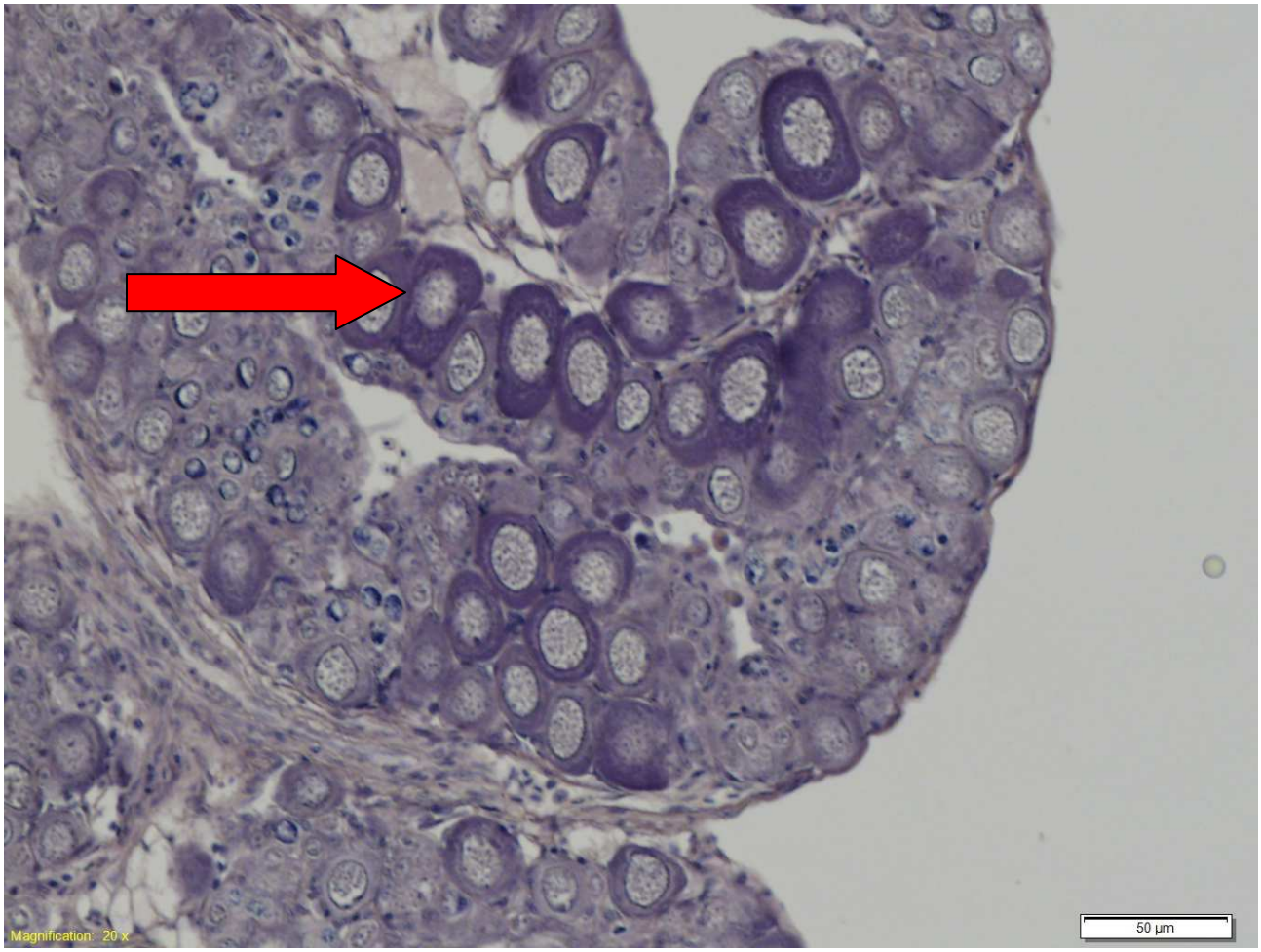


Figure 6: Histological section of female gonad showing oocytes stage 1 (red arrow)

