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Grouper aquaculture: Asian success and Mediterranean trials

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ABSTRACT

1. Grouper are of considerable economic value in tropical and subtropical regions and most particularly in south-east Asia. Moreover, it is in Asia, where this fish is highly prized, that the grouper farming began in the 1980s.
2. The organoleptic qualities of the groupers are not the only characteristics to be appreciated; in culture, their robustness in heavily populated conditions, as well as their rapid growth at elevated temperatures makes them a good species for aquaculture. However, it is, above all, market demand that is outstripping the supply of fish, which is motivating the expansion of grouper aquaculture.
3. At the present time, aquaculture is unable to satisfy demand. The chronic shortage of broodstock means that the alevins, destined for aquaculture, are taken mainly from natural habitats with the aid of fish traps, nets and cyanide, thus contributing to the destruction of south-east Asian reefs. Nevertheless, research is progressing and the number of species whose reproduction is totally controlled is increasing. It is in Taiwan that grouper aquaculture is at its most advanced with a total of five species being raised.
4. Aquaculture is divided into four stages, carried out by different farmers, allowing a more rapid turnover for each farmer and therefore a better financial return. On the other hand, in the Mediterranean at present time, there are no completely controlled grouper captive-breeding programmes. The first attempts at controlled breeding have been carried out since 1995 in Italy, Croatia and Greece. However, farming groupers in captivity, and in particular the control of their reproduction, poses numerous problems.

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INTRODUCTION

Groupers are very widespread in warm and temperate waters of all the seas and oceans of the planet. They are of considerable economic value, especially in the coastal fisheries in subtropical and tropical areas.

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It has been estimated that 90% of the world's harvest of marine food is derived from artisanal fisheries, and groupers are a major component of the artisanal fisheries resource (Heemstra and Randall, 1993). This strong pressure from fisheries could be reduced with the development of large-scale, sustainable grouper aquaculture thus improving the conservation of these species. These fish are much sought after by local and international markets, particularly in south-east Asia (Hong Kong, Singapore) and Japan (Kuo, 1995) where they usually are the most expensive fish. They are highly prized for the quality of their flesh, and most species fetch high market prices (Ottolenghi *et al.*, 2004). Their prices range from 8–11$\$\,kg^{-1}$ for *Epinephelus coioides*, to 15–20$\$\,kg^{-1}$ for *E. fuscoguttatus*, to 30–40$\$\,kg^{-1}$ for *Plectropomus* spp. and *E. akaara*, to 80–95$\$\,kg^{-1}$ for *Cromileptes altivelis* (Williams *et al.*, 2005).

The groupers belong to the Serranid sub-family Epinephelinae which is composed of 15 genera and 159 species principally distributed among the Indo-Pacific region (110 species), the East Atlantic and Mediterranean regions (14 species) and the intertropical American zone (35 species). The most sought-after species belong to the *Epinephelus* and *Mycteropera* genera:

- The genus *Epinephelus* includes 98 species, of which 70 come from Indo-Pacific area, eight from East Pacific region, 11 from West Atlantic region and nine from East Atlantic and Mediterranean zones. In the Mediterranean area, the genus is represented by *E. aeneus*, *E. caninus*, *E. costae*, *E. haifensis* (confusion with *E. marginatus*), *E. marginatus*, and *E. coïoides* recently introduced via the Suez Canal (Parenti and Bressi, 2001).
- The genus *Mycteropera* is of great importance for the commercial and leisure fishing industries. There are 15 species, of which there is one in the East Atlantic area, one in the Mediterranean area, eight in the West Atlantic and five in the East Pacific zones. The Mediterranean species *M. rubra* is also commercially important, but it is less common than *E. marginatus* (Heemstra and Randall, 1993).

Groupers are protogynous hermaphrodites, judging from the few species that have been studied. The majority of these species live on coral reefs, but they are also found in estuaries, on rocky bottoms and less frequently in sandy or silty habitats. Some species occur in depths of 100 to 200 m; however, the majority inhabit depths less than 100 m, and juveniles are often found in tide-pools (Heemstra and Randall, 1993). They are largely piscivorous, but can also feed on crustaceans and cephalopods, hunting from a lair in the reefs.

