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Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 16

1995
pages 131-148

Article available on line / Article disponible en ligne à l'adresse :

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To cite this article / Pour citer cet article

Spedicato M.T., Lembo G., Di Marco P., Marino G. **Preliminary results in the breeding of dusky grouper Epinephelus marginatus (Lowe, 1834).** *Marine aquaculture finfish species diversification .* Zaragoza : CIHEAM, 1995. p. 131-148 (Cahiers Options Méditerranéennes; n. 16)

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Preliminary results in the breeding of dusky grouper *Epinephelus marginatus* (Lowe, 1834)

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SUMMARY - Two research programs on the dusky grouper started in 1992 supported by Italian Ministry of Agriculture-General Direction of Fisheries and Aquaculture. The programs aimed to improve the knowledge on the biology of *Epinephelus marginatus* in order to contribute to the diversification of marine fish culture and to start active restocking programs in protected coastal areas.
This paper presents the problems and the progress in the maintenance of dusky grouper broodstock and the preliminary results on the first trials of induced spawning in females.
E. marginatus is a protogynous species i.e. it matures as females and sex revers into male when it is bigger and older. Difficulties in the formation of a good broodstock are mainly related to the reproductive pattern and thus to the males availability.
Individuals of *E. marginatus* were caught by long-line in two different coastal areas (Ionian Sea and Sicily Channel). Two different broodstocks were formed and reared in two different plants. The difficulty in keeping large groupers alive just after capture required the set up of a technique (hyperbaric treatments) to avoid mortality for gas supersaturation.
After collection the fish were reared in 10 mc tanks supplied with seawater (5-6 volumes/day) and maintained under natural daylength and temperature (stocking density: 4-5 Kg/mc). Groupers were daily fed squid and occasionally trash fish ad libitum.
In 1992-93 natural spawning did not occur either reproductive behaviour or morphological signs appeared. Only following hormone administration females showed characteristic swollen belly and hypertrophic genital papilla. In August 1994 female spawners were selected and treated by hormones on the basis of oocytes size and yolk deposition.
Three injections 24 hours apart at the specific dose of 1000-2000 I.U./Kg of HCG alone or combined with 15-16 μg/Kg of a-LHRH were able to induce maturation of vitellogenic oocytes and partial or full ovulation.

Key words: *Epinephelus marginatus,* broodstock, breeding, hormone induction.

RESUME **-** En 1992 deux programmes de recherche sur le mérou noir, financés par le Ministère de l'Agriculture, Direction Générale de la Pêche et de l'Aquaculture, sont commencés.
Le but de ces programmes était celui d'étendre les connaissances sur la biologie d'*E. marginatus* pour contribuer au processus de diversification productive de l'aquaculture marine et à la réalisation de programmes de repeuplement actif dans les aires côtières protégées.
Cette étude présente les problèmes et les progrès obtenus concernants la maintenance des reproducteurs du mérou noir et les résultats préliminaires des premières expériences d'induction hormonale sur les femelles.
E. marginatus présente un hermaphrodisme protogyne caractérisé par un développment sexuel en 2 étapes: femelle d'abord, mâle ensuite, c'est-à-dire lorsque les animaux deviennent plus grands et plus âgés.
Les difficultés pour la formation d'un parc à géniteurs sont principalement liées au modèle reproductif et, par conséquent, à la disponibilité des mâles.
Les spécimens d'*E. marginatus* ont été capturés, par des palangres de fond, dans deux aires côtières différentes (la Mer Ionienne et le Canal de Sicile).
Ensuite, deux différents parc à géniteurs ont été formés dans deux établissements.
Les difficultés pour maintenir en vie, après la capture, les mérous noirs plus grands ont requis la mise au point d'une technique (traitment hyperbarique) pour éviter la mortalité due à la sursaturation gazeuse.
Après la capture, les reproducteurs ont été élevés dans les bacs de 10mc, approvisionnés d'eau de mer (5-6 volumes/jour), et maintenus (charges: 4-5Kg/mc) à régime de photopériode et de température naturelle. Les spécimens ont été nourris, journellement et ad libitum, de calmars et quelquefois de déchets de pêche.
Pendant la saison reproductive 1992-93 on n'a remarqué ni déposition naturelle ni comportements ou changements morphologiques reproductifs.
Seulement après les traitments hormonales, les femelles ont montré l'abdomen enflé typique et l'hypertrophie de la papille génitale.
En août 1994 les reproducteurs ont été sélectionés et traités avec des hormones selon la taille et l'état de maturation des ovocytes.
Trois injections, à distance de 24 heures, avec la dose spécifique de 1000-2000 UI/Kg d'HCG seule ou combinée avec 15-16 µg/Kg d'a-LHRH, ont induit à la maturation des ovocytes vitellogénétiques et à l'ovulation partielle ou totale.

