Salmo carpio: an Endemic Salmonid of Lake Garda, Northern Italy

Fernando Lunelli*[1], Filippo Faccenda[1], Ivano Confortini[2], Cristina Cappelletti[1] and Francesca Ciutti[1]

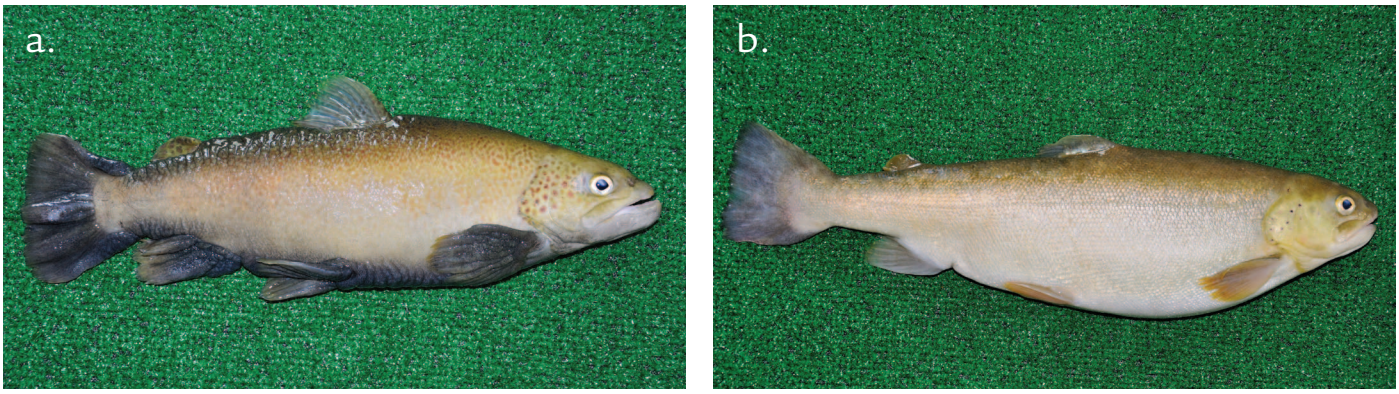

FIGURE 1. *Figure 1. Male (a) and female (b) carpione during the spawning period.*

The carpione *Salmo carpio* is a strictly endemic salmonid of the largest lake in Italy, Lake Garda (volume = 49 km^3, surface area = 368 km^2, maximum depth = 350 m). *Salmo carpio* is a medium-sized trout, attaining a maximum total length of 35 to 40 cm and a maximum weight of approximately 500 g in the natural environment. Record trophy specimens might exceptionally reach 50 cm and one kilogram. The body shape of the carpione is characterized by a relatively small head. The background body color is silvery grey, with small blackish dots on the dorsal area. During spawning seasons, some sexual dimorphism can be observed, with males showing a dark bronze background and blackish fins (Fig. 1). Full sexual maturity of the carpione occurs at three years of age in males and at four years in most females, although some spawning may occur one year earlier (Melotto and Alessio 1990). The species is thought to be gregarious, particularly during seasonal migration between spawning and foraging areas. The diet of the carpione is mostly zooplankton (Alessio *et al*. 1990).

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Genetic diversity of the carpione was investigated by sequence analysis of the complete mitochondrial DNA control region (mtDNA D-loop) on a relatively high number of *S. carpio* and *S. trutta* samples from Lake Garda, prompting us to reconsider the proposed hypotheses on the origin and phylogeny of the taxon (Gandolfi *et al*. 2006). From a genetic viewpoint, extensive molecular screening, including sequencing of the complete mitochondrial DNA control region (mtDNA D-loop) and genotyping of highly-variable microsatellite loci, provide novel insights into phylogeny, population structure and conservation status of a taxon of interest (Gandolfi *et al*. 2006, Gratton and Gandolfi 2011).

The carpione differs from other indigenous salmonids in Italy, such as the marble trout *Salmo (trutta) marmoratus,* on the basis of the temporal and spatial peculiarities of reproductive behavior. Eggs of the carpione are deposited during two spawning seasons, peaking in December-January and July-August, in deep (50-200 m) lakebeds with abundant oxygen. Many authors suggest that each specimen reproduces either in winter or in summer, although the hypothesis of a unique, long spawning period has never been verified in the species' natural environment.