Except for occasional spawning aggregations, most species are represented by solitary fishes, resident on a particular reef for long periods of time. This site specificity and relatively slow growth rate of groupers make them particularly vulnerable to overfishing (Heemstra and Randall, 1993).

The spawning aggregation sites are often heavily exploited during a limited period of several weeks in areas such as the Caribbean (Sadovy, 1999), often targeting single species, e.g. *E. striatus* in Little Cayman, Cayman Islands (Whaylen *et al.*, 2004) and *M. microlepis* in the Gulf of Mexico (Koenig *et al.*, 1996).

FISHERIES

The protogynous mode of reproduction in groupers presents considerable problems for fishery management. Male groupers are usually larger, older and less numerous than females and fisheries are often biased towards the capture of large adults. Hence, males comprise a greater proportion of the catch than they are in local populations (Heemstra and Randall, 1993).

In 1990, according to the FAO (Heemstra and Randall, 1993), a total of 97 kt (kilotonnes) of groupers (*Epinephelus* and *Mycteroperca*) were landed by fisheries in the various oceans of the world (Table 1). These figures are likely to be a significant underestimate owing to the poor reporting of artisanal catches in the statistics. This under-reporting of groupers catches is indicated by the fact that, despite the much greater

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Table 1. Grouper catches in tonnes by geographic area in 1990 (Heemstra and Randall, 1993)

Species	Pacific Ocean	Atlantic Ocean	Mediterranean Sea and Black Sea	Indian Ocean	Total
Epinephelus aeneus		2169	414		2583
Epinephelus akaara	166				166
Epinephelus analogus	18				18
Epinephelus marginatus		1261	3308		4569
Epinephelus morio		2964			2964
Epinephelus striatus		201			201
Epinephelus tauvina	199				199
Epinephelus spp.	21 786	43 241	3547	16 767	85 341
Mycteroperca spp.		1100			1100
Total	2 2169	50 936	7269	16 767	97 141

Table 2. Distribution of grouper catches in percentage by geographic zones (Ottolenghi *et al*., 2004)

Geographic zones	Catches (%)
Pacific	55
Indian Ocean	24
Atlantic	17
Mediterranean and Black seas	4

Table 3. Capture fisheries for groupers: top five country in 2000 (Ottolenghi *et al*., 2004)

Countries	Catches (tonnes)
Indonesia	46 281
China	41 513
Mexico	18 595
Pakistan	16 012
Malaysia	12 174

size and reef area of the Indo-Pacific region, there were more groupers reported in the commercial fisheries statistics from the Atlantic Ocean than from the Indian and Pacific Oceans combined (Heemstra and Randall, 1993).

In 2000, the global catch of grouper showed a 68% increase from 100 724 t in 1991 to 168 943 t and most of them came from the Pacific and Indian Oceans (Table 2). In the same year, Indonesia was the leading country, followed by China (Table 3; Ottolenghi *et al*., 2004).

With the rapidly developing economies of China and south-east Asia, the 'live fish trade' of the Indo-Pacific has expanded rapidly in recent years, and now targets many species. Groupers are the most intensively exploited group in the live fish trade, and the high prices paid by exporters to local fisherman mean that target species may be heavily over-fished. Then, fishermen will go to great lengths in order to catch every fish, and this has already contributed to regional crashes of species (Ottolenghi *et al*., 2004). Signs of over-exploitation have been reported in numerous regions with reductions in the numbers of fish landed (Sadovy, 1999).

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These fisheries are synonymous with destructive fishing practices that damage not only the marine environment but also the economies and fabric of society of coastal fishing communities dependent on coral reef resources (www.enaca.org).