Mots-clés: *Epinephelus marginatus*, parc à géniteurs, élevage, induction hormonale.

INTRODUCTION

This paper reports the preliminary data on the formation of dusky grouper broodstocks and on the first attempt of induced spawning by hormone administration in *Epinephelus marginatus* females, in preparation for the time when males will be available for fertilization trials. The data here reported were collected during the carrying out of two research programs on the dusky grouper started in 1992 and supported by Italian Ministry of Agriculture-General Direction of Fisheries and Aquaculture. The programs aimed to improve the knowledge on the reproductive biology and rearing of *Epinephelus marginatus* in order to contribute to the

diversification of marine fish culture and to start active restocking programs in protected coastal areas.

The rearing of groupers is developed in the world, mainly in Southeast Asia and in the Middle East, where different methods and techniques have been applied for breeding, broodstocks formation and reproduction.

In many cultured species induced spawning has been attempted with HCG alone and in combination with salmon pituitary extract or pituitary gland from the same species (Marte, 1989). A-LHRH was also effective alone or combined with carp pituitary extract (Kungvankij *et al.*, 1986).

Although various combination and doses of hormone have been used, methods for treating most of grouper species have not been standardized, mainly because of the limited number of broodstocks.

The status of the art concerning the groupers culture in the world is hereinafter summarized.

The culture of the groupers in the world

Groupers have been cultured in Southeast Asia for more than 15 years (Tookwinas, 1989). *Epinephelus tauvina* was the first recorded species for farming in Kuwait, Singapore and Thailand, while *E. salmoides* have been reared in Malaysia (Chua, 1978) and Philippines. At present, many grouper species have been cultured in some Asian countries (Table 1). However only *E. tauvina, E. salmoides* and *E. malabaricus* are farmed at commercial scale in Southeast Asia and Middle East. *E. akaara* is reared in Japan and China (Tseng and Poon, 1983).

The major obstacle remaining for all cultured groupers is their larval stage and much of the problems are related to size of the larvae. Encouraging results however have been reported by employing the S-type rotifer strain, enriching the rotifers with *Nannochloropsis oculata* and Artemia with commercial preparations (Tamaru *et al.*, 1993).

Formation and maintenance of the Epinephelinae broodstocks

The success of fish spawning depends greatly on the availability of good condition mature brooders. Therefore efforts to obtain suitable spawners and to improve their quality is of primary concern in broodstock maintenance and development work (Chao and Lim, 1991).

Broodstocks of *Epinephelus* species were usually formed both growing wild juveniles up to sexual maturity (Doi *et al.*, 1991) and collecting adult fish (Kungvankij *et al.* 1986).

Data on broodstock formation and maintenance of the main *Epinephelus* species are summarized in Table 2.

Artificial reproduction techniques

Among grouper, natural spawning was observed in tank or in floating cage for *E. akaara* in Japan (Ukawa *et al.* 1966), *E. tauvina* in Kuwait (Hussain and Higuchi, 1980), *E. malabaricus* in the Philippines and Thailand (Ruangpanit *et al*, 1986), *E. fuscoguttatus* in Indonesia and Singapore (Lim *et al.*1990) and for *E. suillus* in Malaysia (Toledo *et al.*1993). Single or multiple injections of HCG, used alone or in

combination with other hormones (Table 3), have been widely used to induce spawning in most of reared groupers (Chao and Lim, 1991).

The species *Epinephelus marginatus*

Epinephelus marginatus is considered an Atlanto-Mediterranean and amphiatlantic species. Taxonomically it is a Serranid, member of the Epinephelinae sub-family (Tortonese, 1954).

The *E. marginatus* adults preferentially inhabit the rocky bottoms of the shallow water and, around the Italian coasts, their presence was recorded at depth of 9-12 m (Livorno: Bacci, 1969), 10-30 m (Apulia Region: Sarà, 1969), but also at 250 m (Sicily: Bolognari *et al.*, 1971). The juveniles (total length=3-10 cm) are preferentially distributed on the Posidonia or Cymodocea prairies.

