Scientific paper

Reproduction of *Zingel asper* (Linnaeus, 1758) in controlled conditions: an assessment of the experiences realized since 2005 at the Besançon Natural History Museum

by

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Abstract. – The *Zingel asper* or apron is one of Europe's most threatened freshwater fish (classified as critically endangered by the International Union for Conservation of Nature, IUCN). It is also listed in appendices II and IV of the European Habitats Directive (1992) and in appendices II and III of the Berne Convention (1979). The geographical distribution of this percid, endemic to the Rhône basin, is extremely limited, making it particularly vulnerable. Since 2005, the Besançon Natural History Museum has been involved in conservation programmes (LIFE Apron II and a national action plan) to improve the knowledge on its *ex situ* reproduction, the aim being to produce individuals with which to conduct experimental studies (toxicity tests, design of fish passes, etc.), carry out public presentations (aquariums, nature reserves, etc.) and implement pilot reintroduction schemes, without having to systematically take samples from the natural environment. Innovative devices were designed in the Besançon Citadelle Aquarium to enable replication of complete reproduction cycles in artificial conditions. Control of thermal and zootechnical parameters associated with the development of "artificial spawning grounds" have allowed us to understand the different phases involved in reproducing and maintaining this species in captivity. We were able to observe numerous behaviours, which have never been seen in natural environments, in particular during spawning and early life stage development phases. Several experiments were conducted to study how hatching rates varied according to the duration of the winter cold period, known as "vernalization", applied to broodstock. Hatching rates in the order of 80% were obtained with broodstock subjected to a cold period of 120 days at 5°C, from the beginning of November to the end of February. In contrast, hatching rates dropped rapidly for groups of broodstock subjected to vernalization periods of less than 90 days, and with a cold period of only 30 days, the eggs deteriorated before hatching. A precise annual thermal cycle has therefore been identified, corresponding to the different life phases of *Z. asper* in captivity. A particular focus of our work was on breeding of fry to ensure that they achieve survival rates in the order of 80% during the first month, and 90% the following month. The results of these experiments have supported other studies and more than 26,000 juveniles have been released into the Drôme River. Regular genetic monitoring of this reintroduced population has allowed us to ensure that they are reproducing naturally. *Z. asper* born in captivity are also widely used in initiatives to raise public awareness (exhibitions, etc.). To date, the *Z. asper* breeding programme in the Besançon Natural History Museum is the only one of its kind for this percid, but the high sensitivity of broodstock to the intensity and duration of the vernalization period should raise concerns about the sustainability of its populations in the face of climate change.

Résumé. – Reproduction de *Zingel asper* (Linnaeus, 1758) en conditions contrôlées : bilan des expériences réalisées depuis 2005 au Muséum d'histoire naturelle de Besançon.

L'apron du Rhône *(Zingel asper)* est un des poissons d'eau douce européen les plus menacés (classé en danger critique d'extinction par l'Union Internationale pour la Conservation de la Nature, UICN). Il figure également dans les annexes II et IV de la Directive Européenne Habitats, Faune, Flore (1992) ainsi que dans les annexes II et III de la Convention de Berne (1979). La répartition géographique très restreinte de ce percidé endémique du bassin rhodanien le rend particulièrement vulnérable. Depuis 2005, le muséum de Besançon est impliqué dans les programmes de conservation (Life Apron 2 et Plan National d'Action) afin d'améliorer les connaissances relatives à son élevage *ex situ*, l'objectif étant de pouvoir disposer d'individus pour réaliser soit des études expérimentales (essais toxicologiques, conception de passes à poissons…), soit des présentations publiques (Aquariums, Réserves naturelles…) ou encore des réintroductions pilotes, sans avoir systématiquement recours à des prélèvements dans le milieu naturel. Des dispositifs innovants ont été conçus au sein de l'Aquarium de la Citadelle de Besançon afin de permettre la réalisation de cycles de reproduction complets en conditions artificielles. Le contrôle des paramètres thermiques et zootechniques associés à la mise au point de "frayères artificielles", ont permis de maîtriser les différentes phases intervenant dans la reproduction et le maintien de cette espèce en captivité. De nombreux comportements qui n'avaient jamais été observés en milieu naturel ont pu l'être, notamment pendant le frai et le développement des premiers stades de vie. Plusieurs expériences ont permis de mesurer la variation du taux d'éclosion en fonction de la durée de la période froide hivernale, dite de "vernalisation", appliquée aux géniteurs. Ainsi, des taux d'éclosion de l'ordre de 80% ont été obtenus avec des géniteurs soumis à une période froide de 120 jours à 5°C, de début novembre à fin février. Par contre, les taux d'éclosion chutaient fortement pour les groupes de géniteurs soumis à des durées de vernalisation inférieures à 90 jours, et avec une période froide de seulement 30 jours, les œufs périclitaient avant l'éclosion. Un cycle thermique annuel précis a donc été déterminé en adéquation avec les différentes phases de vie des aprons en captivité. Un effort particulier a été porté sur l'élevage des alevins permettant d'atteindre désormais des taux de survie de l'ordre de 80% durant

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le premier mois et 90% le mois suivant. Les résultats de ces expériences ont pu alimenter d'autres études et plus de 26 000 juvéniles ont été relâchés dans la Drôme. Le suivi génétique régulier de cette population réintroduite a permis de s'assurer qu'elle se reproduisait naturellement. Les aprons nés en captivité sont également largement utilisés dans des actions de sensibilisation du public (expositions…). Actuellement, l'élevage d'apron du Rhône du Muséum de Besançon demeure le seul pour ce genre de percidé; la grande sensibilité des géniteurs à l'intensité et à la durée de la période de vernalisation doit nous interpeller sur la pérennité de ses populations face au réchauffement climatique.

The *Zingel asper* (Linnaeus, 1758), or apron, is one of the most threatened freshwater species, and classified by the International Union for Conservation of Nature (IUCN) as critically endangered (Crivelli, 2008). It is also listed in appendices II and IV of the European Habitats Directive (1992) and in appendices II and III of the Berne Convention (1979) (Crivelli, 2008). This Percidae endemic to the Rhône basin is indeed very vulnerable, in particular because of its highly limited geographical distribution (Prolonge-Chevalier, 2007). It currently occupies only 240 km of water course *i.e.* 11% of its distribution in 1900 (LIFE Apron II report – Apron populations assessment, 2009). The last surviving *Z. asper* populations are now confined to three separate geographical areas: the northeast of the Saône basin (Loue and Swiss Doubs), some tributaries of the lower Rhône (Ardèche and Beaume) and the midsection of the Durance basin. These populations should therefore be considered as isolated conservation units.

The causes behind the disappearance of *Z. asper* are directly or indirectly related to human activity. Dams, river flow regulation and pollution are the main causes of its decline (Labonne, 2002). *Z. asper* is a species of community interest, considered to be a sentinel species denoting rivers of high environmental quality (Labonne and Gaudin, 2005). Its presence alone indicates a well-preserved natural environment and efforts made to save it will have a beneficial impact on the whole ecosystem (concept of an umbrella species).

In 1998, a three-year conservation programme known as "LIFE Apron" was started, supported by the European Union and led by the organisation "Réserves Naturelles de France". Biological knowledge of the species was acquired, known populations were monitored, and studies to restore continuity of habitat and examine breeding feasibility were conducted. This resulted in the definition of a conservation strategy and publication of a management guide (Mari, 2001).

In 2004, "LIFE Apron II" was launched, coordinated by the "Conservatoire des Espaces Naturels Rhône-Alpes" (CEN) with the technical support of the "Office National de l'Eau et des Milieux Aquatiques" (ONEMA). The aim of this European programme was to stop the decline of *Z. asper* and safeguard it from extinction. Based on the recommendations of LIFE Apron, a budget of around €3.5 million was largely used to carry out significant civil engineering works to allow the species to cross a variety of obstacles (dams, sills, etc.).

Better intra-population mixing and recolonization of former territories should make it possible to increase the viability of each residual population nucleus. A second objective involved implementing management measures appropriate to conserve *Z. asper* in the affected catchment areas, maintaining favourable habitats and preserving water quality. The final component focused on improving knowledge of *ex situ* reproduction, the aim being to produce individuals with which to conduct experimental studies (toxicity tests, design of fish passes, etc.), carry out public presentations (aquariums, nature reserves, etc.) and implement pilot reintroduction schemes, without having to systematically take samples from the natural environment.

From 2012 to 2016, a national action plan led by the "Conservatoire d'Espaces Naturels Rhône-Alpes" was implemented, enabling the actions undertaken in the previous LIFE Apron programme to be continued.

Z. asper is a benthic percid occupying the grayling (*Thymallus thymallus* Linnaeus, 1758) zone in the south of France and the barbel (*Barbus barbus* Linnaeus, 1758) zone in the rivers of north-eastern France and Switzerland. It is characterised by a subcylindrical body striped with 3 to 4 oblique black bands. Individuals can be identified by the shape and arrangement of these patterns. It has a conical head ending in a rounded snout. Its mouth is in the inferior position (Fig. 1). Rough scales and strong thoracic pelvic fins allow it to cling to the substrate, even in fast-flowing currents. It has two distinctly separate dorsal fins unlike the bullhead (*Cottus gobio* Linnaeus, 1758) and ruffe *(Gymnocephalus cernua* Linnaeus, 1758), two morphologically similar species. Its eyes reflect the light of a torch, making nocturnal location easier.

