

Intra and inter-specific mating options for gynogenetic reproduction of *Carassius gibelio* (Bloch, 1783) in Lake Pamvotis (NW Greece)

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ABSTRACT. Gibel carp *Carassius gibelio* exhibits gynogenetic reproduction by utilising the sperm of other species. Over a period of twelve months, the spawning behaviour of gibel carp in Lake Pamvotis (NW Greece) was monitored. Females almost exclusively (97.7%) composed the population, indicating gynogenesis using the sperm of other species. Reproduction began in March and lasted through April. Contrary to the situation reported in other ecosystems, common carp was not present in the spawning grounds during the spawning season of gibel carp; the most abundant other species present at that time was *Rutilus ylikiensis*. To evaluate the sperm donating results of *R. ylikiensis* as compared with other species of the lake, eggs of gibel carp were fertilised with sperm of *R. ylikiensis* (group 1), *Carassius auratus* (group 2), and *Cyprinus carpio* (group 3). Also, gibel carp eggs attached to natural substrates were collected from the spawning grounds (group 4). Hatching success ranged from 9-29% in group 4, compared to 95-98% in groups 1, 2 and 3. Over a period of 60 days after hatching, mortality ranged from 35% (group 1) to 56% (group 3), and specific growth rate from 2.4% (group 3) to 2.9% (group 1). We conclude that gibel carp can successfully utilise sperm of ylikiensis roach, which was the best available sperm donor option for the gynogenetically reproducing gibel carp of the lake.

KEY WORDS : gynogenesis, reproduction, fish.

INTRODUCTION

Carassius gibelio (Bloch, 1783) can survive and thrive under adverse environmental conditions where other species rarely succeed (MUUS & DAHLSTROM, 1999; HOLCIK, 1980). As a consequence, it is widely distributed and flourishing from Europe, including Greece (ECONOMIDIS, 1991; KOTTELAT, 1997), to the Japanese Islands.

In Greek lakes, gibel carp exhibits rapid growth during the first years of its life and reaches maturity in the second year of its life (LEONARDOS et al., 2001). Recently, a rapid increase in the commercial catch of gibel carp in Pamvotis lake NW Greece has been observed (PASCHOS et al., 2002). Gibel carp became abundant in Lake Pamvotis in the early 1980's. Since then, it gradually increased in numbers and is currently the dominating species in fisheries landings, while landings of species of commercial interest such as common carp, eel and the indigenous south European minnow *Phoxinellus epiroticus* have declined (PERDIKARIS et al., 2003).

According to ECONOMIDIS et al. (2000), the indigenous species of the Lake are : *Anguilla anguilla* (Linnaeus, 1758), *Leuciscus cephalus* (Linnaeus, 1758), *Tinca tinca* (Linnaeus, 1758), *Pseudophoxinus stymphalicus* (Valenciennes, 1844), *Phoxinellus epiroticus* (Steindachner, 1895), *Economidichthys pygmaeus* (Holly, 1929), *Cobitis hellenica* (Economidis, 1991), *Rutilus ylikiensis* (Stepha-

nidis, 1939) and *Barbus albanicus* (Steindachner, 1870). The introduced species are : *Carassius gibelio* (Bloch, 1783), *Carassius auratus* (Bloch, 1783), *Cyprinus carpio* (Linnaeus, 1758), *Silurus aristotelis* (Agassiz, 1856), *Silurus glanis* (Linnaeus, 1758), *Gambusia affinis* (Baird and Girard, 1854), *Hypophthalmichthys molitrix* (Val. 1844), *Ctenopharyngodon idella* (Cuvier and Valenciennes 1844), *Aristichthys nobilis* (Richardson 1845),

It is not clear which factors have contributed to this thriving of the gibel carp population in the lake, but in similar ecosystems environmental degradation and decreased density of predating species have been identified as important causes (HOLCIK, 1980).

Interestingly, gibel carp is one of the few fish species with stocks composed almost exclusively of females, reproducing gynogenetically using the sperm of other species (RIEHL & BAENSCH, 1991; ZHOU et al., 2000). Gynogenetic reproduction has some potential benefits : it allows the biomass of a population to be composed mainly of females, and available ecological resources can be used solely for egg production.

Shallow water and low vegetation characterise the spawning grounds of cyprinid fishes. In similar lake ecosystems, gibel carp and other species utilise the same spawning grounds. This is because of the availability of substrates and oxygen for the eggs, and increased micro-invertebrate densities, which contribute to the survival of

early life stages and enhance recruitment. In a recent pilot study, it became evident that the gibel carp population in Lake Pamvotis is composed mainly of females, and that *Rutilus ylikiensis* was abundant in the spawning grounds of gibel carp, (PASCHOS et al., 2001).