A Critically Endangered Species

Carpione is considered critically endangered according to the Red List of Threatened Species (IUCN 2010), with most important threats being overfishing, habitat deterioration and introduction of exotic competitor species. In detail, the introduction of planktivorous whitefish *Coregonus* spp. in 1918 is generally thought to have promoted the demographic decline of the carpione.

Historically carpione were caught intensively during spawning periods. The commercial catch was more than 20 t per year during the 1950s, but the population of *S. carpio* has been reduced drastically during the last decades (Fig. 2). Currently the only conservation actions concerning the carpione are the prohibition of fishing during spawning periods (15 November — 31 January and 20 June — 20 August), establishment of a minimum harvest size (30 cm), and the regulation of net size used by fishermen (Ciutti *et al*. 2010).

Conservation also goes through *ex situ* multiplication. In

2008, the CARPIOGARDA project was started, funded by the Autonomous Province of Trento. The project aims to define rearing protocols to produce hatchery strains used for rehabilitation, assess the status of spawning areas, and detect the best reintroduction techniques for the species.

Rearing Trials

A fish quarantine facility was specifically created near Lake Garda to evaluate the sanitary state of wild specimens regarding Viral Hemorrhagic Septicemia (VHS) and Infectious Hematopoietic Necrosis (IHN), following European animal health laws (European Parliament and the Council of the European Union 2006). Although there are similarities between carpione and other salmonids, specific hatching and rearing temperatures and artificial diets were evaluated in the quarantine facility.

Rearing tests started from artificial breeding of wild specimens caught in 2006. Previous attempts to catch spawners in the lake were not successful. A light-dark cycle for spawners was tested in a modular production system called ZEFiRe — an acronym for Innovative Zero Energy Fish Farming Research. The ZEFiRe is an energy efficient, modular system for aquaculture that aims to improve energy efficiency, use renewable energy, and have low environmental impact. Water in the system can be reused repeatedly after a treatment process that uses bioremediation and phytoremediation techniques (Fig. 3). A prototype system was constructed to raise fish for commercial purposes. Carpione were reared in the ZEFiRe module in tanks where water temperature and light were set, simulating daily and seasonal natural variations of temperature and light in Lake Garda.

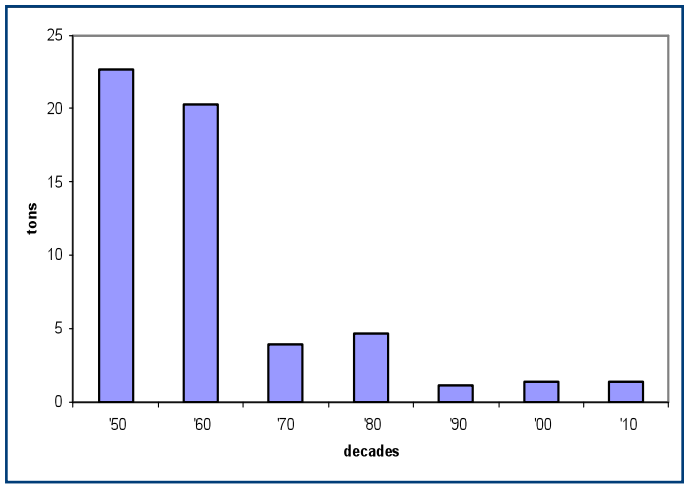

FIGURE 2, ABOVE. *Commercial catch of wild carpione in Lake Garda, Italy.*
FIGURE 3, BELOW. *The ZEFiRe module.*

Environmental factors were monitored and simulated using a multi-parameter smart probe with remote sensing technology.

Improvements of adult feeding and rearing conditions led to a high production of eggs and juveniles. Hatching rates were greater than 60 percent, compared to early artificial breeding (30 percent). There were problems similar to the reproductive disorder in salmon called the M74 syndrome. In carpione, the syndrome was manifest as offspring mortality during the yolk-sac fry phase, but mortality has been reduced by adding dietary supplements.