It is commonly accepted that *Z. asper* is nocturnal; however, a study (Cavalli *et al*., 2009), conducted on a few individuals in the Durance River, showed that their activity can also be diurnal. Its camouflage is adapted to river bottoms scattered with gravel and pebbles, where it occupies riffles and pools at night. During the day, its activity is limited and it hides under larger pebbles in calmer and deeper zones. It is sedentary and territorial.

It is considered a good bioindicator and its presence suggests a high-quality environment. It tolerates a temperature range of between 0°C and 30°C, but the dissolved oxygen content must not be less than 7 mg/l (Perrin, 1988). During winter, *Z. asper* mainly consumes larvae of Diptera (Simulidae and Chironomidae); through the rest of the year,
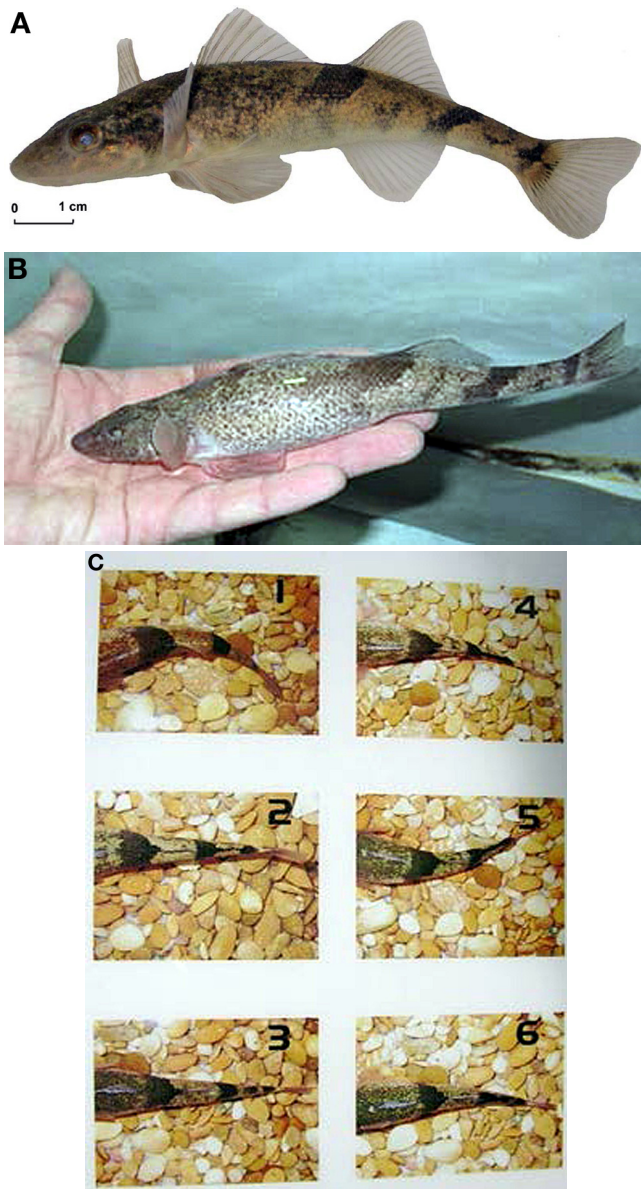
Figure 1. – *Zingel asper* specimens. **A**: Lateral view *Zingel asper*, male, 2 year old specimen born in 2008 in Besançon Natural History Museum; **B**: Lateral view, 4 year old female, size of 21 cm TL (captive born 2008); **C**: Dorsal photo of a *Z. asper* group, each fish can be individually identified thanks to the black stripes on their bodies (1-6).

Ephemeroptera (Baetidae) and Trichoptera (Hydropsychidae) make up the majority of its diet (Cavalli *et al*., 2003; Corse *et al.,* 2017). Sex identification of individuals is only possible during the spawning season, which takes place from February to April (Perrin, 1988) and more specifically during the month of March, when the water temperature is between 10 and 13°C (Labonne, 2002). During reproduction, males stay in fast-flowing areas while females join them briefly but do not remain there (Danancher, 2005). Sexual maturity is reached at 2 or 3 years old and fish of this age can reach sizes

of 15 to 20 cm. According to Danancher (2005), *Z. asper* is short-lived (around 3 years) with a few individuals reaching the age of 4 or 5.

The spawn of the species has never been observed directly in rivers and larvae of less than 2 months have never been located in natural environments.

The first reproduction tests of this species, undertaken in 1988 by J.F. Perrin, were not conclusive. Reproduction experiments conducted at the Ramières nature reserve from 2000 to 2002 by Nicolas Penel (Pers. Comm.) and Vincent Goubier (Institut Rhône Alpes d'Aquaculture, Lyon, France) successfully produced several hundred larvae via an artificial fertilisation procedure using one male and two females caught from the Beaume (a tributary of the Ardèche). Subsequent attempts were, however, unsuccessful. These trials highlighted the difficulties of keeping these fish in captivity, and more importantly, that reproduction of the species in experimental environments was not easy. Because the time of laying is not easily predictable, stripping operations are therefore problematic.

This paper describes the observations and results obtained since 2005 in the only breeding programme of the species at the aquarium of the Besançon Natural History Museum.

EQUIPMENT AND METHODOLOGY

Origins of broodstock

The broodstock used in the reproduction tests came from different locations. However, they all came directly or indirectly from the Beaume River until 2016. The scientific committee of the national action plan for the species had decided to take *Z. asper* from the Durance River because these fish were more genetically diverse (Dubut *et al*., 2016). The idea was that they would gradually replace the "Beaume" stock over time. Table I summarizes the different origins of broodstock.

Reproduction

Previous experiments have demonstrated the difficulties of obtaining viable eggs through artificial fertilisation. The stripping method was therefore initially avoided and a more natural technique developed. It consists of obtaining egg clutches without handling the broodstock, by artificially recreating elements favourable to the emergence of *Z. asper* larvae in rivers. As the fish is small, an "artificial spawning ground" can be created in a tank.

The "artificial spawning ground" technique

Little is known about the characteristics of the spawning grounds used in natural environments by *Z. asper*. However, the gathering of males in certain riffles in March and April has been observed. A riffle is an area where the speed of cur-

Table I. – Experiments conducted from 2005 to 2017 at the Besançon Museum.

Year	Experiments	Number of fish	Origin stock	Birth place	Capture date	Year of birth
2005	First reproduction tests	60	Beaume River	Ramières nature reserve	–	2000
2008	Reproduction with wild broodstock	18	Beaume River	Beaume River	December 2007	unknown
2012	30 and 45 vernalization days	46	Beaume River	Besançon Museum	–	2008
2013	75 vernalization days	45	Beaume River	Besançon Museum	–	2008
2014	90 vernalization days	22	Beaume River	Besançon Museum	–	2008
2015	120 vernalization days	20	Beaume River	Besançon Museum	–	2008
2016	120 vernalization days	30	Durance River	Durance River	September 2015	unknown
2017	120 vernalization days	28	Durance River	Durance River	September 2016	unknown

rent accelerates in proportion to the reduction in water depth. The bottom of this facies is particularly clean and made up of pebbles and gravel. *Z. asper* favours this habitat not only as a nocturnal hunting ground but also for reproduction.

The aim of the "artificial spawning ground" technique is to reproduce these particular conditions, thus encouraging broodstock to lay eggs without direct intervention. Using a sufficiently large tank, it consists of recreating the conditions essential to successful reproductive behaviour of the fish: egg-laying medium, favourable hydrodynamic conditions, appropriate annual thermal regime and nycthemeral cycle. The system is completed with a slow-current zone equipped with hiding places, where *Z. asper* can rest.

The characteristics of the artificial spawning ground are described below. The spawning grounds consist of waterproof mobile trays (45 × 16 × 4 cm) covered with alluvial gravel (grain size of 1-3 cm). The longest edge of the trays is in line with the direction of current. Dividing the spawning ground into independent trays offers several benefits. Firstly, the size of the egg-laying area can be easily modified by adding or removing trays. Identification of trays and analysis of their content can provide information on how egg laying is progressing. The small footprint means that trays can be placed in incubators of a reasonable size. Finally, the water tightness of the trays means that eggs laid can be moved while covered with water. The dimensions of the spawning grounds are 180 × 32 cm. A surface current of 0.5 to 1 m/s washes over the spawning ground. The trays are located at a depth of between 25 and 30 cm. After each lay, all the trays are replaced and the bottom is cleaned, allowing any eggs laid outside the spawning ground to be collected. The remainder of the tank is designed as a rest area. It is arranged with hiding places in the form of tubes (Appendix 1).

Artificial fertilisation procedure

Artificial reproduction techniques were resorted to for experiments, which required batches of homogenous eggs. In 2008, incubation trials at different temperatures were con-ducted on eggs taken from stripping operations. These trials could therefore be conducted using eggs simultaneously fertilised and from the same female. The problem with this method was to determine the female state of maturity. To avoid unnecessary handling, only females that voluntarily visited the spawning ground were used. After anaesthetising the fish, milt and ova were extracted by applying abdominal pressure. The maturity of the ova had to be checked before starting the next phase of the procedure (Fig. 2A). If microscopic examination confirmed maturity of the oocytes, the procedure could continue. If, however, they had not reached this stage, the stripping operation was abandoned. The female was handled first and most of time, three males of different sizes were used to optimise fertilization. Figure 2B, C illustrates how the fish were handled and show where fingers should be positioned during the procedure. The gametes were initially mixed together with a feather while dried (Fig 2E) and then 200 ml of water at 11°C was quickly added while stirring. The eggs were then rapidly spread out on 12 × 12 cm glass plates placed inside a small basin. The latter was then put into an incubator. After an hour, all the eggs had adhered to the glass and this flat and transparent medium facilitated monitoring. This method was efficient. However, as most egg laying takes place at night, only a small number of females have been successfully used. This technique was therefore not applied to broodstock used in experiments on the annual thermal cycle.

