

SPAWNING OF TIGER GROUPERS

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The Value of Groupers



Epinephelus fuscoguttatus
(Tiger Grouper)

Groupers are amongst the most highly priced in the live-reef fish trade in the last two decades especially in South-east Asia.

Most of this demand is met by captured fisheries

of market-size fish. The reliance on wild-caught seed has caused slow growing groupers to be vulnerable to over-fishing with indications that in many areas, overexploitation of groupers has indeed occurred (Cesar et al., 2000). Wild-capture will not only be unsustainable at current levels (Sadovy and Pet, 1998; Quinitio, 1999) but it could also compound the over-fishing of grouper adults by removing fish that might otherwise survive to reproduce and supplement adult stocks (Sadovy, 2000).

In Malaysia, it began with grow-out farms of wild-caught grouper seed and then went on to large hatcheries attempting to generate sufficient and reliable seed in quality and quantity to meet demands. A relatively recent trend is for failed shrimp farms attacked by WSSV disease to convert to marine finfish hatcheries run with foreign assistance mainly from Korea, China, Japan and Taiwan.

The Species *Epinephelus fuscoguttatus*

The literature pertaining to grouper aquaculture commonly misidentifies the species concerned. The common names of *Epinephelus fuscoguttatus* are Flowery Cod, Brown-marbled grouper and more popularly known as Tiger grouper in Asia, due to

the large irregular-shaped dark blotches on the head, back and sides. Its other recorded scientific names are *Serranus fuscoguttatus*, *Epinephelus summana*, *Perca summana fuscoguttata*, *Serranus horridus*, *Serranus taeniocheirus*, *Epinephelus lutra* and *Serranus lutra*. The Tiger grouper is often confused with the Small-toothed Cod *Epinephelus polyphkadion* or *Epinephelus microdon* from more recent authors. *Epinephelus fuscoguttatus* is found in coral and rocky reefs of the Indo-Pacific, the Red Sea, along the east coast of Africa to Mozambique, north to Japan and south to Australia.

Broodstock management

Broodstock should be maintained in good condition. They should be fed well with feed supplemented with Vitamin C, Vitamin E and Omega 3 oil plus minerals. Good quality trash fish feed mixed with supplements is given to broodstock once every two days. Unnecessary handling stress of broodstock is to be avoided. To reduce handling stress during the implantation procedures, anesthetic (clove oil) at concentrations of 20-40 ppm are used. Implantation was carried out with most of the broodstocks' body submerged in the water.

The vehicle- Ovaplant

Commercial use of sustained-release preparations of a potent salmon Gonadotrophin Releasing Hormone analogues (sGnRH_a) began in the mid 1990's and by 1998, Ovaplant, a sustained-release implant containing sGnRH_a was tested and approved for use in Chile (Powell, 2002). This peptide initiates gonad maturation in all species of fish through the fishes' own internal mechanisms. The Ovaplant pellet is implanted into the fish prior to spawning date. The controlled release of the peptide over time ensures the safe induction of spawning. Ovaplant was found to advance maturation dates by 4-6 weeks in populations with a uniform and short spawning period.



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When used in the normal spawning season, using Ovaplant will compress the spawning season to within 1-2 weeks post-implantation. Ovaplant's sustained release formulation is intended to advance maturation, compress spawning, re-start maturation and/or increased milt production in fish. Ovaplant has been successfully demonstrated to advance and synchronize spawning dates in either seawater or freshwater-held broodstock salmon (Powell, 2002); speed up the occurrence of spawning in Channel catfish (Silverstein et. al, 1999); advance and probably re-start maturation in the Mahseer fish (Sena et. al., 2004).

Induced breeding of tiger grouper

In 1989, the same grouper species was reported to produce as many as 403,000 fry (Murayama et al., 1993). In *E. fuscoguttatus* and other grouper 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, Chao and Lim, 1991). A-LHRH was also effective alone or combined with carp pituitary extract (Kungvankij et al., 1986). In this paper, the slow-release Ovaplant is used to treat the tiger grouper *E. fuscoguttatus* species. The work was conducted in The Pulau Sayak marine finfish breeding and research center in Kedah, Malaysia.

The groupers were induced to spawn in 14m³ indoor rectangular concrete tanks with 1.5m water depth. The tanks are flat-bottomed, and the interior coated with grey colour fibreglass. The tanks are provided with strong aeration and continuous supply of filtered seawater with water exchange being 400% per day.