According to some authors reproductive migration toward deeper water may occurr when temperature decreases or during spawning season (Issel, 1928).

Following literature dusky grouper is known as solitary and sedentary species with a certain territorial behaviour (Neill, 1967). It is a very voracious fish with diurnal predatory activity which prefers cephalopods and fish.

As many grouper species, *E. marginatus* is a protogynous hermaphrodite, i.e. it matures as female but transforms into male after a sex reversal (Bruslè and Bruslè, 1975; Bouian and Siau, 1983). The gonads are ovotestis in which the sexual cells are not localized (Tortonese, 1975; Bruslè, 1985). According to Bruslè determination (1985) the fish smaller than 3 Kg are still juveniles and the functional females are more frequent in the size range of 3-9 Kg. Over 9 Kg the number of females is reduced, whereas the males occurrence progressively increases. This process would take place between two reproductive seasons with an ovaric intersexual stage followed by a testicular intersexual phase.

Age and growth data for transitional stages suggest that sex reversal occurs around 9-10 years or 90 cm TL (Bruslè, 1985), although various social and enviromental factors may shift this moment or affect sex inversion process (Shapiro, 1987).

The females reach the sexual maturity mainly at the size of 5 Kg (Bruslè and Bruslè, 1976), though the presence of smaller mature females, which size was 2 or 1.62 Kg, was respectively pointed out by Spartà (1935) and Barnabè (1974). Bruslè and Bruslè (1976) report that the males mature over 9 Kg, but primary males or precocious sex reversal may occurr at smaller size (3-5 Kg).

Data on the seasonal variation of the gonado-somatic index (Bruslè and Buslè, 1976) would indicate the presence of a single spawning period occurring in summer with a peak in July. On *E. marginatus* population of the Tunisian coasts the onset of vitellogenesis was observed in April in oocytes larger than 260 μ (Bouain and Siau, 1983).

Barnabè (1974) reports that mature individuals were caught in July-August when sea-water temperature ranged between 18-23° C.

The eggs of *E. marginatus* were described by Spartà (1935) and Lo Bianco (1969) that respectively indicated a diameter of 810 μ (single oil globule of 200 μ) and of 750 μ (single oil globule of 175 μ).

Despite of the potential of *E. marginatus* for mediterranean aquaculture diversification, no information are available in literature on development and maintenance of *E. marginatus* broodstock, as well as on induced sexual maturity

and spawning in captivity. Ongrowing trials of wild juveniles have been carried out in Spain (Fernandez Vilar *et al.*, 1993)

E. marginatus broodstock development by recruitment and rearing of juveniles is constrained by the growth rate and the age at first sexual maturity of the species. Therefore the capture of adult fish seems to be the more feasible and quick solution for broodstock formation, although the caught in deep waters not always ensures the availabilty of healthy and functional females (Kungvankij, *et al.* 1986).

Moreover the reproductive pattern of the species raises several problems for broodstock management and for artificial breeding, both for the difficulty of prediction of natural sex inversion and for the less frequent availability of male fish.

MATERIALS AND METHODS

Techniques of broodstock development and maintenance

The information reported in this paper are referred to two *E. marginatus* broodstocks (A and B), separately developed and maintained. Broodstock A (n=30, body weight: 2.5-13.5 Kg; total length: 55-88 cm) were caught by long-line at 20-60 m depth in the Ionian Sea from April 1991 to September 1992. To vent gas in eccess, immediately after capture, a 22 hypodermic needle was inserted into the abdominal cavity of each fish (Tucker *et al.* 1991).

Transportation to laboratory, taking 3h at most, was accomplished succesfully using oxigenated 1 mc tanks.

At the arrival to the laboratory each fish was underwent to a sanitary prophylaxis consisted of: the disinfection of the external wounds by mercurochrome for 2-3 days, an antibiotic treatments with furaltadone at the concentration of 30 ppm for the first day and of 20 ppm in the following 5 days, a treatment against the ectoparasites (mainly Caligidae) carried out with formalin (40%) at the concentration of 300 ppm for half an hour.

Fish were than transferred in 10 mc circular tanks supplied with seawater (5-6 volumes/day) and maintained under natural daylenght and temperature at stocking density of 4-5 Kg/mc. Groupers were daily fed squid and occasionally trash fish (i.e. sardina, anchovy) ad libitum.