Annual thermal cycle

From diet to reproduction, water temperature proved to be a determining factor in the success of the breeding programme. The initial years of *Z. asper* reproduction trials led us to closely examine the winter period, and in particular the vernalization period, as many clutches of eggs had been obtained but egg hatching success rates were very low. From 2012 to 2016, five different vernalization periods (between 1 to 4 months) were applied in order to measure the impact of the length of this cold period on reproduction. Several
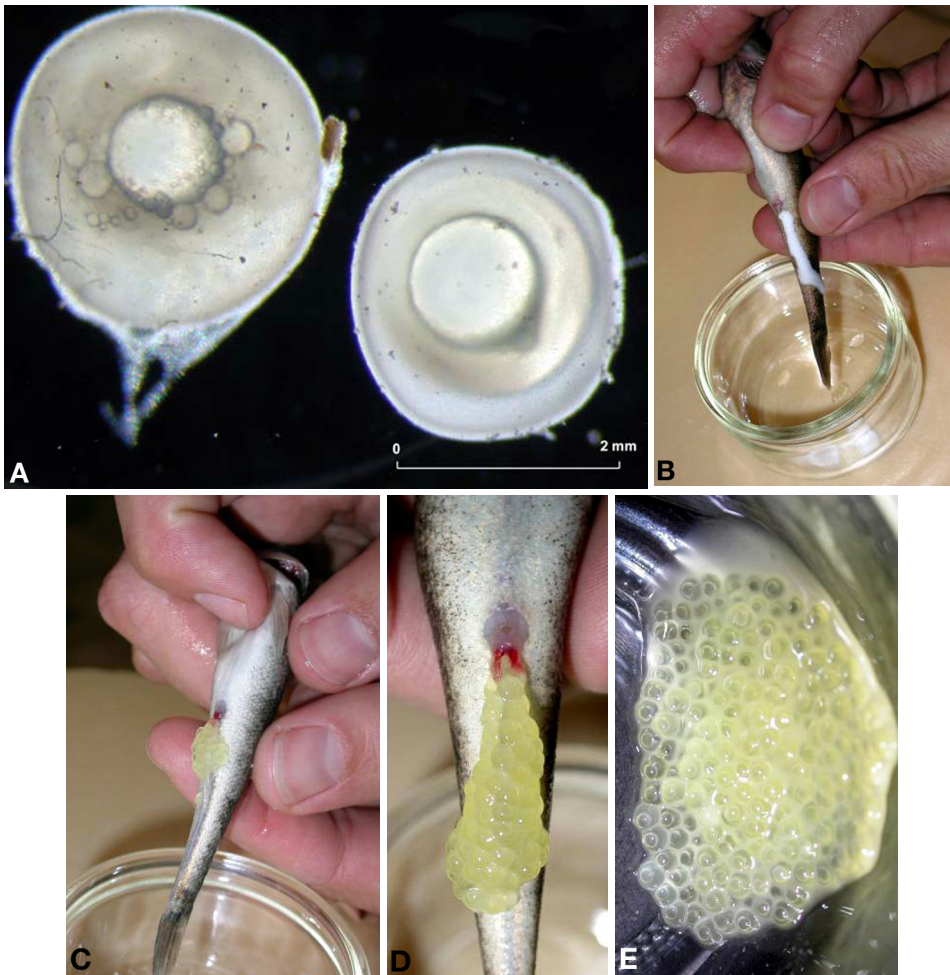
Figure 2. – *Z. asper* eggs. **A**: Mature ovum on the right and immature ovum on the left. **B**: Extraction of milt, this operation is done first. **C**: A low pressure from the abdomen towards the anus with the index finger allows the ova to be extracted. **D**: The yellow colour of the ova is a good indicator of their good quality. **E**: Water can only be added when everything is well mixed.

90 and 120 days, respectively. Finally, two other experiments were carried out, each with 30 wild *Z. asper* from the Durance River, in 2016 and 2017. A period of 120 days at 5°C was applied to these two batches. The results obtained from these final groups therefore allowed us to compare wild and captive *Z. asper* over several years (Tab. II).

Egg incubation

The eggs were incubated in incubators with a water depth of 10 to 15 cm. A surface current and air diffusers ensured maximum oxygenation of the water. Six incubators (Appendix 2) were used to accommodate the lay trays and two others housed the dishes of already sorted eggs. Dividing the spawning ground into moveable trays allowed removal of all the substrate after each lay. To ensure traceability, each tray was identified with a plastic label. The lay number was allocated in chronological order and not by tray to avoid errors. Trays from a same lay were placed in a same incubator. This avoided mixing eggs of different origins.

groups of broodstock, of different composition and origins, were used for these trials (Tab. I). The batch of *Z. asper* born in the Natural History Museum in 2008 was selected to test the influence of vernalization length on hatching rates. This group offered several advantages. These fishes were the offspring of wild broodstock from the Beaume River in the Ardèche department, and 50 of them were taken as samples from the egg clutches obtained. This ensured that a homogeneous batch of fish of satisfactory quality was used for this experiment. In 2012, the batch was split in two to create balanced groups and placed in the DR1 and DR2 tanks (Appendix 1). The group in tank DR1 was subjected to a vernalization period of 30 days but the minimum water temperature was never below 6°C. The group in tank DR2 was subjected to a 45-day period at 5°C. In 2013, the *Z. asper* in tanks DR1 and DR2 were all placed in the latter tank and the broodstock were subjected to a temperature of 5°C for 75 days, while in 2014 and 2015, this period was increased to

After eight to ten days of incubation at 11°C, the eggs were detached from the gravel by rubbing, and collected in a small black basin. Dead eggs (white and opaque) were then separated from living eggs. The latter were then put in small dishes and covered with a fine-mesh net. The dishes were placed in another incubator at a temperature higher than the previous one, at 13°C. A systematic check was then made every two days. After 18 days of incubation, the eggs were transferred to the hatching module. The eggs were counted during each sorting operation and numbers were recorded in a lay log sheet.

Hatching

After a final egg sorting operation of those that had reached maturity, the dishes were placed in small compartments of the hatching modules (Appendix 3). This allowed separate processing of each clutch of eggs. Two different

Table II. – Results of different reproduction trials conducted between 2012 and 2017.

Year	2012	2012	2013	2014	2015	2016	2017
Tank	DR2	DR1	DR2	DR2	DR2	DR1	DR1
Number of females	9	9	18	5	3	9	4
Number of broodstock	22	24	45	22	20	30	28
Broodstock age	4	4	5	6	7	unknown	unknown
Number of breeding experiments carried out with these broodstock	2	2	3	4	5	1	1
Number of vernalization days	30	45	75	90	120	120	120
Vernalization temperature (°C)	6	5	5	5	5	5	5
Number of eggs laid	7310	9935	23754	11563	4150	6188	1496
Average clutch	812	1419	2639	2313	1383	774	374
Minimum clutch	40	221	192	876	181	14	168
Maximum clutch	3111	2891	7091	3757	2517	1820	678
Number of surviving eggs at 10 days of incubation	9	775	7948	7956	3400	5550	1194
Number of surviving eggs before hatching	4	740	6805	7307	3361	5398	1175
Number hatched	1	659	4177	4717	3166	5106	1141
Average survival rate at 10 days (%)	0	8.5 ± 19.7	32.3 ± 13.6	66.5 ± 23.8	80.1 ± 3.8	89.1 ± 5.6	80.3 ± 18.9
Average survival rate before hatching (%)	0	8.2 ± 18.9	28.7 ± 13.8	59.4 ± 22.2	79.5 ± 3.3	87.1 ± 6.3	79.1 ± 20.4
Average hatching rate (%)	0	7.4 ± 17.5	19.5 ± 9.8	36.2 ± 14.5	73.7 ± 5.6	83.6 ± 5.3	77.6 ± 20.5
Number of positive lays (at –1 larvae)	1	4	9	5	3	8	4
Number of clutches	9	7	9	5	3	8	4
Number of broodstock dead during repro. (March to May)	1	0	19	2	1	4	7
Number of females unable to lay	1	0	5	0	0	0	0
Duration in days between first lay and last lay	12	9	12	10	3	34	36
Number of lays discarded following a technical problem	0	0	0	0	0	1	0

techniques were developed for this stage. The first uses a surface current to stimulate hatching. The second replicates the principle of Zoug jars with use of an ascending current. The hatching thermal parameters used were based on the temperatures recorded in rivers in April, namely, 13 to 15°C.

Rearing larvae

Two techniques were developed. The first was for pelagic larvae less than a month old and the second for benthic larvae. Once counted, freshly hatched larvae were reared in small 8-litre tanks fitted with a filtered water inlet and strainers. These items were also installed in the incubators, which were gradually transformed into rearing tanks. However, an additional installation was built for young larvae (Appendix 4). Larvae older than one month were then released into the tanks fitted with strainers and hiding places. At one month, the larvae were transferred to larger tanks. The incubators used to house trays of eggs were then adapted to accommodate benthic larvae. At 2 months, hiding places were installed on the bottom of the tanks. The first feeds were made using *Artemia nauplii* and temperatures varied from 14 to 18°C during the first month. After three weeks, pieces of

Chironomidae were distributed, and after 1.5 month, larvae fed on complete specimens of Chironomidae. At this stage, the water temperature was 20°C.