Table I: Results of oocyte diameter measurements

	N°1	N°7	N°15	N°16	N°17	N°18
Mean diameter µm	43.52	33.33	28.83	49.83	20	45.5
Standard deviation (SD)	5.1	6.43	2.94	5.85	3.33	2.23
Standard error (SE)	1.61	2.03	0.93	1.85	1.05	0.7
Length (Ls) cm	8.9	7.3	7.1	8.2	7.2	7.6

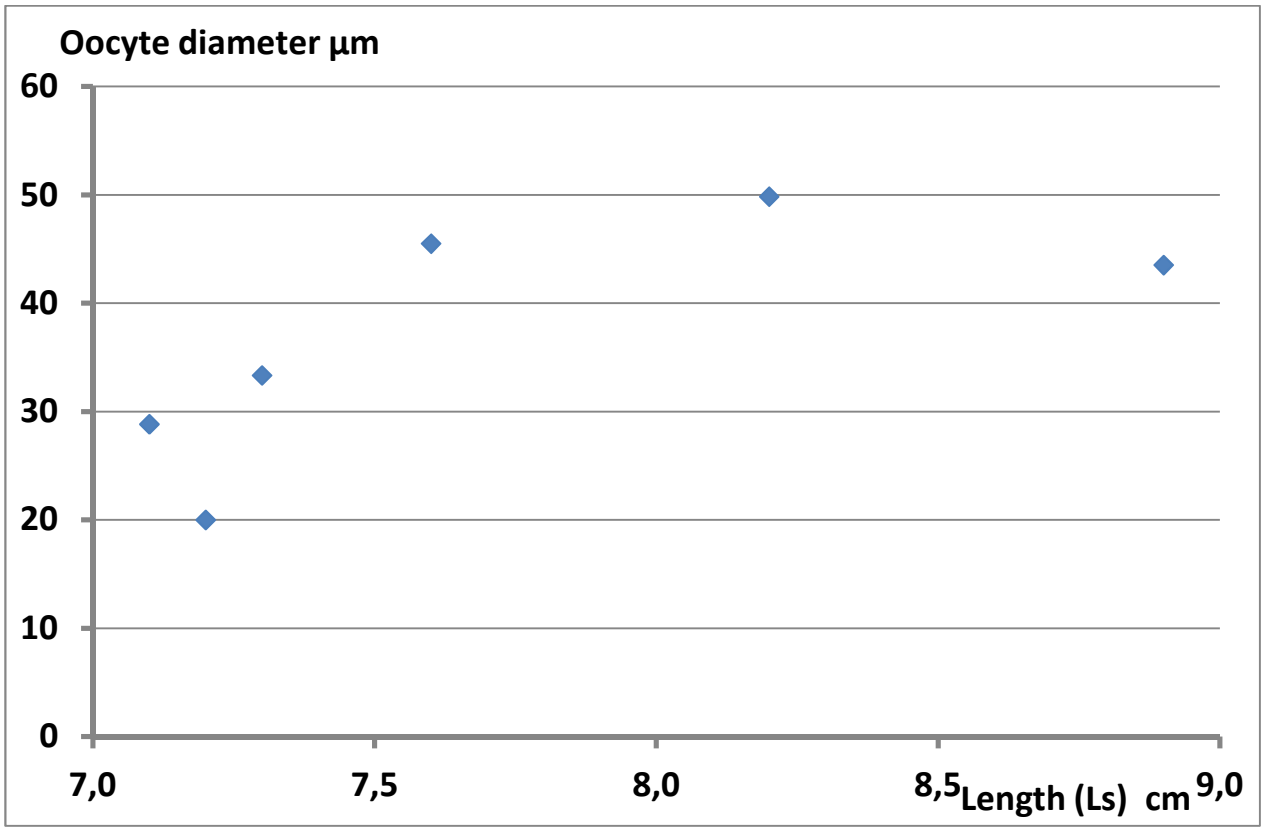


Figure 7: Oocyte diameter plotted against length

2.2.2 Males

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 8).

