# USE OF PRUSSIAN CARP, *CARASSIUS GIBELIO*, PITUITARIES IN THE ARTIFICIAL PROPAGATION OF FEMALE COMMON CARP, *CYPRINUS CARPIO*

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**Background.** The usage of common carp pituitaries has been associated with very high costs, resulting from sacrificing the valuable broodstock. A close taxonomic proximity between the Common carp, *Cyprinus carpio* L., and Prussian carp, *Carassius gibelio* (Bloch, 1782), reflected in the presence of high levels of the homologous carp LH hormone in Prussian carp pituitaries, was recently demonstrated. This prompted the present authors to evaluate the Prussian carp pituitaries as a less expensive alternative to carp pituitaries, for inducing spawning in common carp.

**Materials and Methods.** Prussian carp were fished in Lake Pamvotis (NW Greece) on April 4, 2007 and 204 pituitaries were extracted and processed. Accordingly, the prepared pituitary liquid extract was injected in 30 female common carp (experimental group) in the State Carp Hatchery in Psathotopi, (western Greece). Common carp pituitary extract was used for the hypophysation of female common carp in the control group ($n$ = 30). In both groups, standard hatchery procedures were followed concerning broodstock handling during the artificial propagation of common carp. Fertilized eggs were incubated in 7-L Zugar jars after removing the sticky substrate and hatching percentage was calculated.

**Results.** Spawning success was 73.3%, total egg yield was 5.94 kg, total relative fecundity was 112.5 $g \cdot kg^{-1}$ of body weight (150.0 $g \cdot kg^{-1}$ of body weight based on fertile broodstock), and hatching percentage reached 85.2 %. Statistically, all the above performance values were not significantly different compared to the controls.

**Conclusion.** Taking advantage of a natural resource practically unexploited, the use of Prussian carp pituitaries in the artificial propagation of common carp was equally effective to common carp pituitaries, contributing to lower running costs in the hatchery.

**Keywords:** common carp, Prussian carp, gibel carp, artificial propagation, pituitaries, spawning

## INTRODUCTION

Fish reproduction is dependent on the coordinated actions of various hormones along the brain–hypothalamus–pituitary–gonad axis with the gonadotropins, follicle stimulating hormone (FSH), and luteinizing hormone (LH) having a central role (Van Der Kraak et al. 1992). Steroidogenesis is stimulated by LH and results in the final maturation and ovulation of the females and sperm production by the males (Yaron et al. 2003). According to the salmonid model, synthesis and release of gonadotropins show considerable variations throughout the reproductive cycle. Early in this cycle, a high FSH level stimulates the first stages of gametogenesis and steroidogenesis. Eventually, during the final stages of the oocytes maturation, FSH levels decrease concurrently with an increase in LH levels, especially before spawning (Gomez et al. 1999, Swanson et al. 2003).

Hypophysation have been used increasingly during the last 70 years in fish farming (Bromage 1995). Pituitary extracts have become a standard practice in cyprinids (common carp, grass carp, Chinese carps, and Indian minor carps), catfish and sturgeon hatcheries, throughout the globe (Woynarovich and Horváth 1980, Zohar 1989, Horváth et al. 2002). In the case of common carp

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(*Cyprinus carpio* L.), the use of pituitary extracts (in dose of 3–4 mg · $kg^{-1}$ of body weight) in order to enhance final oocyte maturation and synchronize the ovulation of female broodstock (Yaron et al. 1984, Yaron 1995, Paschos 2004), is practically indispensable.

Commercially available pituitary preparations are in many cases the compounds of choice, even though synthetic hypothalamic compounds were proved to be effective in various degrees (Peter et al. 1988, Drori et al. 1994, Peter and Yu 1997, Yaron et al. 2002, Brzuska 2006). However, the need to sacrifice valuable broodstock and the increased production targets for many freshwater species are the main reasons for the high prices of these commercial preparations ($199–432 per g) (Perdikaris et al. 2007). In the case of freshwater farms in Europe, the annual requirements are well above 100 g, since one fish farm alone in Hungary produces 40–50 g (Andras Ronyai personal communication). Therefore, assessing alternative sources of suitable pituitaries would help to reduce the running costs of hatcheries.