Fish belong to Broodstock B (n=17, body weight: 3-18 Kg; total length: 59-98 cm) were caught by long-line in Pelagie Island (Sicily Channel) from April to August 1994 at 50-70 m depth. To avoid serious diseases due to diffuse gas sovrassaturation, fish immediately after collection underwent to a hyperbaric recompression at sea in a 500 l container for six hours. Fish were then temporarily stocked in a submergible cage at depth of 8 m and then successfully transferred in a commercial plant. Fish were stocked in 9 mc square tanks with running seawater supply (2-3 volumes/day). Stocking density, feeding and prophylaxis were as described for group A.

Sampling at sea of wild *E. marginatus*

In order to carry out studies on reproductive biology of *E. marginatus,* monthly samples were collected from April to October 1994 by fishing survey in Pelagie Island. A total of 138 fish were sampled. The total and standard length (in cm), and body weight (in Kg) were measured. The sex of *E. marginatus* is not detectable by

visual examination. Therefore gonads were sampled and examined for sex and maturity stage determination according to De Moussac data (1986) partially modified for stage II. Gonad weight was measured at the nearest 0.1 g.

Protocol of the first trial on hormone induced spawning

Breeders of Group A were conditioned for 4 months under an experimental regime consisted of natural photoperiod and increased temperature from 16 to 23°C.

In August 1994, spawners from group A and B were selected for hormonal treatments on the basis of oocytes size and yolk deposition. Oocytes were extracted from the ovaries of anesthetized fish (MS 222 Sandoz, 100 ppm) by cannulation with polyethylene tubing (diameter: 2 mm) and examined under microscope for identification of vitellogenic stages (150-620 μ).

Two hormone treatment protocols were tested on 7 females from group A and on 4 females from group B, using single or multiple intramuscolary injections of Human Chorionic Gonadotropin (HCG- Profasi, Serono) alone or in combination with a-LHRH (buserelina-Suprefact, Hoechst) at interval of 24 hours.

Among various hormone preparations, HCG alone was used according to the successfull results achieved in *E. akaara* (Tseng and Ho, 1979), in *E. fario* (Kuo *et al.*, 1988) in *E. salmoides* (Kungvankij *et al.*, 1986), in *E. tauvina* (Chen *et. al.*, 1977) and in *E. striatus* (Tucker *et al.*, 1991). Also a-LHRH was effective on *E. salmoides* according to Kungvankij *et al.* (1986). On the basis of the traditional methods of induced spawning of cultured freshwater fish in China (Peter et. al, 1988), and positive results got in coho salmon with gonadotropin plus a-LHRH (Van Der Kraak *et al.* 1982) also the combination of HCG and a-LHRH was tested.

Oocytes were extracted and measured at 24, 48 and 72 hours from the first injection and 72 and 120 hours after spawning. 50 oocytes were measured in each sample at the above intervals.

RESULTS

The dusky grouper seems to bear rather well the influence of captivity, mainly as concerns the feeding behaviour and the survival rate in rearing conditions. Indeed, mortality occurred only just after capture.

Preliminary data on the sex-ratio of the wild *E. marginatus* sampled at sea (Pelagie Island) are summarized in Table 4. By these samples the occurrence of males seems more frequent in the classes with individuals larger than 12.8 Kg. First results on the seasonal variation of gonado-somatic index (50 samples collected in the Ionian Sea), showed in Fig. 1, would indicate the presence of a single spawning time in summer season and mainly in late July. Nevertheless further investigations are necessary to deeeper clarify the reproductive biology of the wild populations.

As concerns the reproductive activity in reared brooders, during the summer 1992 and 1993, spontaneous spawning did not occurr in the broodstock A, either reproductive behaviour or morphological signs appeared. Only following hormones administration, females showed characteristic swollen abdomen and hypertrophic genital papilla.

The trials on the effects of HCG, a-LHRH on induced ovulation achieved some degree of success, mainly conditioned to initial oocytes stage. The details on

hormone treatments, initial and final oocytes diameter and particular remarks are given in Tables 5 and 6.

Among the 11 treated females, four subjects presented most of oocytes at 150-300 μ corresponding to lipid vesicles and yolk protein I and II stage (unpub.data), five subjects presented oocytes ranging from 420 to 650 μ (yolk protein III stage and mature oocytes respectively) and two fish showed hydrated oocytes of 800 μ.