For carpione held in captivity, anticipated sexual maturity was 16-18 months for males and 24 months for females. Fish reared in captivity can spawn twice per year. This is undoubtedly an exceptional occurrence when compared to wild specimens, representing a peculiarity in the reproductive biology of salmonids. On the other hand, gregarious behavior has also been observed also in rearing tanks, where fish tend to move in shoals.

Reared carpione are susceptible to some common bacterial diseases, such as furunculosis (*Aeromonas salmonicida*, *Aeromonas hydrophila*) and flavobacteriosis (cold water disease, *Flavobacterium psychrophilum*); water mold infections, such as *Saprolegnia* spp.; and parasites (*Icthyobodo necator*, *Gyrodactylus* spp.). Preliminary results of experimental infection of carpione revealed high resistance to VHS, while tests for sensitivity to IHN and Infectious Pancreatic necrosis (IPN) will be conducted in the near future.

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At present we have achieved a good production of eggs, fry and juveniles, with an increasing number of broodstock (Table 1).

Study of Reproductive Sites

Spawning areas of carpione are characterized by the presence of gravel and cobbles free of mud, mainly located in proximity to river estuaries or recent landslides from mountains. Historical maps show the distribution of spawning areas, mainly located in the northwestern part of the lake for the winter reproductive period and in the southern part for the summer one (Fig. 4).

The functionality and conservation status of reproductive sites was assessed during spring 2010 by direct and indirect measurements. Transects of the lake coast (24.5 km) were made with an underwater robotic camera operated at depths between 50 and 250 m. Sonar sensor measurements were conducted to investigate the geologic and stratigraphic profile of the lake floor.

Gravel stratification was connected with weak streams, which might indicate functional reproductive sites. Indirect measurements were able to distinguish two morphological classes of spawning areas: submerged alluvial fans and coastal cliffs (Fig. 5) (Lunelli 2010).

FIGURE 4. *A map from 1725 of reproductive sites in Lake Garda, Italy.*

Reintroduction Techniques

Stocking of high numbers of alevins in past decades did not lead to an effective increase in commercial harvest of carpione. In deep reproductive areas, reintroduction will be conducted by positioning Vibert boxes containing eyed eggs. Thereby, eggs also will be protected from predation by burbot *Lota lota*, a species introduced to Lake Garda around 1858. The species is a voracious predator on eggs and can reach great depths in the lake, as observed during underwater surveys.

The Future of Carpione

The production of elevated quantities of eggs, fry and juveniles indicate the promise of future *ex situ* multiplication and reintroduction of carpione. This represents an encouraging result, considering this is the first attempt to rear carpione. We are aware, however, that a viable hatchery strain of *S. carpio* should be reinforced repeatedly by wild spawners. This will be a major challenge in the future, given the scarcity of wild carpione.

We observed good reproductive sites in our study and lake monitoring surveys showed a trophic condition ranging from oligotrophy to mesotrophy (Salmaso *et al*. 2009). Accordingly, these data allow us to hypothesize that the most severe threat factors, which led to the drastic decline of the carpione, are not related to environmental degradation but primarily to overfishing during spawning periods and competition of the carpione with introduced exotic fish species. Furthermore, the occurrence of wild fish prone to reproduction outside the fishing prohibition period highlights the inefficiency of fishing period restrictions.

Finally the available information regarding the biology of *S. carpio* are based on only a few studies, in which few individuals were analyzed. As a consequence, many aspects have not yet been investigated. Traditional and innovative methodologies should be combined, such as radio tracking to study migration behavior, stomach content analysis to investigate food competition between the carpione and exotic fishes, and histological analysis of gonads to reveal details of reproductive biology. Unfortunately the small number of individuals caught in recent years has constrained new research endeavors. Hopefully our research efforts are not too late!

TABLE 1. Available carpione at present.