There is a strong link between fishing activity and the capture-based seed used for farming, with declines in premium species from the overfishing of grouper adults. However, the reasons for this decline cannot be evaluated without carefully controlled studies, as falling catches may in fact be due to a combination of different causes, e.g. over-fishing of the adults which produce the juveniles, habitat degradation and pollution, destructive fishing techniques, high export demand (Ottolenghi *et al.*, 2004). Responsible management of these fisheries is needed to ensure sustainable use of marine resources and the conservation of coral reefs for the benefits of future generations; thus developing alternatives to the harvesting of wild, live, reef food fish such as hatchery-based aquaculture (for example) is very important (www.enaca.org).

AQUACULTURE

Grouper mariculture is most developed in Asia, principally because of the high commercial value of these fish in the markets of Hong Kong, Singapore and Taiwan in particular. Their robustness in overcrowded conditions as well as their rapid growth at high temperatures, making them good species for aquaculture, and the insufficient supply of wild caught fish needed to satisfy the strong demands of the market, is motivating the expansion of aquaculture. Most grouper are cultured in floating net cages either in the open sea or at the seaward end of estuaries (Sim *et al.*, 2005b).

Sixteen grouper species are raised in south-east Asian fish farms, the dominant species varying depending upon the country of origin (Sadovy, 2001). However, the most frequently encountered wild-caught species intended for aquaculture, or those being raised in hatcheries are *E. coioides*, *E. malabaricus*, *E. bleekeri*, *E. akaara*, *E. awoara*, *E. areolatus*, *E. amblycephalus*, *E. fuscoguttatus*, *E. lanceolatus*, *E. sexfasciatus*, *E. trimaculatus*, *E. quoyanus*, *E. bruneus*, *Cromileptes altivelis*, *Plectropomus leopardus* and *P. maculatus*.

In south-east Asia grouper mariculture is not well-organized; it is largely based on the grow-out of wild-caught grouper seed (i.e. fry, fingerlings and juveniles) which are often in insufficient quantity and of unreliable quality to meet demand, and is confounded by a series of problems that hinder its expansion (Sadovy, 2000). The most pressing problem appears to be the shortage of, and reliance on, wild-caught seed. Not only may wild-capture be unsustainable at current levels but it could also compound the overfishing of adult groupers by removing fish that might otherwise survive to reproduce and supplement adult stocks (Sadovy, 2000). The volume of seed caught each year exceeds hundred of millions of individuals (Sadovy, 2000). When seed catches are compared to the numbers of marketable fish produced, the results are astonishing and strongly suggest crude and wasteful culture practices. To produce 23 000 t of table-size live fish about 60 million seed are necessary (see Sadovy (2000) Hong Kong section for details of the calculation). Yet, crude estimates of trade around the region suggest that the international trade of many hundreds of millions of seed occur annually and it is clear that there is an enormous mortality and wastage of biomass involved in the process (Sadovy, 2000). The depletion of wild seed stock has led to the development of grouper hatchery technology in the region, particularly in China, Indonesia, Malaysia, Taiwan and Thailand. Hatchery survival rate for many grouper species is still low, ranging from less than 2% to around 10%. However, the very high fecundity of grouper species compensates for this low survival, helping to make hatchery operations viable (Sim, 2005). In 2002, the Krabi Coastal Fisheries Research and Development Centre in Thailand reported its first success in breeding and larviculture of *E. fuscoguttatus* with a survival rate of 2% to 70-day-old juveniles. This centre has also succeeded in producing *E. coioides* fingerlings for some years and now provides 100 000–200 000 fingerlings per year to Thai farmers (Sim *et al.*, 2005a).

The major destinations of the trade routes for grouper seed are Hong Kong, China and Taiwan, while the major sources are the Philippines, Thailand and, to a lesser extent, Indonesia, Malaysia and Taiwan.

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Two-thirds of the production from Taiwan is based on the grow-out of hatchery-reared fry; this country also exports both hatchery-produced seed, and imports and re-exports capture-based seed (Ottolenghi *et al*., 2004).

