

# **PERSPECTIVES IN MARICULTURE**

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**Observations on  
fattening, growth and  
sex reversal of the  
greasy grouper  
*Epinephelus tauvina*  
(Forsk.)**

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**ABSTRACT**

*This paper deals with the experiments conducted on the development of broodstock, rearing of fingerlings and sex conversion of the greasy grouper, *Epinephelus tauvina* under controlled conditions. Groupers collected from the wild and harvested from the culture ponds were raised to broodstock in 10 x 5 x 2.3 m (net water capacity of 100 tons) R.C.C. tanks by feeding with freshly killed sardines, cod liver oil and vitamin tablets. Experimental*



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rearing of fingerlings was carried out in 30 ton tank. In broodstock rearing, the growth rate varied from 10.1mm/106.8g to 13.1mm/178.3g for the wild and 16.6mm/116.8g to 21.1mm/271.5g for the pond harvested stock with a feed conversion ratio of 4.6-7.7 and 3.2-3.5 respectively. Maximum growth increments of 27.2mm/404g and 25.5mm/305g have been realised for the wild and pond harvested stock

respectively. In general, pond harvested groupers showed higher growth with less conversion ratio. The fingerlings registered a growth rate of 24mm/42g with a conversion ratio of 10. Preliminary experiments on sex reversal using androgen hormone (Testoviron Depot containing Testosterone Enanthate) revealed the possibility of converting female to male by the indication of regression in ovary size and disappearance of oocytes.

### **Introduction**

Although there are several reports on the breeding and larviculture of groupers, controlled breeding and hatchery production of fingerlings for large scale commercial farming is scarce due to several technical and hatchery constraints and poor larval survival (Leis, 1987; Kohno *et al.*, 1988; Tookwinas, 1990; Al-Thobaiti and James, 1996). Among several species of groupers, *Epinephelus coioides* (previously reported as *E. tawina*) was the first species to be cultured in the Middle East and the natural spawning of this species in captivity was reported by Hussain and Higuchi (1980) in Kuwait. The artificial spawning and larval rearing of *E. tawina* in Singapore and *E. salmonoides* in Philippines have been reported by Chen *et al.*, (1977) and Kunvankji *et al.*, (1986) respectively. In Japan, Tawada (1989) described on the breeding and larval rearing of the camouflage groupers, *E. polyphekadion* as *E. microdon* with poor larval survival. Debas *et al.*, (1989) reported the fully mature *E. polyphekadion* caught all year round in polynesian waters and focused in the identification of the life cycle of reproduction and sex reversal phenomena of this hermaphrodite species. Successful year round natural spawning of *E. polyphekadion* under captive conditions and hatchery rearing of larvae has been reported in the hypersaline waters along the Red Sea coast of Saudi Arabia by James *et al.*, (1997).

Most of the grow out culture systems for groupers depend on the seeds collected from the wild (Quinitio and Toledo, 1991). The availability of grouper fingerlings from the wild for farming are very erratic and inconsistent due to seasonal and environmental conditions. A more reliable source is to produce the seed in hatchery under controlled conditions. An important aspect in controlled breeding is broodstock raising and fattening which enters on promoting sexual maturation and enhancing broodstock quality to ensure better quality of eggs and sperm. This paper reports the results of experimental fattening and growth of the grouper *Epinephelus tauvina* conducted from June to December 1998 in captive conditions and sex reversal using androgen hormone.

### **Material and methods**

Grouper, *E. tauvina* used for this study were collected from the wild by traps, gillnets and stake net ('patti') and were transported to the experimental site by jeep. They were segregated into three size groups such as below 1 kg, 1-2 kg and above 2kg and stocked separately in 10 x 5 x 2.3m R.C.C. tanks F, H and G respectively (net water capacity 100 tons) at the rate of 1 kg biomass/cubic metre. Besides, cultured groupers, harvested from the ponds were transported from Tuticorin and used for the investigation. These stocks were screened out as below 0.5 kg, 0.5-1 kg and above 1 kg and stocked separately in another R.C.C. tanks (L, K and J) of same size and at the same density. Fingerlings of *E. tauvina*, collected from the wild along Tuticorin coasts were transported and stocked in 30 ton capacity R.C.C. tank. The stocks in 100 ton tanks were fed with freshly killed sardines (*Sardinella* spp) at the rate of 2-6% of the body weight of the biomass. Apart from this, sardines injected with Seven Seas cod liver oil and orally implanted vitamin E tablets were provided for the stock weighing above 2 kg. The fingerlings were fed with chopad sardines at the rate of 5-10% of the body weight.

