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Domestication and brood stock development of the orange spotted grouper, *Epinephelus coioides* (Hamilton, 1822) in open sea cage off Visakhapatnam coast

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ABSTRACT

Aimed at domestication and broodstock development of the orange spotted grouper (*Epinephelus coioides*), 63 nos. of the species of average weight 4.06 ± 0.81 kg were collected. Out of these, 54 nos. were stocked in two 6 m dia HDPE floating cages installed in Bay of Bengal off Visakhapatnam coast. Nine fishes were stocked in 5 t FRP tanks @ 1 kg per ton of water. After acclimatisation for a period of four months, 20 fishes from the cages and four fishes from the tanks were implanted with 17 α-methyl testosterone aimed at developing male brooders. The fishes were fed twice a day @ 5% body weight with *Decapterus russelli*, sardine and squids. Vitamin E, cod liver oil and mineral supplements were also given along with feed, twice a week. The survival rate was 100% and 94.11% in tanks and cages respectively. The final average weight of fishes at the end of the experiment was 6.99 (± 0.93) kg, registering a growth rate of 243.33 g and 206.02 g in terms of body weight per month in cages and tanks respectively. Female gonad development started four months after stocking in the cage. In one year, the mature females were cannulated and intra-ovarian egg size ranged from 400 to 600 µ. However, fishes stocked in FRP tanks did not show any sign of ovarian development till the end of the experiment. The cage reared hormone implanted fishes were oozing milt after slight pressure on abdomen whereas none of the fishes reared in tanks were found with oozing milt. The water quality parameters (ammonia and nitrite) in the tank water were significantly ($p < 0.05$) higher than water samples collected from the cage site. The mature females and sex reversed males from the cages were successfully induced to spawn. However, fertilized eggs were obtained only in the trial conducted in cage using hapa whereas only unfertilized eggs were obtained from the tank trial. The results of the present experiment showed that open sea floating cages are more ideal for domestication and broodstock development of the greasy grouper.

Keywords: Broodstock, Cage, Grouper, *Epinephelus coioides,* Sex reversal, Spawning

Introduction

Groupers of the genus *Epinephelus* are major candidate species for commercial finfish farming. About 49 species of this genus have been reported from the seas around India. Most of them inhabit coral reefs and rocky habitats, while others prefer sea grass beds, muddy and sandy bottoms. Juveniles of some species occur in lower reaches of estuaries, occasionally ascending upper reaches also. Their robustness under heavy stocking conditions, good feed conversion rate, high adaptability to different culture systems, rapid growth at elevated temperatures and the excellent flesh quality make them potential species suitable for aquaculture. Above all, its market demand is the main factor, which motivates the expansion of grouper aquaculture (Pierre *et al*., 2008). These fishes are much sought after by local and international markets, particularly in South-east Asia (Hong Kong and Singapore) and Japan (Kuo, 1995) where they usually are the most expensive in the live fish market. Groupers are cultured in either ponds or cages and being euryhaline in nature, they can thrive well in brackishwater environments. However, they are mostly cultured in floating net cages either in the open sea or near the seaward end of estuaries (Sim *et al*., 2005).

Groupers are farmed in many South-east Asian countries, including Indonesia, Malaysia, Philippines, Taiwan, Thailand, Hong Kong, South-east China and Vietnam as well as south-eastern USA (Tucker, 1999). More recently, other countries like Sri Lanka, Saudi Arabia, South Korea and Australia have also started grouper farming (Ottolenghi *et al*., 2004). At present, growth and development of grouper farming industry in India has been constrained by inadequate supply of juveniles for stocking. The existing supply of wild-caught juveniles cannot meet the demand of the expanding grouper culture industry Groupers are

farmed in many South-east Asian countries, including Indonesia, Malaysia, Philippines, Taiwan, Thailand, Hong Kong, South-east China and Vietnam as well as south-eastern USA (Tucker, 1999). More recently, other countries like Sri Lanka, Saudi Arabia, South Korea and Australia have also started grouper farming (Ottolenghi et al., 2004). At present, growth and development of grouper farming industry in India has been constrained by inadequate supply of juveniles for stocking. The existing supply of wild-caught juveniles cannot meet the demand of the expanding grouper culture industry. Therefore the development of this industry is reliant upon the successful hatchery production of grouper juveniles. For artificial propagation of this species in hatchery, development of mature broodstock in captivity is the major bottleneck which is expensive and time consuming. Broodstock management generally comprises collection, selection and domestication of brooders; control of maturation as well as spawning, egg collection and incubation (Liao *et al.,* 2001).

