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Correlation and assessment of the adequacy and productivity of the GnRH treatment and counterfeit propagation of the *Labeo parvus*

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Abstract

The effectiveness of hormonal treatments to induce gamete emission in *Labeo parvus* was investigated in two spawning seasons (2007 and 2008). Brood fishes measuring 15 to 30 cm were gill netted in the Ouémé River (Benin) during their migration to their spawning habitat and then held in captivity (ponds). Captive fish (N = 30 males and N = 37 females) were treated with GnRHa combined with a dopamine antagonist during their natural spawning season from July through October. Ovulation was stimulated in four experimental groups of females by injecting Dagin (0.2 to 0.3 mlkg^{-1} body weight) and Ovaprim (0.5 to 0.6 mlkg^{-1} body weight). No female ovulated either in the groups receiving normal (as recommended by the manufacturers) dose injections of Dagin and Ovaprim (0.2 and 0.5 mlkg^{-1} body weight respectively) or in the group treated with a Dagin injection of 0.3 mlkg^{-1} body weight. Successful ovulation was only obtained with Ovaprim in the group treated with 0.6 mlkg^{-1} body weight. Latency times ranged from 10 to 14 h after hormone injection. *L. parvus* eggs were nonadhesive and transparent at ovulation. Fertilisation rates ranged from 74.0 to 97.7%. Hatching occurred after 10 to 14 h of incubation at 27 to 30°C and lasted 5 to 7 h. Hatching rates ranged from 70.0 to 83.3%. Larvae were 3.0 ± 0.4 mm long (total length) at hatching. In the two reproductive seasons, fertilisation rates and hatching were not significantly different (P > 0.05). The water temperature was shown to significantly affect spawning latency and incubation time. Our study demonstrated that captive *L. parvus* could be successfully induced to produce sperm and ova using injection of a synthetic gonadotropin-releasing hormone coupled with a dopamine antagonist (Ovaprim). Their ova could also be artificially fertilised in an experimental hatchery at water temperatures ranging from 27 to 30°C. The results obtained indicate that Ovaprim is the most effective agent for inducing artificial reproduction in *L. parvus*.

Keywords: GnRH analogues, Induced spawning, *Labeo parvus*.

INTRODUCTION

Fishing pressure continues to increase in the inland waters of Benin even though studies and reports have revealed over-exploitation of some of the most important species (Lalèyè et al., 2003, 2007; Montchowui et al., 2008). Many fish species are in decline and some have become endangered due to a combination of over-exploitation, aquatic pollution and habitat modification due to river-valley projects, excessive water abstraction for irrigation and siltation due to clearing.

Labeo parvus is one of the most exploited fish species and one of the more popular food fish in Benin (Montchowui, 2009). The available data on this species show that it is particularly vulnerable due to over fishing and environmental condition changes that influence its

reproductive strategy (Skelton et al., 1991). This strategy includes first a compulsorily whole egg-laying for the majority of fish species and, seconds a migration in group to adjacent flooded areas or in connected water course where they are dependant to rain falls and other environment conditions (Bowmaker, 1973; Tomasson et al., 1984). In addition, eggs, embryo and larva of *Labeo* are particularly sensitive to sudden flooding and to associated silting up that can significantly reduce the lava survival and recruitment (Skelton et al., 1991). Then, total spawner *Labeo* as well as *L. parvus* are the most vulnerable species. The impact of these different disturbing factors reduces *L. parvus* populations and there is a risk of a drastic decline (Skelton et al., 1991). Thus, Skelton et al. (1991) suggested artificial multiplication of these species in captivity in order to sustain natural stocks. Captive breeding programs have become one of the principal tools used in attempts to compensate for declining fish populations and simultaneously to supplement and enhance yields for fisheries (Fleming, 1994). It is also an approach solution for fish conservation by means of restocking their natural habitat with hatchery-reared individuals (Philippart, 1995; Poncin and Philippart, 2002), and a viable alternative to capture fisheries in providing a sustainable source of proteins for fishing communities and local populations.

The mastery of artificial reproduction is a key aspect as it permits intensive production of a given species in controlled conditions. This allows continue production of juveniles for restocking natural water courses, artificial lakes or fish breeding ponds (Awaïs et al., 1993). The present study aims to establish biological techniques for artificial reproduction of *L. parvus* in captivity that can serve as basis for future programs both for restocking of natural populations and for fish breeding for human consumption.

