Brook chub, *Squalius lucumonis* (Pisces, Cyprinidae) conservation aquaculture: first attempt at artificial reproduction and larval rearing

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ABSTRACT

Many recovery programs for endangered species rely on the release of hatchery-reared juveniles to support threatened populations or to re-build new ones. In the framework of a conservation plan for the Italian critically endangered fish (*sensu* IUCN Red List) *Squalius lucumonis*, artificial reproduction and larval rearing was carried out for the first time. Wild breeders were collected during reproductive period (May-June) by electrofishing from Fosso Corese, a small tributary of Tiber River (Central Italy). *In situ* stripping of gametes followed by manual fecundation was carried out. *Ex situ* incubation of eggs and larval rearing were conducted in the hatchery of LESA (University of Tor Vergata). With the aim of producing fingerlings with "wild-like" behavior, a larval rearing technique in a "green water" large volume tank was applied. For the first time, about 400 fingerlings (87 days old) of brook chub were obtained from about 2,300 free embryos (17% survival rate). This result is promising as it demonstrates the technical feasibility of brook chub conservation aquaculture, the first step for an *in situ-ex situ* recovery plan for this species based on reared fingerlings' restocking.

Keywords: green waters, endangered fish, conservation aquaculture, enhancement aquaculture, fish biodiversity.

1. Introduction

The use of reared fingerlings for restocking and enhancing natural fish stock has a long history worldwide. Restocking is often based on *ex situ* production of juveniles in aquaculture facilities. This type of aquaculture technique, also known as “enhancement aquaculture” or “conservation aquaculture”, has proven to be successful for a variety of freshwater fish species. Conservation aquaculture can contribute to the protection and restoration of endangered natural fish populations through the provision of juveniles for releasing and restocking (i.e., trouts, sturgeon, shads) (Hundt, 2015; Ireland et al., 2002; Romanenko et al., 2005; Schreier et al., 2012). The restocking with reared autochthonous juveniles is recommended for two main reasons: i) compensation for some shortage of recruitment (so stabilizing the population size); ii) re-introduction for re-building a population already extinct or at the brink of extinction.

As already demonstrated in previous experiments mainly carried out on Mediterranean marine fish species (Boglione et al., 2009; Cataudella et al., 2011; Russo et al., 2009), larval rearing in the “green waters” system and in semi-intensive conditions (i.e., large volumes, low density, a large range of wild live preys) has been demonstrated to be particularly effective for producing juveniles with “wild-like” behavior in order to better tackle natural environment conditions (Cataudella et al., 2002). In fact, this rearing technique is aimed at recreating some environmental conditions of natural nursery grounds, i.e. ecological dynamics, a large spectrum of live preys. The use of drugs and/or disinfectants is avoided.

Recently, a growing interest in conservation aquaculture for freshwater fish restocking has characterized various EU LIFE NATURE projects: i.e., LIFE-Projekt Maifisch (The re-introduction of allis shad *Alosa alosa* in the Rhine System, 2007); LIFE BARBIE (Conservation and management of *Barbus meridionalis* and *Barbus plebejus* in the Emilian

52 tributaries of Po river, 2014); LIFE + TROTA (Trout population recovery in central Italy,
53 2012); LIFE92 NAT/E/014400 Pego Oliva/samaruc (First phase of an action programme for
54 the conservation of two wetlands and the creation of a reserve network for *Valencia*
55 *hispanica*, 1992).
56 The target species is the brook chub, *Squalius lucumonis*, firstly described by Bianco
57 (1983) and genetically confirmed as species by Ketmaier et al. (1998), Rossi et al. (2012)
58 and Tancioni et al. (2013). It is an Italian endemic cyprinid of the Latium-Tuscany district
59 which inhabits tributaries of the main Thyrrenian basins (i.e. Tiber, Arno, Ombrone, Magra-
60 Vara) (Bianco and Taraborelli, 1984; Bianco and Ketmaier, 2003, Ciuffardi et al., 2010;
61 Tancioni et al., 2013; Bianco et al., 2013; Bianco, 2014; Tancioni and Lorenzoni, 2016).
62 This species shows small size and fragmented local populations, which are strongly
63 threatened by extreme meteorological events (i.e., prolonged droughts and abnormal
64 floods), by potential competition with allochthonous species (Tancioni and Lorenzoni,
65 2016), habitat degradation and predation from ichthyophagous birds (Bianco, pers. comm.).
66 This species has an estimated area of occupancy (AOO) inferior to 500 km². It is listed in
67 Annex II of Habitat Directive, as species of European interest for conservation, and in IUCN
68 International Red List as Endangered Species (Crivelli, 2006). Recently, due to the
69 progressive decline of populations, the species obtained the status of Critically Endangered
70 in the Italian IUCN Red List of Vertebrates (Bianco et al., 2013; Rondinini et al., 2013;
71 Bianco, 2014). The brook chub is a small moderately rheophilic and thermophilic cyprinid
72 which often cohabits with the congeneric Italian chub, *Squalius squalus* (Bonaparte, 1837)
73 (Bianco et al., 2013; Giannetto et al., 2013; Tancioni and Lorenzoni, 2016). The ecological
74 interactions between these species seem limited, due to the diverse ecological traits (i.e.,
75 habitat preference) as highlighted in the Tiber river (Giannetto et al., 2013). Nevertheless,
76 genetic interactions between the two species, with possible hybridization, have been