In India, grouper culture practices are entirely supported by the supply of seeds collected from the wild (Nammalwar *et al.,* 1998). Even though techniques for seed production, larval rearing and culture of groupers have been developed internationally (Pierre *et al.,* 2008), research needs to be intensified in this direction in India, for developing viable technology for successful seed production and ensuring uninterrupted supply of grouper juveniles for farming. Shrimp farmers in the country are looking for alternate species for aquaculture, in view of the decrease in shrimp production mainly because of diseases and environmental deterioration. The orange spotted grouper (*Epinephelus coioides*) is one of the promising grouper species for farming in India, owing to its faster growth rate, local availability and market demand. Greasy groupers can reach 1.78 kg within 11 months of culture period (Somasekharan Nair *et al.*, 2005). Keeping these facts in view, broodstock development programmes were initiated aimed at captive seed production of *E. coioides*, at the Visakhapatnam Regional Centre of the Central Marine Fisheries Research Institute. The present paper discusses the successful development of captive broodstock of *E. coioides* in open sea cage at Visakhapatnam

Materials and methods

Fabrication and installation of sea cages

The site for installation of sea cages was selected at a distance of about 500 m from the shore in the Bay of Bengal off Visakhapatnam (between 17°42’43.62" N; 83°19’43.96" E and 17°42’38.92" N; 83°19’37.90" E). The site was selected after conducting a detailed assessment of environmental parameters such as water current, wave pattern, water quality and physical profile of sea bed. A minimum of 8 m water depth during low tide was ensured at the site to facilitate efficient water exchange. Two circular HDPE cages of 6 m inner ring and 8 m outer ring dia was fabricated and launched for stocking adult groupers. Each cage was moored with 32 m length of 16 mm alloy steel chain attached to a 4 t capacity gabion box. The collar of the cage was made up of HDPE pipe of 140 mm dia. The outer and inner rings were interconnected with horizontal base pipes. The outer ring of the frame was fitted with HDPE net bag of 50 mm stretched mesh size and the inner ring was fitted with HDPE net bag of 20 mm stretched mesh size (which was elevated up to the rim of the hand rail to prevent fishes jumping out from cages), with 5 and 4 mm twine thickness respectively (Fig. 1). The depth of the inner net cage was 4.5 m. A bird net was tied on the top of rim to avoid bird menace. A circular ballast pipe of 30 kg weight was fixed on the bottom rim of inner net to keep the net bag stretched in cylindrical shape.

Fig. 1. Broodstock cage for greasy grouper, installed in Bay of Bengal off Visakhapatnam

Broodstock development

Fishes (*E. coioides)* obtained from the commercial hook and line operations were transported live in 300 l tanks to the mariculture hatchery of the Regional Centre of Central Marine Fisheries Research Institute (CMFRI) at Visakhapatnam, Andhra Pradesh. After arrival at the hatchery, the fishes were recompressed by inserting a needle through their anal openings to relieve barotrauma stress and then treated with 200 ppm formalin for 30 min, followed by a freshwater dip for 5 min. After prophylactic treatment, fishes were stocked in FRP/cement tanks filled with filtered seawater. The sexes were identified by live ovarian biopsy using flexible catheter of 1 mm inner and 2 mm outer diameter and found that all collected fishes were females. The fishes were subsequently transferred and stocked in sea cages installed off Visakhapatnam. A total 63 nos. of *E. coioides* (4.06 ± 0.81kg) were collected and out of these 54 nos. were stocked in two 6 m dia HDPE floating cages. Nine fishes were stocked in 5 t FRP tanks @ 1 kg per ton of water. Following acclimatisation for a period of four months, 20 fishes from the cages and 4 fishes from tanks were implanted with 17 α-methyl testosterone aimed at developing male brooders. The fishes were fed twice a day @ 5% body weight with *Decapterus russelli,* sardine and squids. Vitamins, cod liver oil and mineral supplements were also given twice in a week along with feed, in order to complement any possible nutritional deficiencies in their diet. All fishes were tagged (PIT TAG FS 2001) for identification and to maintain the history of individual fish.

