Natural spawning of three species of grouper in floating cages at a pilot broodstock facility at Komodo, Flores, Indonesia

Sudaryanto[1], Trevor Meyer[1] and Peter J. Mous[1]

Abstract

Broodstock of mouse grouper, *Cromileptes altivelis*, tiger grouper, *Epinephelus fuscoguttatus*, and estuary grouper, *E. coioides*, are commonly housed in shore-based tanks. Often, hormone injections are used to induce spawning. Broodstock of a pilot fish culture project in the Komodo area (Flores, Indonesia) were kept in floating fish cages with a surface area of 16 m^2 and a depth of 6 m, where they reproduced naturally without hormonal treatment. The grouper species stocked at Komodo were found to typically spawn around the period of new moon. Duration of spawning varied from 3–14 days. Spawning occurred in groups (*E. fuscoguttatus*) or in discrete pairs (*E. coioides*, *C. altivelis*). Time of spawning varied between dusk (*E. coioides*) to beyond midnight (*C. altivelis* and *E. fuscoguttatus*). Possibly, natural spawning was facilitated by the broodstock compartment's water depth, which was about two times greater than in the shore-based tanks that are most commonly used. The greater water depth of floating fish cages appears to facilitate pre-spawning behaviour ("dancing") and spawning itself. Furthermore, cage systems also allow for the provision of good water quality, ambient and stable water temperature and reduced stress, presumably leading to improved fecundity from the broodstock.

Introduction

Indonesia, a large equatorial island republic of more than 220 million people with a total coastline of over 81,000 km, is the prime source of live groupers for the Hong Kong-based reef fish trade. However, overexploitation by legal fishing methods and by the widespread use of illegal fish anaesthetics such as cyanide has led to a dramatic decrease in the wild population of groupers. This has forced the trade to source groupers from remote destinations such as the West Coast of Africa and the Pacific islands (Hughes et al. 2003). Grouper culture has the potential to make this profitable trade sustainable (see Anonymous 2003a; 2003b).

Groupers (family Serranidae, subfamily Epinephelinae) are popular food fish now widely cultured in net cages and earthen ponds throughout Southeast Asia. However, growth and development of the grouper farming industry has been constrained by an inadequate supply of fish juveniles for stocking (Chao and Lim 1991). The existing supply of wild-caught juveniles cannot meet the demand of the expanding grouper culture industry, so the development of this industry is reliant upon the successful hatchery production of grouper juveniles.

At least 23 species of serranids have spawned naturally in captivity, mostly during the natural spawning periods under ambient temperatures and partial or full levels of natural light. Optimum rearing conditions and feed are critical to induce natural spawning in captivity (Tucker 1994). Captive spawning of *Epinephelus fuscoguttatus* has been reported by Kohno et al. (1990) and Lim and Chao (1990) and natural spawning in captivity has been reported by Chao et al. (1993). Artificial spawning by hormone injection of *Epinephelus coioides* has also been reported by Chao and Lim (1991).

Females of some species of epinepheline serranids are capable of spawning more than once during a season, often very frequently. A single caged female *Epinephelus coioides*, kept with two males, was reported to have spawned 5–10 times per month over a period of 4 months (Lim and Chao 1990). Ten female and ten male *Epinephelus fuscoguttatus* kept in a cage spawned 2–5 times during each of nine periods of 2–6 days, usually starting between the lunar last quarter and new moon (Lim and Chao 1990). Forty female and nine male *Epinephelus coioides* spawned almost continuously for 50 days during April to June 1976 (Hussain and Higuchi 1980). *Cromileptes altivelis* have spawned voluntarily in net cages in Singapore, in tanks at Gondol, Bali and Situbondo, Java, and in 50-m^3 tanks in Lampung, Sumatra.

Although broodstock are often collected and conditioned in floating cages, most hatcheries are reliant upon egg production from broodstock housed in

1. The Nature Conservancy, Southeast Asia Center for Marine Protected Areas, Jl. Pengembak 2, Sanur, Bali, Indonesia.
Email: pmous@tnc.org

shore-based tanks (Ruangpanit 1993). This paper describes the spawning activity and husbandry methods for three protogynous grouper species (*C. altivelis*, *E. coioides* and *E. fuscoguttatus*) maintained in floating cages as a low-cost alternative to holding broodstock in shore-based tank systems.