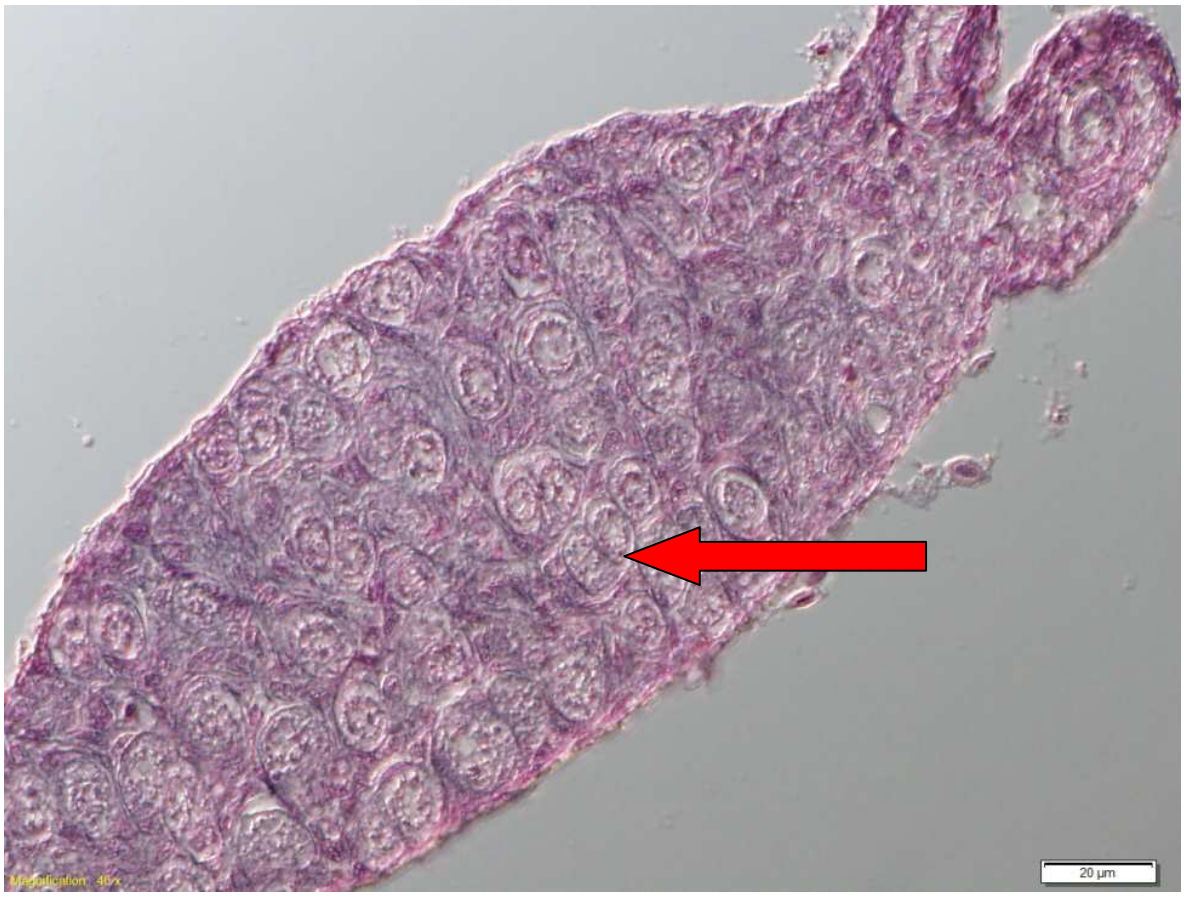


Figure 8: Male gonad with numerous spermatogonia, and figure of mitosis (red arrow).

2.3 Samplings carried out in 2013

Samplings took place the 5 and 6 June 2013.