Tanks were cleaned twice in a week and 100% water exchange was done with water drawn directly from the sea through pumping in

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addition to 50% daily change. One third portion of tank on the top was covered by mats made out of coconut leaves to provide shade for the fishes. Considering the behaviour of the fish, hide outs were provided with asbestos sheets and fibre glass tanks at the bottom. The injured/diseased fish were given antibiotic dip treatments using malachite green, methylene blue, chloramphenicol and oxytetracycline. Hydrological parameters such as water temperature, salinity, dissolved oxygen and pH were monitored regularly. Stocks were sampled at an interval of thirty days and the measurements of length and body weight of broodstock and fingerlings were done to ascertain the growth rate. For the experiments on sex reversal, the androgen hormone, a derivative of testosterone was used as inducing agent. Fish are regularly monitored for gonadal condition by ovarian biopsy.

### **Results**

**Growth:** The growth progress and increments in length and weight for the broodstock of each size group and fingerlings reared in R.C.C. tanks are presented in Tables. 1-4

Table 1. Rearing of wild grouper, *Epinephelus tauvina* during June - December 1998 in R.C.C. tanks

Days	Tank F (Below 1.0 kg)		Tank H (1.0-2.0 kg)		Tank G (above 2.0 kg)	
	Average length (mm)	Average weight (gm)	Average length (mm)	Average weight (gm)	Average length (mm)	Average weight (gm)
Initial	378.2	742	472.3	1515	560.0	2600
30	382.0	763	480.0	1681	562.1	2765
60	400.4	948	495.4	1785	568.8	2817
90	427.1	1100	496.3	1833	586.0	2942
120	429.3	1180	522.6	2237	588.6	3200
150	438.3	1341	535.0	2400	600.4	3495
180	442.6	1383	550.9	2480	620.4	3670

Observations on fattening, growth

Table 2. Rearing of pond harvested stock of grouper, *Epinephelus tauvina* during August-December 1998 in R.C.C. tanks

Days	Tank L (Below 0.5 kg)		Tank K (0.5 -1.0 kg)		Tank J (Above 1.0 kg)	
	Average length (mm)	Average weight (gm)	Average length (mm)	Average weight (gm)	Average length (mm)	Average weight (gm)
Initial	298.8	324	389.7	755	481.8	1534
30	306.6	395	402.8	876	502.8	1800
60	338.2	584	422.8	1128	521.9	2105
90	363.7	726	442.4	1289	540.3	2394
120	383.2	791	456.1	1485	551.7	2622

Table 4. Rearing of fingerlings of grouper, *Epinephelus tauvina* during August 1997 - May 1998 in R.C.C. tanks

Days	Average length (mm)	Average weight (g)	Growth increments	
			Length (mm)	weight (g)
Initial	69.8	4.5	-	-
30	126.0	42.4	56.2	38.1
60	152.0	62.2	26.0	19.8
90	210.6	117.7	58.6	55.5
120	211.2	175.1	0.6	57.4
150	247.9	204.5	30.7	29.4
180	257.5	224.1	15.6	19.6
210	271.4	252.7	13.9	28.6
240	280.5	327.0	9.1	74.3
270	284.6	377.0	4.1	50.0

Table 3. Growth increments of broodstock of *Epinephelus tauvina* in R.C.C. tanks