Groupers are cultured in many south-east Asian countries, including Indonesia, Malaysia, Philippines, Taiwan, Thailand, Hong Kong, south-east China and Vietnam, as well as other parts of the tropics in the south-eastern USA (Tucker, 1999) and the Caribbean. More recently, other countries have joined them: India, Sri Lanka, Saudi Arabia, South Korea and Australia (Ottolenghi *et al*., 2004). The principal producer countries in 2000 were: Taiwan (5053 t yr^{-1}), Thailand (1250 t yr^{-1}), Malaysia (1217 t yr^{-1}) and Indonesia (1159 t yr^{-1}).

According to FAO statistics, the annual aquaculture production increased from approximately 2000 t in 1991 to 9300 t in 2000 (Ottolenghi *et al*., 2004). However, the actual figures of grouper production in south-east Asia are reported by Sadovy (2000) to be far higher at 23 000 t, with perhaps 20% of this production based on hatchery-produced fry, and the remainder from wild seed. Kongkeo and Phillips (2002) estimated Asian production to be around 15 000 t. In each case, these figures are significantly higher than the official statistics published by FAO (Ottolenghi *et al*., 2004). Accurate information is difficult to obtain; hence the production estimates vary significantly, depending on the author's sources with data coming from a combination of interviews, questionnaires and literature reviews gathered by each author. When information is collated from a variety of sources, e.g. Government offices, private producers, traders, middlemen, exporters and importers, and from fishing communities, academic institutions and hatcheries, etc., it is not easy to obtain reliable estimates (Ottolenghi *et al*., 2004).

A DETAILED EXAMPLE: TAIWAN

More than 52 grouper species are found in the waters of Taiwan. Grouper are the most expensive fish on the Taiwanese market, and have generated a great deal of interest amongst breeders and research organizations. In 2002, the production of commercial-sized groupers (between 0.6 and 1 kg) amounted to 5000 t with a value of 848 million Taiwanese dollars (approximately 20 million euros). The species bred were: *E. coioides*, *E. malabaricus*, *E. lanceolatus*, *E. tauvina* and *E. fuscoguttatus* with a controlled reproduction cycle (Su, 1999), and *E. akaara*, *E. awoara*, *E. quoyanus*, *E. trimaculatus*, *Cromileptes altivelis* and *Plectropomus leopardus* with wild caught seed used.

It takes around 15 months to grow groupers to commercial size involving different breeders in four stages: (i) production of fertilized eggs, (ii) hatchery, (iii) nursery and early grow-out and (iv) grow-out. This division of the activities results in a more rapid turnover for each farmer and therefore greater profitability.

Twenty thousand broodstock were required for the production of 20 million fingerling (2 to 3 cm) in 1995 (Teisson, unpublished data). The average survival rate from the larval stage to the fingerling is estimated to be between 0.1% and 0.15%. From the fingerling to the early-fattened juveniles (7 cm) the survival rate is estimated to be 23%. The following data come from information collected during field visits in *E. coïoides* farms (Pierre, 2004; personal work; Teisson, 1995; unpublished data).

Broodstock and egg production

In general, the broodstock are kept in large ponds ($50 \times 50 \times 1.5$ m) at a low population density. Pumping stations provide the ponds, not far from the coast, with water taken from the sea, with settling tanks and water treatment/purification facilities being sometimes available. The quality of the coastal water in Taiwan is very poor, which is why treatment of the water entering in the ponds is essential (Teisson, 1995). Aeration is assured by the use of paddle wheels. The temperature of the water is fairly stable throughout the year at

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between 27 and 30°C. Groupers are carnivorous and have a diet high in fish protein; the broodstock are fed daily with 'trash fish'. The term 'trash fish' is inaccurate in that these fish species would not necessarily otherwise be wasted and alternative uses might include being used as a protein source for other agricultural commodities or even human food, consumed directly or as fish sauce. Despite the apparent abundance and availability of trash fish in many areas, there are several major issues and problems related to its use in fish farming (e.g. very short storage life, availability often highly variable and seasonal) (Sim *et al.*, 2005a). The continued use of trash fish as a feed source for groupers should be discouraged because of the risk of disease transfer and the environmental problems associated with its use (Suwirya and Giri, 2005).