Data analysis

Data collected from 2012 to 2016 were statistically tested to determine whether hatching rates are comparable between years. Given the small sample size, the Kruskal-Wallis non-parametric test was used (variance analysis). It was completed by the Wilcoxon-Mann-Whitney test (two-sample comparison test).

RESULTS

Behaviours and needs of *Z. asper* in captivity
Acclimatisation

Wild *Z. asper* acclimatise quickly once in captivity. Care should be taken to ensure that each fish has a hiding place. They are initially fed on small earthworms. Two weeks after their arrival, they start eating dead Chironomidae. Since 2007, five batches of wild *Z. asper* have been added to the

aquarium in the Besançon Natural History Museum. Only a few individuals died during the acclimatisation phase.

Behaviour

The behaviour of *Z. asper* in captivity can be summarised as passivity during the day and first signs of activity at the end of the day. This very low level of diurnal activity is interspersed with short periods of movement to find hiding places.

We noticed rapid eye movements and sensitiveness to nearby peripheral disturbance. Despite the fish are permanently monitoring their environment, they can be easily caught if hand movement is relatively slow. *Z. asper* therefore adopt passive behaviour. Escaping is only used as a last resort but *Z. asper* are capable of sudden acceleration, which can propel them out of the water. If the tank walls are not sufficiently high, they can easily escape. The edges of the tank must rise above the surface of the water by at least 20 cm and a lid on each tank is necessary to prevent *Z. asper* from jumping out of the tank at night.

It should be noted that each *Z. asper* occupies the same area for several weeks but, one month before the reproduction period, males return to the riffle and stay there until May. Females only go to the riffle to lay their eggs.

By ensuring that there is at least one hiding place for each fish, up to 40 adult individuals per m² can be assembled in winter and 30 individuals per m² in summer.

Diet and growth

Z. asper have strict dietary requirements both in terms of quantity and quality (Corse *et al*., 2017). They will continue feeding at temperatures of 5°C. They favour frozen Chironomidae and live maggots and earthworms. Fish were fed *ad libitum* and portions readjusted in accordance with water temperature (Fig. 3B) and growth of the fish. Food was distributed three times a week for adults and daily for juveniles under the age of 6 months. Removal of detritus and unconsumed food appeared to be essential. Aspiration of the bottom of the tanks before feeding ensures a sufficient degree of cleanliness.

A group of 50 *Z. asper* born in the Besançon Museum was monitored for nine years. At 1 year old, the average size (from snout to end of caudal fin) was 11.3 ± 1.1 cm, with an average weight of 12.9 ± 3.3 g. Some individuals already measured 13 cm in length and a few males had milt. At 2 years old, some fish could not be sexed but all *Z. asper* were mature at 3 years and monitoring of both sexes was possible (Fig. 3A). Broodstock were therefore measured and weighed in February before reproduction to facilitate sexing of the fish. *Z. asper* size increases rapidly up to the age of 6 years old and then the growth curve levels out. Since the third year, females are significantly larger than males that rarely exceed 17 cm in length. In contrast, females can reach 19 cm
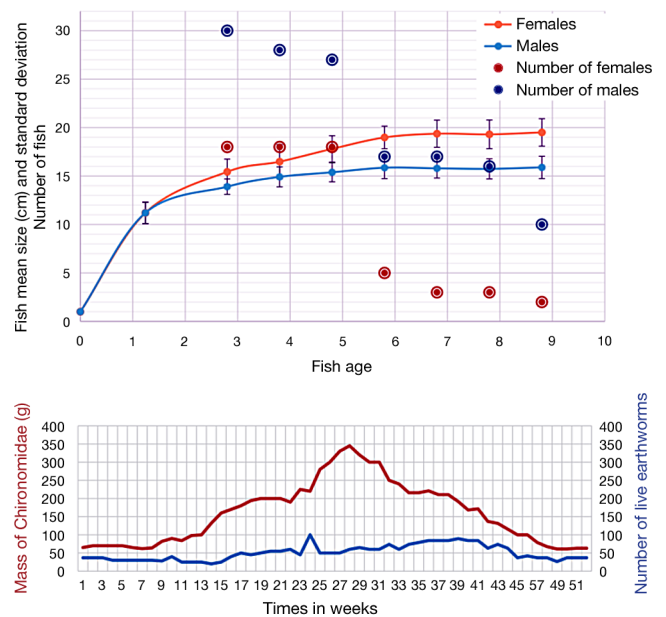

Figure 3. – **A**: Growth curve of the *Z. asper* group born in captivity (2008) descended from wild *Z. asper* from the Beaume. At 2 years old, some fish could not be sexed but all *Z. asper* were mature at 3 years and monitoring of both sexes was possible. At 5 years old, females are significantly longer than males. **B**: Quantity of food per week for 20 *asper* (Beaume 2008) in 2015. When the temperature reaches 14 to 15°C in May, *Z. asper* consume 3 times the quantity of food eaten at 5 to 10°C. From 20°C in July, consumption levels are more than 5 times higher than in the winter.

in length since their fourth year. One female reached 21 cm in length (Fig. 1B).

Reproduction

No egg laying or reproduction has yet been observed in the natural environment, although broodstock gathering in areas of current have been seen in March and April. In contrast, certain reproduction behaviours were easily observed since the first reproduction tests in aquarium. Moreover, the implementation of nocturnal video monitoring (in 2008) allowed to film most egg laying events. From 2005, using a broodstock individual recognition system (Fig. 1C), we found that certain females (in particular the largest ones), could stagger egg laying over several nights at 24- or 48-hour intervals. But most of time, eggs were laid in a single night. On 12 March 2008, a female laid eggs during seven hours, from 9:00 to 16:00. All stages were observed and filmed (Béjean and Maillot, 2008). Altogether, these observations made in artificial environments since 2005 teach us the following: (1) spawning activity can mobilise one to 20 males and one to two females simultaneously; (2) however, most of the time, only two or three males accompany one female (Fig. 4); (3) depending on their size, females can produce 300 to 3000 ova and expel them in batches of around 40 oocytes; (4) most of the time, eggs are expelled onto gravel,

Figure 4. – Female (in front) with 3 males in the current just before the expulsion of ova.

and a small proportion of them may be carried by the current and settle further downstream; (5) most ova are laid on clean gravel in fast-flowing current, although some clutches of eggs are found in areas of weak current (between 0.4 and 0.8 m/s); (6) the females can take up to seven hours to expel all her ova; (7) egg laying can take place from late February to mid-May at temperatures between 8 and 12°C; (8) maximum activity is observed during the months of March and April at temperatures of 10 to 11°C; (9) males do not leave the spawning ground from early February to the end of May, while females only go there to spawn; (10) egg size is 2 mm

Incubation and hatching

Full embryonic development (Fig. 5A-C) requires between 250 and 380 degree-days. This corresponds to an incubation time of 20 to 30 days at a temperature varying from 10 to 13°C. The "eyed" stage appears after around 10 days. The length of the development period varies widely, even between eggs subjected to identical incubation conditions.

Many trials were conducted and the method selected consisted of applying a temperature of 11°C for the first seven days. After a first sorting operation, the eggs were subjected

to a temperature of 13°C until the 19th day. They were then placed in the hatching module at 15°C. With this procedure, full embryonic development requires 280 degree-days. However, incubation success rates fluctuate greatly depending on the thermal cycle the broodstock were subjected to, and special attention was paid to the influence of winter temperatures on hatching rates (see below).

Hatching takes place in just a few minutes. However, some larvae extricate themselves in two stages: initially, the envelope is broken but the head and abdomen remain inside it. Several hours later, or even the following day, the larva manages to free itself. In some cases, however, it dies without completing its exit. Before this occurs, it can be removed from its envelope using very fine tweezers. Several hatching techniques were tested. The most effective consisted of leaving the eggs to float freely in a gentle current. In 2016, this method achieved a 95% success rate in 5,398 eggs. The results and zootechnical parameters are summarised in table I.

Larvae

The first few weeks of larvae development can be broken down into 4 phases: post-hatching phase until the first intake of food (Fig. 6A); pelagic phase (Fig. 6B); benthic phase (Fig. 6C) and juvenile phase (Fig. 6D).

Particular behaviours are seen at each stage and they determine the rearing parameters used. Just after hatching, the larvae stay immobile on the bottom or close to the substrate. They can already move around, even in the current. The larvae only attempt to reach the surface to fill their swim bladder after several hours. They gather together in the lightest corner of the tank. At this stage, they work their way into the slightest crack or slit, where they can stay hidden in. The larvae were placed in small rearing tanks at a concentration of 25 per litre with a water temperature of 15°C. After two to five days, the larvae colonise the entire water mass and start to feed. It is easy to catch them using a pipette and the transparency of their body allows to check if they have eaten or not. They are fed three times a day with *Artemia* nauplii.