Materials and methods

The Komodo fish culture project

The Nature Conservancy, a global environmental organisation, works closely with the Indonesian Park Authority (PHKA, Balai Taman Nasional Komodo) to protect the marine biodiversity of Komodo National Park and to safeguard the park's function as a source of recruits for surrounding fishing grounds. One of The Nature Conservancy's alternative livelihood projects is based on the development of a sustainable fish culture industry that provides alternative income to local fishers. The project rationale is to establish a multi-species marine fish hatchery that supplies reef fish juveniles and training on fish husbandry to village-based cage grow-out units that are being developed around Komodo National Park (Anonymous 2003c). A secondary aim of the project is to contribute to the transformation of the live reef fish trade from an unsustainable, capture-based industry to a sustainable, culture-based industry.

Based at Loh Mbongi, near Labuan Bajo on the West coast of Flores, the project maintains broodstock housed in cages to provide eggs and larvae for hatchery production.

Broodstock maintenance and development

Three species of grouper are maintained as broodstock, namely mouse grouper (*Cromileptes altivelis*), tiger grouper (*Epinephelus fuscoguttatus*) and estuary grouper (*Epinephelus coioides*). The fish culture project also maintains broodstock of Asian seabass (*Lates calcarifer*) and mangrove jack (*Lutjanus argentimaculatus*), but these species are not discussed in this paper.

All broodstock were captured from surrounding waters by trap and hand line during late 1997 and 1998, and as such are of locally pure genetic stock. Size at capture varied between a few grams and several hundred grams. Fish were on-grown for a period of three years, on a diet of fresh fish at 5–10 per cent of body weight per day. These mostly immature fish were stocked in cages of 3-m diameter, containing nets initially 3 m in depth. Stocking density varied between 30 and 100 fish per cage.

By August 2000 some of the brooders were found to be mature and ready for spawning. Mature fish were transferred to simply constructed wooden square floating cages of 16-m^2 surface area, fitted with nets of 6-m depth. The cages were arranged into a platform holding 24 cages, grouped as four rows of six cages. The cages were moored in a sheltered bay, about 150 m from the shore in waters about 18 m deep. The 20-mm-mesh-sized nets were cleaned every four weeks to ensure high water exchange and thus optimum water quality within the cage. The stocking density of the broodstock was maintained at 25 fish per cage. The sex ratio at the start of the observations was approximately 1 male to 3 females for mouse grouper and tiger grouper, but for estuary grouper, females vastly outnumbered males. By 2003, the sex ratio changed due to females changing sex to become males. The species composition, sex ratio and average body weight of the broodstock in August 2003 are summarised in Table 1.

Mature broodstock were fed with fresh fish and squid at 4–5 per cent of body weight every other day. Fish used for feeding were caught by the local pelagic fishery and included purse-eyed scad, *Selar crumenophthalmus*, mackerel scad, *Decapterus macarellus*, spotted halfbeak, *Hemiramphus far*, and hound needlefish, *Tylosurus crocodilus*. Feed fish were immersed in freshwater for 30 minutes prior to feeding to remove external parasites. To ensure high egg quality, the fresh fish used for feeding were enriched with a commercial preparation containing essential fatty acids and vitamins A, B and E. The feed fish were cut into appropriate sized morsels prior to feeding.

Table 1. **Species composition, sex ratio and mean body weight of broodstock in August 2003.**

Species	Number of males	Number of females	Total number	Mean body weight (kg)
C. altivelis	15	24	39	2.0
E. coioides	22	131	153	7.5
E. fuscoguttatus	39	39	78	10.0

Fine-meshed skirts (0.5 mm mesh size), measuring 3.75 m x 3.75 m x 3 m depth were fitted to cages that contained gravid females and males in spawning coloration to prevent eggs drifting out of the cages. Fertilised eggs may be positively or neutrally buoyant; unfertilised eggs are negatively buoyant (Rimmer 2000). Within an hour after spawning, the buoyant eggs were collected by seine scoop-net with 0.5-mm mesh for transport in 15-litre plastic buckets to the incubation tanks at the hatchery.