The sex ratio in the fishponds is around one female to two males. As sperm production is very low compared to the production of eggs by the females, the farmers place a greater number of males in the ponds in order to achieve a better rate of fertilization. However, the sex ratio very often remains difficult to determine owing to the fact that the females can change sex. Masculinization trials were carried out by researchers by inducing sexual inversion with the aid of implants containing 17 α-methyltestosterone or a combination of three forms of androgen hormone in equal proportions: testosterone, testosterone propionate and 17 α-methyltestosterone (Yeh *et al.*, 2003). However, the quality of spermatozoa of these males is not good, and the quality of the eggs obtained after fecundation is poor. The stress that is generated by manipulations (their significant size make their handling difficult) means that the broodstock need to be replaced every year.

Broodstock reproduction is carried out naturally and follows the lunar cycle in the majority of species. Spawning can last for several days, or even several months and occurs in general in the spring but the exact timing of spawning cannot be predicted (Teisson, 1995). Harvesting of the fertilized eggs, which float in all species, is carried out by shallow skimming with the help of nets and the water current produced by the paddle wheel. During the spawning period the nets are checked every morning. The farmers can harvest up to 50 kg of fertilized eggs per night; the current selling price for fertilized eggs is approximately 125 euro kg^{-1} (5000 NT$ kg^{-1}; Taiwanese dollars).

Hatchery

Hatching of the eggs (D0) is temperature-dependent and occurs approximately 24 h after spawning. The eggs are held in tanks of sea water which is filtered and sterilized by a system of mechanical, biological and UV filters under conditions of natural light and at a temperature maintained between 28 and 30°C. There are numerous aerators in the tanks so that homogeneous aeration can be achieved without too much agitation of the water. The larvae live on their vitelline reserves for 3–4 days, and then open their mouths by D3 and start feeding. At this point, the tanks are provided with phytoplankton, oyster eggs and small trochophore mussel larvae. By D6-7 the larvae feed on live prey such as rotifers and copepods, and finally by D15–16 they feed on artemias. The most critical stage of metamorphosis occurs at D30–40 owing to problems such as cannibalism, viral infections and parasites (Teisson, 1995); these are minimized by maintaining a larval density of 3 larvae L^{-1} (controlled by counting). This stage lasts for approximately 40 days, with a sale price of the 1–2 cm fingerling being 0.80 euro (33 NT$) each.

Nursery and early grow-out

The rearing of 0.1 g to 10 g (1–2 cm to 7 cm) groupers is carried out under natural light conditions in circular tanks (1 to 2 m in diameter) containing sea water that is filtered and sterilized as it enters the tanks. The fingerlings are fed upon an artificial food based on fishmeal in the form of pellets. The protein content of the pellets must be high in order to prevent cannibalism. The major problem encountered at this stage is white spot disease (protozoal), especially when the temperature is too high. This stage lasts for around 30 days, with the sale price of a 7 cm (10 g) juvenile being 1.35 euro (55 NT$).

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Grow-out

The commercial size of groupers in Taiwan is 25–30 cm for a weight of 600 g to 1 kg. At this stage the growth rate is quite high with the juveniles growing from 7 cm to 30 cm in only a few months. For example, *E. lanceolatus* reaches commercial size within 6 months, *E. malabaricus* within 12 months and *E. coioides* between 12 and 15 months (Su, 1999). The fattening of the groupers is traditionally carried out in extensive pond systems made of earth or concrete and is rarely carried out in polyculture or in cages. The stocking density at the end of grow-out is around 4 kg m^{-3}. Once they have reached a marketable size, groupers are removed with a seine net. It is rare to empty a pond of grouper in a day, with more often than not a partial removal of individuals being carried out. Mortality at this stage is low, with a survival rate of approximately 80%. Every 6 months the ponds are emptied and left to dry in order that any pathogenic agents will be completely removed. The fish are sold to a wholesaler who collects the fish from the grow-out farm. They are transported alive in a lorry, equipped with tanks and oxygen, to the town where they are sold to fish traders, who in their turn sell them, still alive, to the consumer. Dead fish are of little commercial value; they are sold at a lower price than live fish.

