The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),
University of Hawaii at Manoa Library
and
University of Hawaii Aquaculture Program in association with
AquacultureHub
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

Pituitary Collection from Gibel Carp *Carassius gibelio* (Bloch 1782) in Lake Pamvotis (Greece): Prospects for Use in Carp Reproduction

Costas Perdikaris[1]*, Berta Levavi-Sivan[2], Athanasios Chantzaropoulos[1], Cosmas Nathanailides[1], Evangelia Gouva[1] and Ioannis Paschos[1]

[1] Department of Aquaculture and Fisheries, Technological Educational Institute of Epirus, 46100 Igoumenitsa, Greece

[2] Department of Animal Sciences, Hebrew University of Jerusalem, 76100 Rehovot, Israel

(Received 20.11.06, Accepted 10.5.07)

Key words: gibel carp, pituitaries, cLH levels, Lake Pamvotis, Greece

Abstract

Gibel carp (*Carassius gibelio* Bloch, 1782) is regarded as a pest species in most aquatic ecosystems where it occurs and eradication methods have not been very promising. However, because of the close taxonomical proximity between gibel carp and cultured cyprinids, the pituitary of gibel carps could be exploited for hormonal induction of artificial propagation in aquaculture. Thirty-two mature female gibel carps were electrofished in Lake Pamvotis (northwest Greece) in March 2005 and their pituitaries were collected and processed. The carp luteinizing hormone (cLH) levels in 20 pituitaries were assayed and found to correlate with total body weight, total length, dry pituitary weight, and gonadosomatic index. The cLH levels were in the same range as cLH levels in pituitaries of common carp (*Cyprinus carpio* Linnaeus, 1758) used in Israeli hatcheries for artificial propagation. Hence, it may be possible to use gibel carp pituitaries for hormonal induction in cyprinids, with total weight of the gibel carp as a field criterion for selecting pituitary donors.

Introduction

Carps represent 52% of the total fishery production of the world's inland waters, making them by far the largest group of cultured aquatic organisms. More than 26 cyprinid species have been cultured with the Chinese carps, common carp, and major Indian carps dominating global culture. In Greece, aquaculture production of carps is still quite low and in 2004 reached only 1220 tons plus about 1 million common carp and grass carp fry produced in two hatcheries and used mainly to stock lakes (Paschos, 2004).

* Corresponding author. E-mail: kperdik@otenet.gr

Reproduction in fish depends on the coordinated actions of hormones along the brain-hypothalamus-pituitary-gonad axis with gonadotropins, follicle stimulating hormone (FSH, formerly GtH-I), and luteinizing hormone (LH, formerly GtH-II) occupying major roles (Yaron et al., 2003). Gonadotropins are produced in distinct pituitary cells. According to the salmonids model, synthesis and release of gonadotropins vary throughout the reproductive cycle. Early in the cycle, a high FSH level stimulates the first stages of gametogenesis and steroidogenesis while LH is undetectable. Towards spawning, FSH decreases while LH increases, especially just before final oocyte maturation and ovulation (Gomez et al., 1999; Swanson et al., 2003).

In culture systems, hormonal induction is used to complete the final stages of oocyte maturation and ovulation and to synchronize the release of spawn, processes which are not successfully completed in captivity (Mylonas and Zohar, 2000). The use of pituitaries in the artificial reproduction of carp is common throughout the world (Yaron, 1995). Pituitary extracts with high levels of LH can be administrated intramuscularly or intraperitoneally. These are commercially available preparations containing whole acetone-dried glands or pulverized glands (Zohar, 1988). So far, the use of common carp pituitaries has produced positive results in many freshwater species (Zohar, 1989; Horvath et al., 2002). However, the need to decapitate mature donor fish to obtain the pituitary increases the cost of these commercial preparations to US$199-432 per gram. The use of pituitaries from mass-collected pest species, such as the gibel carp, could dramatically reduce the cost.

The gibel carp (*Carassius gibelio*) has acclimated and spread to many aquatic systems in Greece where they can be found in great numbers (Economidis et al., 2000). In some locations, such as Lake Pamvotis, it has displaced native species (Paschos et al., 2004). Since the gibel carp is regarded as a pest and is not a target species for fishermen, it could be exploited as a pituitary donor. The efficiency of a pituitary depends on the taxonomic distance between the donor and the recipient species (Zohar and Mylonas, 2001). Thus, the pituitary of the gibel might be compatible to that of other cyprinids. Despite the currently low demand from Greek hatcheries, development of a method for collecting pituitaries from gibel carp could be an important item for the international industry and, in time, generate interest in growing carps for the domestic market.