To our knowledge, little is known about the reproduction of *L. parvus*; therefore research is needed to develop and standardise techniques for its artificial multiplication. The specific objectives of the study were:

(1) To induce ovulation in African carp by hormonal intervention under artificial conditions; (2) To compare and evaluate the effectiveness and efficiency of the GnRH treatment; and (3) To clarify whether combination with a dopamine antagonist is necessary to induce ovulation in this species with GnRH analogue treatment.

MATERIAL AND METHODS

Studies were carried out in July to October 2007 and July to October 2008 at the experimental fish-culture facility of the Unit of Training and Research in Fish Farming (Hydrobiology and Aquaculture Laboratory, University of Abomey Calavi, Benin). *L. parvus* broodfish were caught using gill nets in the Ouémé River. The time for collection of ripe fish was predicted based on cyclic variations of the gonadosomatic index and the prevalence of oocytes in advanced stages of development (Montchowui et al., 2007). Ripe fish were obtained over the period of the year characterised by high gonadosomatic indices and a dominance of tertiary yolk vesicle oocytes and fresh post-ovulatory follicles. Sexually mature fish were caught during their migration to their spawning habitat. After capture, fish were transferred into an oxygenated plastic container and transported to the fish culture facility in Cotonou. The fish were placed in rectangular concrete tanks supplied with gravity-fed spring water. The tanks were covered with nets to prevent fish from jumping out. Fish were held in captivity and fed with artificial Tilapia feed twice daily.

Hormonal treatment

Two spawning-inducing agents, Dagin and Ovaprim, were tested for their efficacy in stimulating ovulation in *L. parvus*: Dagin, an Israeli agent for spawning induction in fish, combines a superactive GnRH analogue ([Arg^6, Pro^9NEt]-sGnRH) and the dopamine receptor antagonist, metoclopramide. Each dose calculated for 1 kg body weight of fish contains 10 µg of the analogue and 20 mg of metoclopramide, which is directed to block the inhibitory effect of the endogenous dopamine on gonadotropin release from the pituitary of the fish (Kulikov et al., 1995). Dagin was dissolved in 0.7% NaCl prior to injection.

Ovaprim was supplied in liquid form and contained 20 µg of the analogue and 10 mg of domperidone in 1.0 ml. The preparation contained its active ingredients dissolved in propylene glycol.

Selection of mature males and females

Prior to hormonal treatment, fish were tested for readiness to spawn by gentle pressure applied on the abdomen after anaesthesia in 2-phenoxyethanol to minimise trauma and stress to the fish. Females were selected for induction when they presented mature oocytes that were greenish-yellow in colour. As criteria in the selection of females, external signs of maturity (protruding genital papilla, dilated and bulging abdomen) were also taken into consideration. Males were selected from the wild population, and milt was released after application of a gentle pressure on the abdomen.

Experiments

Several experiments were carried out to determine the optimal dosages of Dagin or Ovaprim which allowed the successful and highest rate of ovulation and the suitable latency time between injection and stripping to be obtained.

In a first experiment conducted in October 2007, 12 females were kept captive in separate tanks. This stock of females was divided into two groups of six individuals and each group was placed in a separate tank. The ovulation stimulation began with the injection of Ovaprim to females of group 1 and of Dagin to those of group 2. Single doses of hormone were administrated to the fish. The dose of hormone used, as Ovaprim, was 0.5 ml/kg^{-1} body weight and as Dagin 0.2 ml/kg^{-1} body weight, as recommended by the manufacturers.

In a second experiment carried out in September 2008, the same number of females was used and divided into two groups of six individuals. The first group received Ovaprim and the second Dagin. The doses of Ovaprim and Dagin used were 0.6 and 0.3 $mlkg^{-1}$ body weight, respectively. This experiment was inspired by those conducted on *Tor khudree* and *Clarias batrachus* (Nandeesha et al., 1993; Mahapatra, 2004), where the recommended doses by the distributors were increased by 0.1 ml.