77 demonstrated in local populations cohabiting some small watercourses within the range of
78 brook chub (Martinoli, 2017). The spawning period of brook chub starts when the water
79 temperature reaches about 20 °C, in the spring (April - June). Each reproductive event may
80 extend over a range of days (Bianco et al., 2013; Tancioni and Lorenzoni, 2016).
81 Our study is aimed at identifying and applying an experimental rearing protocol for the
82 artificial reproduction and fingerlings production of brook chub for restocking.
83
84 2. Materials and methods
85 2.1. *Breeders and gametes collection*
86 The catching of breeders was carried out during the reproductive period, in June 2015, by
87 electrofishing. A total of 27 adults of brook chub (sex ratio 2:1, standard length range
88 (SL_{range}) males: 8.3–14.5 cm, total length range (TL_{range}) males: 9.8–16.5 cm, SL_{range}
89 females: 9–15 cm, TL_{range} females: 11–17.1 cm) were collected in the Fosso Corese (RI)
90 stream, a tributary of Tiber river, located 25 Km north of Rome (Central Italy). The
91 maturation stage was checked by soft abdomen squeezing. Soon after capture, specimens
92 were lightly anesthetized with a 2-phenoxyethanol solution (5 mg/L) and the species
93 confirmed according to external morphology described in Bianco (1983) and Bianco and
94 Recchia (1983). A total of 12 mature brook chub were identified. Dry fecundation was
95 applied: four females (15-17.1 cm TL) were gently stripped to collect eggs into a clean,
96 sterilized dish, and sperm from 8 males (10-15 cm TL) was added. Soon after, we added a
97 small quantity of fertilizing solution (18 g of carbamide and 20 g of non-iodinated salt added
98 to 5 L of distilled water) for elongating the activity of the sperm and hardening the eggs. The
99 same solution was used to remove the sticky layer around the eggs after hydration (Billard,
100 1995; Woynarovich and Horváth, 1980). Then, filtered fresh water was added and renewed
101 several times to wash the fecundated eggs. Eggs were carried in plastic bags with

oxygenated and filtered freshwater to the LESA indoor aquaculture facility. The fish sampling procedure was carried out in agreement with relevant legislation (CEN EN 14011/2003 - Water quality - Sampling of fish with electricity), without the sacrifice of the animals and allowing the release of the specimens immediately after gamete collection.

2.2. *Eggs incubation, larvae rearing, live preys culture and larval ontogeny*

2.2.1. Eggs incubation

The incubation of eggs was carried out in two California-type incubation troughs, with the perforated bottom (diameter 0.5 mm) made from a mosquito net, in an open flow system. The eggs (diameter range: 0.32 – 1.76 mm) were distributed homogeneously in one single layer into each trough. Each incubator was filled with filtered water from an artesian well (constant water temperature 16.5 - 17°C), equipped with air stone diffusers. To check the eggs' early development, samplings were carried out eight times/day, for the first 50 hours.

2.2.2. Larval rearing

After hatching, larvae were transferred to one indoor rearing tank of 2,000 L of volume, in an open flow system, at a density of about 1 larva/L. The tank was provided with freshwater both from the artesian well and the green water from an outdoor phyto-zooplankton culture tank and aerated with an air pump connected to an air stone. Temperature, pH, and oxygen levels were all monitored daily. Water exchange rate was 2 – 3/day.