The aims of the present study were (a) to determine the LH level in the pituitary of sexually mature gibel carps and (b) to study the correlation between LH level, dry pituitary weight, total length, total weight, and GSI, to assist in developing a method for selecting pituitary donors.

Materials and Methods

In order to obtain fish with the highest possible LH contents, mature female gibel carps were collected from the southeastern part of Lake Pamvotis at the beginning of the reproductive season (late March 2005) just before spawning, as they approached spawning grounds. The sexually mature females (n = 32) were captured with a backpack electrofishing device (Hans Grassl IG 200/1), transferred to the Freshwater Aquaculture Laboratory of the Department of Aquaculture and Fisheries at the Technological Educational Institute in Epirus, and killed with a high dose of anesthetic (MS-222).

Total weight, total length, and gonad weight were measured and the gonadosomatic index (GSI) was calculated as GSI = 100(gonad wt/total wt). The fish were decapitated, the brain was exposed, and the pituitaries were detached from the skull with sterilized forceps. The pituitaries were placed in acetone (15:1 v/v) in Eppendorf vials. The acetone was renewed one and ten hours after collection according to Lutz (2001). After 14 more hours in acetone (24 h total), the pituitaries were left to dry at room temperature, weighed on a precision balance (Metter Toledo AB54), and stored in airtight sterilized vials.

Carp luteinizing hormone (cLH) levels were determined in 20 pituitaries by homologous competitive ELISA based on the method described by Mananos et al. (1997) for striped bass and Aizen et al. (2007) for tilapia. The

ELISA was based on cLH for the standard curve and specific polyclonal antibodies against cLHβ subunit (Levavi-Zermonsky and Yaron, 1986). Pituitary samples were homogenized by sonication in ELISA buffer (10 mM Na_2PO_4, 2 mM KH_2PO_4 [pH 7.4], 140 mM NaCl, 3 mM KCl, 0.05% Tween 20) and diluted according to the concentration of the sample. Parallelism was used to compare cLH, Israeli carp pituitary extract (CPE), and gibel carp pituitary, using the standard curve of cLH, CPE prepared according to Yaron et al. (1984), and gibel carp no. 9 (pituitary wt = 0.0026 g, total wt = 849 g, total length = 36 cm). We used analysis of covariance (http://home.ubalt.edu/ntsbarsh/Business-stat/otherapplets/ANOCOV.htm) to test for parallelism between regression lines. The intra and inter-assay CV were 7.2 and 14.8, respectively, and the sensitivity of the assay was 0.65 ng/ml.

Results

The mean total weight, total length, and GSI of the fish (n = 32) were 697.3±38.1 g, 33.7±0.7 cm, and 11.4±0.6%, respectively. The mean pituitary dry weight was 1.9±0.1 mg (n = 32). The mean dry weight of the 20 pituitaries tested for cLH was 2.2±0.1 mg and the cLH level in these fish ranged 429.9-871.2 µg (mean 591.8±22.07 µg).

In the parallelism test, the slope of the displacement curve obtained with CPE (-0.218± 0.060) did not significantly differ from that obtained with gibel carp no. 9 (-0.188±0.094), indicating that both pituitary extracts contain a substance, probably cLH, that has an affinity to the antisera against cLHβ (Fig. 1).

Pituitary dry weight and cLH levels correlated better with total weight and total length than with GSI (Table 1, Figs. 2,3). The pituitary dry weight correlated well with the cLH level (Fig. 4). The negative correlations between cLH content and total weight, total length, and pituitary weight were statistically significant.

Discussion

Pituitaries of Israeli carp contain around 350 µg cLH per kg fish (Yaron et al., 1984; Levavi-Zermonsky and Yaron, 1986). Spawning in carp is induced by two injections: the first (primer) containing 70 µg cLH (equal to 20% of a pituitary) and the second 350 µg cLH (equal to one pituitary) per kg fish (Levavi-Zermonsky and Yaron, 1986). Pituitaries of gibel carp contain 591.8±22.07 µg/pituitary. Therefore, besides the immunological similarity between gibel carp pituitaries and standard carp cLH, the gibel carp pituitary contains a greater amount of cLH. This could be decisive when used for carp hypophysation as the

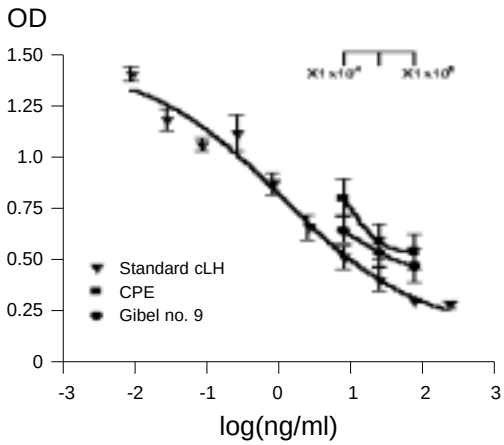

Fig. 1. Parallelism between the standard curve of carp luteinizing hormone (cLH), serially diluted concentrations of carp pituitary extract (CPE) of fish collected in Israel, and the pituitary of gibel carp no. 9 collected in Greece.