Slow sand filtered seawater was used in the brood stock development tanks throughout the experimental period and round the clock aeration was provided in all the tanks. The fishes were fed at 08.00 and 15.00 hrs in tanks and at 0700 and 1600 hrs in cages. Unconsumed feed were removed from the tanks was daily, after 2 h of feeding. Manual cleaning of tanks and siphoning off faecal matter were done every day in the morning. Daily about 50% water was exchanged in the experimental tanks and replaced with filtered fresh seawater. This procedure

was followed throughout the experimental period of one year Water quality parameters such as salinity, temperature, dissolved oxygen, ammonia and nitrite in grouper rearing tanks as well as sea cage site were analysed weekly. The salinity and temperature were measured using a refractometer and thermometer respectively. Dissolved oxygen, ammonia and nitrite were estimated following the standard methods of APHA (1998).

Growth of the fishes was monitored regularly by taking body weight. The fishes were cannulated every month on new moon days for checking gonadal development. The fishes were anaesthetised using 200 ppm phenoxy ethanol before cannulation. Gonad tissue samples were preserved in Bouin's fixative for further studies as per (Yamamoto, 1956). Ova diameter measurements were taken using ocular micrometer in a calibrated microscope.

Induced spawning

For induced spawning experiment, female fish with intra-ovarian eggs of around 450 µ dia were selected. Trials were conducted using mature females and sex reversed males in the ratio 1:1, which were initially injected with human chorionic gonadotropin (HCG) at the rates of 1600 and 1200 IU per kg body weight (BW) respectively in four split doses at intervals of 24 h each, followed by luteinising hormone releasing hormone analogue (LHRHa) @ 40 µg kg BW-1 after 24 h of the final HCG injection in the first two induction trials. In the subsequent trials, HCG alone was used to induce male as well as female. All trials were undertaken in FRP tanks except the last trial of induced spawning where the brooders were kept in the cage itself.

Results and discussion

The survival, growth and maturity status of fishes in the experimental cages and tanks were recorded for a period of one year. The survival rate was higher in tanks (100%) compared to cages (94.11%) The salinity varied from 20 - 36 ppt during the experimental period. At the end of the study period, the final body weight range of fishes were 5.6-8.9 kg with average weight increase at the rate of 243.33 and 206.02 g per month in cages and tanks respectively. Nammalwar *et al*., (1998) recorded fast growth (2-5 kg in 3 months) for *E. coioides* stocked in cement tanks. The present study indicated that the growth of the greasy grouper is better in cages than in tanks. Stocking the wild collected fishes in sea cages would have helped to stimulate their natural environment leading to faster growth of fishes stocked in cages as compared to the fishes stocked in tanks. During the present study, development of female broodstock was successful in cages, whereas in tanks the females did not mature within the study period. Gonadal development of female brood fishes started after 4 months of stocking in cage (Fig. 2; Table 1) and by 9 months mature ova of size 400-600 µ were observed during cannulation (Fig. 3, Table 1). Within one year in several females, 86% of the cannulated ova were above 400 µ size and were found to be in ideal condition for spawning induction. The cage reared and hormone (17 α-methyl testosterone) administered fishes became ripe males and were found oozing milt whereas none of the tank reared fish were found to ooze milt even after slight pressure was applied on abdomen. Reports are available

Table 1. Progression of gonadal development and percentage (mean±SD) of cannulated ova from female greasy grouper (4.84±0.23 kg) stocked in sea cage in different months (n = 10)

Ova dia (µ)	Months (post-stocking in cages)								
	1	2	3	4	5	6	7	8	9
0-100	HC	HC	HC	HC	97.38±0.42	79.11±1.20	-	-	-
100-200	-	-	-	-	2.63±0.42	13.49±0.82	25.6±1.95	-	-
200-300	-	-	-	-	-	7.4±0.82	52.2±1.83	11.6±1.02	-
300-400	-	-	-	-	-	-	22.2±1.69	51.9±3.61	13.5±1.21
400-500	-	-	-	-	-	-	-	36.5±3.67	71.8±1.82
500-600	-	-	-	-	-	-	-	-	14.7±1.57

HC – Honey comb stage

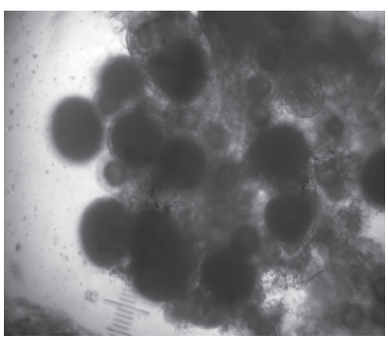

Fig. 2. Vitellogenic oocytes (stage 3 and 4) of varying size (100 – 300 µ) from cage stocked fish