In all experiments, the males were treated with a dose of 0.2 ml/kg body weight in a single injection given at the same time as the female injection. Prior to injection, fish were anesthetised with 2-phenoxyethanol (Fluka-Chemie-Sigma Aldrich), measured and

Table 1. Results (mean ± SE) from 2 years of experiments on artificial reproduction of captive *Labeo parvus* females

Spawning season	Treatment	NO of fish	Mean weight (g)	Fish stripped (%)	Latency time (h)	Mean fecundity	Mean fertilisation (%)
2007	Ovaprim (0.5 mlkg^{-1})	7	142.9± 22.8 ^{a}	0	-	-	-
	Dagin (0.2 mlkg^{-1})	6	141.5± 23.5 ^{a}	0	-	-	-
	Ovaprim (0.6 mlkg^{-1})	6	58.8 ± 12.8 ^{b}	100	11.0 ± 0.0 ^{a}	10756 ± 1436^{a}	84.8 ± 3.7 ^{a}
	Dagin (0.3 mlkg^{-1})	6	58.1± 7.8 ^{b}	0	-	-	-
2008	Ovaprim (0.6 mlkg^{-1})	6	150.6 ± 4.0 ^{a}	83.3	12.2 ± 0.8 ^{a}	48198 ± 3705^{b}	96 ± 0.8 ^{a}
	Dagin (0.3 mlkg^{-1})	6	160.9 ± 3.1 ^{a}	0	-	-	-

Values followed by the same superscript are not significantly different (P > 0.05) from others in the same column.

weighed. Males and females received an intramuscular injection (between the dorsal fin and the lateral line) of the inducing agent. Females were individually examined for successful ovulation by a gentle abdominal massage starting 8 h after the hormonal injection, and then every 2 h until ovulation.

When ovulated eggs were present, the female was stripped after drying the papilla with a paper towel and ova were collected into dry plastic containers. The sperm was collected by stripping the males in a clean dry pot before female stripping. Males not releasing milt after abdomen massage were sacrificed. The testes of these males were taken in a clean pot and ground. Ovulated eggs were gently mixed with milt for 2 to 3 min, and then fertilising water (3 g of urea plus 4 g of NaCl in 1 L water) was added according to Woynarovich and Horvath (1981). Fertilised eggs were rinsed three or four times with fertilising water and then transferred to a hatchery. The fertilised eggs were discernable by a dark spot of the developing embryo in the transparent vitelline membrane.

Data and statistical analysis

After stripping, the absolute fecundity of each female was determined by randomly taking a representative sample by weight of eggs from the total mass of eggs laid. The total number of eggs in the sample were counted and then extrapolated to the total weigh t of eggs released. The fertilisation rate of eggs was determined after 2 h fertilisation by randomly taking a sample of approximately 100 eggs in a Petri dish and fertilised eggs having an intact nucleus were counted for calculating the fertilisation percentage.

The quantitative results were examined by analysis of variance (ANOVA 1). When ANOVA indicated significant treatment differences, a Fisher PLSD test was applied to compare means at the P < 0.05 level of significance. Percentages were log-transformed prior to statistical analysis. All statistical analyses were performed using Staview software (Version 1992-98, SAS Institute Inc.).

RESULTS

Ovulation and fertilisation

Results on the response to hormonal induction of ovulation, absolute fecundity, latency time and fertilisation rate for the different experiments are summarised in
Table 1.

No female ovulated either in the groups receiving normal (as recommended by the distributors) dose injections of Dagin and Ovaprim (0.2 and 0.5 mlkg^{-1} body weight, respectively) or

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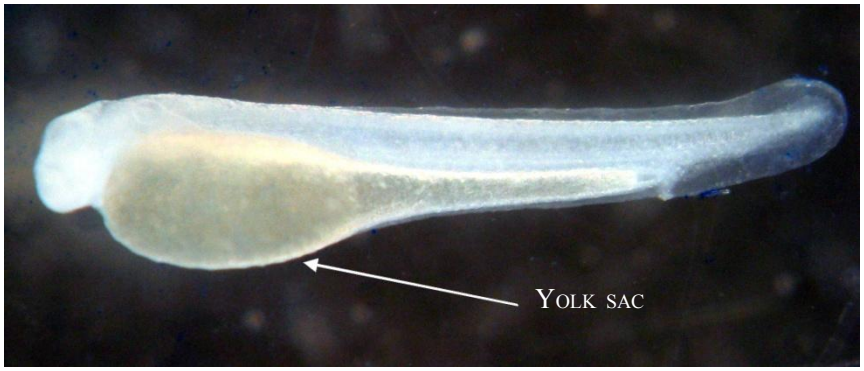

Figure 1. Newly hatched larvae of *L. parvus.*

Figure 2. Newly hatched larvae of *L. parvus* after 20 h post-hatching.