STATE OF THE ART FOR DUSKY GROUPER MARICULTURE IN THE MEDITERRANEAN

The dusky grouper, *Epinephelus marginatus*, is a common wide-ranging species, occurring in the east Atlantic Ocean, along the coast of southern Brazil and throughout the Mediterranean Sea (Heemstra and Randall, 1993). Among the six species of the genus *Epinephelus* found in the Mediterranean, the dusky grouper is the most in demand and recreationally important and is considered a potential candidate for commercial aquaculture and coastal zone management programmes (Marino and Mandich, 2001).

The dusky grouper is a protogynous species and in the wild, the females are considered to be mature between 2.5 kg ($\sim$ 38 cm = 5 yr) and 11 kg ($\sim$ 57 cm = 7 yr); the sexual inversion occurs between 10 (around 80 cm) and 16 years of age (around 110 cm).

Although no specific fishery statistics are available, the dusky grouper is believed to be over-exploited in most Mediterranean areas and was recently listed as an endangered species in Annex 3 of the Bern Convention (1995) and in Annex 3 of the protocol for specially protected areas and Mediterranean biodiversity of the Barcelona Convention (1995) (Marino and Mandich, 2001).

The first attempts to reproduce dusky grouper under controlled conditions were made in 1995 by research groups in Italy, Croatia, Spain and Greece (Marino and Mandich, 2001). Larval rearing trials have been carried out by Glamuzina *et al.* (1998a) up to the 10th day, by Marino (unpublished data) up to the 25th day and by Spedicato *et al.* (1998b) up to the 50th day after spawning but not further. Water temperature has been proposed as a key element in the reproduction of Mediterranean groupers and is thought to influence the duration of the breeding season and spawning activity (Zabala *et al.*, 1997). Feeding protocols and environmental conditions for larval rearing used with other marine species are of no use in groupers, which are characterized by their slow development and long larval period. At the present time dusky grouper reproduction in captivity still poses many problems.

Broodstock formation

There are three methods of successfully breeding broodstock of protogynous grouper species: raising of wild juveniles to sexual maturity, collection of already sexually mature wild adult broodstock, and the development of techniques for hormone-induced sexual inversion (Marino and Mandich, 2001). The collection of wild adult fish is in general the preferred method, even if the increasing scarcity of these fishes around the coasts, as well as the difficulties in catching them alive makes this method relatively complex.

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Males development

Hormonally induced sex change was recognized as being an indispensable tool to the development of a population of males, as well as influencing the sex ratio (Marino and Mandich, 2001). Sexual inversion protocols were developed on juveniles and young females by different research groups (Glamuzina *et al*., 1998a; Marino *et al*., 1998a; Spedicato *et al*., 1998a). Males were obtained by the oral administration of the synthetic hormone, 17α-methyltestosterone (17α-MT); the sperm motility of all sex-reversed males was 40–95%, and fertility rates varied between 50% to 95%. Six months after the end of 17α-MT administration, sex-inverted fish changed back to females (Marino *et al*., 1998a), thus indicating that gonad changes are not stable and prolonged 17α-MT treatment is required (Marino *et al*., 2000).

Maturation of the females

In captivity, the majority of females appear unable to complete vitellogenesis and ovocyte maturation, whilst inappropriate environmental and welfare conditions can disrupt the natural spawning process (Marino *et al*., 2000). Artificial maturation of the females is achieved by treatment with gonadotrophins (GnRHa or hCG): human chorionic gonadotrophin (hCG) either alone or in combination with seabass hypophyseal hormone.