The purpose of the present work was to determine the relative densities of potential sperm-donating fish species in the spawning habitat of gibel carp in Lake Pamvotis. Subsequently, this information was used for experimental evaluation of gynogenetic reproduction and for assessing the degree of potential "sexual parasitism" of gibel carp in Lake Pamvotis.

MATERIAL AND METHODS

This study was carried out in Lake Pamvotis NW Greece (Fig. 1), which is a natural, eutrophic, holomictic lake. It is relatively shallow (mean depth about 4 meters and maximum depth not exceeding 8 meters) with a total area of 355 km², and is thermally stratified from April to August (KAGALOU et al, 2001).

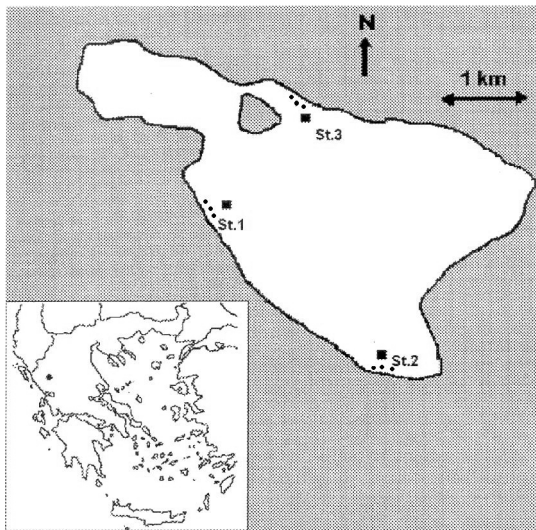


Fig. 1. – Map of Pamvotis Lake. Sampling sites (St.1, St.2, St.3) of gill nets (squares) and locality of spawning grounds (circles).

Typical spawning grounds of gibel carp and other members of the Cyprinidae family are characterised by shallow depth and the presence of vegetation (VARLEY 1967; LAGLER 1972; MUUS & DAHLSTROM 1999). To monitor the spawning migration of gibel carp and identify the species that can act as sperm donors for female gibel carp, sampling was, therefore, carried out from January 1999 to January 2001, using gillnets (100m long, mesh size 40-50 mm) positioned at 100 meters away from the shallows and parallel to the shoreline of three separate spawning locations (Fig. 1). Stationary fishing traps (fyke-nets, mesh size 30mm) were also placed in typical shallow spawning grounds of the species with depth from 0.5 m to 1m. Temperature was monitored throughout the period of study using max-min thermometers.

Determination of the spawning period was based on monitoring of the catches in the shallows and examination of the gonadosomatic index ($GSI = GW \cdot 100 / NW$,

where GW is the gonad weight, NW is the eviscerated weight), staging of gonadal development according to Kesteven's scale (BAGENAL, 1978), and finally by the degree of pressure required to obtain eggs from captured females. Condition factor (K) was calculated according to the following equation $K = (BW / L^3) \times 100$, where BW is the eviscerated weight, L is total length.

In addition to the gill and fyke nets, electrofishing was carried out in the shallows when, for the first time, gibel carp was found in the shallows exhibiting typical spawning behaviour of Cyprinidae (MICHAELS, 1988). All individuals of other species present in the shallows during the spawning of gibel carp were thus captured with electrofishing or fyke nets.

When spawning of gibel carp became evident, ten pieces of natural substrate (branches, leaves, vegetation, rough dimensions trimmed to 0.5 × 0.4m) with eggs were collected randomly from the three different sampling sites of gibel carp spawning grounds. These were taken to the lab and placed in aquariums (0.9 × 0.6 × 0.5m) supplied with Lake Water. From each substrate and group, 100 larvae were collected and placed in four trays (1.0 × 0.25 × 0.25m).

In addition, eggs from three sexually mature female gibel carp were fertilised with sperm from three male goldfish *Carassius auratus*, three ylikiensis roach, *Rutilus ylikiensis*, and three carp *Cyprinus carpio*. From each cross, a total number of 2000 eggs were placed in incubators (zugars having total volume 7lt). The rate of embryonic development, hatching percentage and growth rate were monitored for a period of 60 days for each group.