In the inland waters of Greece, Prussian carp (known also as gibel carp), *Carassius gibelio* (Bloch, 1783), is considered as invasive species (Economidis et al. 2000) and its known distribution pattern extends to at least twenty hydrographic basins (Economou et al. 2007). Prussian carp is distributed in 31 countries (Froese and Pauly 2008). It is widely considered as pest species in most European aquatic ecosystems (Kottelat 1997) due to gynogenetic reproduction (Fan and Shen 1990, Gui 1996, Zou et al. 2001) and its flexible interspecific mating strategy for sperm exploitation (Paschos et al. 2004). This reproductive strategy and the antagonistic behaviour for food and space in spawning grounds have created visible negative effects on indigenous species and in trophic complexes in various ecosystems (Paschos et al. 2004, Perdikaris et al. 2005, Tsoumani et al. 2006). On the other hand, common carp is a valuable fishery resource in many lakes in western and northern Greece. However, total catches (28 t in 2001 compared to 139 t in 1991 according to the FAO statistics; Anonymous 2008) have been seriously reduced the last decades due to overfishing, pressure from non-indigenous species and various anthropogenic impacts. This pattern has been clearly shown in the case of Lake Pamvotis, characterized by proliferation of Prussian carp population (Prussian carp production increased by 37.5 percentage points between 1995 and 2000 against common carp catches that reduced by 92 percentage points between 1990 and 2000) (Paschos et al. 2004, Perdikaris et al. 2005).

Given that successful eradication methods of Prussian carp are practically not available, it would be useful to assess the possibility of exploiting this natural resource. Mature Prussian carps can be fished in great numbers using gillnets, particularly when they approach the shallow spawning grounds. Since the close taxonomic proximity between common carp and Prussian carp was actually reflected on hormonal level (LH type and profile just before spawning) (Perdikaris et al. 2007), Prussian carp would be useful as pituitary donor, contributing to reduction of running costs in carp hatcheries.

The aim of the present work was to assess the effectiveness of Prussian carp pituitaries in the artificial propagation of female common carp, in a commercial hatchery.

## MATERIALS AND METHODS

In order to obtain pituitaries with the highest possible levels of LH, mature Prussian carps were fished at the beginning of the reproductive period (4 April 2007) (when fish approach the spawning grounds; Paschos et al. 2001) using gillnets in the spawning grounds of the species in Lake Pamvotis (near the city of Ioannina, NW Greece). Only alive specimens ($n$ = 204) were selected for decapitation.

After the application of excess amount of anaesthetic (MS-222), total weight, total length, and gonad weight were measured and gonadosomatic index was calculated as GSI = 100 (gonad weight/total weight). Sex ratio was 98.5% in favour of females and all females were fully mature. Mean total body weight of Prussian carp was 301.7 g (± 91.8 g), mean total length was 27.3 cm (± 2.0 cm) and mean GSI was calculated to 9.2% (± 3.9%).

Fish were decapitated, the brain was exposed and pituitaries were detached from the skull of each fish using sterilized forceps. The fresh pituitaries were processed with acetone according to the protocol proposed by Lutz (2001) (detailed application process in Perdikaris et al. 2007) and processed glands were transferred to the hatchery and stored in sterile vials ready for use.

On 17 April 2007, mature broodfish of common carp were fished with gillnets in earthen ponds, selected, and moved to indoor cement tanks (six 3-$m^3$ tanks for the females and one 3-$m^3$ tank for the males) in the State Carp Hatchery in Psathotopi (western Greece). Water temperature in earthen ponds fluctuated throughout the year between 10 and 20°C, dissolved oxygen levels between 5.2 mg · $L^{-1}$ and 7.0 and mg · $L^{-1}$ and pH between 7.2 and 7.9 (Table 1). Females were weighted, marked with coded metal fin clips, and divided in two groups, the experimental and the control group, each consisted of 30 individuals. Total body weight of females was 52.8 kg and 56.7 kg in the experimental and control groups, respectively ($P > 0.05$) (Table 2). The group of males was kept in separate indoor tank, consisted of eight individuals with total body weight of 15.7 kg. Same amounts of sperm mixture from this group were used for insemination in both female groups.