Hybridization trials

Hybridization trials were also attempted between *E. marginatus* and *E. aeneus* (Glamuzina *et al*., 1999) and between *E. marginatus* and *E. costae* (Glamuzina *et al*., 2001). These trials resulted in good fertilization and survival rates during the embryonic phase as well as in a good development of the yolk sac, with data comparable to the results of trials carried out on *E. marginatus* alone (Glamuzina *et al*., 1999).

E. marginatus trial protocol (Table 4)

Broodstock husbandry and spawning

Maturation and natural spawning by the broodstock is dependent upon the *in situ* conditions of the geographical location in which they are reared, in particular, a temperature above 20°C such as occurs in

Table 4. *Epinephelus marginatus* trial protocol (Gracia Lopez and Castello-Orvay, 1995; Glamuzina *et al*., 1998b; Spedicato and Boglione, 2000)

	T°C	S‰	Food	Tank/pond volume	Density	Water renewal
Broodstock	16–18	34	Cephalopods (squid), fresh fish	16–40 m^3	2–5 $kg\,m^{-3}$	5–6 vol. day^{-1}
Eggs	23	38	×	120–300 L	20 eggs L^{-1}	25%
Larvae	20	38	D1: microalgae (*Nannochloropsis oculata*) D3: cryopreserved oyster trocophore larvae (20–40 μm), marine copepods (20–60 μm) D4: small rotifers (SS type, 135 μm) D5–D15: rotifers D15: *Artemia nauplii* D30: progressively sized adult artemia	500 L	16–17 ind. L^{-1}	25%
Grow-out juveniles	20.4–25.9	20–35	Frozen octopus and artificial pellets	500 L	3 $kg\,m^{-3}$	

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North Africa at the beginning of June and in the north Adriatic at the end of September. The artificial induction of spawning is carried out by the use of the hormones HCG (2000 IU kg^{-1}) on the males and GnRHa or LHRHa (200 μg kg^{-1}) on the females administered by two injections with an interval of 24 h. Eggs are expressed from the female by hand stripping 16 h after the second injection. Fertilization is obtained by mixing the eggs with the milt of the inverted males. The fertilized eggs floating on the surface of the water (750 to 885 μm in diameter, with a single oil globule) are incubated under strict environmental conditions.

Larval rearing

The duration of incubation is variable and dependent upon temperature (Glamuzina *et al*., 1998b). It can vary from 25 h after fertilization at 25°C in darkness, to 34 h at 20°C and 50 h at 18°C. Hatching takes around 2 h with 85% to 90% of the eggs giving rise to larvae of 1.4 to 2.1 mm in length. Mouth opening occurs after 70–85 h at 25°C in the dark or 120 h at 20°C.

Massive mortality has been reported at the mouth opening stage and currently there are no grouper hatcheries able to overcome this. Grouper larvae are small in size, they have few reserves and the size of the mouth when it opens is around 70–100 μm; consequently the size of the prey during their initial feeding is extremely important (<40% of the mouth) (Glamuzina *et al*., 1998b). However, this is not the only problem, as larval feeding with oyster trochophores and small rotifers (SS-type) is not appropriate, resulting in massive mortality during the initial rearing phases (Marino *et al*., 1998b). It would appear that grouper larvae identify their food by sight and choose their prey by size and not by taste (Spedicato and Boglione, 2000). Other problems can occur, such as the formation of crystals in the urinary bladder, from D2 (before the opening of the mouth) as well as amongst all the larvae that have started to eat, and spinal deformation at early larval stage (Glamuzina *et al*., 1998b).

Grow-out

Gracia Lopez and Castello-Orvay (1995) reported on the effect of food, salinity, temperature and the availability of artificial hiding space on the growth and feeding efficiency of dusky grouper. Wild grouper fingerlings were collected and stocked in 500-L tanks; fish fed on artificial pellets grew faster than those fed on frozen octopus, those kept in water of 20‰ and 27‰ salinity grew more slowly than those at 35‰, and those kept at temperature of 25.9°C grew more quickly than those at 20.4°C. In optimal conditions the fish grew from 7 to 458 g within a 15-month period.

