

## Grouper culture in Brazil

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In Brazil, mariculture is a well developed activity, with farming focused largely on shrimp, clams, oysters and mussel culture. Brazilian shrimp culture is represented by *Penaeus vannamei* farming in more than 16,000 hectares and malacoculture by *Perna perna*, *Crassostrea gigas*, *Crassostrea rizophorae* and *Nodipecten nodosus* farming.



Dusky grouper (*Epinephelus marginatus*).

The first investigations on marine fishes in Brazil were conducted in the middle 1970s by researchers from Universidade Federal de Pernambuco studying mullets (*Mugil liza*)<sup>1</sup>. Investigations on the dusky grouper (*Epinephelus marginatus*) began in 1980, by Eduardo Fagundes Netto and Daniel Benetti, researchers from the Instituto de Pesquisas da Marinha, in Arraial do Cabo<sup>2</sup>. Nowadays, many institutions maintain research groups studying marine fish species suitable for farming, including the Instituto de Pesca, Universidade Federal de Santa Catarina, Fundação Universidade do Rio Grande and the Universidade Federal do Ceará.

The most studied species in Brazil are the snook (*Centropomus parallelus* and *C. undecimalis*), the flounder (*Paralichthys orbignyana*), the groupers (*Epinephelus marginatus* and *Mycteroperca microlepis*) and the snappers (*Lutjanus synagris* and *L. analis*). For many reasons, the activity had a low profile for many years, but at present marine fish farming is facing a big expansion, particularly with enterprises dedicated to cobia (*Rachycentron canadum*) farming.

Many grouper species are considered to have a high potential for farming and are threatened in the wild due to overfishing and destruction of coastal environments. The most commonly encountered species that occur in Brazil<sup>3</sup> are: *Epinephelus marginatus* (Lowe, 1834), *Epinephelus morio* (Valenciennes, 1828), *Epinephelus niveatus* (Valenciennes, 1828), *Epinephelus flavolimbatus* (Poey, 1865), *Epinephelus itajara* (Lichtenstein, 1822), *Epinephelus adscensionis* (Osbeck, 1765), *Epinephelus striatus* (Bloch, 1792), *Epinephelus nigrilus* (Holbrook, 1855), *Epinephelus mystacinus* (Poey, 1852), *Epinephelus guttatus* (Linnaeus, 1758), *Epinephelus drummondhayi* (Goode & Bean, 1879), *Mycteroperca acutirostris* (Valenciennes, 1828), *Mycteroperca microlepis* (Goode & Bean, 1880), *Mycteroperca interstitialis* (Poey, 1860), *Mycteroperca tigris* (Valenciennes, 1833), *Mycteroperca bonaci* (Poey, 1860) and *Mycteroperca venenosa* (Linnaeus, 1758).



Giant grouper (*Epinephelus itajara*).

Among these, outstanding species include the dusky grouper (*Epinephelus marginatus*), printed on the Brazilian one hundred reais bill, black grouper (*Mycteroperca bonaci*), gag grouper (*Mycteroperca microlepis*) and the giant grouper (*Epinephelus itajara*), the largest species in the genus, reaching more than three hundred kilograms.

### Conservation

The reduction of some of Brazilian grouper populations reinforces the concept of developing economically feasible, environmentally sustainable and socially responsible options to reduce the extractive pressure on the fishing stocks. In that sense, the development of marine fish culture

of these endangered species may represent a strategic implement for conservation, reducing wild capture as cultivated fish is offered, making restocking possible. One measure that may contribute to this is the implementation of a semen bank for such species.

Considering the occurrence of many species of serranids in Brazil and the situation of their stocks, their economic importance and the possibility of generating rearing technology for these species, the Instituto de Pesca has conducted studies upon the culture of these fishes since 2005. This work, known as Serranidae Project, is conducted in Ubatuba, Brazil, and has brought important advances in serranid culture. The employed system is "near shore" in 8 m<sup>3</sup> net cages and fishes are fed with trash fish. After twelve months rearing dusky grouper present a mean weight of 1.0 to 1.2 kg and giant grouper after only ninety days presented a mean weight of 1.3 kg.

The project has demonstrated that grouper farming in net cages has an internal rate of return of 15.05% to 36.74% for two selling prices (US\$ 7 and US\$ 9), is economically feasible and possible to be conducted by small scale undertakings and artisanal fishermen.



Reproduction of dusky grouper.

Although showing aptitude for farming, the reproduction of serranids has a great complexity due to a curious characteristic. The members of the sub-family Epinephelinae are protogynic hermaphrodites, what means that they initially mature as females and in a given moment of their growth have a definitive sexual reversion, becoming males. With that complex reproductive strategy, to obtain males for the reproduction in captivity is a problem. To



*Semen collection from dusky grouper.*

get males in the natural environment is very difficult for many reasons: they are rare (serranids form harems with few males and many females), they are big fishes (difficult to manage) and normally occur in depths of more than 30 meters, making capture difficult and with a low survival rate.

An alternative for this question, already shown by many authors, is the sex inversion in captivity, using masculine hormones. This treatment provokes the development of testes, but six months after interrupting the administration of androgens, the fishes return to their initial condition of females, making the process laborious and expensive. Even being an interesting strategy, few studies have focused on the importance of semen cryopreservation of these fishes. Cryopreservation and stockage of the semen of fishes submitted to sexual reversion may reduce the complexity of the process, lowering difficulties and costs to obtain males for reproduction of serranids.

Recently, the researchers of Serranidae Project succeeded, for the first time in Brazil, to obtain and cryopreserved semen of the dusky grouper, defining a protocol of cryopreservation and formation of a bank of semen, which resulted of the first fishes gotten from stocked semen. Cryopreservation also made unnecessary the constant processes of sexual reversion to obtain males. The creation of a bank of semen of many serranid species may also provide an important implement for conservation and commercial production of these species, contributing to conservation of these fishes for the future generations.

Considering the history of Brazilian marine fish culture, the bank of semen contributed decisively for the success of the reproduction of the dusky grouper in captivity, making possible the first commercial production of juveniles from these species in Brazil. Presently, the reproduction of other serranids such as gag grouper, black grouper and jewfish is already in course.



*Dusky grouper semen (400 X).*

### The process of semen cryopreservation

The process begins with the sex inversion of females. As soon as that is completed the collection of semen starts. It is extracted individually from each specimen, after a soft pressure on the abdomen, being collected with the help of a plastic syringe, which aids to avoid the accidental contamination with blood and urine. The semen is then analysed in its qualitative aspects, determining parameters of sperm mobility (percentage of cells with movement in the sample), time of sperm mobility (durability of cell movement, in seconds) and sperm concentration (number of cells/ml of semen).

Only semen with sperm mobility higher than 90% is utilised for freezing. The semen is mixed with an extender



*Dusky grouper fingerlings.*

and a cryoprotector and is frozen in nitrogen vapor using cryogenic slats, and subsequently transferred to liquid nitrogen for long term storage (-196°C).

When it is intended to use the semen in the reproductive process, the cryogenic slats are thawed in water (26°C) for 2-3 minutes, sperm mobility and time of mobility are checked and then the sperm are used utilised in the fertilisation of the oocytes.

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*Dusky grouper juvenile.*