Observations on the timing of spawning behaviour of *Cromileptes altivelis*, *Epinephelus coioides* and *E. fuscoguttatus* were made over the period October 2000 to June 2003.

Results

Natural spawning of mouse grouper, tiger grouper and estuary grouper in the floating cages was first observed in late 2000. Spawning was preceded by pairing of the fish and a brief "dance" leading to spawning itself. During spawning, the sperm and eggs were released into the water column and fertilisation occurred externally.

Spawning of mouse grouper

Spawning of *C. altivelis* stocked in two separate cages was observed over the period October 2000 to July 2003 (Table 2).

Mouse grouper spawned in discrete pairs, following a period of "dancing" during which a pairing was established. Just prior to spawning, the pair swam together whilst maintaining physical contact between their heads. The fish then swam in a circular upward motion (presumably to remain within the confines of the cage) up to the water surface, where the eggs and sperm were released.

Spawning occurred from the third quarter to the first quarter of the moon, with 57 per cent of spawning observations made during the fourth quarter, 24 per cent during the first quarter and 19 per cent during the third quarter. No spawning activity was recorded during the second lunar quarter. Eighty-one per cent of spawning observations were made between 2100 h and 2300 h, with the remainder taking place between 2300 h and midnight. These observations show that spawning within a single cage can continue for at least eight consecutive days.

Spawning of estuary grouper

Spawning of *E. coioides* stocked in five separate cages was observed over the period December 2000 to July 2003 (Table 3).

Table 2. Observed natural spawning events of mouse grouper, *C. altivelis*, housed in two cages.

Cage	Date	Lunar day*	Time
1	29 Oct 2000	2	2140
1	30 Oct 2000	2	2120
1	22 Nov 2000	24	2125
1	23 Nov 2000	25	2150
1	24 Nov 2000	26	2130
1	25 Nov 2000	27	2105
1	26 Nov 2000	28	2200
1	27 Nov 2000	29	2215
1	28 Nov 2000	30	2145
1	29 Nov 2000	1	2150
1	15 Dec 2000	17	2110
1	16 Dec 2000	18	2205
1	17 Dec 2000	19	2240
1	18 Dec 2000	20	2135
1	19 Dec 2000	21	2155
1	1 Sep 2001	2	2230
1	7 Oct 2001	20	2120
1	18 Oct 2001	1	2105
1	11 Nov 2001	23	2130
1	6 Feb 2002	23	2100
1	8 Feb 2002	25	2130
1	9 Feb 2002	26	2300
1	7 Mar 2002	22	2200
1	8 Mar 2002	23	2100
1	8 Oct 2002	1	2230
1	2 Dec 2002	27	2330
1	6 Dec 2002	1	2100
1	28 Mar 2003	24	2150
1	28 Mar 2003	24	2135
1	29 Mar 2003	25	2215
1	29 Mar 2003	25	2300
1	30 Mar 2003	26	2235
1	30 Mar 2003	26	2115
1	4 Apr 2003	19	2235
1	3 May 2003	19	2230
1	24 Jun 2003	23	2300
2	24 Jun 2003	23	2300
1	25 Jun 2003	24	2315
2	25 Jun 2003	24	2345
1	27 Jun 2003	26	2250
2	27 Jun 2003	26	2250
1	1 Jul 2003	1	2400

* Lunar day 1 represents new moon, while lunar day 14 represents full moon.

E. coioides spawned in pairs. Typically the male searched the stock of females for a suitable partner, whilst the females remained relatively inactive on the cage floor. Once the pair was established, spawning commenced as the pair swam together from the cage floor up to the water surface, where eggs and sperm were released.

Spawning was concentrated in the fourth quarter of the moon, when 64 per cent of all spawning events were observed. Onset of spawning occurred later than for *C. altivelis* (lunar day 21 as opposed to lunar day 17), but it continued up to lunar day 9 in the second quarter, whereas *C. altivelis* spawned only up to lunar day 2. *E. coioides* spawned much earlier in the evening than *C. altivelis*, with 84 per cent of spawning observations made between 1700 h and 1800 h.