DISCUSSION

Marine finfish culture has been practised by Asian farmers for a long time, although the techniques employed have largely been dependent on wild seed. Normal practice has been to buy grouper fingerlings from fishing boats and to fatten them in a cage or pond before resale. Some farmers catch their own grouper fingerling from the wild and culture them at a low density so that the production is low and not well targeted to market demand (Thai, 2005). These fisheries are synonymous with destructive fishing practices and over-fishing which damage not only the marine environment but also the economies and social fabric of coastal fishing communities dependent on coral reef resources. The longer-term negative impacts of unmanaged live reef food fish (LRFF) fisheries on fish stocks and traditional fishing communities that target them are now apparent in many countries (www.enaca.org).

A few years ago grouper farming began to expand as an industry, creating increased demand for wild grouper fingerlings and a shortage of supply (Thai, 2005). The move from wild-caught to cultured live reef food fish as a source of market product is seen as a significant way of taking pressure off species,

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particularly those that are highly fished or endangered. These activities are seen to have their own sustainability issues (www.enaca.org).

To ensure that farms can get an adequate supply of high-quality seed from a sustainable source, it is necessary to implement grouper hatchery technology and to develop a seed supply chain that will service the industry. To promote hatchery technology the Australian Centre for International Agricultural Research (ACIAR) has developed a project (FIS/97/73) to improve hatchery and grow out technology for grouper aquaculture in the Asia Pacific region (Sim *et al*., 2005b). The recent development of technology for large-scale hatchery production of grouper fry, largely as a result of research carried out in this collaborative ACIAR grouper project, is producing a plentiful supply of grouper fingerlings for aquaculture on growing.

At the time that the ACIAR grouper project began in 1999, information on the nutritional requirements of groupers for grow out from fry to market was almost non-existent (Williams *et al*., 2005). To address this need, the ACIAR grouper project collaborators in Australia, Indonesia, the Philippines and Vietnam embarked on grow-out feeds research with the primary aim of developing cost-effective pelleted grouper grow-out feed. The ultimate goal is to develop pelleted grouper feeds as a more sustainable, less polluting and more cost-effective alternative to the feeding of fresh fishery by-catch. If achieved, this would provide a more sustainable and profitable way of culturing groupers and at the same time reduce competition between man and aquaculture for a dwindling supply of fishery catch and lessen aquaculture's impact on the surrounding environment (Williams *et al*., 2005).

Wholesale marine fish prices in Hong Kong in September 2006 were: 22.28\$ kg^{-1} for *E. polyphekadion*, 15.95\$ kg^{-1} for *E. fuscoguttatus*, 14.08\$ kg^{-1} for *E. areolatus* and 11.67\$ kg^{-1} for *E. coioides* (www.enaca.org).

Although many claim that the taste of cultured grouper and wild grouper cannot be differentiated by professional tasting panels, consumers from China, Hong Kong, Singapore and Malaysia still prefer wild grouper. Could this be merely the taste that consumers preferred or could it be the perception that wild products taste better and are healthier than the farmed products? (www.enaca.org).

Asian successes are a testimony to what is possible and are an encouragement to continue European research. It is true that the grouper family is composed of species of mainly tropical and subtropical waters, and that in Europe these fish are almost only found on the Mediterranean and the Portuguese Atlantic coasts, but the situation in these areas is already alarming and requires, immediately, that protection measures be put in place. Repopulation is only really occurring on the protected sea floor of marine parks. It is now time to move on to the next phase and to dedicate the necessary time and resources to this problem, while promoting, for example, more regional collaboration and exchange in order to further develop grouper aquaculture.

With increased grouper aquaculture capacity, the demands of the market could be more easily met, thus reducing the potential financial benefits to be gained from poaching and other illegal fishing practices. The development of rearing techniques could also result in the capacity to introduce large numbers of grouper fingerlings into the wild, thus enhancing wild populations.

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