All fish were kept according to standardised procedures (HORVATH et al., 1984). Initially, food was solely zooplankton, collected daily from the lake. From the 20th day, dry trout food was provided (DABROWSKI et al., 1986; KAUSHIK 1986). Feeding was *ad libitum* in all groups. Mortality, length and growth rates were monitored every 20 days, for a period of two months after hatching. Specific growth rate (SGR) was calculated according to the following equation $SGR = \ln BW_2 - \ln BW_1 \text{ days}^{-1}$, where BW₁ is the initial body weight and BW₂ is body weight after 20 days of rearing.

RESULTS

Catch Data

In total 598 gibel carp individuals were captured over the period of the study : 392 in gillnets and 206 in fyke-nets. The largest portion of the annual gibel carp catch with gillnets, was in March (19.9%). With the exception of November, December and January, when fishing was fruitless, about 10% of the total annual catch was taken in each other month. In fyke nets, gibel carp catches occurred only during March and April, with 42% and 58% of the total catch each month, respectively (Fig. 2). Females comprised 97.7% of all gibel carp captured in the spawning grounds. Over the period of the study, temperature ranged between 7°C and 27°C. Peak capture of gibel carp, with gill and fyke nets, coincided with temperatures above 12°C (Fig. 2).

Mean total length of captured females was 28.06 cm (min 13.2, max 35.5 cm), mean body weight was 464.9 g (min 88.5, max 894 g) and condition factor was 0.93 (± 0.17). The gonadosomatic index of the female fish was 12.6 and the fish were in the last stage of sexual maturation. Initially the larger fish spawned, and smaller individuals followed.

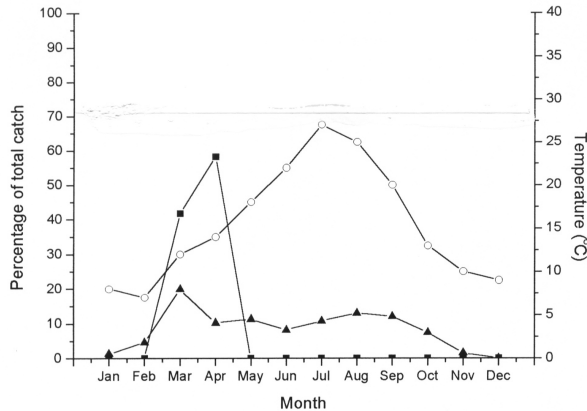


Fig. 2. – Catch data of gibel carp from Lake Pamvotis, over a period of 12 months. Number of fish caught in the spawning grounds (squares) and at 100 meters away from the shoreline (triangles), and water temperature in the shallow region of the lake (circles).

Catch data of other species in the spawning grounds

During March and April, when gibel carp spawned in the shallows, 206 fish were captured in the three sampling sites. In all sampling sites, gibel carp and ylikiensis roach were always present. All individuals of other species captured in the shallows were found to be sexually mature and the majority readily released their gametes. Gibel carp (89%), followed by ylikiensis roach (10.5 %) exhib-

ited the highest relative abundance. The captured ylikiensis roach comprised sexually mature males (26%) and females (37%), and the rest were not sexually mature. During electrofishing operations in the shallows, *R. ylikiensis* males and females were caught in the act of mating and spawning, while simultaneously gibel carp females were observed to release their eggs.

Hatching percentage of eggs from natural substrate, under hatchery conditions

The number of eggs ranged from 118-1520 per substrate collected from the spawning grounds. Hatching occurred in all groups after three days at 14°C, and the number of larvae ranged from 58-380. Hatching coefficient varied between samples from 7.2-29% ($\chi^2=203$, DF=9, $P<0.01$).

Artificial fertilisation of gibel carp eggs with sperm of other species

Viable larvae were obtained from gibel eggs fertilised with sperm from the ylikiensis roach (CrXR), goldfish (CrXG) and common carp (CrXC). Hatching occurred after three days at 14°C in all groups. Hatching coefficient ranged from 95-98% (Table 1) and there was no significant difference between the crosses in terms of hatching coefficient (Chi-square test, $\chi^2=0.024$, DF=2, $P>0.05$).

Rearing of fish

Fish from the three groups of crosses (CrXR; CrXG; CrXC) and from the natural substrates exhibited similar mortality (Table 2, $\chi^2=5.38$, DF=3, $P>0.05$), but the cross between gibel carp and ylikiensis roach exhibited the lowest mortality of all. The specific growth rate was similar in all groups (Table 2, $\chi^2=0.058$, DF=3, $P>0.05$).