Twenty shads were sampled after euthanasia in a concentrated bath of eugenol.

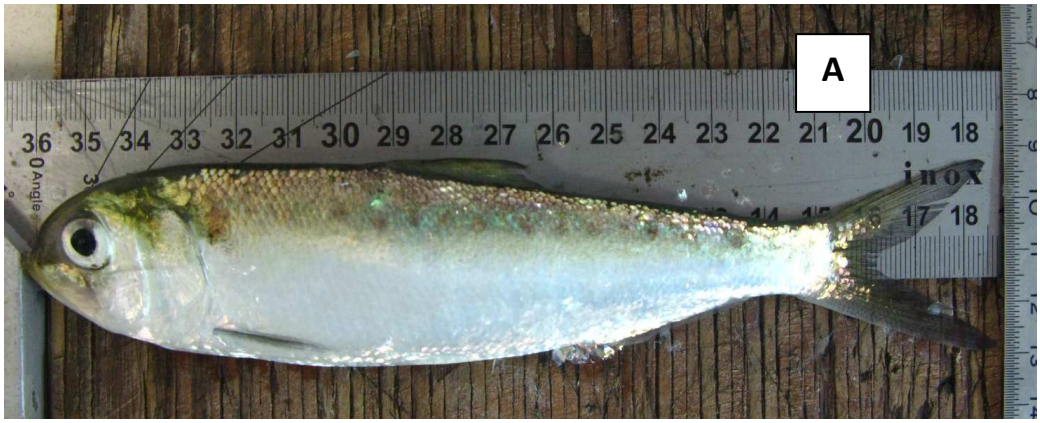
Following parameters and samplings have been recorded and made (Figure 8):

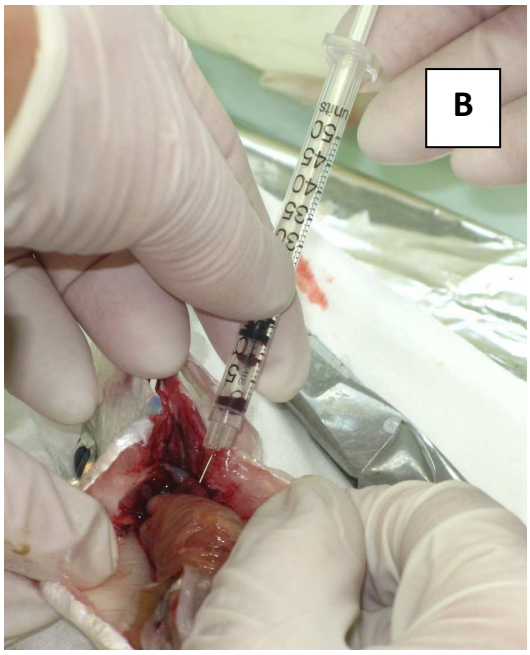
- Length, weight, height, weight of the digestive tract, gonad and liver
- Blood, gonad, digestive tract and liver

In addition, samples of gills, brains and pituitary have been realized for future analysis.

Blood samples have again been taken directly in the heart due to the small size of the individuals. After centrifugation, plasma were kept frozen at -20°C.

Gonad and liver samples were fixed in Bouin fixative solution for histological studies.