Incubation and hatching

The first hatching occurred 10 to 14 h after incubation and lasted about 5 to 7 h depending on the water temperature. Incubation took a significantly ($P < 0.05$) (Table 1) shorter time to occur in the first spawning season (in October the mean water temperature was 29.7 ± 0.4°C) than in the second spawning season (in August and September, the mean water temperature recorded was 27.6 ± 0.2°C).

Hatching rates averaged 73.8 ± 4.8%, ranging from 70 to 80% in the first spawning season. In the second spawning season, hatching rates were similar to those in the first spawning season and ranged from 73.8 to 83.3%, with a mean value of 77.9% (Table 1). These hatching rates were not statistically different ($P > 0.05$) between the two seasons. The highest embryo mortality occurred between 4 and 6 h after incubation. Newly hatched larvae had a mean total length of 3.0 ± 0.4 mm and were very transparent with a small yolk sac attached (Figure 1). Immediately after hatching, newly hatched larvae exhibited particular movements (about 10 cm vertical movements to the water surface and back to the bottom) at a rate of about 8±1.0 movements/min. The yolk sac resorption lasted about 20 to 24 h after hatching. Most larvae observed with the magnifying glass at 20 h had their yolk sac empty (Figure 2).

DISCUSSION

The present study has demonstrated that *L. parvus* can be successfully induced to produce sperm and ova using an intramuscular injection of Ovaprim (sGnRHa + DOM). This success achieved with Ovaprim may be owing to the linpe method (Peter et al., 1988), which consists in treating fish with a combination of gonadotropin-releasing hormone analogue (GnRHa) plus a dopamine antagonist. The successful use of Ovaprim in inducing ovulation in cyprinid fish is well documented (Pandey and Singh 1997; Mijkherjee et al., 2002; Sharma and Singh, 2002; Szabó, 2003; Sarkar et al., 2004). *L. parvus* ovulation in our study was obtained with a dose of 0.6 $mlkg^{-1}$, which was found to be the minimal dose for spawning induction in that species because all the fish injected with a dose lower than 0.6 ml of Ovaprim did not ovulate. This dose of 0.6 $mlkg^{-1}$ was higher than those generally recommended by the distributors (0.5 $mlkg^{-1}$) and used for other cyprinids. Increased doses of Ovaprim needed for spawning induction have been reported for many

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species: *Mystus gulio* (2.5 $mlkg^{-1}$) (Mijkherjee et al., 2002), *T. khudree* (0.8 $mlkg^{-1}$) (Nandeesha et al., 1993) and *C. batrachus* (0.75 to 2 $mlkg^{-1}$) (Mahapatra, 2004). According to Billard et al. (1984) and Peter et al. (1986), differences between species in the Ovaprim requirements may stem from varied levels of dopamine activity between the species of fish concerned.

It should be noted that no female ovulated in the fish groups treated with the different doses of Dagin tested. This result is similar to what was reported by Szabó (2003) in northern pike *Esox lucius* L., where no ovulation occurred in the group treated with Dagin. The failure of Dagin to induce ovulation might be explained by an insufficient stimulation of pituitary GtH release likely to be linked to an insufficient action of dopamine receptor antagonist. In many cyprinids, the endogenous inhibitory impact of dopamine is so strong that it severely compromises the effectiveness of externally applied GnRH to increase GtH release, which would lead to ovulation and subsequent spawning (Peter et al., 1991; Driori et al., 1994).

Based on our result, it may be presumed that dopamine activity is high in *L. parvus*, since it has responded to only a high dose of Ovaprim.