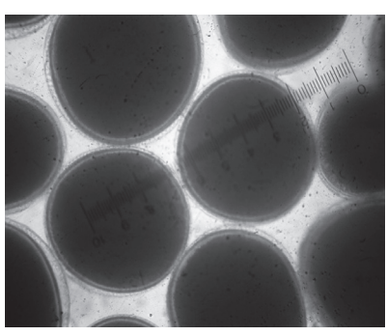

Fig. 3. Oocytes in the late stage 4 (400 – 600 µ) from brood fish stocked in cage, ready for hormonal induction for spawning

Table 2. Physicochemical parameters of the rearing water in cage and FRP tank during the experimental period

Parameters	Cage	FRP tank
Temperature (°C)	28.42± 0.31	28.67 ± 0.72
Salinity (ppt)	32.00 ± 0.71	29.42 ± 1.40
pH	7.89± 0.04	7.86 ± 0.04
Dissolved oxygen (mg l^{-1})	4.16 ± 0.08	4.56 ± 0.06
Total ammonia (mg l^{-1})	0.029*± 0.005	0.217 ± 0.034
Nitrite (mg l^{-1})	0.005*± 0.001	0.024 ± 0.004

**Indicates significant (p<0.05) difference between treatment means (independent sample t-test)*

on successful maturation of marine fin fishes such as cobia, grouper, pompano, red seabream, Japanese flounder and yellow croaker in cages. (Hong and Zhang, 2003, Gopakumar *et al*., 2011). Although the water quality parameters such as salinity, temperature, pH, dissolved oxygen and feeding pattern in terms of quality and quantity of tanks were same as cage (Table 2), the adult fish stocked in tanks did not show much progress in gonadal development. Higher levels of total ammonia ($p< 0.05$) and nitrite in tank water might have added to the overall stress for the tank reared fishes as compared to cage reared fishes. However , the total ammonia and nitrite levels in the tanks were observed to be with in safe limits as reported by Vatanakul *et al.* (2001) for the giant grouper, *Epinephelus lanceolatus*, which matured in water with ammonia–N and nitrite–N levels of 0 - 0.237 and 0 - 0.277 ppm respectively. The permissible limits of total ammonia–N and nitrite–N levels reported for Asian sea bass are 2.0 and 1.0 ppm respectively (FAO, 1989). Marino *et al.* (2000) reported that majority of females of *Epinephelus marginatus* in captivity were unable to reach vitellogenesis and oocyte maturation. Many reports are there in several other species of fishes where females failed to undergo final oocyte maturation in captive conditions (Zohar, 1988; 1989a, b; Peter *et al.,* 1993). The observation from the present study that the same group of fish when stocked in cages matured within one year, indicated that open sea floating cages are better for domestication, conditioning and broodstock development of greasy groupers than indoor tanks. The broodstock rearing in cages with adequate feeding seems to provide suitable conditions for maturation and a large number of brooders can be stocked in a limited water volume (Cacot *et al.*, 2002). In addition, broodstock development in sea cage is advantageous as it helps to save the additional costs involved in maintaining broodstock in land-based tanks, primarily the energy costs towards seawater supply and aeration.

Several factors, including hormone dose, administration method and degree of ovarian development, have been reported to influence the efficacy of gonadotropic agents in stimulating ovulation (Mylonas and Zohar, 2001). Human chorionic gonadotropin (HCG) was used for induction as it is reported more appropriate because it acts much faster, via direct stimulation of the gonad, in inducing final oocyte maturation (FOM), spermiation and spawning (Hodson and Sullivan, 1993). The dosage of HCG in the breeding experiments was determined after conducting trials with different dosages. HCG is often given in a single dose, which ranges between 100 and 4000 IU per kg BW-1 (Zohar and Mylonas, 2001). A single and relatively low dose of HCG (275 IU BW-1) was found to be enough to induce ovulation in fish with post-vitellogenic oocytes (Caylor *et al.*, 1994). In the present study, HCG and LHRHa were tried for spawning induction in *E. coioides* . In the first two trials, fishes were induced by four split doses of HCG and last dose with LHRHa. Later fishes were induced to spawn employing 2 split doses of HCG hormone alone. In all the induced spawning trials, fishes were successfully spawned but the eggs were unfertilized (except in the last trial). This could be due to failure of males to respond synchronously with the females or sex reversed males might not have converted completely into productive males. In the last trial, fertilized eggs were obtained when a female and sex reversed male were hormone induced and maintained in cage inside a hapa (Fig. 4). These results indicate that sea cage is a better option for broodstock development, induced spawning and domestication, of the greasy grouper. Husain *et al.* (1975) reported natural and unstimulated spawning of *E. coioides* in tank using spawners caught from wild whereas Chen *et al.* (1977) succeeded in induced spawning of cage reared broodstock of the species in Singapore.