Before hormone treatment most of fish showed oocytes both in healthy (20-40%) and degenerated (80-60%) condition. Only in A6 fish, 100 % of healthy oocytes were observed.

Independently from doses and number of injections, HCG alone or combined with a-LHRH, were ineffective to induce oocytes maturation when oocytes of recipient fish did not reach the end of esogenous vitellogenesis (A4, A5, B1 and B3). In these females, treatments were interrupted after 1-2 injections, as no oocytes maturation was observed .

In fish with oocytes ranging from 420 to 650 μ, HCG alone or with a-LHRH were able to induce final maturation and hydratation (820-850 μ) in all treated fish (A1, A2, A3, A6, A7).

Natural releasing of healthy eggs was only observed after a dose of 2000 IU/Kg HCG + 16 μg/Kg a-LHRH in triple injections (A6). Ovulation occurred 12-15 hours after final treatment. Released healthy eggs, with a mean diameter of 888μ, (C.V. 3.9) were spherical, transparent, buoyant with one oil globule (166μ). Hormone treatments (independently from type and dose) induced partial ovulation in females and ovarian catheterization at 72 and 120 hours showed the presence of 8-20cc serous matrix containing both healthy and opaque eggs irregular in shape (850-960μ).

Females with hydrated oocytes of 800μ (B2 and B4) after administration of HCG alone or combined with a-LHRH showed overripening condition.

From the above described results three injections 24 hours apart at the specific dose of 1000-2000 I.U/Kg of HCG alone or combined with 15-16μg/Kg of a-LHRH are able to induce maturation of vitellogenic oocytes and partial or full ovulation in *E. marginatus.*

DISCUSSION

This is the first study aimed to test the effectiveness of HCG and of a-LHRH on inducing oocytes final maturation and ovulation in *E. marginatus* in captivity.

The treatments were generally performed administrating a lower priming dose followed by a higher resolving dose of hormone (Marte, 1989). The timing injections, 24 h apart, was applied according to most of the trials performed on groupers. HCG dosages were administrated according to Kuo *et al.* (1988).

As observed in *E. striatus* (Tucker *et al.* 1991), ovarian catheterization in *E. marginatus* should be carried out with care. Injury and infection may occur as the genital papilla is not yet hypertrophic and oviduct is not completely pervious before hormone treatments.

All females in which partial or full ovulation occurred, swollen abdomen and irrorated hypertrophic papilla was observed 24-48 hours after the first injection.

According to Bruslè (1985) mature females occurred at 3 Kg of total weight and probably intersexual status might occurr in fish weighting 9 and 11.1 Kg, in which negative response to hormone injection was recorded.

In this study successfull spawning was induced by administration of both HCG alone or combined with a-LHRH, with the response of mature females depending more upon stage of sexual maturity than upon hormone and dose treatment. Hormone effectiveness seem to be related to oocytes size as only fish with vitellogenic oocytes at the tertiary yolk globules stage (>420μ) or mature oocytes (>500μ) positive responded to treatments. Conversely, no significantly difference in size were recorded in oocytes at lipid vesicle stage and at yolk protein stage I and II (<420μ) after injections. This data are in accordance with the outcomes of Kuo *et al.* (1988) and Huang *et al.* (1986), which stressed in *E. fario* and in *E. salmoides* different response to HCG treatment of yolk protein stage III oocytes with respect to lipid and other yolk stages. In such species different dose and number of injections were required depending on oocytes maturation stages.

The results of induced spawning attempts in dusky grouper indicate that ovulation could de induced with multiple injection of 1000-2000 I.U./Kg of HCG alone or combined with a-LHRH (15-16 μg/Kg) when oocytes diameter of recipient fish is larger than 420μ. Time of ovulation in *E. marginatus* seems to be quite similar to that reported for most of grouper species, being 12-15 hours after final injection (Chen *et al.* 1977; Kungvankij *et al.* 1986).

Hormone treatments on oocytes with an initial mean diameter of 800μ might induce an overripening condition. This response could be likely due to the inhibition of egg releasing related to stress condition of brooders, captured and transported few days before treatments. A negative response to HCG, resolving in overmature eggs, is also described in wild *E. striatus* brooders injected 3-7 hours after capture (Tucker *et al.* 1991). More generally a lower or negative response to gonadotropin administration is reported in many fish in stress condition for handling, confinement and transportation (De Montalembert *et al.* 1978; Pickering, 1981).