The pre-weighted amount of dried glands of Prussian carp were pulverized and processed (as for carp pituitaries), using 1–2 mL sterilized commercial saline solution (0.7%) per fish, in order to produce the injectable pituitary preparation, according to protocols proposed by Woynarovich and Horváth (1980) and Paschos (2004). For control and the male group, commercially available pulverized carp pituitary preparation, were used. The doses used for the intramuscular applications were 4 mg · $kg^{-1}$ of body weight in females for both preparations and 3 mg per kg of body weight in males. Spawning was induced by two injections in both groups after deep anaesthesia with MS-222 (1 g in 10 L of water); the first dose (primer) was

10% 24 h before the expected time of ovulation and the second (resolving) was 90% of the total dose, 12 h later. The genital pore was sewed before the second injection. Males were injected only once, at the same time with the second injection in the females. Physicochemical parameters of water were identical in all tanks (Table 1).

Sperm was collected 12h after the injection from all males ($n = 8$), pooled together in sterilized containers and stored on ice (4°C). The amount of eggs collected in each group, were separately weighted, fertilized with the same volume of sperm mixture in both eggs using freshwater and the sticky substrate was removed with urine–saline and tanin solutions (Anonymous 1985, Paschos 2004). Fertilized eggs from each fish were separately incubated in 7-L Zugar jars. Temperature, dissolved oxygen levels, pH, conductivity and salinity were continuously monitored in the jars (Table 1). Hatching initiated 2.5 to 4.0 days post-hatching and the mean hatching percentage was estimated under a dissecting microscope before hatching commenced, by taking three random samples ($n = 100$) from each group (as the percentage of fully eyed eggs against unfertilized eggs). Finally, hatchlings were transferred in 500-L Zugar jars and after yolk-sac absorption were fed ad libitum with egg and artemia nauplii for two and five days, respectively, before stocked in outdoor polyester tanks.

Two-way analysis of variance (ANOVA) tests were applied to compare statistically spawning and hatching performance in experimental and control groups. Normality of data was verified using the Shapiro–Wilk's test.

## RESULTS

Eggs in both groups were collected 210 degree-hours from the first injection (soon after the sperm collection), after removing the stitches from the genital pore. With respect to spawning percentage of common carp, 22 fish responded positively to hypophysation with Prussian carp pituitaries (73.3%), compared to 20 fish in control group (66.7%). Total amount of eggs produced in the experimental group was 5.94 kg compared to 5.32 kg in the controls, without significant difference between the two groups ($P > 0.05$). Accordingly, relative fecundity either calculated based on the total number of fish in each group (112.5 g of eggs per kg of broodstock in the experimental group and 93.8 g in the controls; $P > 0.05$) or based on spawning fish (150.0 g of eggs per kg of broodstock against 144.56 g; $P > 0.05$) showed non-significant differences. Hatching rate was 85.2% in the experimental group and 89.6% in the control group, again without statistically significant difference ($P > 0.05$) (Table 2). The above results in both groups are summarized in Table 2.