Live preys were daily added to the larval rearing tank, starting from the 2nd day after hatching (hereafter DAH).

The larval rearing lasted about 90 days, up to the reaching of the juvenile stage. Moreover, starting from 40 DAH, small quantities (1–3 g of weight) of artificial starter food (Skretting Nutra MP T) were daily administrated to integrate larvae diet. The starter (2-3 mm) was crumbled with blender and sieved to a particle size of 500-700 μm, before distribution to larvae.

127 *2.2.3. Live preys culture and administration*

128 Wild zooplankton was caught in the LESA semi-natural wetland and then cultured in a large
129 outdoor tank (25,000 L; diameter 6 m), in green waters condition.

130 Green waters production system consisted of:

131 • filling about one-quarter of the useful volume of the tank with water and initial
132 fertilization (20 g $NaNO_3$ + 4 g K_2HPO_4 / 5000 L) (Russo et al., 2005);

133 • inoculation with 500 L of green (i.e., containing phytoplankton) water obtained by
134 filtering at 60 µm water from the LESA wetland;

135 • raising the water level to reach the half of the total tank volume;

136 • introduction of wild zooplankton obtained by filtering at 60 µm water from the LESA
137 wetland containing Rotifera, Copepoda (larvae and adults), Diptera (Chironomidae)
138 larvae; Cladocera (*Bosmina* sp. and *Daphnia* sp.);

139 • supplying yeast to enhance zooplankton production (about 1 g yeast/million of
140 organisms/2 time week).

141 Zooplankton was daily filtered with a mesh of different sizes from the large volume and
142 administered to the larvae. Daily controls were carried out on randomly sampled larvae to
143 verify the presence and the taxon of ingested preys. After the larval yolk sac absorption,
144 rotifers (collected by filtration at 60-120 µm) were administered to the larvae several times
145 a day, to maintain a density of around 8 rotifers/ml in the tank. From the 6^{th} up to the 12^{th}
146 DAH, rotifers, *Bosmina longirostris* and copepod larvae, filtered at 120-400 µm, were
147 selected. Starting from the 13^{th} day, live preys were mainly copepodites and cladocerans
148 (*Bosmina longirostris* and juveniles of *Daphnia magna*), filtered at 250-700 µm, in
149 quantities sufficient to maintain a tank concentration of at least 5 ind/ml. From 40^{th} to 60^{th}
150 DAH a small amount of artificial food (1–2 g) was daily administered in addition to live
151 preys. From the 61^{st} DAH onward, adults of *Daphnia magna* and chironomids larvae were

also added (Tab. 1) at a constant density of 5 ind/ml; artificial food administration increased from 1 – 2 g to 3 g/day.

Zooplankton in the tank was identified (and quantified) at stereomicroscope (LEICA MZ 12) using the dichotomic keys of Błedzki and Rybak (2016) for Cladocerans and Copepods; Braioni and Gelmini (1983) for Rotifers and Ward and Whipple (1959) for the other taxa.

2.2.4. Larval development, growth, and survival

Every day, between 9 am and 10 am, 1-3 individuals were randomly sampled, photographed using a digital camera (Nikon D90) and measured (TL; mm).

Instantaneous (specific) Growth Rate (G) was calculated every ten days, according to the following formula (Ricker 1979):

$$G = \frac{Log_e\, YT - Log_e\, Yt}{T - t}\, x\, 100$$

where *YT* is the final size at time *T*, *Yt* is the size at time *t*, *e* is the base of natural logarithms.

Final survival rate was computed by subtraction of the total number of dead (daily checked) to the total initial number of larvae.

2.2.5. Water quality

Chemical and physical parameters were monitored: temperature (°C), pH and dissolved oxygen (%) were daily measured with a multi-parameter probe (Eutech Instruments, mod. PCD650) and nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+) and phosphates (PO_4^{3-}) concentrations were weekly measured using a spectrophotometer (Hach, mod. DR/2000), always at the same hour of the day (9:00 am).

3. Results

3.1. Water quality

Values of the primary chemical-physical water parameters, measured in the larvae and juvenile rearing tanks from beginning to the end of the breeding, were: NO_2^-: range: 0.001 –

176 0.06 mg/L; NO_3^-: 6.5 – 16.2 mg/L; NH_4^+: 0.004 – 0.05 mg/L; PO_4^{3-}: 0.05 – 2.75 mg/L;
177 temperature: 17 – 20°C; pH: 6.7 – 7.7 and O_2%: 97 - 162.