HCG and a-LHRH were effective also in the releasing of degenerated eggs, which presence seems not dependent upon treatments, as occurrence of degenerated eggs did not differ significantly before and after injections.

Since no data are available in the literature on the reproductive status of reared *E. marginatus,* is difficult to couple the presence of degenerated oocytes to captive conditions or to the unsuitable time of hormone injections. Further investigations are needed to better clarify the reproductive cycle in this species, in order to set up more specific protocol of induced spawning and proper time of operation.

ACKNOWLEDGEMENTS

This study is supported by a grant of Ministry of Agriculture - General Direction of Fisheries and Aquaculture. Special thanks are due to Prof. Stefano Cataudella of II University in Rome, who promoted the research project idea and contributes to the study development with precious suggestions.

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Tab. 1 - Listing of major groupers cultured in the world (from Tookwinas, 1989 revised)

SCIENTIFIC NAME	COMMON NAME	PRINCIPAL CULTURE LOCATION	COMMERCIAL OR EXPERIMENTAL
E. akaara	red-spotted grouper	Japan, China, Hong kong	Commercial
E. fasciatus		Japan	Experimental
E. microdon	polynesian grouper	Japan, Polynesia, Indonesia, Taiwan	Commercial (*)
E. salmoides	brown spotted grouper	Japan, Taiwan, Philippines, Thailand, Malaysia	Commercial
E. fario	blue-spotted grouper	Taiwan	Experimental
E. tauvina	greasy grouper	Kuwait, Malaysia, Indonesia, Taiwan	Commercial
E. striatus	nassau grouper	Caribees	Experimental
E. fuscoguttatus	brown-marbled grouper	Malaysia, Indonesia, Taiwan	Commercial (*)
E. malabaricus	black spotted grouper	Thailand, Philippines, Japan	Commercial
E. suillus		Malaysia	Experimental

(*) wild seed

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Tab. 2 - Broodstock formation and mainteinance of major Epinephelus sp.

Species	Broodstock origin (wild/reared)	Size (Kg)	Stock density	Sex-ratio	feeding feeding ratio	Rearing techniques	References
E. fuscoguttatus			0.8 ind/mq	1:1	trash fish enriched (2,5% body weight)	cage	Chao and Lim, 1991
E. salmoides	wild	3-15	1-2 ind/mc		trash fish (5% body)	concrete tanks	Kungvankij *et al* ., 1986
E. malabaricus	reared	6-12			trash fish enriched/ ad libitum	cages/pouds/ outdoor tanks	Doi *et al* ., 1991
E. suillus	reared	6-12			trash fish enriched/ ad libitum	cages/pouds/ outdoor tanks	Doi *et al* ., 1991
E. tauvina	reared		10 Kg/mc		trash fish (5%)	cage	Chen *et al* ., 1977
E. akaara	wild/reared	0.3-0.5	2 ind/mc		trash fish, squid, pellets	cage/tanks	Fukuhara, 1989

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Tab. 3 - Summary of hormone protocols for induced spawning in the major grouper species

Species	Hormone	First dose	Second dose	Third dose	Interval (h)	Time of ovulation/ spawning (h)	oocyte diameter/stage	References
E. akaara	HCG	1000 IU/fish	500 IU/fish		24	14-23		Tseng and Ho, 1979
E. fario	HCG	1000 IU/Kg	1000 IU/Kg	1000 IU/Kg	24	24-48	<0.5 mm	Kuo *et al.*, 1988
	HCG	1000 IU/Kg	2000 IU/Kg		24	24 (+)	0.6 mm/tertiary (*)	
	HCG	1000 IU/Kg	1000 IU/Kg		24	24 (+)	0.5 mm/tertiary (*)	
E. salmoides	HCG+PG	500 IU/Kg +3 mg	1000 IU/Kg +3 mg		24	12 (+)		Kungvankij *et al* ., 1986
	HCG+PG	500 IU/Kg +3 mg	500 IU/Kg +3 mg	500 IU/Kg +3 mg	12	12-15		
	HCG+PG	500 IU/Kg +3 mg	500 IU/Kg +3 mg		24	12-15		
	LHRHa	10 µg/Kg	10 µg/Kg	10 µg/Kg	12	12-15		
E. tauvina	HCG+SPE	250 IU/Kg+ 10 mg/Kg	250 IU/Kg +10 mg/Kg	250 IU/Kg +10 mg/Kg	18-24	8-10	0.4 mm/tertiary (*)	Chao and Lim, 1991
	HCG+SPE	500 IU/Kg	500 IU/Kg	500 IU/Kg +9 mg/Kg	24	13	>0.380 mm/ tertiary (*) and mature	Chen *et al* ., 1977
	HCG+SPE	500 IU/Kg	700 IU/Kg +10 mg/Kg		24	12		
E. striatus	HCG	1000 IU/Kg	1000 IU/Kg		24	24-27	mature	Tucker *et al* ., 1991
	HCG	0.7-0.8 IU/Kg	0.7-0.8 IU/Kg					