Days	Wild stock						Pond reared stock					
	Below 1.0 kg		1.0 -2.0 kg		Above 2.0 kg		Below 0.5 kg		0.5-1.0 kg		Above 1.0 kg	
	Length (mm)	weight (g)	Length (mm)	weight (g)	Length (mm)	weight (g)	Length (mm)	weight (g)	Length (mm)	weight (g)	Length (mm)	weight (g)
30	3.8	21.8	7.7	166	2.1	165	7.8	71	13.1	121	21	266
60	18.4	184.8	15.4	104	6.7	52	31.6	189	20.0	252	19.1	305
90	26.7	161.9	0.9	48	17.2	125	25.5	142	19.6	161	18.4	289
120	2.2	70.0	26.3	404	2.8	258	19.5	65	13.7	197	11.4	228
150	9.0	161.0	12.4	163	11.6	295	-	-	-	-	-	-
180	4.3	42.0	15.9	80	20.0	175	-	-	-	-	-	-

### *Observations on fattening, growth*

**Wildstock:** The stocks weighing below 1 kg in Tank F have grown to an average size of 400.4 mm (948g) in 60 days, 429 mm (1180g) in 120 days and 442.6 mm (1383g) in 180 days. The growth was observed to be better at 60,90 and 150 days period. The growth increments varied from 2.2-26.7 mm in length and 21.8-184.8g in weight. The overall monthly average growth rate was 10.7 mm and 106.9g in length and body weight respectively. The feed conversion ratio was 7.7 : 1.

In Tank H (1-2 kg), the fishes grew to 495.4 mm (1785g), 522.6 mm (2237g) and 550.6 mm (2480g) in 60, 120 and 180 days respectively. Encouraging growth was observed at 30 and 120 days period. The growth increments ranged from 0.9 mm to 26.3 mm and 480-404g in length and weight respectively. The average growth increment per month was 13.1 mm in length and 160.8 g in weight. The feed conversion ratio was 4.8 : 1.

The fishes in Tank G weighing above 2 kg showed the growth progress of 568.8 mm (3200g) in 120 days and 620.4 mm (3670g) in 180 days. The growth was fast upto 150 days. The growth increments varied between 2.1 and 20 mm in length and 52 and 225g in weight with an average growth rate of 101 mm and 178.3g in length and body weight respectively. The feed conversion ratio was found to be 4.6 : 1.

**Pond harvested stock:** In Tank L (below 0.5 kg) the fish were found to grow to an average size of 338.5 mm (584g) in 60 days and 383.2 mm (791g) in 120 days. Better growth was observed upto 90 days. The growth increment ranged from 7.8-31.6 mm in length and 65-189g in body weight. The monthly average growth rate was 21.1 mm and 116.8g in length and body weight respectively. The feed conversion ratio was 3.2 : 1.

The stock weighing between 0.5 and 1 kg in Tank K progressed to 422.8mm (1128 g) and 456.1mm (1485g) in 60 and 120 days respectively. The growth of fish was encouraging throughout the rearing period. The growth increments ranged from 13.1-20.0 mm and 121-252g in length and weight respectively. The average growth increment was 16.6 mm and 182.5g. The feed conversion ratio was 3.3 : 1.



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In Tank J (above 1 kg), the fishes have attained an average size of 521.9 mm (2105g) in 60 days and 551.7 mm (2622g) in 120 days. The growth of fish was found to be encouraging throughout the rearing period. The monthly growth increments varied from 11.4-21 mm in length and 228-305g in weight. The average growth increment was 17.5mm and 271.5g in length and weight respectively. The feed conversion ratio was 3.5 : 1.

**Fingerlings:** The fingerlings progressed to 152.0 mm (62.2g) in 60 days, 211.2 mm (175.1g) in 120 days, 257.5 mm (224.1g) in 180 days, 280.5 mm (327g) in 240 days and 284.6 mm (377g) in 270 days. Encouraging growth was observed at 30, 90 and 150 days. In the rest of the period, increase in body weight was pronounced rather than length. The growth increments varied from 0.6-58.6 mm in length and 19.6-74.3g in weight. The average increment was found to be 24.0 mm and 42.0g. The feed conversion ratio was 10:1.