Table 1. Correlation between pituitary dry weight, pituitary cLH level, total fish weight, total fish length, and gonadosomatic index (GSI) .

	Pituitary wt (n = 32)		*cLH content (n = 20)*	
	r	*p*	*r*	*p*
Total fish wt	0.75	<0.001	0.57	<0.01
Total fish length	0.68	<0.001	0.34	>0.05
GSI	0.11	>0.05	0.09	>0.05
Pituitary wt			0.47	<0.05

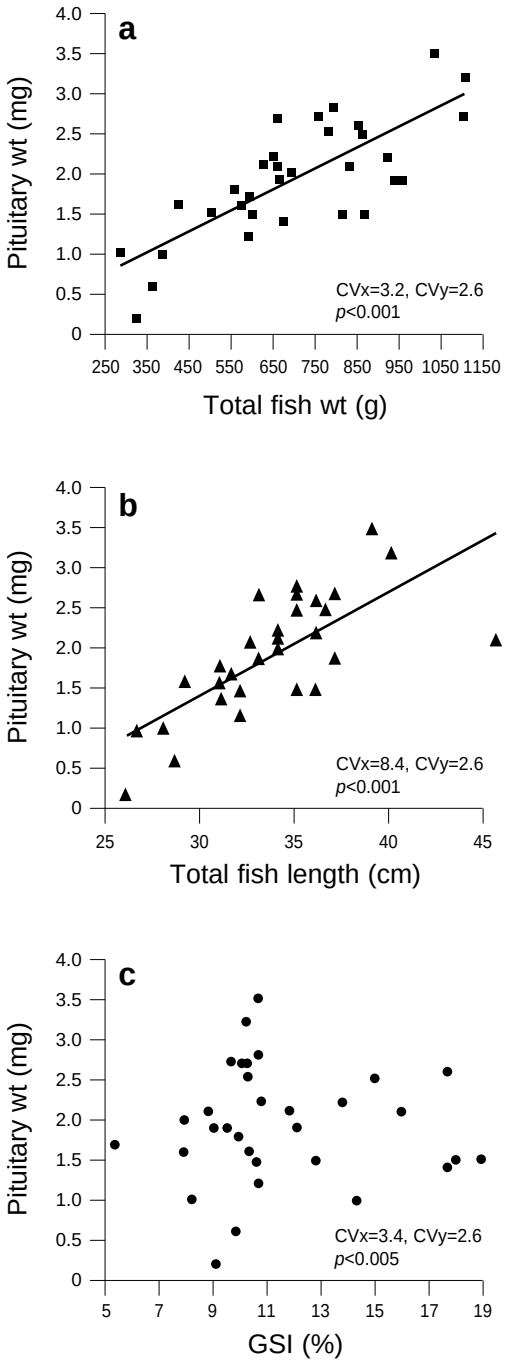

Fig. 2. Correlation between pituitary dry weight (n = 32) and (a) total fish weight, (b) total fish length, and (c) gonadosomatic index (GSI).

amount in a single pituitary of gibel carp is more than sufficient to induce spawning in a one kilogram carp (Yaron et al., 1984).

Total weight more strongly correlated to the pituitary dry weight and LH level than total length. Therefore, total weight can be used to select fish with potentially high hormone levels and fishermen can easily collect donors by discarding fish under 500 g. The overall cost for such collection is low and requires moderate expertise. Gibel carp can be collected in mass with fyke net traps or electrofishing in shallow waters and the cost of the fish is negligible. The costs of extracting and processing the gland, i.e., chemicals (acetone) and glassware (Eppendorf vials), are the same for gibel carp as for common carp. Accordingly, the difference in overall cost is mostly dependent on the capture per unit effort (CPUE) of the fishing method and the scale of the fishing operation. Gibel carps could be exploited as a source of income for local fishermen or by hatcheries that want to reduce operational costs.