(+) natural spawning
(*) tertiary yolk globule stage

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Tab. 4 - Sex-ratio of wild *E. marginatus* sampled at sea (Pelagie Island)

	Classes Weight Total length	Total fish	Juveniles N.	%	Females N.	%	Males N.	%	Intersex N.	%
I	0.46-1.0 kg 31.5-44.0 cm	15	15	100	0	-	0	-	0	-
II	1.1-3.0 kg 42.5-62.0 cm	24	14	58.3	10	41.7	0	-	0	-
III	3.2-6.0 kg 59.0-76.0 cm	22	0	-	22	100	0	-	0	-
IV	6.4-8.5 kg 73.0-87.0 cm	13	0	-	13	100	0	-	0	-
V	9.1-12.0 kg 83.0-95.0 cm	22	0	-	22	100	0	-	0	-
VI	12.8-15.0 kg 84.0-99.0 cm	7	0	-	6	85.7	1	14.3	0	-
VII	15.4-17.8 kg 90.0-99.0 cm	16	0	-	7	43.75	7	43.75	2	12.5
VIII	18.5-21.0 kg 98.0-109.0 cm	12	0	-	4	33.3	8	66.7	0	-
IX	21.1-23.0 kg 104.0-120.0 cm	7	0	-	2	28.6	5	71.4	0	-
		138	29		86		21		2	
			Females: 115 = 83.3%				Males: 23 = 16.7%			

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Tab.5 - Summary of hormonal treatments for induced spawning trials of broodstock A females

Fish	Hormone	TL (cm)	BW (Kg)	IOD* (µm)	Injection dose	Specific dose	Total dose	FOD** (µm)	Results and Remarks
A1	HCG	76.0	8.6	500	1st 4300 I.U. 2nd 4300 I.U.	1000 I.U./kg	8600 I.U.	820	**Partial ovulation**
A2	HCG	79.0	7.7	440-480	1st 2600 I.U. 2nd 2600 I.U. 3rd 5200 I.U.	1340 I.U./kg	10400 I.U.	850	**Partial ovulation**
A3	HCG	59.0	4.0	500-650	1st 2000 I.U. 2nd 2000 I.U. 3rd 4000 I.U.	2000 I.U./kg	8000 I.U.	850	**Partial ovulation**
A4	HCG+LHRH	81.0	9.0	150	1st 4500 I.U. + 30µm	500 I.U./kg+3.3 µg /kg	4500 I.U. + 30µg	150	No response
A5	HCG+LHRH	83.0	11.1	150	1st 5500 I.U. + 37µg 2nd 5500 I.U. + 75µg	991 I.U./kg+10 µg/kg	11000 I.U.+112µg	150	No response
A6	HCG+LHRH	75.5	7.5	420-590	1st 3800 I.U. + 25µg 2nd 3800 I.U. + 50µg 3rd 7600 I.U. + 50µg	2000 I.U./kg+16µg/kg	15200 I.U.+125µg	820	**Full ovulation**
A7	HCG+LHRH	62.0	4.5	500-520	1st 1500 I.U. 2nd 1500 I.U. 3rd 1500 I.U. + 67 µg	1000 I.U./kg+15µg/kg	4500 I.U. + 67µg	820	**Partial ovulation**

***IOD: Initial oocyte diameter; **FOD: final oocyte diameter**

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Tab. 6 - Summary of hormonal treatments for induced spawning trials of broodstock B females