Table 3. **Observed natural spawning events of estuary grouper, *E. coioides*, housed in five cages.**

Cage	Date	Lunar day	Time
1	19 Dec 2000	21	1910
1	17 Feb 2001	21	1945
1	18 Feb 2001	22	1905
1	19 Feb 2001	23	1940
1	20 Feb 2001	24	1950
1	11 Sep 2001	24	1810
1	11 Oct 2001	24	1740
2	11 Oct 2001	24	1820
1	12 Oct 2001	25	1755
2	12 Oct 2001	25	1730
1	10 Feb 2002	27	1900
1	9 Sep 2002	1	1900
2	8 Oct 2002	1	1700
1	2 Dec 2002	27	2300
2	2 Dec 2002	27	2300
1	28 Feb 2003	26	2000
1	1 Mar 2003	27	1800
1	2 Mar 2003	28	1620
2	2 Mar 2003	28	1825
2	4 Apr 2003	21	1750
2	3 May 2003	9	1820
3	4 May 2003	9	1815
4	5 May 2003	9	1755
5	5 May 2003	23	1805
2	3 Jul 2003	3	1800

Spawning of tiger grouper

Spawning of *E. fuscoguttatus* stocked in three separate cages was observed over the period November 2000 to July 2003 (Table 4).

Tiger grouper spawned in pairs, but different pairs would often spawn at the same time, thus giving the effect of a "group spawning". Typically, a male displayed or "danced" to establish a pairing with a chosen female, who lay relatively inactive on the cage floor. Once the pair was established, spawning behaviour started with the pair swimming together up from the cage floor to the water surface, whereupon both fish released their eggs and sperm.

Spawning was concentrated in the fourth quarter of the moon, when 97 per cent of all spawning events were observed. This shows that *E. fuscoguttatus* exhibits a much more confined spawning period than mouse grouper and estuary grouper. Ninety-four per cent of spawning events were recorded between 2100 h and midnight.

Table 4. **Observed natural spawning events of tiger grouper, *E. fuscoguttatus*, housed in three cages.**

Cage	Date	Lunar day	Time
1	22 Nov 2000	24	2200
1	23 Nov 2000	25	2130
1	24 Nov 2000	26	2135
1	25 Nov 2000	27	2215
1	26 Nov 2000	28	2150
1	27 Nov 2000	29	2205
1	28 Nov 2000	30	2145
2	22 Nov 2000	24	2230
2	23 Nov 2000	25	2245
2	24 Nov 2000	26	2300
2	25 Nov 2000	27	2315
2	26 Nov 2000	28	2210
2	27 Nov 2000	29	2150
2	28 Nov 2000	30	2315
1	16 Oct 2001	29	2320
1	6 Feb 2002	23	2300
2	8 Feb 2002	25	2330
1	1 Dec 2002	26	2100
2	1 Dec 2002	26	2100
3	1 Dec 2002	26	2100
1	2 Dec 2002	27	2100
2	2 Dec 2002	27	2100
3	2 Dec 2002	27	2100
1	29 Mar 2003	25	2210
2	29 Mar 2003	25	2130
3	29 Mar 2003	25	2210
1	30 Mar 2003	26	2120
2	30 Mar 2003	26	2310
3	30 Mar 2003	26	2240
1	24 Jun 2003	23	2300
2	24 Jun 2003	23	2300
3	24 Jun 2003	23	2300
2	28 Jun 2003	27	0100
2	2 July 2003	2	1900

Discussion

These observations show that naturally spawning mouse grouper, estuary grouper and tiger grouper can be maintained in floating cage systems, and that the broodstock can be managed so as to supply fertilised eggs for hatchery rearing. This is advantageous in that the additional costs of maintaining broodstock in land-based tanks, primarily the energy costs of seawater supply and aeration, can be avoided. The use of cages allows a much larger holding volume of water, which allows the grouper more space to conduct spawning behaviour. Furthermore, ambient physicochemical conditions reduce stress to a minimum.