TABLE 1
Hatching performance of the crosses

Group	Hatching (days)	Hatching (%)
<i>C.gibelio X R. ylikiensis</i>	3	98
<i>C.gibelio X C. carpio</i>	3	97
<i>C.gibelio X C. auratus</i>	3	95
<i>C.gibelio</i> Embryos from natural substrates	3	18

TABLE 2
Specific growth rate and cumulative mortality (M) of the reared fish at 20, 40 and 60 days after hatching

Days After 1 st feeding	20		40		60	
	SGR%	M%	SGR%	M%	SGR%	M%
<i>C.gibelio X R. ylikiensis</i>	2.4%	19%	3.4%	25%	2.9%	35%
<i>C.gibelio X C. carpio</i>	2.4%	30%	2.4%	44%	2.4%	56%
<i>C.gibelio X C. auratus</i>	2.2%	21%	3.2%	31%	2.7%	41%
<i>C.gibelio</i> from the lake	1.4%	18%	3.6%	33%	2.5%	47%

DISCUSSION

Spawning activity of gibel carp in Lake Pamvotis peaked during March and April. Peak capture of gibel carp with gill nets, positioned parallel to the shoreline of the spawning sampling sites, occurred during March. In spawning grounds, gibel carp were captured only during March and April. (Fig. 2). During March and April, sexually mature gibel carp exhibiting typical spawning behaviour of Cyprinidae were captured with electro-fishing, as well as with the fyke nets,

The field data indicate that spawning of gibel carp and ylikiensis roach coincided. Female gibel carp caught with gillnets during March were probably migrating towards the shallow spawning grounds. Both gibel carp and ylikiensis roach captures in the shallows (with fyke nets and electrofishing) peaked during March and April. In fact ylikiensis roach was the most abundant other species present in the spawning grounds of gibel carp. In these typical shallow spawning grounds for cyprinidae species, gibel carp were caught in the act of releasing their eggs during the spawning of ylikiensis roach.

Timing of spawning is a significant element of the reproductive strategy of all fish species. According to the field data (catch in the shallows), the spawning period of gibel carp and ylikiensis roach overlap. This period is also a period of spawning for Epirus minnow, an endemic fish species that is on the verge of extinction in the Lake (PERDIKARIS et al., 2003). It can be assumed that these three mentioned fish species of the lake have the same timing strategy for spawning.

Optimal conditions for hatching and growth of larvae early in the season are very important for survival and recruitment. For example, temperature and food abundance can significantly influence survival during early life stages of development (HEGGERGET, 1988; HUTCHINGS, 1991). Initiation of spawning activity of gibel carp in the lake coincided with a critical increase of temperature from 7°C in February to 13°C in March; 13°C appears to be on the low edge of the thermal limit of goldfish embryonic development (WIEGAND et al., 1989). Successful reproductive investment and recruitment of young fish are largely influenced by environmental conditions in the nursery grounds. The timing of spawning may enhance gibel carp larval viability and growth through the increased temperature and primary and secondary productivity that is evident at the beginning of spring in the lake (KAGALOU et al., 2001). Gibel carp exhibits rapid growth and development under favourable thermal conditions. For example gibel carp in Lake Lysimachia, a neighbouring Lake in West Greece, reach 41.2% of the maximum length exhibited by the population, within the first year of life (LEONARDOS et al., 2001).

There was some evidence of temperature related spawning migration of gibel carp in the lake. Gibel carp distribution in the shallows peaked during March and April, associated with temperature ranging from 12-14°C. At this stage individuals were sexually mature and exhibited spawning behaviour. According to the catch data from the deeper parts of the lake, when temperature was below 12°C or above 16°C, gibel carp moved towards the deeper parts of the lake and returned to the

shallows when temperature was between 12 and 16°C (Fig. 2). This is in agreement with information collected from local fishermen about seasonal distribution of the species in the lake.

Contrary to the situation seen in other ecosystems (MUUS & DAHLSTROM 1999), we found no evidence in Lake Pamvotis that common carp was present on the spawning ground of gibel carp. Common carp was found to be present in the shallows during May, with water temperature between 16 and 18°C, but by this time gibel carp had released their eggs and were absent from the spawning grounds.

The laboratory results indicate lack of paternal effects on the gynogenetically reproduced gibel carp fry. Irrespective of sperm origin (goldfish, common carp or ylikiensis roach), post-hatch growth and survival were similar. In the same manner, growth and survival did not vary between fish originating from eggs collected in situ or eggs artificially fertilised in the laboratory (Table 2). This contrasts with some paternal effects observed in gibel carp crosses (ZOU et al., 2001).