Fully mature females, just prior to spawning, were captured at the beginning of the reproductive period when the cLH level was expected to be the highest (Yaron et al., 1984; Yaron and Levavi-Zermonsky, 1986; Tyler and Sumpter, 1996). However, GSI cannot be used as a selection criterion as it did not correspond well with the LH level. Hormone contents should be analyzed in other seasons of the year to determine the best season for harvesting gibel carp pituitaries. Sampling should start when the water temperature reaches a minimum of 12-14ºC. At this point, mature or nearly mature gibel carps approach shallow spawning grounds (Paschos et al., 2001).

As the ultimate test of the efficiency of using gibel carp pituitaries to induce spawning in cultured cyprinids is the success in artificial reproduction, the use of gibel carp pituitaries should be thoroughly evaluated. As a cyprinid, gibel carp is taxonomically close to other cyprinids and it is possible that this will increase compatibility. Further research is required to validate its biological potency in carp spawning.

An additional benefit is that the intensive fishing of mature gibel carps, together with

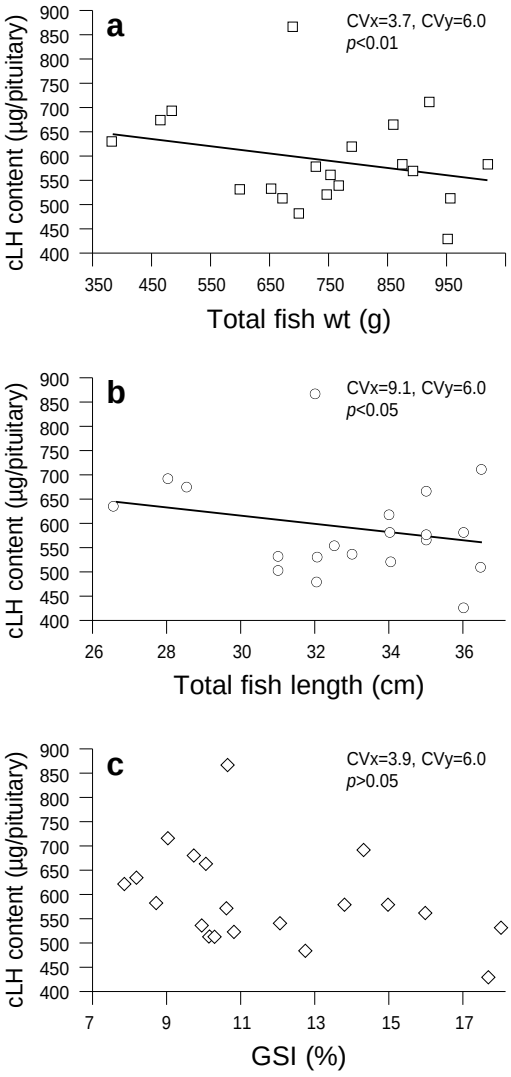

Fig. 3. Correlation between pituitary cLH content (n = 20) and (a) total fish weight, (b) total fish length, and (c) gonadosomatic index (GSI).

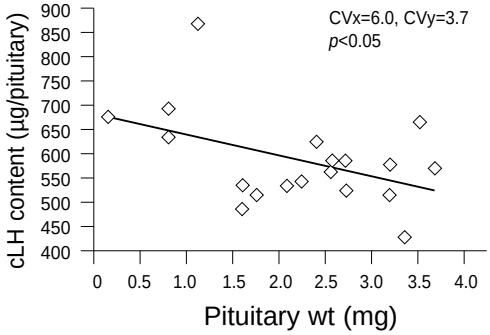

Fig. 4. Correlation between pituitary (dry) weight and pituitary cLH content.

population restocking and habitat restoration, may help balance ecosystems in favor of endemic fish (Perdikaris et al., 2005).

Acknowledgements

The authors would like to thank Mr. Tasos Haratsiaris for his valuable help during electrofishing and Ms. Paraskevi Anagnostou and Ms. Ioulia Bonarou for the collection and processing of the pituitaries. We also thank Dr. Andras Ronyai and Dr. Lazlo Varadi for information concerning pituitary demands in Europe.

References

Aizen J., Kasuto H., Golan M., Zakay H. and B. Levavi-Sivan, 2007. Tilapia follicle-stimulating hormone (FSH): Immunochemistry, stimulation by gonadotropin-releasing hormone, and effect of biologically active recombinant FSH on steroid secretion. *Biol. Reprod.*, 76:692-700.