178 *3.2. Larval survival rate*

179 In Fig. 1 the survival rate of brook chub is shown, starting from the 1st DAH (initial number
180 of just hatched larvae = 2,300). The survival rate showed a first critical drop on the 6th DAH,
181 followed by a peak of dramatic mortality around the 15th DAH. After, the mortalities lowered
182 considerably, and at 87 DAH the survival rate was 17%.

183 *3.3. Larval growth*

184 In Fig. 2 the linear growth (TL, cm) of *S. lucumonis* from hatching up to 87 DAH is shown.

185 G values trend is shown in Fig. 3: values were highest in the first ten DAH (around 12), then
186 drastically decreased between 10 - 20 DAH, with the lowest value (G = 0.8) at 40 DAH.
187 After, at the beginning of feeding on copepods (from 40 to 50 DAH), a slight increase (G=
188 2.4) was observed. The trend remained almost constant up to 87 DAH.

189 *3.4. Hatching and larval development*

190 The timing of the larval development steps is based on observations carried out on photos
191 taken *in vivo* and stereomicroscopy.

192 Hatching occurred after four days of incubation, about 104 HAF (hours after fecundation), at
193 an average temperature of 18.8°C.

194 At hatching, brook chub larvae showed a curved body, with a large yolk sac and the
195 embryonic finfold extended from the dorsal mid region up to reach the posterior (caudal)
196 edge of the yolk sac. The head was curved and adhering to the anterior region of the yolk
197 sac. At this stage, larvae were almost inactive on the bottom of the tank and the caudal fin
198 was straight and symmetrical (protocercal type).

199 The mouth started to open in some 2 DAH larvae. At this age, the head region was raised
200 and pointed forward, while the yolk absorption started in the anterior (craniad) region. The

alimentary tract was still an undifferentiated tube and alimentation was completely lecithotrophic.

At the 3rd DAH, the posteriormost chamber of the gas bladder was inflated and the mouth was wholly opened in the 40% of larvae observed *in vivo*.

At the 4th DAH, the larvae were in the notochord preflexion stage: the notochord tip curved upward, and hypurals started to be identifiable below it. At *in vivo* examination, the yolk reserves seemed completely reabsorbed, the alimentary tube detectable, but food items are detected only in one of the observed samples.

The 5th DAH larvae (6.5 mm<TL<8.5 mm) moved from the bottom of the tank to adhere to walls. Some rare rotifers were visible in the alimentary tract.

Starting from the 6th DAH, an opened mouth was detectable in all the larvae and the activity of larvae continued to increase. At this age, the first massive mortality was registered.

In randomly samples collected on the 7th DAH, preys larger than 200 µm (mainly bosmins and nauplii of copepods) were found in the intestine in all the samples for the first time.

In 9th DAH brook chub larvae (8.3<TL<9.6 mm), the second (posteriormost) chamber of the swim bladder was externally detectable. Dorsally it was covered by melanophores.

In 10th-12th DAH larvae, the embryonic finfold is reabsorbed in the anus area and at 13th - 16th DAH (larvae 9,1 mm<LT<9,7 mm) the flexion of the notochord tip took place, the embryonic finfold was completely reabsorbed and the differentiation of dorsal and anal fins started. Adult copepods and small cladocerans were detected in the alimentary tube.

The 20th -30th DAH brook chub (11.5 mm<TL<14.6 mm) had still a transparent body, with melanophores mainly concentrated on head and mesenteries. Around 30th DAH, fins differentiated: at this stage, the caudal fin was already fully formed while the dorsal and the anal ones were in differentiation. The differentiation of the pectoral fins was observed after the 31st DAH.