The benthic phase starts after 15 to 20 days. The larvae then abandon the open water, and gradually colonise the bot-
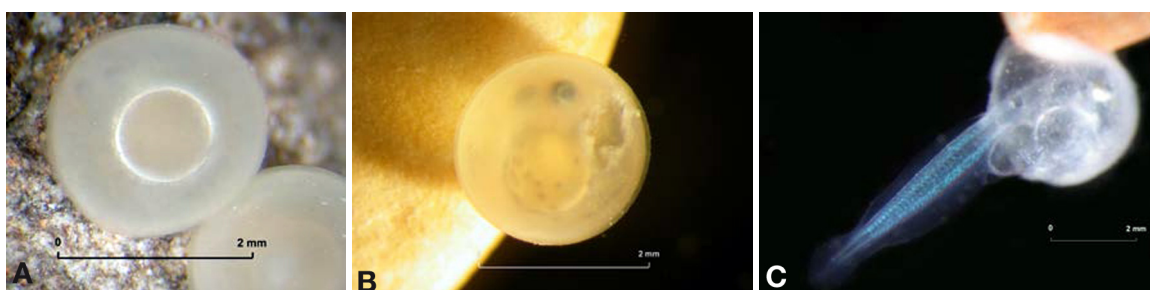

Figure 5. – Development of *Z. asper* before hatching. **A**: Embryo at 4 days; **B**: Embryo at 12 days; **C**: Hatching at 20 days.
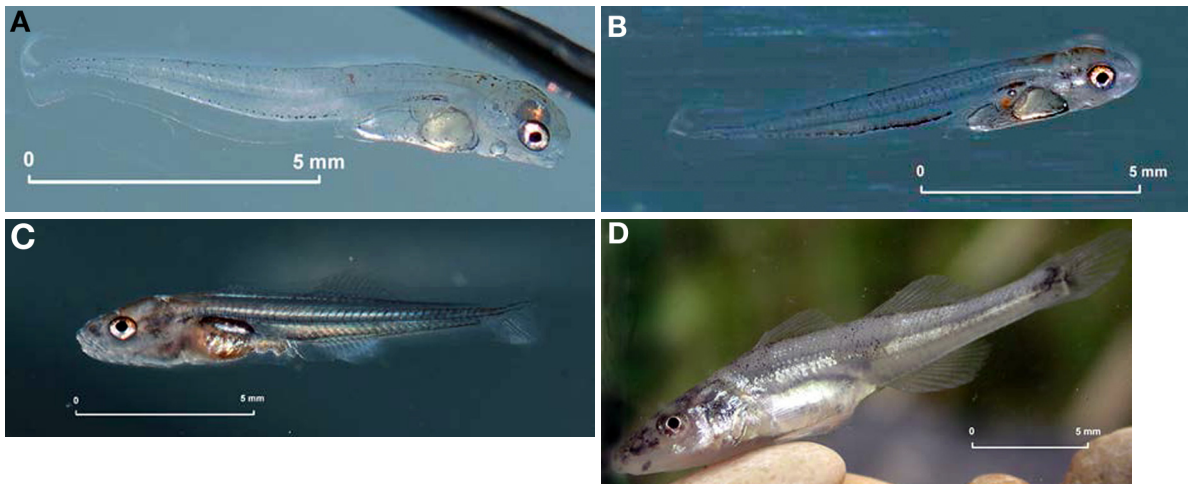
Figure 6. – Development of *Z. asper* after hatching. **A**: Larvae post-hatching; **B**: Pelagic larvae at 5 days; **C**: Benthic phase at 15 days; **D**: Juvenile phase at 40 days.

tom as well as the vertical walls of the tank. However, they systematically avoid placing themselves on the glass panels. They start to eat pieces of bloodworm and pigmentation commences. One-month survival rates are around 80% in these conditions at temperatures varying from 15 to 18°C. The appearance of nostrils is the signal to move the larvae to a larger tank at a concentration of 400 to 500 fish per m². The water temperature may then exceed 20°C.

The juvenile phase starts 40 to 50 days after hatching, as small *Z. asper* acquire their definitive morphology and eat whole bloodworms. At this stage, they are nocturnal and adopt the same behaviour as adults. From this point onwards, losses are very rare and rearing them no longer presents any difficulties, unless they are raised in aquariums made entirely of glass. At 2 months, they measure between 35 and 40 mm and we chose this size to transfer them to their natural environment. To transport them, the fry were packaged in batches of 300 in 50-litre bags containing one-third water and two-thirds pure oxygen. They were able to survive in these conditions at least nine hours.

In 2016, several rearing trials using larvae aged between 1 and 2 months old were conducted with concentrations varying from 315 to 559 individuals per m². The average survival rate in these experiments was 93 ± 5.8%. The range of concentrations tested does not allow to relate the survival rate and the original larvae concentrations ($R^2 = 0.0049$). However, the average survival rate and that obtained with the higher concentration (94.9%) indicates that rearing 1-month-old larvae at a concentration of about 500 larvae per m² does not cause any supplementary loss.

Up to the age of 1 year, small *Z. asper* were reared at a concentration of 100 per m². At 1 year old, they were treated as adults. They measured between 7 and 13 cm.

Sensitivity

Although this species sometime copes with unfavourable physicochemical conditions (nitrite concentrations of 0.2 mg/l measured via twice-weekly monitoring), it appears to be sensitive to excess organic matter, even in small amounts. Surplus quantities over long periods can promote
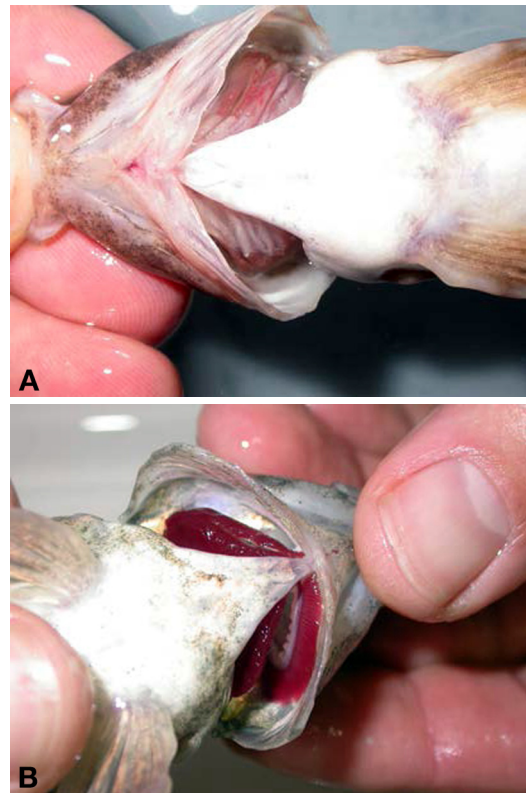

Figure 7. – Health of gills. **A**: Early signs of mycosis on gills; **B**: Healthy gills.

Table III. – Medical treatments for *Z. asper* at the Besançon Museum.

Drug to be dissolved in tank water	Dosage	Treatment time	Time between treatments	Number of treatments	U.V. sterilizer
NaCl	5 g/l	24 h	10 days	2	
Malachite green oxalate	0.1 mg/l	24 h	10 days	2	
Praziquantel	3 mg/l	24 h	10 days	2	Stop
Cholramine T	20 mg/l	24 h	10 days	2	
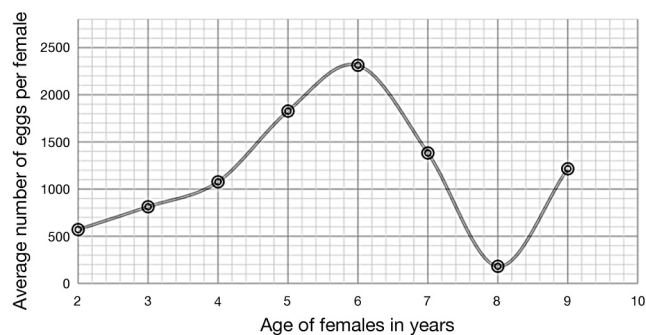
Figure 8. – Egg production as a function of age. These results were obtained from the number of eggs laid throughout the breeding season divided by the number of females that laid. The same bloodstocks (born in captivity in 2008) were used for 9 years. The maximum production was obtained at 6 years. The number of females was 17 at the beginning and 2 at the end (Tab. II).

the development of mycosis in the gills (Fig. 7A, B). The first symptoms are fast breathing, the fish then keeps its mouth open and eventually dies through asphyxiation. This sensitivity is increased during the reproduction period when the majority of deaths is due to mycosis. Treatment is possible using chloramine-T or malachite green (Tab. III). In 2017, *Z. asper* from the natural environment were infested by *Dactylogyrus* parasites. After seven months in captivity, they caused fatalities by weakening the fish. A course of treatment with praziquantel successfully eradicated this widespread infestation in the gills and at the base of the fins.

Longevity, fertility and fecundity

In captivity, some *Z. asper* live over than nine years. Observation of a group of *Z. asper* born in 2008 at the Besançon Museum and monitored until 2017 allowed us to study changes in the number of ova per female as a function of age (Fig. 8). The number of eggs per female starts reducing after the fifth year. The number of eggs per female then diminishes rapidly, reaching very low production in the 8th year. This led us to stop using the data from this group for interpretations of the experiment on winter temperatures presented below. However, it is worth noting that for two remaining females, egg production started again in the ninth year. Regarding the average hatching rate, at the age of 7 years old, it was still close to 74%. At 8 years, the rate dropped to 45% and at 9 years it rose to 64%, without any

obvious explanation, as the environmental conditions were the same. By the 7th and 8th year, there were only three females, and two in the 9th year. By the end of 2017, seven broodstock were still alive in this group, including one female.