Swollen genitalia

Twelve breeders weighing between 4 to 10 kg were reared in the tank. They are individually identified with electronic tags. Periodically, breeders are treated with formalin to remove parasites before being transferred to another tank. The breeders feeding behaviour, body colouration change and swelling of the abdomen were observed regularly especially during the new and full moon phases. Tagging is done to monitor the broodstock to avoid implanting the same fish twice in a row. With proper handling and minimal stress, the broodstock could be induced to spawn once every month.

Mature females are externally identified by their round, bulging and soft abdomen. Swollen genital papilla shows the female to be very ripe and in the state of readiness. Before induction of spawning, cannulation is done. Cannulation using a polyethylene catheter, 1mm inner diameter size, is done to confirm sexuality of fish and to observe egg size and condition. If the eggs are opaque, loose (does not clump together) and more than 400µm, it is considered a good indication that the females



Cannulation

are mature. Males with a soft belly that extrude sperm easily when the abdomen is pressed slightly are considered ready for implantation. Male tiger groupers, when mature, extrude milt readily.

Implantations were done on soft spots along the dorsal sinus, or on a soft area in front of dorsal spine. Two or three scales are removed to facilitate the implantation. The needle is inserted at an angle of about 30 degrees so that the implant pellet is imbedded well in the flesh almost under the skin.



Milt from a ready male

Ovaplant is implanted two to five days before the new moon and usually eggs are produced two days after implantation. Implanted breeders are returned into the spawning tank which is fitted with egg collecting hapa nets.

Tiger groupers commonly spawn over four to six consecutive nights on the new moon. Spawning occurs in the early morning, usually between 12 midnight to 2am. Eggs hatched between 5-6pm. The tiger groupers at the research station are implanted about 2-5 of days before new moon and they usually



Implanting in the dorsal sinus

lay eggs 2-9 days after implant.

Mating behaviour

After hormonal induction male tiger grouper shows a very territorial behaviour and swims close to its chosen mate.



Colour changes in induced fish

Tiger groupers tend to pair up, with males being more aggressive than the females. The female is sedentary and lies quietly at the bottom of the tank within the paired males' territory. The body color

becomes lighter and the blotches on its body are more clearly seen. The male instigates a courtship display by continuously nudges the female with its snout. At one point, the male will puts its head tightly against the female and the female, half pushed, and half swimming will swim up and rush out of water together with the male. As the groupers swim up the female lays eggs and the male releases a gush of milt to fertilize the eggs. The courtship behavior is attributed to scarcity of sperm. Water levels in the tank/cage must be more than 2m deep to warrant completion of this grasping and rushing movement.

Tiger grouper Eggs

Eggs of *E. fuscoguttatus* take 15–19 hours to hatch. Grouper eggs and newly hatched larvae are very sensitive to stress and handling (Predalumpaburt & Tanvilai, 1988; Caberoy & Quintio, 1998). Handling mortality is minimised by handling eggs only at neurula-stage (after the formation of the optic vesicles) and by stocking eggs into the culture tanks two hours before hatching so that the larvae need not be handled (Lim, 1993; Tamaru *et al.*, 1995; Caberoy & Quintio, 1998). Good fertilized eggs float near the water surface while the unfertilized or spoiled eggs sink to the bottom of the hapa net then discarded. The floating eggs are harvested, numbers estimated and distributed into hatching cum rearing tanks. The eggs were collected 7-8 hours after fertilization or after the eggs had passed the gastrula subdivision stage. Fecundity averages 12 million egg per spawning. Grouper larvae are very weak during first few days after hatching. They tend to be stationary with heads pointing towards the tank floor. They slowly sink and occasionally wriggle up towards water surface. Some larvae get caught on the water surface and die. Grouper larvae need to go to water surface to ingest air in order to inflate their air bladder. As a precaution,

drops of fish oils are applied to the larvae tank water to reduce the surface tension. At the research station, the larvae (mouth size 200µm) are initially fed on SS type rotifer at a density of 5 individuals/liter, 3-5 days after hatching. 6-10days old fry were then introduced to S type rotifer at the density of 10 individuals/liter. Rotifer density is monitored and remedied daily. The rotifers were supplemented with baker's yeast with sardine/cod oil which is high in unsaturated fatty acid (HUFA- an essential component in a fish larvae diet). Larvae supplemented with HUFA enriched feed grow faster. Larvae that were fed with rotifer supplemented with *Nannochloropsis* high in EPA/DHA allowed for a higher survival rate. 3-4 week larvae also had an increased survival rate with thyroid hormone treatment through immersion or feed with bioencapsulated rotifers. Major obstacles in culturing grouper larvae are related to the quality of hatchlings, suitable size and the nutritional quality of live feed.

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