226 At the 87th DAH brook chub reached the juvenile stage, identifiable by the differentiation of
227 the scales.
228
229 4. Discussion and conclusions
230 LESA's aquaculture plant has successfully achieved the artificial reproduction of the brook
231 chub for the first time, by *in situ* gametes stripping and dry fecundation, and *ex-situ* eggs
232 incubation and larval rearing. The macroscopic observation of brook chub ovary that
233 allowed the oocytes distinction on a dimensional basis and a numerical estimate (around
234 6,000 oocytes/year in one female weighing 39.6 g; SL: 135 mm; TL: 155 mm), confirmed
235 that this species can have multiple or polycyclic depositions (multispawner species) with a
236 possible further reproductive event at the end of summer. In each spawning event, each
237 female seems to release just around three thousands of eggs (Tancioni pers. comm.).
238 Multispawner habits are often associated to polyandry and polygyny, survival strategies
239 adopted by several cyprinid species, in Italy at least *Barbus tyberinus, Squalius squalus*
240 and *Sarmarutilus rubilio* (Tancioni et al., 2001; Picariello et al., 2004), living in extreme
241 natural habitat conditions, especially the pioneer ones. Thanks to these adaptations, just a
242 few reproductive adults are able in a short time to recolonize the aquatic habitat subjected
243 to temporary droughts, heavy pollution and overflow disasters. Multiple spawning and
244 promiscuity when performed by a limited number of individuals guarantee a maximum
245 genetic diversity in the progeny, partially avoiding the bottleneck effect (Bianco and
246 Ketmaier, 2015). This original result could be of great help in programming the production
247 cycle.
248 A certain quantity of "wild-like" (*sensu* Cataudella et al., 2002) fingerlings was produced by
249 semi-intensive rearing coupled with "green waters" technique, as recently successfully used
250 to produce fingerling of chub (*S. squalus*, Bonaparte, 1839), barbel (*Barbus plebejus*,

Bonaparte, 1839) and perch (*Perca fluviatilis*, Linnaeus, 1758) in restocking aquaculture in Italy by Marino et al. (2009) and Russo et al. (2005). The chosen rearing methodology here used was aimed at recreating as closely as possible the trophic items of natural nurseries (i.e., significant availability of different live preys' *taxa*), thus stimulating larvae to develop a more natural behavior, at least for the searching and selection of food. The RAS (recirculating aquaculture system) facility of the LESA, based on recycling and reuse of wastewater by phytodepuration and lagunage systems (artificial wetland), made it possible to carry out the outdoor phyto-zooplankton culture starting from wild zooplankton.

The developmental stages of the brook chub followed the same sequence described for the congeneric European chub, *S. squalus*, by Çalta, 2000; Economou et al., 1991; Kupren et al., 2011. Some functional steps, such as swim bladder insufflation, hypural differentiation, and fins differentiation, were reached earlier in *S. lucumonis* than in *S. cephalus*. Such differences in the differentiation timing should be firstly linked to differences in the temperature at which the development occurred. This seems to not be the case, taking into account that even if the above-mentioned authors carried out the larval rearing of European chub at different average temperatures (i.e., Çalta, 2000: 17 °C; Economou et al., 1991: 25 °C; Kupren et al.,2011: 19 °C), the reported differentiation timing was the same. Thus, the earlier insufflation of the swim bladder and fins differentiation observed in *S. lucumonis* should be considered as a species-specific feature.

The larval growth exhibited a higher growth rate in the first ten days after hatching, followed by a significant decrease (due to concomitant dramatic mortality peaks) between the 6^{th} and the 20^{th} DAH. The causative factor of this impressive mortality is hard to be clearly individuated. No data are available in the literature on trophic ecology of brook chub larvae and juveniles, to our knowledge. Adults feed on invertebrates (Crivelli, 1996) and spawn in shallow riffle habitats, in fast-flowing water (Kottelat and Freyhof, 2007). According to

Kottelat and Freyhof (2007), brook chub is sympatric with the congeneric European chub in fast-flowing water with Mediterranean water regimes. The only difference was in the preference of the former towards smaller and shallow than larger riffle habitats. However, even for European chub there is no literature on larval development and trophic ecology, and comparison with other species belonging to Cyprinidae is useless due to massive eco-devo diversity among cyprinids. The first mortality peak was observed at 6 DAH, soon after larvae migration from the tank bottom toward the walls. In this first experience, we carefully checked numbers and dimensions of zooplanktonic organisms but we did not analyze the actual concentration and typology of aufwuchs present in the tank. If we take into consideration that only rare rotifers (which are mobile and not adhering to a substratum) were detected in the alimentary tube, we cannot exclude that rotifers could be occasional preys and larvae presumably looked for the periphyton or zooplankton stuck on the walls, at least at this early stage. Thus, inadequate food intake could be the factor promoting the first mortality peak. This could be solved by establishing an even more trophic environment (than the green waters we used) where more small adhesive items (i.e., phyto-, zooplankton and benthonic bacteria, protozoans, fungi) are available in the first 15 DAH.