Physical characteristics

Each fish can be individually identified thanks to the black bands on its body since their patterns are sufficiently distinctive. Dorsal and lateral photographs (Fig. 1C) allow to produce an accurate register for each cohort. This identification proved extremely useful during observation of reproductive behaviour. It was gradually abandoned as the number of *Z. asper* in the breeding programme increased. However, this technique was successfully used to count the wild *Z. asper* population in the Swiss part of the Doubs River (Bonnaire, 2012).

A characteristic of the species is the ability of its eyes to reflect torchlight. This property was used from 1998 onwards to count the wild populations in the first LIFE programme. Observations in aquariums have shown that pupil dilation in *Z. asper* is affected by light levels.

Influence of winter temperature on reproduction

The broodstock used to study the influence of vernalization period length came from 2 sources. The first group was reproduced in 2008 from wild broodstock from the Beaume River, and 50 fries were taken as samples from the egg clutches obtained in 2008 to take part in the experiment. These *Z. asper* fish of the "Beaume" stock were used from 2012 to 2015. The second group consisted of two batches of 30 wild *Z. asper* from the Durance River (Tab. I), caught in the frame of the reintroduction programme in the Drôme River in 2015 and 2016. The *Z. asper* fishes of the "Durance" stock (used in 2016 and 2017) were replaced between the two years. Tanks DR1 and DR2 (Appendix 1) were selected for this experiment as they were equivalent and allowed easy monitoring of reproduction. Feeding and lighting conditions were similar.

More than 64,000 eggs were collected during these trials and all egg clutches were accurately counted at four key moments of the incubation process: on laying, at 10 days, at transfer for hatching and at hatching. Average survival rates up to hatching differed as the experiment conditions changed (Fig. 9A) (Tab. II).

Firstly, the broodstock subjected to clement winter temperatures produce egg clutches of mediocre quality, or even no egg at all in the case of the group subjected to no more than a 30-day vernalization period at 6°C. From the first development stages, egg mortality declines as the length of the winter cold period increases. After 10 days of incu-
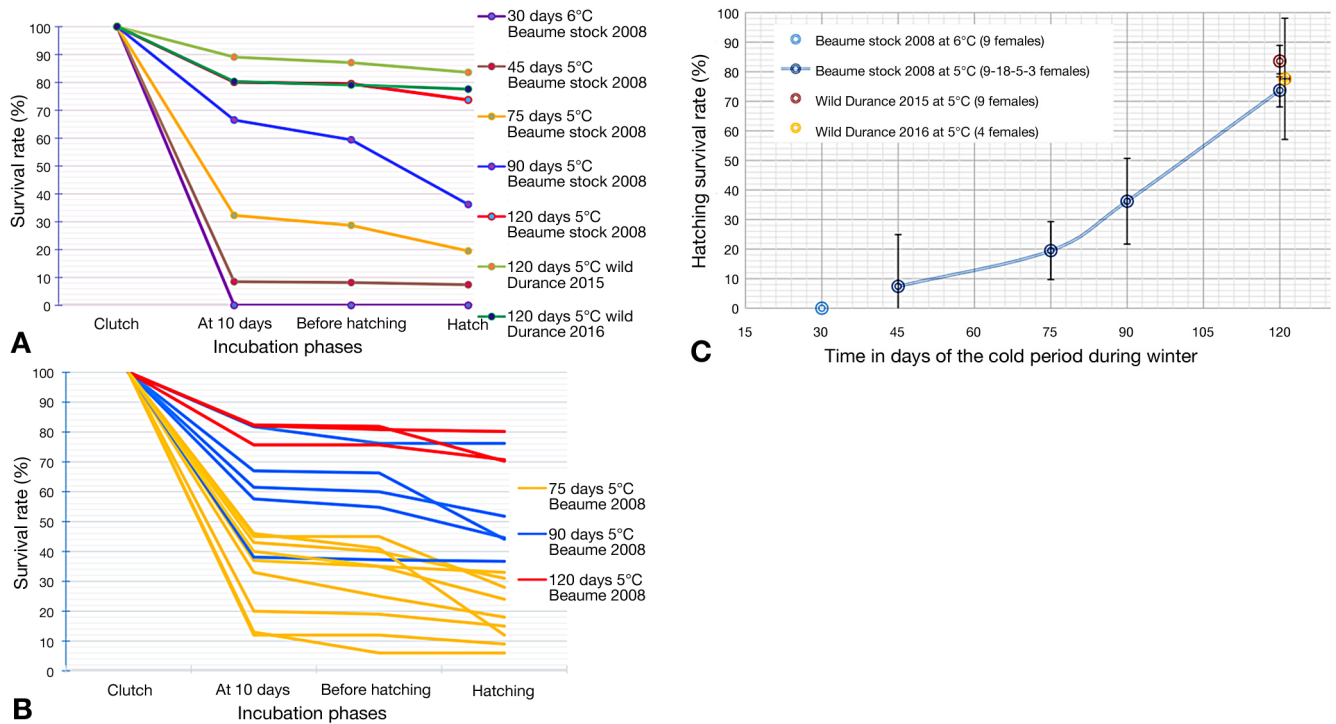
Figure 9. – **A**: Comparison of incubation results as a function of cold period length from 30 to 120 days. More than 64,000 eggs were collected during these trials and all egg clutches were accurately counted at four key moments of the incubation process: on laying, at 10 days, at transfer for hatching and at hatching. From the first development stages, egg mortality declines as the length of the winter cold period increases. **B**: Clutch by clutch detail of the incubation survival rate of eggs for batches of "Beaume" broodstock subjected to 3 different temperature periods. The egg clutches produced during the same experiment are identified by an identical colour. Within the same group, survival rate results sometimes varied widely. The hatching rates for broodstock subjected to 90 days of vernalization are the most variable. This wide variability suggests that the 90-day period of cold temperatures creates a boundary, which determines whether reproduction is successful or not. **C**: Variation in hatching rate (mean and standard deviation) as a function of vernalization period length. Unlike those from the "Beaume" stock, *Z. asper* of "Durance" stock we used in 2016 and 2017 (captured in 2015 and 2016), were replaced between the two years. The hatching rate varies in accordance with the duration of the cold period experienced by the broodstock during the winter. For the "Beaume" broodstock, Kruskal-Wallis tests show that comparisons between the results obtained with the 120-day period and the other periods are significant ($p < 0.05$). The length of the vernalization period clearly has an influence on the hatching rate. This test was also applied to data from the experiments involving both sets of stock and 120-day periods. As this test was not significant ($p = 0.07$), it demonstrates that both sets of stock reacted similarly to this vernalization period.

bation, a significant improvement in the survival rate is observed when the vernalization period exceeds 90 days. However, survival rates continue to moderately decrease over the next stages. Within the same group, survival rate sometimes varies widely. The graph in figure 9B illustrates, clutch by clutch, survival rates for the tests conducted on the "Beaume" broodstock born in captivity. The egg clutches produced during the same experiment are identified by an identical colour. The hatching rates for broodstock subjected to 90 days of vernalization are the most variable. They range from 18 to 52.4% with an average of 36.2% ±14.5%. This great variability suggests that the 90-day period of cold temperatures induce a boundary, which determines whether reproduction is successful or not. For this period, four out of five females produced egg clutches with 10-day survival rates of 70 to 88%; the rate dropped dramatically between 29 and 52% at hatching. The survival rates of egg clutches subjected to a vernalization period shorter than 90 days show a

very sharp drop before the tenth day and then later stabilised. In contrast, the experiment with a cold period of more than 90 days produced very good results, with survival rates of between 70 and 80% from the tenth day up to hatching.

These tests were completed with two experiments using wild broodstock of various ages from the Durance River in 2015 and 2016. Variation in survival rates of egg clutches from wild broodstock is similar to those of broodstock born in captivity with the same vernalization period of 120 days. The influence of vernalization period length on the hatching rate is illustrated in the graph in figure 9C. The hatching rate varies in accordance with the duration of the cold period experienced by the broodstock during the winter. For the "Beaume" broodstock, Kruskal-Wallis tests show that comparisons between the results obtained with the 120-day period and with the other periods are significant ($p < 0.05$) and that only the test between the data of the 75-days and the 90-day periods is not significant ($p = 0.06$). The length of the
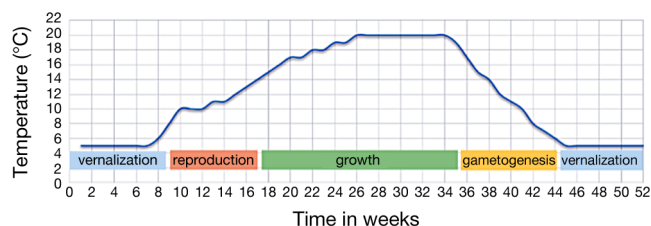
Figure 10. – Annual thermal cycle for different life phases of *Z. asper*. The "gametogenesis" phase starts when temperatures fall in September and continues throughout the winter but slows down in November when the temperature reaches 5°C. This period of time at 5°C, indicated as "vernalization" on the graph, lasts from November to February. The duration of this period is very important because it directly conditions the success of the reproduction. Rapid and regular rising of temperatures to 10°C triggers reproduction from the beginning of March and continues until the end of April.

vernalization period has a marked influence on the hatching rate. This test was also applied to data from the experiments involving both sets of stock and 120-day periods and show that both sets reacted similarly to this vernalization period (p = 0.07). The Wilcoxon-Mann-Whitney test was applied to these data and confirmed the results of the previous statistical test.