The latency time varied between 10 and 14 h, the difference observed between the two spawning seasons clearly being attributed to a water temperature approximately 2°C higher in experiment 1 than in experiment 2. Fertilisation rates obtained in the present study were higher than 90%, suggesting the stripped eggs produced after Ovaprim treatment were of high quality. The mean number of stripped eggs obtained was significantly higher in the second reproduction season than in the first simply because of a substantial difference between the weights of the females used. Moreover, females used in the first reproduction season came directly from the Ouémé River and could have lost eggs when they were gill netted.

The hatching rates obtained during the two reproduction seasons were better (73.8 to 77.9%) with rapid embryo development. The significant differences in incubation time and duration of hatching between the two spawning seasons might be attributed to water temperature differences in the two periods. For the first spawning season, the experiments were carried out in October when the mean water temperature was 29.7 ± 0.4°C. The repeated experiments for the second spawning season were conducted in August to September when the mean water temperature recorded was 27.6 ± 0.2. Rutaisire and Booth (2004) reported the same observation in *Labeo victorianus* where the same factor is thought to have caused prolonged hatching.

The high mortality of embryos recorded after 4 and 6 h incubation may partly be attributed to the hatchery system. Indeed, after fertilisation the eggs were incubated in small circular baskets (Ø = 18 cm) covered with a very fine mosquito mesh net and placed in a closed recirculating circuit. This system does not allow undulation of eggs which tend to settle on the bottom of the basket. The swelling of the vitelline membrane of fertilised eggs put in contact with water confined these eggs and caused certain developing embryos to die by smothering before hatching. According to Fryer and Whitehead (1959), the swelling of eggs after fertilisation on contact with water is an adaptation to ephemeral flood conditions by providing a standard microenvironment to the developing embryo, effectively safeguarding it from a wide variety of environmental conditions. The swelling of the vitelline membrane of the *L. parvus* eggs has also been reported to occur in *Labeo mesops* and *Labeo victorianus* (Msiska, 1990; Rutaisire and Booth, 2004). In future experiments, Zug incubation bottles should help reduce the high embryo mortality recorded in our study. This system will allow better buoyancy of eggs, which will be better oxygenated.

In conclusion, this study demonstrates the development of a reliable method to induce breeding in captive *L. parvus*. High fecundity, high fertilisation and hatching rates make *L. parvus* a species of great potential for African aquaculture.

REFERENCES

Billard R, Bieniarz K, Peter RE, Sokolowska M, Weil C, Crim LW (1984). Effects of LHRH and LHRH-a on plasma GtH levels and maturation/ovulation in common carp, Cyprinus carpio, kept under various environmental conditions. Aquaculture, 41: 245-254.

Bowmaker AP (1973). Potadromesis in the Mwenda River, Lake Kariba. In: Man-Made Lakes: Their Problems and Environmental Effects, W.C. Ackermann, G.F. White, E.B. Worthington & J.L. Ivens (eds.), Geophysical Monograph, American Geophysical Union, Washington, pp. 159–164.

Driori S, Ofir M, Levavi-Sivan B, Yaron Z (1994). Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature. Aquaculture, 119: 393–407.

Fleming IA (1994). Captive breeding and the conservation of wild salmon populations. Conserv. Biol., 8: 886-888.

Fryer G, Whitehead PJP (1959). The breeding habits, embryology and larval development of *Labeo victorianus* Boulenger. (Pisces: Cyprinidae). Rev. Zool. Bot. Afr., 59:33–49.

Lalèyè P, Niyonkuru C, Moreau J, Teugels GG (2003). Spatial and seasonal distribution of the ichtyofauna of Lake Nokoué, Bénin, West Africa. Afr. J. Aquat. Sci., 28: 151-161.

Lalèyè P, Ezin A, Vandewalle P, Philippart JC, Teugels GG (2007). La pêche dans le fleuve Ouémé (Bénin). J. Afrotrop. Zool. Special issue, pp. 137-148.

Mahapatra BK (2004). Conservation of the Asiatic catfish, *Clarias batrachus* through artificial propagation and larval rearing technique in India. Aquaculture Asia, 9: 8-9.

Mijkherjee M, Praharaj A, Das S (2002). Conservation of endangered fish stocks through artificial propagation and larval rearing technique in West Bengal, India. Aquaculture Asia, 7: 8-11.