**Sex reversal :** Six female fishes measuring 60-68 cm in length and 3.4-5.4 kg in body weight were given intramuscular injection with methyl testosterone (Testoviron Depot containing Testosterone Enanthate) at the dose of 2 mg/kg body weight. The injection was given twice in a week. The fish were found healthy and surviving with the injections for 3 months. The intake of food was poor and as a result the body weight decreased. After receiving a total dose of 40-60 mg, 4 fishes were observed to be disease affected and found sluggish but did not survive. On examination of the body cavity of these fishes, it was observed that the ovary has reduced in size and oocytes disappeared.

### **Remarks**

In broodstock rearing, a maximum weight increment of 404g for the wild and 305 g for the pond harvested stock has been observed for the fishes weighing above 1.0 kg. The growth of fish weighing below 1.0 kg was found to be good in pond harvested stock. It has also been revealed from the study that pond harvested groupers raised in R.C.C. tanks showed

better growth with less conversion ratio when compared to wild groupers. The reason may be attributed due to their domestication in the pond environment from the fingerling stage itself.

Anon (1986) stated that grouper fingerlings of 75-100 mm and 120-150 mm in size are stocked at the rate of 100-150 and 44 per sq.m. in hapa and nursery nets respectively for culture. In the present study, 49-87 mm size fingerlings of *E. tauvina* were stocked in R.C.C. tank at the rate of 1 per sq.m. Panbanpaew and Sakara (1990) reported that fingerlings of *E. malabaricus* have grown to 90-100 mm after 60 days in concrete tanks from the initial size of 43 mm and 141 mm (49.9g) in net cages 75 days from the size of 57 mm (3.1g). In the present observation, fingerling of *E. tauvina* registered the monthly growth rate of 24.0 mm (42.0g) by growing to 284.6 mm (377g) in 270 days.

Most of the grouper species are protogynous hermaphrodite, meaning that they are females during early stage of their life cycle and become males during later phase (Shapiro, 1987). Abdullah *et al.* (1983) stated that sex transmission in *E. tauvina* occurs in fish measuring 55-75 cm in length under natural condition. Tan-Fermin *et al.* (1994) showed that 2 year old *E. coioides* weighing 1.2 kg can be sex reversed when 0.5-5.0 mg/kg body weight Methyl Testosterones (MT) injection at fortnight intervals for 5-6 months. Marte *et al.* (1995) reported that bi-monthly implantation of MT (4 mg/kg body weight) or MT (4 mg/kg body weight) + Luteinizing hormone-Releasing hormone (20 µg/kg body weight) were more effective than bi-weekly injection of MT (1 mg/kg body weight). In the present investigation, androgen hormone injection was given twice in a week at the dose of 2 mg/kg body weight. The androgen hormone used was a local derivative of testosterone (Testoviron Depot containing Testosterone Enanthate). The reason for slow development of sex reversion to male may be due to the quality of the product used. There is possibility of quick sex conversion of male from female with original MT. However, the reduction in ovary size and disappearance of oocytes confirm the possibility of sex reversion from female to male.