Economidis P.S., Dimitriou E., Pagoni R., Michaloudi E. and L. Natsis, 2000. Introduced and translocated fish species in the inland waters of Greece. *Fish. Manag. Ecol.*, 7:239-250.

FAO, 2006. *Yearbook of Fisheries Statistics 2004*. Aquaculture Vol. 98/2, Rome.

Gomez J.M., Weil C., Ollitrault M., Le Bail P.Y, Breton B. and F. Le Gac, 1999. Growth hormone (GH) and gonadotropin subunit gene expression and pituitary and plasma changes during spermatogenesis and oogenesis in rainbow trout (*Oncorhynchus mykiss*). *Gen. Comp. Endocrinol.*, 113(3):413-428.

Horvath L., Tamas G. and C. Seagrave, 2002. *Carp and Pond Fish Culture*. Fishing News Books, Oxford. 170 pp.

Levavi-Zermonsky B. and Z. Yaron, 1986. Changes in gonadotropin and ovarian-

steroids associated with oocytes maturation during spawning induction in the carp. *Gen. Comp. Endocrinol.*, 62:89-98.

Lutz C.G., 2001. *Practical Genetics for Aquaculture*. Blackwell Sci., Oxford, 256 pp.

Mananos E.L., Swanson P., Stubblefield S. and Y. Zohar, 1997. Purification of gonadotropin II from a teleost fish, the hybrid striped bass, and development of a specific enzyme-linked immunosorbent assay. *Gen. Comp. Endocrinol.*, 108:209-222.

Mylonas C.C. and Y. Zohar, 2000. Use of GnRHa-delivery systems for the control of reproduction in fish. *Rev. Fish Biol. Fish.*, 10: 463-491.

Paschos I., 2004. *Freshwater Aquaculture*. Ioannina, Greece. 293 pp. (in Greek).

Paschos I., Nathanailides C., Samara A., Gouva E. and M. Tsoumani, 2001. Presence of gibel carp (*Carassius auratus gibelio*) in Lake Pamvotis: spawning behaviour, characteristics and prospect for controlling the population. *Proc. 10th Hellenic Congress of Ichthyologists*, 18-20 October 2001, Chania, Greece (in Greek with English abstract).

Paschos I., Nathanailides C., Tsoumani M., Perdikaris C., Gouva E. and I. Leonardos, 2004. Intra and inter-specific mating options for gynogenetic reproduction of *Carassius gibelio* (Bloch, 1783) in Lake Pamvotis (NW Greece). *Belg. J. Zool.*, 134(1):55-60.

Perdikaris C., Nathanailides C., Gouva E., Karipoglou C. and I. Paschos, 2005. Collapse of Epirus minnow, *Phoxinellus epiroticus* (Steindachner, 1895) fisheries in lake Pamvotis, Greece. *Ichthyol. Explor. Freshw.*, 16(4):371-374.

Swanson P., Dickey J.T. and B. Campbell, 2003. Biochemistry and physiology of fish gonadotropins. *Fish Physiol. Biochem.*, 28:53-59.

Tyler C.R. and J.P. Sumpter, 1996. Oocyte growth and development in teleosts. *Rev. Fish Biol. Fish.*, 6:287-318.

Yaron Z., 1995. Endocrine control of gametogenesis and spawning induction in the carp. *Aquaculture*, 129:49-73.

Yaron Z. and B. Levavi-Zermonsky, 1986. Fluctuations in gonadotropin and ovarian-steroids during the annual cycle and spawning of the common carp. *Fish Physiol. Biochem.*, 2:75-86.

Yaron Z., Bogomolnaya A. and B. Levavi, 1984. A calibrated carp pituitary extract as a spawning-inducing agent. *Eur. Maricul. Soc. Spec. Publ.*, 8:151-168.

Yaron Z., Gur G., Melamed P., Rosenfeld A., Elizur A. and B. Levavi-Sivan, 2003. Regulation of fish gonadotropins. *Int. Rev. Cytol.*, 225:131-185.

Zohar Y., 1988. Gonadotropin releasing hormone in spawning induction in teleosts: basic and applied considerations. pp 47-62. In: Y. Zohar, B. Breton (eds.). *Reproduction in Fish: Basic and Applied Aspects in Endocrinology and Genetics*. INRA Press, Paris.

Zohar Y., 1989. Fish reproduction: its physiology and artificial manipulation. pp. 65-119. In: M. Shilo, S. Sarig (eds.). *Fish Culture in Warm Water Systems: Problems and Trends*. CRC Press, Boca Raton, FL, USA.

Zohar Y. and C.C. Mylonas, 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture*, 197:99-136.