Montchowui E. 2009. The reproductive biology and artificial breeding of Labeo parvus (Pisces: Cyprinidae). Ph. D. thesis, University of Liège, Belgium, 151 p.

Montchowui E, Lalèyè P, Philippart JC, Poncin P (2007). Reproductive biology of Labeo parvus Boulenger, 1902 (Cypriniformes: Cyprinidae) in the Ouémé River Benin (West Africa). Cah. Ethol., 22: 61-80.

Montchowui E, Tobaba P, Chikou A, Lalèyè P (2008). Characteristics and fishing impact on exploitation of Labeo senegalensis Valenciennes 1842 in the lower Ouémé River valley, Benin. Int. J. Biol. Chem. Sci., 2: 478-489.

Msiska OV (1990). Reproductive strategies of two cyprinid fishes in Lake Malawi and their relevance for aquaculture development. Aqua. Fish. Manage., 21: 67–75.

Nandeesha MC, Bhadraswamy G, Patil JG, Varghese TJ, Sarma K, Keshavanath P (1993). Preliminary results on induced spawning of pondraised mahseer, *Tor khudree*. J. Aqua. Tropics. 8: 55-60.

Pandey AC, Singh RN (1997). Breeding of *Catla catla* (Bhakur), *Labeo rohita* (Rohu) and *Cirrhinus mrigala* (Nain) by ovaprim injection for seed production. J. Adv. Zool., 18: 38-41.

Peter RE, Chang JP, Nahorniak CS, Omeljaniuk RJ, Sokolowska M, Shih SH, Billard R (1986). Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. Recent Prog. Horm. Res., 42: 513–548.

Peter RE, Lin HR, Van Der Kraak G (1988). Induced ovulation and spawning of cultured freshwater fish in China: advances in application of GnRH analogues and dopamine antagonists. Aquaculture, 74: 1–10.

Peter RE, Trudeau VL, Sloley BD, Peng C, Nahorniak CS (1991). Actions of catecholamines, peptides and sex steroids in regulation of gonadotropin-II in the goldfish. Proceedings of the 4th Symposium on the Reproductive Physiolology of Fish. Fish Symp, 91: 30–34.

Philippart JC (1995). Is Captive breeding an effective solution for the preservation of endemic species?. Biol. Conserv., 72: 281-295.

Poncin P, Philippart JC (2002). The role of aquaculture in fish conservation: a case study of *Barbus barbus* in Belgium. In: Conservation of Freshwater Fishes: Options for the Future, M.J. Collares-Pereira, M.M. Coelho & I.G. Cowx (eds), Fishing News Books, Blackwell Science Ltd, London, UK. pp. 402-413.

Rutaisire J, Booth AJ (2004). Induced Ovulation, Spawning, Egg Incubation, and Hatching of the Cyprinid Fish *Labeo victorianus* in Captivity. J. World. Aqua. Soc., 35: 383-391.

Sarkar UK, Negi RS, Deepak PK, Singh SP, Srivastava SM, Roy D (2004). Captive breeding of vulnerable Indian carp *Cirrhinus reba* with Ovaprim for conservation of wild populations. Aquaculture Asia, 9: 5-7.

Sharma AP, Singh VK (2002). Induced breeding response of Indian major carps, viz. *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* using ovaprim and carp pituitary extract. Indian. J. Anim. Sci., 72: 351-354.

Skelton PH, Tweddle D, Jackson PBN (1991). Cyprinds of Africa. In: Cyprinid Fishes. Systematics, Biology and Exploitation, IJ Winfield & JS Nelson (eds), pp. 211-239. Chapman & Hall, London.

Szabó T (2003). Ovulation induction in northern pike *Esox lucius* L. using different GnRH analogues, Ovaprim, Dagin and carp pituitary. Aqua. Res., 34: 479-486.

Tomasson T, Cambray JA, Jackson PBN (1984). Reproduction biology of four large riverine fishes (Cyprinidae) in a man made lake, Orange River, South Africa. Hydrobiologia, 112: 179-195.

Woynarovich E, Horváth L (1981). The artificial propagation of warm-water finfishes: a manual for extension. FAO Fisheries Tech. Pap. 201, 191 p.