The second critical period was detected from the 15 (beginning of fins differentiation) up to 55 DAH. This period was the most critical one in brook chub ontogenesis: in the antecedent fourteen days the brook chub larvae differentiated digestive apparatus and completed the swim bladders insufflation, preluding crucial changes in alimentation that becomes exclusively exotrophic. They move from the tank walls and actively swim in search of food items in the water column. With the progressive differentiation of fins, occurring between the 15^{th} and the 31^{st} DAH, even the locomotor ability changes, from the so-called anguilliform swimming (viscosity-dominated swimming mode with typical C bending of the anterior body region) of the early larvae to the sub-carangiform motion (inertia-dominated

swimming mode with only important undulations of the tail and the tail fin) of bigger larvae and juveniles (Osse and Van den Boogart, 1999). According to Osse and Van den Boogart (1995), the ability of active swimming movements generally precedes exogenous feeding during fish ontogenesis, but we observed the inverse sequence in brook chub. This could be related to the fact that this species spawn in fast-flowing waters, thus they do not need to search for mobile preys because they can rely on ambush instead of active predation, at least in the first days of exotrophic feeding. However, the possibility that some delay in fins differentiation could occur in our brook chub as a consequence of some alimentary disequilibrium determined previously (i.e. around 5-6 DAH) cannot be ignored and further experiences are necessary to definitely individuate the real ontogenetic sequence. Another possible cause for the second mortality peak can be individuated in the absence of suitable quantities, quality and "behavior" of the food items available in the rearing tank, not adequate to satisfy energetic needs of larvae at first feeding. Further, one should also take into account the hypothesis that the energetic (i.e., proteins, fatty acids) characteristics of administered zooplankton could have been partially lost during the tank culture or that they were insufficient to satisfy the larvae's energy need (Hagiwara et al., 2017). Thus, nutritional limitations are the most probable responsible of the observed heavy mortalities. The offering a broadest range of live preys, including a more significant number of cladocerans and, particularly, copepod species (Bush et al., 2010; Karlsen et al., 2015; Kotani et al., 2017), could improve the survival rates. The administration of live zooplankton, in fact, is in line with that reported in wild juveniles of cyprinids (i.e., *S. cephalus*, *Leuciscus leuciscus*), ranging from rotifers in the first phase, to cladocerans in the intermediate to the final stage, with prevalence of cladocerans and chironomids at the end of larval rearing (Garner, 1996; Mark et al., 1987; Nunn et al., 2012; Nunn et al., 2007a,

2007b). However, it should be stressed that authors only look for zooplanktonic and not phytoplanktonic organisms.

Our observations allowed us to formulate some other recommendations:

- too high water fluxes should be avoided up to the notochord flexion stage (15-17 DAH) for enhancing phytoplankton blooms;
- scales are not differentiated up to 87 DAH, thus the re-stocking of juveniles in the native stream should be carried out later.

In conclusion, this first experimental rearing permitted us to establish the basis for the reproduction and a rearing protocol based on *in situ* - *ex situ* strategy: i) *in situ* gametes production, in order to minimize domestication effects that can derive by the management of broodstock in captivity; ii) *ex-situ* wild-like fingerlings production, based on simulation of natural nursery grounds in the environmental rearing conditions.

Due to experimental reproduction' novelty, the larval rearing of brook chub was inevitably carried out using an empirical approach, based on periodical morpho-functional observations, mainly related to alimentation capability (i.e., *in vivo* mouth opening and, observation of intestinal contents), hydrostatic equilibrium (activation of the swim bladder) and swimming ability (fins development). At the same time, we were able to identify the main bottlenecks and critical periods during the larval rearing and to find some tentative solutions for reaching higher survival rates.

The whole body of knowledge gained through this work, together with new insights into population genetic and Management Units (MUs) (*sensu* Moritz, 1994), highlighted by means of molecular approach (Martinoli, 2017), can contribute to identifying priority interventions for the protection of this endangered species, and to develop a Specific Action Plan for Conservation, in agreement with the technical guidelines of IUCN and the Habitat Directive and its application at national and local levels.