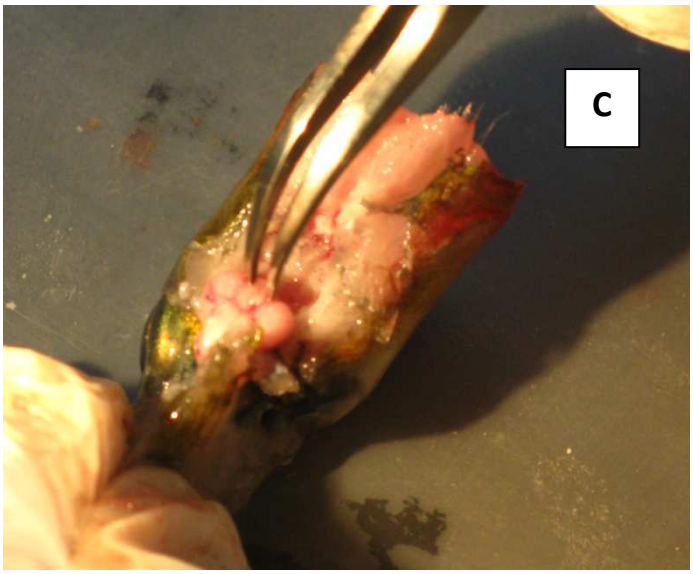


Figure 8: Length measurement, A – blood sample, B – brain sample, C – ovary, D – testis, E

During the dissection, ovaries (larger size and orange colour) could be distinguished from testis (smaller size and white colour). Histological analyses to be performed during the next reporting period will allow to assess these observations.

Based on these preliminary observations (to be confirmed), among the 20 fish sampled, 10 would be females and 10 males. They would not differ by their body length (18.38 ± 0.59 cm for females *versus* 18.45 ± 1.61 for males) nor by their body weight (47.15 ± 4.77 g for females *versus* 49.43 ± 5.79 g for males). In contrast, they differ by their gonadosomatic index (0.89 ± 0.05 % in females versus 0.13 ± 0.01 % in males)

3. Action D 7

Following a technical problem on 24 August 2012, the 8 shads in the exposition aquaria died with all the other fish (sardine, mackerel and common scad).

Because shads of the 2011 batch were not larger enough, new fish were not introduced immediately. The transfer to the exposition aquaria is planned for September 2013.

4. Technical and management meetings

A first meeting was held in Aquarium La Rochelle on 15 November 2012. Growth measurements were realized at this occasion (Figure 9).

A presentation of the Life+ Alosa alosa project has been realized on 17 April 2013 at the Conseil Regional Aquitaine on the occasion of the visit of a Delegation of Hessian Members of Parliament (presentation in annex).

A half day Life+ project meeting was held at Aquarium La Rochelle on 6 June 2013, with Borea Team and Pierre Morinière from Aquarium La Rochelle. Members of Borea Team presented results of the histological analysis performed on the 2012 samples. Discussions focused also on the schedule for the next season.

Figure 9: Shads in the anesthetic bath

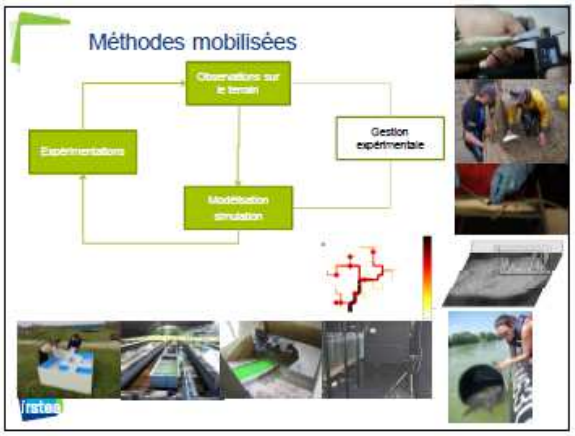
A management meeting was held in Bruch on 25 June 2013. Middle results have been presented and discussed.

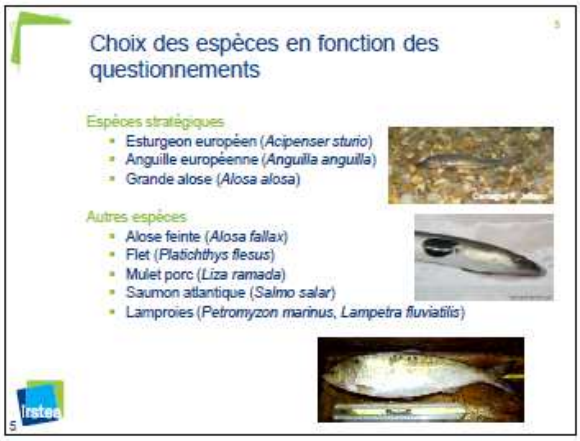
Annex


Irstea Bordeaux
Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture
Unité de recherche
Ecosystèmes estuariens et Poissons migrateurs amphihalins
Philippe Jatteau, Equipe Poissons Migrateurs