Fish	Hormone	TL (cm)	BW (Kg)	IOD* (μm)	Injection dose	Total dose	Specific dose	FOD** (μm)	Results and Remarks
B1	HCG	74.0	5.0	300	1st 2500 I.U. 2nd 5000 I.U.	7500 I.U.	1500 I.U./kg	300	No response
B2	HCG	62.0	3.0	800	1st 3000 I.U. 2nd 3000 I.U.	6000 I.U.	2000 I.U./kg	≈ 800	Overmature condition
B3	HCG+LHRH	71.0	4.8	300	1st 2400 I.U. + 48μg 2nd 4800 I.U. + 48μg	7200 I.U. +96μg	1500 I.U./kg+20μg/kg	300	No response
B4	HCG+LHRH	65.0	4.0	800	1st 2000 I.U. + 40μg 2nd 4000 I.U.+ 40μg 3rd 4000 I.U. + 40μg	10000 I.U. +120μg	2500 I.U./kg + 30μg	≈ 800	Overmature condition

***IOD: Initial oocyte diameter; **FOD: final oocyte diameter**

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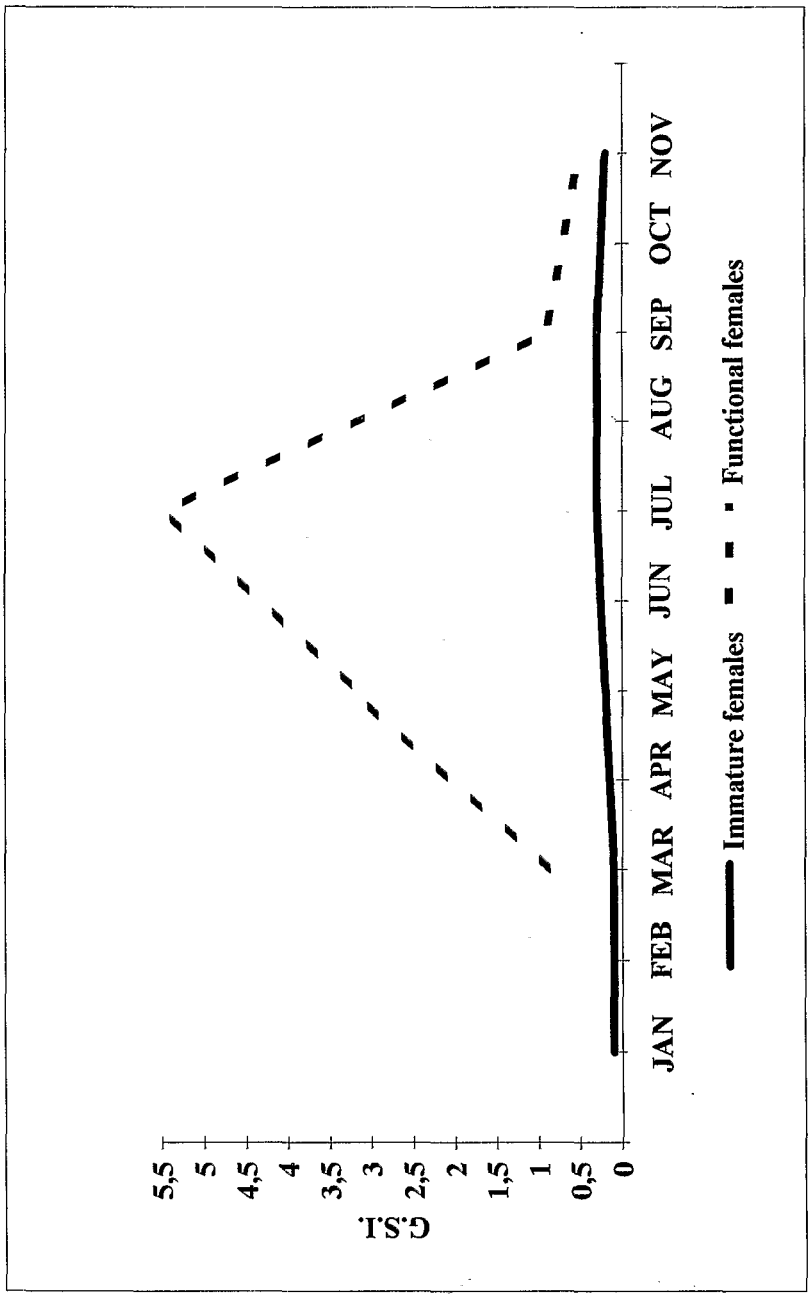

Fig. 1 Seasonal variation of gonado-somatic index.