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### **References**

- Abdullah, M.A.S., S. Akaten, K.M. Al-Abdul-Elah, and B.K. Teng, 1983. *Annu. Res. Rep. KISR* : 55-57.
- Al-Thobaiti, S. and C.M. James, 1996. Developments in grouper culture in Saudi Arabia. *INFOFISH International* 1/96 : 22-29.
- Anon. 1986. Manual on floating netcage fish farming in Singapore. Fisheries Handbook No.1. *Prim. Prod. Dept. Republic of Singapore* : 17 pp.
- Chen, F.Y., M. Chow, T.M. Chao, and R. Lim, 1977. Artificial spawning and larval rearing the grouper, *Epinephelus tauwina* (Forsk.) in Singapore. *Singapore Journal of Primary Industry* 5 : 1-21.
- Debas, L., A. Fostier, J. Fuchs, M. Weppe G. Nedlec, A. Benett, C. Cauty, B. Jalabert, and Aquacop, 1989. The sexuality of cultured hermaphrodite fish species: analysis of morphological and endocrinological features in a protogynous hermaphrodite, *Epinephelus microdon*, as a basis for further research to control reproduction in the grouper. In : *Advances in Tropical aquaculture* (Ed. J. Barret). pp. 543-557. AQUACOP, IFREMER. Actes de Colloque 9. Tahiti, French Polynesia.
- Hussain, N. A. and M. Higuchi, 1930. Larval rearing and development of the brown-spotted grouper, *Epinephelus tauwina* (Forsk.), *Aquaculture*, 19 : 339-350.
- James, C.M., S.A. Al-Thobaiti, B.M. Rasem and M.H. Carlos, 1997. Breeding and larval rearing of the camouflage grouper *Epinephelus polyphekadion*

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(Bleeker) in the hypersaline waters of the Red Sea coast of Saudi Arabia. *Aquaculture Research*, **28** : 671-681.

Kohno, H., M. Duray, and J. Juarlo, 1988. State of grouper (lapu-lapu) culture in the Philippines. *SEAFDEC Asian Aquaculture*, **10** : 4-9.

Kungvankij, P., L.B. Tiro, B.P. Pudadera and I.O. Potestas, 1986. Induced spawning and larval rearing of grouper (*Epinephelus salmoides* Maxwell), p.663-666. In J.L. Maclean, L.B. Dizon and L.V. Hostillos (Eds). *The First Asian Fisheries Forum*. Asian Fisheries Society, Manila, Philippines.

Leis, J.M. 1987. Review of the early life history of tropical groupers (Serranidae) and snappers (Lutjanidae) In: *Tropical Snapper and Grouper: Biology and Fisheries Management* (Ed. J.J. Polovina & S. Ralston). pp. 189-237. Westview Press, Boulder, Colorado USA.

Marte, C.L., G. Qunitio, and N. Caberoy, 1995. Spontaneous spawning of sex inversed groupers *Epinephelus coioides* administered 17 -alpha methyl-testosterone implants. A paper presented at the *Fourth Asian Fisheries Forum*. 16-20 October 1995, Beijing, PROC.

Panbanpaew, S. and W. Sakaras, 1990. Experiment on sea grouper *Epinephelus malabaricus*. Technical paper No.38/1990. Rayong Brackishwater Fisheries Station, Dept. of Fisheries, 17 pp.

Qunitio, G.F. and J.D.Toledo, 1991. Mariculture techniques for *Epinephelus* spp. in the Philippines, p. 94-106. In : R.D. Guerrero III and M.P. Garcia, Jr. (Eds.) *Advances in Finfish and Shellfish Mariculture : Proceedings of the First Phillippine-French Technical Workshop on Advances in Finfish and Shellfish Mariculture*. 24-26 October 1990, Los Banos, Laguna, Philippines. Philippine Council for Aquatic and Marine Research Development; French Embassy in the Philippines.

Shapiro, D.Y., 1987. Reproduction in groupers, p.295-327. In : J.J. Polovina and S. Ralston (Eds). *Tropical Snappers and Groupers : Biology and Fisheries Management*, Westview Press, Inc., Boulder, Colorado.

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- Tan-Fermin, J.D., L.M.B. Garcia, and A.R. Jr. Castillo, 1994. Induction of sex inversion in juvenile grouper, *Epinephelus suillus* (Valenciennes) by injections of 17 $\alpha$ -methyl testosterone. *Japan J. Ichthyol.* **40** : 413-420.
- Tawada, S., 1989. Spawning of broodstock of the grouper *Epinephelus microdon* (Bleeker). *Suisanzoshoku* III. 105-108.
- Tookwinas, S. 1990. Review of knowledge on grouper aquaculture in Southeast Asia. In: *Advances in Tropical Aquaculture* (Ed. J. Barret). pp. 429-435. AQUACOP IFREMER, Tahit, French Polynesia.