Implantations et effectifs
Deux implantations
Centre de Cestas (bureaux, laboratoires)
Station d'expérimentations de Saint-Seurin sur Isle
50 personnes
20 (+ 3 contractuels) ingénieurs et chercheurs
4 doctorants
6 (+ 6 contractuels) techniciens
4-8 stagiaires


Thématiques, disciplines
Equipe Ecosystèmes estuariens (P. Boët)
Approche écosystémique, bio-indication
Ecologie des communautés
Modélisation
Equipe Poissons migrateurs amphihalins (P. Lambert)
Approche populationnelle, biologie de la conservation
Ecologie des populations
Dynamique des populations
Modélisation


Méthodes mobilisées
Observations sur le terrain
Expérimentations
Gestion expérimentale
Modélisation simulation


Choix des espèces en fonction des questionnements
Espèces stratégiques
Esturgeon européen (Acipenser sturio)
Anguille européenne (Anguilla anguilla)
Grande alose (Alosa alosa)
Autres espèces
Alose feinte (Alosa fallax)
Flet (Platichthys flesus)
Mulet porc (Liza ramada)
Saumon atlantique (Salmo salar)
Lamproies (Petromyzon marinus, Lampetra fluviatilis)
5


Programme Life Alose 2007-2010
Objectif
Réintroduire la grande alose dans le bassin du Rhin
Les principales actions Cemagref
Transfert de technologie (Cemagref → Migado)
- reproduction contrôlée
- élevage larvaire
Mise au point d'une méthode de marquage
Les autres actions
Production de larves
Transfert et lâchers dans le Rhin – suivi des larves
Recensement des frayères potentielles

Programme Life+ Alose 2011-2015

Objectif

Poursuivre les actions de repeuplement
Installer une unité de production de larves en Allemagne
Contribuer à la conservation de la population de Gironde

Les principales actions

Poursuite de la production de larves et lâchers dans le Rhin
Transfert de technologie France → Allemagne
Etude de la franchissabilité des obstacles
Suivi de la dévalaison des larves et juvéniles
Mise en place de la production pilote à Asslar

Action d'Irstea

Mettre au point une méthode d'élevage ex situ
Suivre la maturation des géniteurs en captivité

La production de larves

	Life			Life+	
	2008	2009	2010	2011	2012
Nb larves relâchées	480 000	1 745 000	2 590 000	2 200 000	900 000

Stimulation hormonale

Bac d'élevage

Conditionnement pour le transport

Les lâchers en Allemagne

Lâcher et suivi des larves déversées

Etude de la franchissabilité des obstacles

Implantation d'un émetteur dans le tube digestif

Lâcher des aloses équipées en aval du barrage de Golfech (Garonne) ou Tuilière (Dordogne)

Suivi de leur comportement face à l'obstacle
Efficacité du dispositif de franchissement

Suivi de la dévalaison (Garonne et Dordogne)

Mieux connaitre l'âge, la cinétique de la dévalaison ; les habitats colonisés

Transfert de compétences

Formations des techniciens allemands par Migado:
élevage larvaire
alimentation larvaire

Mise en place d'une unité pilote à Asslar


Suivi de la maturation en captivité
Objectif : préserver le stock de géniteurs sauvages
Mise au point de la méthode d'élevage de la larve à l'adulte (Aquarium La Rochelle)
Suivi de la croissance et de la maturation sexuelle en captivité


Ensemble pour le retour de l'alose dans le Rhin !


Vielen Dank für Ihre Aufmerksamkeit