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Tab. 1. Zooplankton groups and integrative artificial food supplied larvae during the different phases. Filter size, estimated density of plankton and dry food in the tank, DAH, and TL of the larvae (mean ± SD) are shown.

Zooplankton taxa	Filter size	Administered individuals/ml or dry food weight	DAH	Larvae TL (mm)
Rotifera *Brachionus angularis* *Brachionus urceolaris* *Brachionus calicyflorus* *Keratella quadrata* *Polyarthra* sp.	60 - 120 µm	8	2 - 5	4.6 ± 2.6
Copepoda nauplii Cladocera *Bosmina longirostris* (juv)	120 - 400 µm	5	6 - 12	8.34 ± 1.3
Cladocera *Bosmina longirostris* *Daphnia magna* (juv) Copepoda *Cyclops* sp.	250 – 700 µm	5	13 - 60	11.43 ± 7
Artificial food	500 µm	1 – 2g	40 - 60	18.65 ± 5.4
Chironomidae larvae *Daphnia magna*	500 – 2,000 µm	5	61 - 87	27.33 ± 3.2
Artificial food	700 µm	3g		

LEGEND OF FIGURES

Fig. 1. Survival rate (%) of *S. lucumonis* during the rearing period.

Fig. 2. Linear (TL) larval growth. Vertical bars = standard error of measurements.

Fig. 3. Instantaneous (specific) Growth Rate (G) values calculated every ten days during the experimental rearing period.

Fig. 1.

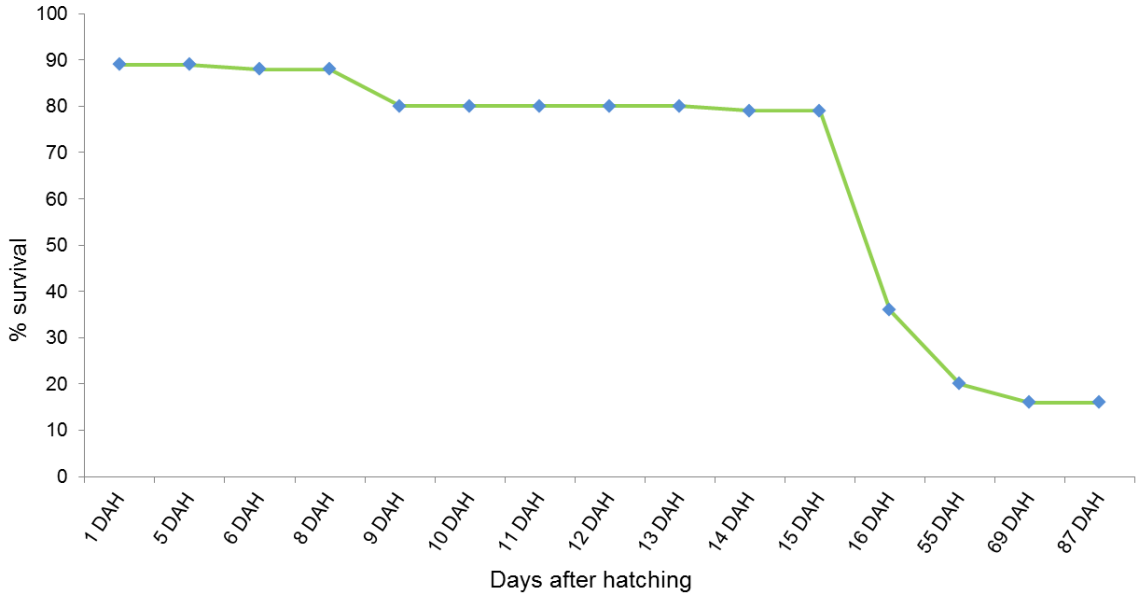

100
90
80
70
60
50
40
30
20
10
0
% survival
1 DAH
5 DAH
6 DAH
8 DAH
9 DAH
10 DAH
11 DAH
12 DAH
13 DAH
14 DAH
15 DAH
16 DAH
55 DAH
69 DAH
87 DAH
Days after hatching

Fig. 2.