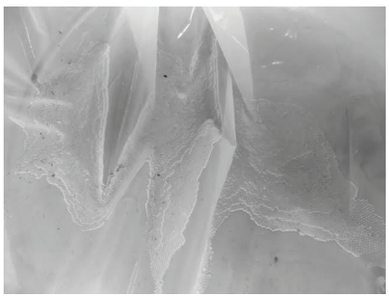

Fig. 4. Fertilized eggs collected after induced spawning

The global aquaculture production of groupers has been increasing from the year 1990 onwards in which the major contributors were Taiwan and Thailand (Pierre *et al.,* 2008). The current status of grouper culture in Asia (15000 t) clearly indicates that farming of this species has a bright future (Kongkeo and Phillips, 2002). It is well understood that only continuous supply of grouper seeds can lead to the development of grouper aquaculture in India. In this context, the present success in the broodstock development of the greasy grouper in sea cage is a major step towards development of successful seed production technology for groupers in captivity and grouper farming in India.

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References

APHA 1998. *Standard methods for the examination of water and wastewater*, 20th edn. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D. C., p. 45.

Cacot, P., Legendre, M., Dan, T. Q., Tung, L. T., Liem, P. T., Mariojouls, C. and Lazard, J. 2002. Induced ovulation of Pangasius bocourti (Sauvage, 1880) with a progressive HCG treatment. *Aquaculture*, 213: 199-206.

Caylor, R. E., Biesiot, P. M. and Franks, J. S. 1994. Culture of cobia (*Rachycentron canadum*): cryopreservation of sperm and induced spawning. *Aquaculture*, 125: 81–92.

Chen, F. Y., Chow, M., Chao, T. M. and Lim, R. 1977. Artificial spawning and larval rearing of the grouper, *Epinephelus tauvina* (Forsskal) in Singapore. *Singapore J. Pri. Ind.,* 5:1-21.

FAO 1989. P*ropagation of seabass, Lates calcarifer in captivity.* Seafarming Development Project (Jakarta, Indonesia).

Banchong Tiensongrusmee, UNDP/FAO Seafarming Development Projec, t. 110 pp.

Gopakumar, G., Abdul Nazar, A. K., Tamilmani, G., Sakthivel, M., Kalidas, C., Ramamoorthy, N., Palanichamy, S., Ashok Maharshi, K., Rao, S. and Rao, G. S. 2011. Broodstock development and controlled breeding of cobia *Rachycentron canadum* (Linnaeus 1766) from Indian seas. *Indian J. Fish.,* 58(4): 27-32.

Hodson, R. and Sullivan, C. V. 1993. Induced maturation and spawning of domestic and wild striped bass, *Morone zaxatilis* (Walbaum), broodstock with implanted GnRH analogue and injected HCG. *Aquacult. Fish. Manage.,* 24: 389-398.

Hong, W. and Zhang, Q. 2003. Review of captive bred species and fry production of marine fish in China. *Aquaculture*, 227: 305-318.

Husain, N., Saif, M. and Ukawa, M. 1975. *On the culture of Epinephelus tauvina (Forsskal).* Kuwait Institute for Scientific Research, Kuwait, 12 pp.

Kongkeo, H. and Phillips, M. 2002. Regional overview of marine seafarming with an emphasis on groupers and current/planned NACA activities. In: *Report of the regional workshop on sustainable seafarming and grouper aquaculture*, Medan, 17–20 April 2000, APEC/BOBP/ NACA , NACA, Bangkok, p. 35–42.