The duration of this cold period probably also impacts on broodstock mortality. In 2013, a large number of fishes of the "Beaume" broodstock died (Tab. II). This increase in mortality could have been caused by a higher concentration of fish. However, it was primarily females that died, either just after laying or without having laid eggs. During the 2013 reproduction period, 70% of the females died. We noticed that the clutches contained eggs 1.5 times larger than normal. This anomaly was also presented by the ova of the females that were not able to lay. In contrast, no large eggs were seen in the clutches of broodstock subjected to 120 days of vernalization. It seems that overly clement winter temperatures caused early maturation of the ova and ultimately adversely affected their expulsion during spawning, leading to significant mortality among females.

The best hatching rates were therefore obtained with a vernalization period of 120 days. However, it is important to apply this period at the correct time. The graph in figure 10 summarises the different life phases of *Z. asper* in captivity, as a function of temperature. Four key moments in the life of *Z. asper* can be identified where water temperature plays an important role. When the temperature reaches 14°C to 15°C in May, *Z. asper* consume triple the quantity of food eaten at 5°C to 10°C. From 20°C in July, consumption levels are more than 5 times higher than in the winter. The fish take advantage of the summer season to grow and build reserves for the production of gametes. This time period with temperature of 14°C to 20°C (and higher) is the section shown as "growth" on the graph (Fig. 10). The "gametogenesis" phase starts when temperatures fall in September and con-

tinues throughout winter but slows down in November when the temperature decreases and reaches 5°C. This time period with temperature around 5°C ("vernalization" in Fig. 10) lasts from November to February. Rapid and regular increase of temperature to 10°C triggers reproduction from the beginning of March until the end of April.

DISCUSSION AND CONCLUSION

Twelve years of reproduction trials on *Zingel asper* have allowed us to rear it successfully. Although behavioural observations made in captivity cannot be directly transposed to the natural environment, the work conducted at the Besançon Natural History Museum has allowed us to significantly increase our knowledge of the biology of this species. Few publications are devoted to the other *Zingel* species but they allow some comparisons. First of all, the two other species of this genus produce sticky eggs with a diameter of 2 mm (Patzner *et al.*, 1994; Kovac, 2000). An artificial reproduction test was carried out with *Zingel streber* by Kovac (2000) and the fish gonads were ripe on April. Fertilization, incubation and larvae maintenance were carried out at temperatures of 8°C, 12°C and 17°C, respectively. This is similar to the settings applied to *Z. asper*. However, the embryonic period is shorter for *Z. streber* (176 to 302 degree-days). *Z. asper* therefore has a much longer embryonic phase (250 to 380 degree-days) and, indeed, one of the longest among freshwater fish (Teletchea *et al.*, 2009). The larva size is slightly smaller in *Z. streber* (6 mm at hatching and 15 mm at 50 days). Nevertheless, the morphology of the larvae of the two species is very similar (Kovac, 2000).

No information is available for the other species of the genus *Zingel* about the thermal requirements during the gametogenesis. Studies conducted on the Eurasian perch show the negative influence of excessively high temperatures during the gametogenesis, on the quality of gonads and eggs since too high temperature, or lack of cooling down may induce egg resorptions and other gonad malfunctions during autumn and winter (Luksiene, 1986; Lapina, 1991; Luksiene and Sandström, 1994; Sandström *et al.*, 1995; Migaud *et. al*, 2002). A seasonal rhythm with low winter temperatures is needed for uninterrupted vitellogenesis. Moreover, females of yellow perch *Perca flavescens* Mitchill need a minimum period of temperatures below about 6°C for about 6 months for optimal and complete gonad maturation (Hokanson, 1977). The results obtained for *Z. asper* are similar and this specie is to be added to the list of the species sensitive to winter temperatures.

The direct connection between a significant drop in hatching rates and the length of vernalization period should raise concerns about future risks in the frame of climate change. If the temperature regime of aquatic environments is

no longer suited to their biological rhythms, species such as *Z. asper* may rapidly extinct. Analysis of thermal data under the national action plan (Action 15) has shown that, over the last few years, optimal vernalization conditions where not meted in the watercourses inhabited by *Z. asper*. The thermal cycles applied for protocol purposes to the experiment conducted at the Besançon Museum are therefore not exactly equivalent with what happens in rivers. In natural conditions, temperature variations are much more marked and a period of 120 consecutive days at 5°C is rarely observed. Maazouzi and Olivier (2017) attempted to connect the thermal and hydrological parameters recorded in rivers that currently still contain the *Z. asper* populations with their development. Numerous thermal and hydrological long-term datasets have been collected over the course of different conservation programmes and the results of the experiments conducted at Besançon Museum provide guidelines for further research. Unfortunately, insufficient data on reproduction of wild *Z. asper* and incomplete thermal datasets prevent to establish a link between the development of the populations of wild *Z. asper* and environmental parameters.

In addition to the intrinsic interest of the results obtained for keeping and reproducing *Z. asper* in captivity, the individuals reared and kept in the Besançon Museum can be used for other conservation initiatives, to improve knowledge on their biology and to raise public awareness on climate change impact on patrimonial species and more generally on biodiversity.

As an example of scientific interest, in 2005, *Z. asper* specimens from Besançon Museum breeding programme were used in a study to experimentally define the characteristics of a fish pass adapted to the species (Gomes *et al*., 2005). Today, most of the structures built to improve the ecological continuity of watercourses in the Rhône basin are inspired by the recommendations provided in this study. Also, as part of the LIFE Apron II programme, reintroduction trials were planned so that capacity was available to support wild populations if they continued to decline. Between 2008 and 2017, more than 26,600 larvae (2-month old) born in the Besançon Museum have been reintroduced into the Drôme River. The *Z. asper* released are regularly monitored, and for the first time in 2015, genetic analysis showed that reproduction in the natural environment had taken place (Dubut *et al*., 2016). The definitive conclusions of this initiative will be available in 2020. Moreover, in 2012, forty *Z. asper* were given to the SPYGEN research laboratory to conduct detection tests of the species in rivers using environmental DNA (eDNA). The results of the experiments were conclusive: this species can now be easily detected using this method and this allowed identification of three new research stations, *i.e*. the Verdon, the Bléone and the Doubs rivers.

Finally, around 1000 *Z. asper* are on permanent display in the installations of the aquaculture farm of the Besançon Museum, where the public can learn about the difficulties encountered by the species in the natural environment. Each year, 270,000 people visit the farm and numerous school groups take part in activities offered by the mediation department. The *Z. asper* breeding programme of the Besançon Museum is currently the only one of this kind for this genus. However, five organisations are regularly provided with specimens of the fish for display such as the "Gare des Ramières reserve" (France, Drôme) and the Aquatis Aquarium (Switzerland, Lausanne).

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Appendix 1 Broodstock tanks
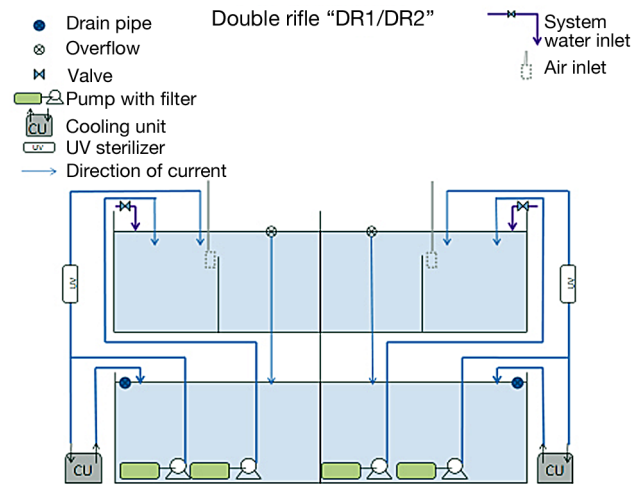
Double riffle "DR1 and DR2"

Top view of reproduction module "DR1/DR2"

Gravel tray

PVC tube

⊗ Overflow

Direction of current

DR1   DR2

0   100 cm
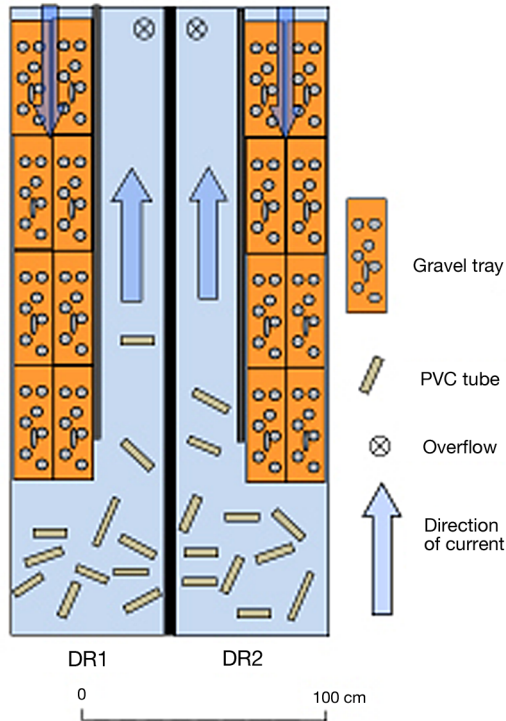
Top view of DR1/DR2 double riffle, each section contains a spawning ground made up of eight gravel-filled trays, a rest area. The "double riffle" was designed to accommodate two groups from 25 to 50 specimens of broodstock in strictly identical conditions. The spawning grounds are equipped with waterproof, motion-sensing cameras with infrared night vision, connected to a 1000 Gb recorder. The diurnal and nocturnal activities of the two groups can therefore be simultaneously recorded over a long period.