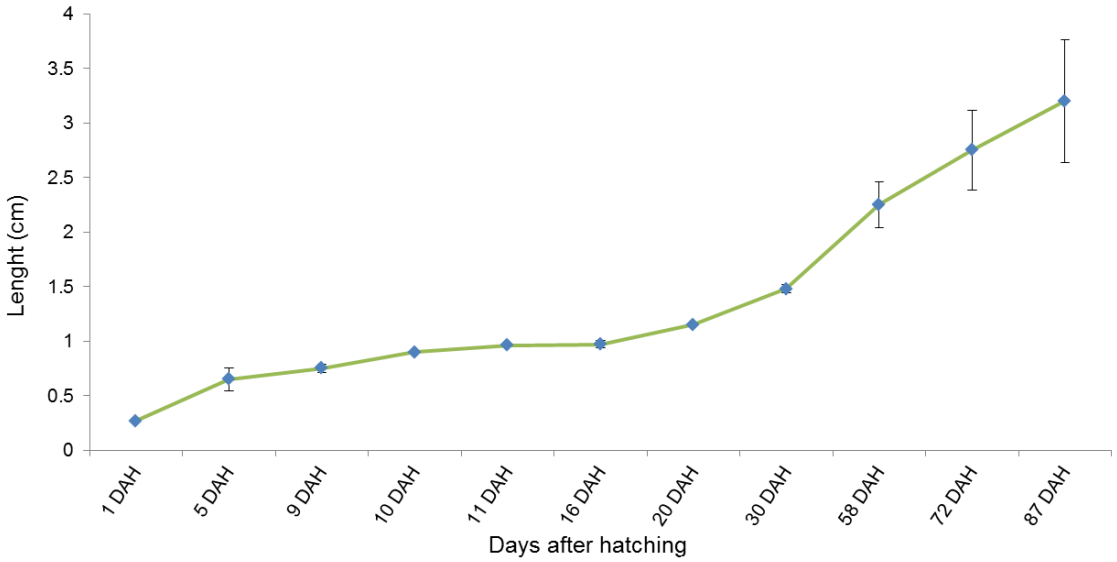
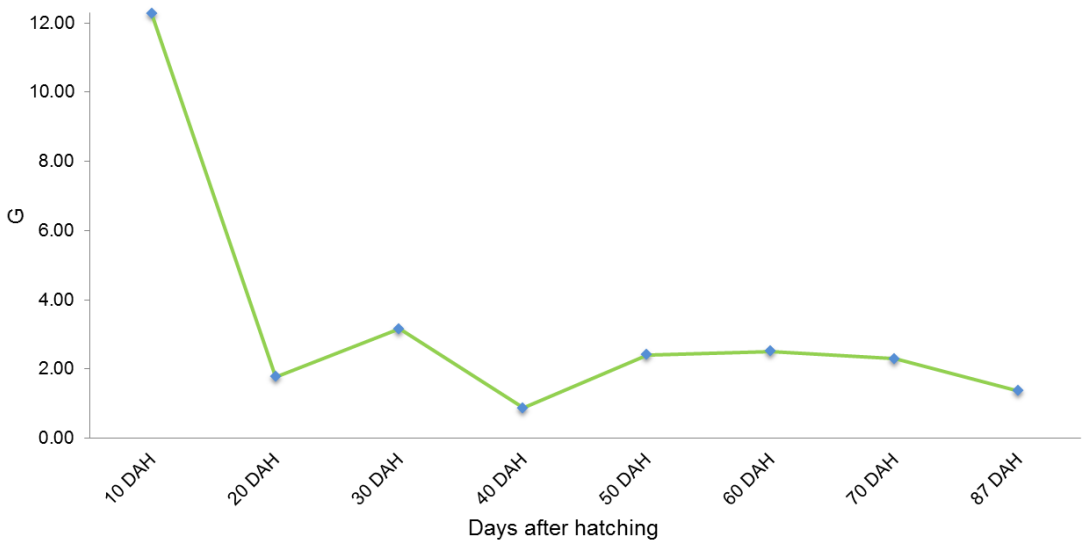

Lenght (cm)
4
3.5
3
2.5
2
1.5
1
0.5
0
1 DAH
5 DAH
9 DAH
10 DAH
11 DAH
16 DAH
20 DAH
30 DAH
58 DAH
72 DAH
87 DAH
Days after hatching

Fig. 3.


12.00
10.00
8.00
6.00
4.00
2.00
0.00
G
10 DAH
20 DAH
30 DAH
40 DAH
50 DAH
60 DAH
70 DAH
87 DAH
Days after hatching