Kuo Ching-Ming 1995. The groupers in world animal sciences. In: Nash, C. E. and Novotny, A. J. (Eds.), *Production of aquatic animals: fishes*, Elsevier, Amsterdam, p. 305–317.

Liao, I. C., Su, H. M. and Chang, E. Y. 2001.Techniques in finfish larviculture in Taiwan. *Aquaculture*, 200: 1-31.

Marino, G., Azzurro, E., Finoia, M. G., Messina, M. C., Massari, A. and Mandich, A. 2000. Recent advances in induced breeding of the dusky grouper *Epinephelus marginatus* (Lowe, 1834). In: Chioccioli, E. (Ed.), *Proceedings of the Seminar of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM)*, CIHEAM and FAO, 24–27 May 1999, Zaragoza, Spain,. Cahiers Options Mediterrane´ennes vol. 47, CIHEAM/FAO, Rome, p. 215–225.

Mylonas, C. C. and Zohar, Y. 2001. Endocrine regulation and artificial induction of oocyte maturation and spermiation in basses of the genus Morone. *Aquaculture*, 202: 205-220.

Nammalwar, P., Marichamy, R., Regunathan, A. and Kandasamy, K. 1998. Prospects of grouper culture in India. In: Sakthivel, M., Vivekanandan, E., Rajagopalan, M., Meiyappan, M. M., Paulraj, R., Ramamurthy, S. and Alagaraja, K. (Eds.), *Proceedings of the workshop on national aquaculture week.* The Aquaculture Foundation of India, Chennai, p. 144-148.

Ottolenghi, F., Silvestri, C., Giordano, P., Lovatelli, A. and New, M. B. 2004. *Capture-based aquaculture: the fattening of eels, groupers, tunas and yellowtails,* Rome. FAO, 308 pp.

Peter, R. E., Lin, H. R., Van der Kraak, G. and Little, M. 1993. Releasing hormones, dopamine antagonists and induced spawning. In: Muir J. F. and Roberts R. J. (Eds.), *Recent advances in aquaculture.* Blackwell Scientific, Oxford, p. 25–30.

Pierre, S., Gaillard, S., Prevot-Dalvise, N., Aubert, J., Rostaing-Capaillon, O., Leung-Tack, G. and Grillasca, J. 2008. Grouper aquaculture: Asian success and Mediterranean trials. *Aquatic Conserv. Mar. Freshw. Ecosyst.,* 18: 297-308.

Sim, S. Y., Rimmer, A., Williams, K., Toledo, J. D., Sugama, K., Rumengan, I. and Phillips, M. J. 2005. *A practical guide to feeds and feed management for cultured groupers.* NACA, ACIAR, Publication No. 2005-02 of the Asia-Pacific Marine Finfish Aquaculture Network, 18 pp.

Somasekharan Nair, K. V., Manoj Kumar, P. P., Said Koya, K. P. and Suresh, V. K. 2005. Experiments on grow-out culture of groupers, *Epinephelus malabaricus* (Schneider) and *Epinephelus tauvina* (Forsskal). *Indian J. Fish*., 52(4): 469-475.

Tucker, J. W. 1999. *Species profile grouper aquaculture.* Southern Regional Aquaculture Center; SRAC Publication no. 721, 11 pp.

Vatanakul, V., Kongkumnerd, J., Rojanapitayakul, S., Yashiro, R. and Panichasuke, P. 2001. Broodstock development of giant grouper, *Epinephelus lanceolatus*. *Grouper News,* 11: 1-6.

Yamamoto, K. 1956. Studies on the formation of fish eggs: 1. Animal cycle in the development of ovarian eggs in the flounder, *Liopsetta obscura*, J. *Fac. Sci., Zool., Hokkaido Univ.,* 12: 362-373.

Zohar, Y. 1988. Gonadotropin releasing hormone in spawning induction in teleosts: basic and applied considerations. In: Zohar, Y. and Breton, B. (Eds.), *Reproduction in fish: basic and applied aspects in endocrinology and genetics.* INRA Press, Paris, p. 47–62.

Zohar, Y. 1989a. Fish reproduction: its physiology and artificial manipulation. In: Shilo, M. and Sarig, S. (Eds.), *Fish culture in warm water systems: problems and trends.* CRC Press, Boca Raton, p. 65–119.

Zohar, Y. 1989b. Endocrinology and fish farming: aspects in reproduction growth, and smoltification. *Fish Physiol. Biochem.,* 7: 395–405.

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

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