Under the controlled conditions and procedures of artificial egg fertilisation and incubation (mixing of eggs and sperm, eggs treated to become non sticky, optimum incubation conditions) the fertilisation ability of sperm and embryonic viability may be increased compared to natural reproduction and incubation conditions (HORVATH et al., 1984). This may partially explain the fact that hatching rate did not vary significantly ($P > 0.05$) between the artificially produced crosses, whereas between different egg substrates collected from the spawning grounds, hatching rate ranged from 7.2-29% and varied significantly ($P < 0.01$).

The small percentage (2.3%) of male gibel carp captured during spawning indicates that, in addition to gynogenetic reproduction, bisexual reproduction is to some extent an option for the Pamvotis gibel carp. Nevertheless, the population was almost exclusively composed of females. Gynogenetic reproduction appears to be the main mode of reproduction for gibel carp in the lake. This is in agreement with reports about other aquatic ecosystems (MIGDALSKI & FICHTER 1976; RIEHL & BAENSCH, 1991; ZHOU et al., 2000).

Ylikiensis roach can contribute significantly to the gynogenetic reproduction of gibel carp in the lake. Based on the laboratory data, it can be concluded that gibel carp can potentially utilise the sperm of ylikiensis roach, which according to the field data, was the most obvious sperm donor. In fact, the results of the artificial fertilization indicate that ylikiensis roach was equally as good as the sperm of common carp or goldfish. Furthermore, based on the relative abundance data collected in the field, it can be concluded that ylikiensis roach was at least statistically the most obvious potential sperm donor for gynogenetic reproduction of gibel carp in lake Pamvotis. To our knowledge this is the first record of gynogenetic reproduction between these two fish species, and contrasts with the situation reported from other lakes, where common carp (*C. carpio*) is the principal sperm donor.

The prospects for restricting the population of gibel carp in the lake are limited. Larvae exhibit high viability and the species is almost exclusively composed of

females, which grow rapidly and spawn massively. During the last five years fisheries landings of gibel carp tripled while fisheries landings of other species were significantly reduced (PERDIKARIS et al., 2003).

Considering the energetic cost of sperm production in small fish such as the ylikiensis roach, it could be argued that any sexual parasitism of gibel carp on ylikiensis roach may have limited but negative consequences for the donating species. The male fish of roach species arrive first in the spawning ground and await the arrival of the female, when mating is initiated and spawning takes place (MUUS & DAHLSTROM, 1999). Field observations indicate that gibel carp waits for the mating of ylikiensis roach to occur for releasing its eggs on the spawning grounds. It is, therefore, possible that some sperm of ylikiensis roach is lost because of the sexual parasitism of gibel carp, but further field work would be necessary to verify this hypothesis.

Gynogenetic reproduction may have contributed to the increased gibel carp population in the lake. Nevertheless, population size is a complex product of birth, recruitment, natural and fishing mortality, carrying capacity, ability to compete for food, ecophysiological fitness or adaptation, and environmental conditions. At least two factors contribute to the thriving of the gibel carp population in the lake. One factor is the tolerance to environmental degradation (for example, reports emerged recently in local newspapers, about pollution of the lake that occurred on several occasions as a result of an overflow of the sewage pipelines of Ioannina city). Another factor is reduction in abundance of predating species (PERDIKARIS et al., 2003) or reduced competition for food from other species (HOLCIK & ZITMAN, 1978).

The thriving of gibel carp populations is further supported by its ability to survive in adverse environmental conditions (HOLCIK & ZITMAN 1978; HOLCIK, 1980). For example gibel carp fry originating from gynogenetic reproduction with sperm of *R. ylikiensis*, exhibited specific growth rates of 3.14% and 0.91% at NH₃ concentration 0.51 mg/l and 8 mg/l respectively (NATHANAILIDES et al., 2003).

In conclusion, gibel carp females have little opportunity for mating with gibel carp males. Ylikiensis roach is the best available sperm donor option for the gynogenetically reproducing gibel carp of the lake.

As to the loss of biodiversity by the expansion of the gibel carp population, as is the case with other aquatic ecosystems (HOLCIK & ZITMAN, 1978; HOLCIK, 1980), a combination of biological and environmental factors appears to be responsible for the thriving of gibel carp in Lake Pamvotis. It would be reasonable to take action to improve ecological conditions and counteract environmental degradation, particularly in the spawning grounds.

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Received : July 15, 2003

Accepted : December 29, 2003