Stage	*Number of individuals*
Eggs/Fry 2012	100,000
Juveniles 2011	15,000
Broodstock 2008-2010	400

Notes

[1] Fondazione Edmund Mach–Istituto Agrario di San Michele all'Adige. Via Edmund Mach, 1 I- 38010 San Michele all'Adige (TN), Italy

[2] Provincia di Verona, Servizio tutela faunistico ambientale, Verona, Italy

Corresponding author: fernando.lunelli@fmach.it

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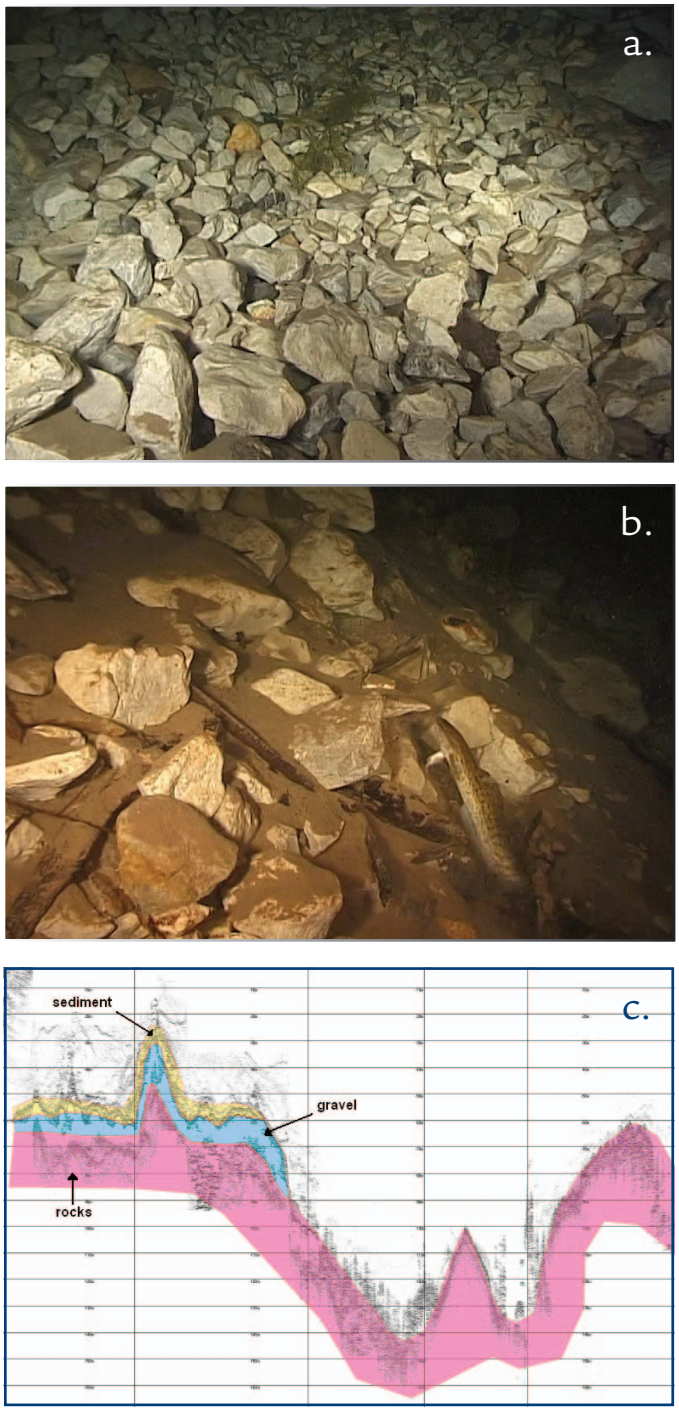

FIGURE 5. *Suitable (a) and unsuitable (b) substrate for spawning and sonar sensor profile of the lake bottom (c).*

TRADITIONAL AND INNOVATIVE METHODOLOGIES SHOULD BE COMBINED, SUCH AS RADIO TRACKING TO STUDY MIGRATION BEHAVIOR, STOMACH CONTENT ANALYSIS TO INVESTIGATE FOOD COMPETITION BETWEEN THE CARPIONE AND EXOTIC FISHES, AND HISTOLOGICAL ANALYSIS OF GONADS TO REVEAL DETAILS OF REPRODUCTIVE BIOLOGY. UNFORTUNATELY THE SMALL NUMBER OF INDIVIDUALS CAUGHT IN RECENT YEARS HAS CONSTRAINED NEW RESEARCH ENDEAVORS.