The collection of eggs is reliant upon staff manning the broodstock facility throughout the night and manually collecting eggs after spawning. Egg collection should not be delayed by more than two hours post-spawning, since the grouper eggs are rapidly consumed by small fish swimming into the cages through the mesh of the nets.

Fecundity and spawning frequency of the three grouper species discussed in this paper were found to vary through the year, with a seasonal low occurring during the months of June, July and August. This low availability of fertilised eggs through part of the year may cause bottlenecks in hatchery production. Spawning frequency and fecundity during the low season can probably be increased by administration of hormones to feed or by injection.

This production system does have some disadvantages. Photoperiod control, used to induce spawning in other cultured fish species, is not possible. Inclement weather can disturb and postpone spawning of broodstock. Furthermore, broodstock are vulnerable to the spread of infectious disease, since the cages cannot be isolated and supplied with filtered and sterilised water in the way that tank-based systems can. Consequently, it is recognised that this method of husbandry may be more appropriate for small-scale fish culture projects in remote areas than for areas where large-scale production takes place and where diseases are a constant threat.

Broodstock that are maintained in floating cages may also benefit capture fisheries in surrounding waters. Uncollected eggs will disperse through the cage nets into the surrounding waters. It is estimated that the three species of grouper held by the Komodo Fish Culture Project may, together, naturally produce in excess of 200 million eggs per month. Since no more than 1 million eggs per month are required by the project, this represents a significant contribution of eggs to surrounding waters. In this way, the broodstock facility at the Komodo Fish Culture Project may be contributing to the natural re-stocking of depleted stocks of grouper in and around Komodo National Park. In many areas in Indonesia, badly managed fisheries have extirpated high-value grouper stocks (Johannes 1997; Mous et al. 2000). For instance, in 80 hours of reef fish population surveying using scuba in Sangihe-Talaud, a heavily fished archipelago stretching 400 km between North Indonesia and the Philippines, only 8 coral trout (*Plectropomus* spp.) and no mouse grouper were sighted (Mous 2002). In such situations captive broodstock may contribute to a faster recovery of the stocks after more effective capture fishery management measures have been put in place. The downside is that if broodstock of non-native species or races of fish are kept in cages, these fish may rapidly establish themselves in the local environment, becoming pests or causing genetic pollution.

Acknowledgements

The authors would like to thank The Nature Conservancy for encouraging research work at the Fish Culture Project in Loh Mbongi. The authors also thank the Gondol Research Centre for Mariculture (Bali) and the Komodo National Park Authority for their support and assistance in the development of the Komodo Fish Culture Project.

References

Anonymous. 2003a. Fish farming: The promise of a blue revolution. The Economist, 7 August 2003.

Anonymous. 2003b. The blue revolution: A new way to feed the world. The Economist, 7 August 2003.

Anonymous. 2003c. When fishing grounds are closed: Developing alternative livelihoods for fishing communities. MPA News 5(2):1–4.

Chao, T.M. and Lim L.C. 1991. Recent developments in the breeding of grouper (*Epinephelus* spp.) in Singapore. Singapore Journal of Primary Industries. 19(2):78–93.

Chao, T.M., Lim L.C. and Khoo L.T. 1993. Studies on the breeding of brown-marbled grouper *E. fuscoguttatus* (Forsskal) in Singapore. Tungkang Marine Laboratory Conference Proceedings 3:143–156.

Hussain, N.A. and Higuchi M. 1980. Larval rearing and development of the brown-spotted grouper, *Epinephelus tauvina* (Forsskal). Aquaculture 19:339–350.

Hughes, T.P., Baird A.H., Bellwood D.R., Card M., Connolly S.R., Folke C., Grosberg R., Hoegh-Guldberg O., Jackson J.B.C., Kleypas J., Lough

J.M., Marshall P., Nyström M., Palumbi S.R., Pandolfi J.M., Rosen B. and Roughgarden J. 2003. Climate change, human impacts, and the resilience of coral reefs. Science 301:929–933.