Bottom of DR1 tank, the sides of the module are fitted with glass panels which allow natural light from a window to enter the tank, and the observer to view the behaviour of the broodstock. This device ensures easy viewing and checking of the broodstock, facilitates management of feeding and allows effective monitoring of reproduction.

● Drain pipe   Double rifle "DR1/DR2"   System water inlet
⊗ Overflow   Air inlet
◄ Valve
▭ Pump with filter
CU Cooling unit
UV UV sterilizer
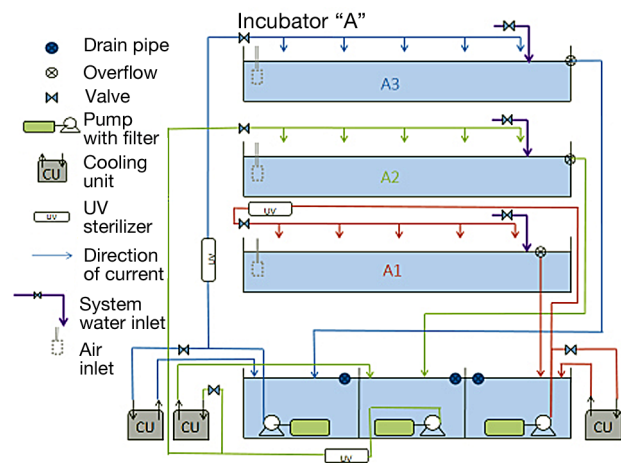→ Direction of current

Operating diagram of DR1/DR2 double riffle; it consists of two independent sections (DR1 and DR2), each containing 630 litres of water and measuring 2.5 x 0.6 m. Each section contains a filtration system separate from the fish, a cooling unit and an ultraviolet sterilizer. An 80 W UQL lamp completes the lighting of the module lit during the day.

Appendix 2 Incubators

The eggs were incubated in incubators with a water depth of 10 to 15 cm. A surface current and air diffusers ensured maximum oxygenation of the water. Six incubators were used to accommodate the lay trays and two others housed the dishes of already sorted eggs. They were all fitted with independent filtration devices, cooling units and UV sterilizers. Built specifically for this experiment, two different designs of incubator were used.
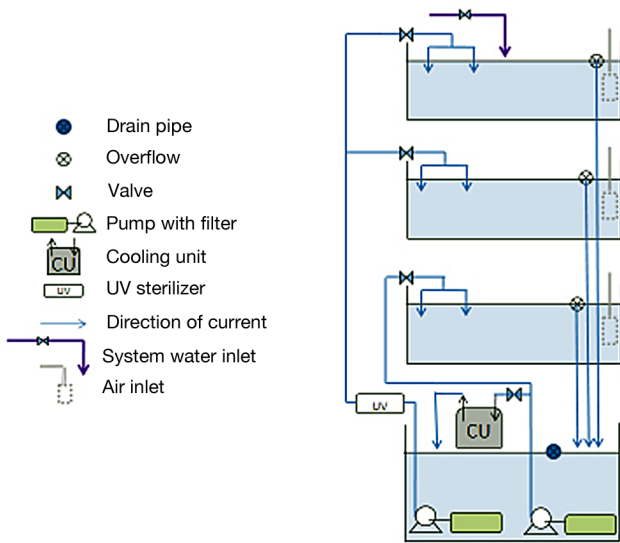
Incubation module for eggs on trays

● Drain pipe   Incubator "A"
⊗ Overflow
◄ Valve
▭ Pump with filter
CU Cooling unit
UV UV sterilizer
→ Direction of current
System water inlet
Air inlet

Operating diagram of the incubator, two tiered modules contain six independent incubators. Three shallow hatching are (220 × 60 × 17 cm) stacked on top of each other to create a compact assembly in which each tier functions independently. Eighteen trays covered with eggs can be placed in each tier, allowing the simultaneous incubation of seven to nine lays.

Incubators for sorted eggs

Incubator "IF"

- ● Drain pipe
- ⊗ Overflow
- ⋈ Valve
- Pump with filter
- CU Cooling unit
- UV UV sterilizer
- → Direction of current
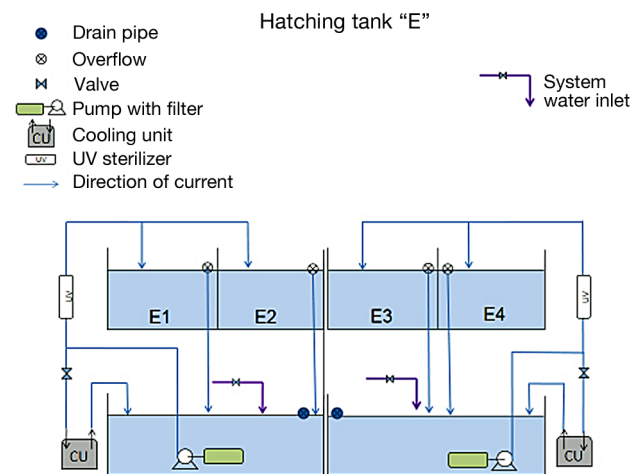- System water inlet
- Air inlet

Operating diagram of IF incubator, two identical incubators were used for eggs that were already sorted. They are made up of an isothermal enclosure and contain three tiers (100 × 60 × 17 cm). The water circulating in each tier comes from the same filtration, cooling and sterilisation device. As a result, the eggs placed in the different tiers are subject to the same temperature regime.

Appendix 3 Hatching modules

Hatching module "E"

The "ME" hatching module can be used to observe hatching progress without having to remove the dishes, thus avoiding premature hatching caused by handling. For this reason, the surface of the water is entirely uncovered to allow easy egg-sorting and larvae-sampling.

Hatching tank "E"

- ● Drain pipe
- ⊗ Overflow
- ⋈ Valve
- Pump with filter
- CU Cooling unit
- UV UV sterilizer
- → Direction of current

System water inlet

Operating diagram of hatching module, this module consists of two clearly separated sections, each consisting of two long tanks (2 × 0.2 × 0.2 m) designed to accommodate hatching boxes, a filtration tank and an independent water circulation pump with a cooling unit and UV sterilizer. This allows simultaneous monitoring of 16 batches of eggs.
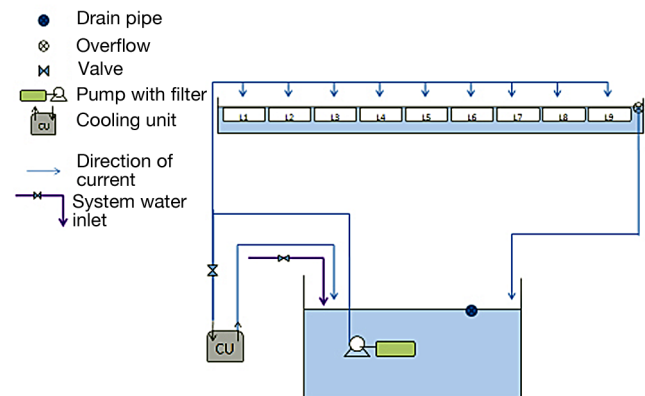
Zoug jar module "BZ"

Zoug jar incubator
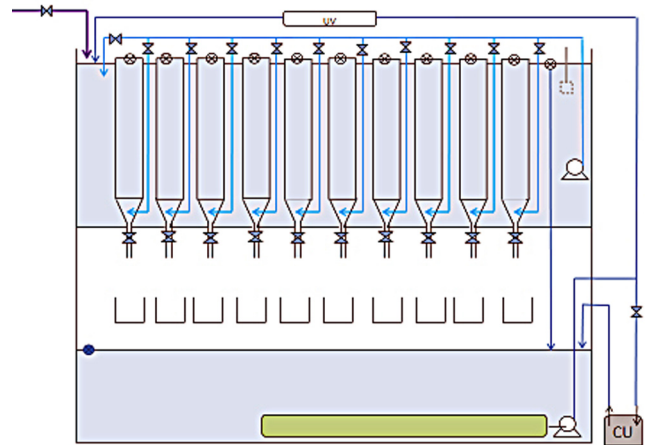
Operating diagram of hatching module in Zoug jars, this system consists of a 300-litre temperature-controlled isothermal enclosure containing 10 one-litre Zoug jars, each able to accommodate several hundred eggs. An ascending current holds the eggs in suspension and carries the larvae to the surface. Another bottle connected to this device collects the larvae. The water circulating in the jars is independent of that used in the filtration circuit. A cooling unit and UV sterilizer complete the installation.

Larvae rearing module "L"

Larvae module "L"

- ● Drain pipe
- ⊗ Overflow
- ⋈ Valve
- Pump with filter
- CU Cooling unit
- → Direction of current
- System water inlet

Operating diagram of larvae hatching module, this installation was used to determine the optimum larvae load during the rearing process and provided additional space for rearing several thousand larvae. It consists of nine 20-litre tanks with a glass panel along the front. They are fitted with an inlet supplying filtrated water at a rate of 100 l/h and an individual air inlet.