**Table 1**

Basic physicochemical parameters of water in indoor tanks used to keep the broodstock before hypophysation and during incubation in 7-L Zugar jars (mean values ± standard deviation)

| Parameter | Cement tanks | Zugar jars |
|---|---|---|
| Temperature [°C] | 21.4 ± 0.41 | 21.5 ± 0.52 |
| pH | 7.9 ± 0.10 | 7.6 ± 0.12 |
| Conductivity [$\mu S \cdot cm^{-1}$] | 667 ± 31.5 | 655 ± 22.4 |
| Salinity [‰] | 0.3 ± 0.1 | 0.3 ± 0.09 |
| Dissolved oxygen [$mg \cdot L^{-1}$] | 7.2 ± 0.41 | 7.0 ± 0.5 |

**Table 2**

Spawning and hatching performance in experimental and control groups

| Parameter | Experimental group | Control | Significance |
|---|---|---|---|
| Females | | | |
| $n$ | 30 | 30 | — |
| Broodstock weight [kg] | 52.8 | 56.7 | $P = 0.47$* |
| Pituitary weight [mg] | 211.2 (PCP) | 226.8 (CP) | — |
| Spawning success [%] | 73.3 | 66.7 | — |
| Spawned broodstock weight [kg] | 39.6 | 36.8 | $P = 0.52$* |
| Total egg production [kg] | 5.94 | 5.32 | $P = 0.81$* |
| Total relative fecundity [g of eggs per kg of body weight] | 112.5 | 93.8 | $P = 0.85$* |
| Relative fecundity of fertile broodstock [g of eggs per kg of body weight] | 150.00 | 144.56 | $P = 0.90$* |
| Hatching percentage [%] | 85.2 | 89.6 | $P = 0.23$* |
| Males | | | |
| Pituitary weight [mg] | 24 (CP) | | — |

* level of significance $P < 0.05$; PCP, Prussian carp pituitary; CP, common carp pituitary.

## DISCUSSION

Spawning induction of final oocyte maturation and ovulation is necessary in controlled reproduction of common carp, since fish will not spawn in captivity due to lack of certain environmental cues. As this process is controlled by the secretion of gonadotropins from the pituitary, exogenous sources of gonadotropins, or synthetic hypothalamic hormones have become standard practise. Carp pituitary extract (CPE) have been used in most hatcheries, however the increased production targets and the cost of this biological material led to consider alternative approaches (Yaron 1995).

The biological potential of Prussian carp pituitaries in carp spawning was unknown (Perdikaris et al. 2007). During the present study, hypophysation of female common carp with Prussian carp pituitaries was proved to be successful, compared to common pulverized carp pituitary preparations, as spawning percentage was higher in the experimental group, compared to the controls. Differences in egg production, relative fecundity and hatching percentage were not statistically significant and cannot be attributed to the use of Prussian carp pituitary, but to individual traits of each individual broodfish. Spawning performance of the experimental group (73.3%) was comparable to results from other carp hatcheries using CPE. Early work by Horváth and Lukowicz (1982) suggested that average ripening of hypophysed females reaches 80%. Bieniarz et al. (1985) reported complete ovulation in 59% of the broodstock when fish were injected early in the morning and according to Billard et al. (1995), the expected ovulation performance after hypophysation practically range between 60% and 90%. Concerning hatching performance, hatching rate of 85.2% was slightly lower compared to 95% reported by Horváth and Lukowicz (1982) and 90%–100% reported by Billard et al. (1995). This could be attributed to overripening of some individuals, since the experiment was performed late in the reproductive period, in order to secure the spawning response in fully mature fish.

Concerning the drawbacks of pituitary extracts compared to purified LH preparations, there is still a potential for transmission of diseases to recipient broodfish, LH content varies depending on size, sex, age, and time of pituitary collection and the presence of additional hormones in the pituitary may affect physiologically the recipient fish (Zohar and Mylonas 2001). Nevertheless, the advantage of replacing or even substituting the commercially available CPE preparations affects the running costs of the hatcheries, since the overall cost is significantly lower and depends mainly to the catch per unit effort (CPUE) of the fishing method and the scale of the fishing operation (Perdikaris et al. 2007).

Future work should be focused on the use of Prussian carp pituitaries in common carp males and in the artificial propagation of other cyprinid species, since the application in goldfish seems to be promising (Perdikaris, unpublished). Moreover, hormone contents should be analyzed in other seasons of the year to determine the effectiveness of pituitaries harvested in different time periods.

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