Johannes, R. 1997. Grouper spawning aggregations need protection. SPC Live Reef Fish Information Bulletin 2:13–26.

Kohno, H., Philip and Imanto T. 1990. Reproductive performance and early life history of the grouper, *E. fuscoguttatus*. Buletin Pen. Perikanan. Special Edition I:27–29.

Lim, L.C. and Chao T.M. 1990. Observation on the breeding of brown-marbled grouper *E. fuscoguttatus* (Forskal). Singapore Journal of Primary Industrie. 66–84.

Mous, P.J., Pet-Soede L., Erdmann M., Cesar H.S.J., Sadovy Y. and Pet J.S. 2000. Cyanide fishing on Indonesian coral reefs for the live food fish market – What is the problem? SPC Live Reef Fish Information Bulletin 7:20–27.

Mous, P.J. 2002. Draft Report on the rapid ecological assessment in Sangihe-Talaud, North Sulawesi, Indonesia. Report from The Nature Conservancy Coastal and Marine Program – Indonesia, Bali.

Rimmer, M. 2000. Broodstock maintenance and spawning. Department of Primary Industry, Queensland:1–4.

Ruangpanit, N. 1993. Technical manual for seed production of grouper (*Epinephelus malabaricus)*. National Institute of Coastal Aquaculture, Dept. of Fisheries, Thailand and the Japan International Cooperation Agency.

Tucker, J.W. 1994. Spawning by captive serranid fishes: A review. Journal of the World Aquaculture Society 25:345–359.

Toward MAC certification of Hawaiian Islands collectors: A project update

Rezal Kusumaatmadja[1], John Parks[2], Scott Atkinson[3] and Jan Dierking[4]

Background

Over the past 30 years, the marine aquarium trade in the Hawaiian Islands has quadrupled. Annually supplying USD 3.2 million in live reef organisms, primarily to US and European markets (Dierking 2002), at present the Hawaiian marine aquarium fishery is a flourishing business in the islands and the source of hundreds of people's livelihoods. Over the past decade, the majority (58 per cent average annually, Miyasaka 2000) of organisms exported are captured off of the Kona (west) coast of the Big Island of Hawaii (see Fig. 1). Public concern over the sustainability of the trade has been voiced for a quarter century, highlighting the need for increased study and careful industry regulation. Decreasing reef fish abundance and overall reductions in the West Hawaii coral reef community health are increasingly being blamed by the public on current levels of aquarium trade operations, and these suspicions are supported by at least one recent study (Tissot and Hallacher 1999; Tissot 1999).

In response to growing public concern, the State of Hawaii Division of Aquatic Resources has strengthened the management of the West Hawaii aquarium fishery by developing new fishery regulations, enhancing monitoring and enforcement of such regulations, and regularly conducting scientific research on the state of West Hawaii's coral reefs and fish populations to help clarify what levels of catch are sustainable. One of the most important of these new state-led management efforts has been the establishment of nine Fish Replenishment Areas in 2000. These areas prohibit marine aquarium organism collection within approximately 30 per cent of the Kona coast's nearshore habitat.

MAC efforts initiated in Hawaii

As a result of a reduced collection area without a corresponding decrease in the number of collectors, there is now increased fishing effort in West Hawaii waters remaining open to collection. While the Hawaiian industry largely uses non-destructive collection techniques, overharvesting of target species is therefore an increasing concern that is difficult to control alone through the use of no-take zones and state-mandated catch reporting. Specifically, since the mid-1990s there has been increasing interest in how market incentives,

1. Author to whom correspondence should be addressed; Marine Aquarium Council, 923 Nu`uanu Avenue, Honolulu, HI 96817, USA. Tel: +1 (808) 550-8217. Email: info@aquariumcouncil.org
2. Community Conservation Network, 212 Merchant Street, Suite 200, Honolulu HI 96813, USA.
3. The Nature Conservancy of Hawaii, 923 Nu`uanu Avenue, Honolulu, HI 96817, USA.
4. Dept. of Zoology, U. of Hawai`i, 2538 McCarthy Mall, Edmondson 152, Honolulu, HI 96822, USA.