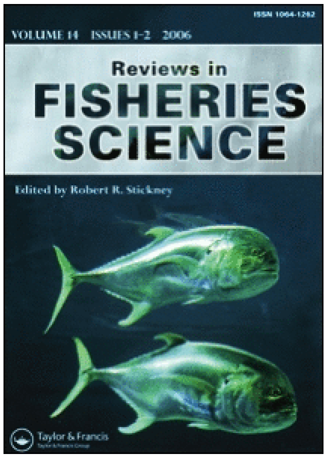

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Koichi Okuzawa [a]; Ronald J. Maliao [b]; Emilia T. Quinitio [c]; Shelah Mae A. Buen-Ursua [c]; Ma. Junemie Hazel L. Lebata [c]; Wenresti G. Gallardo [d]; Luis M. B. Garcia [e]; Jurgenne H. Primavera [c]

[a] Fisheries Research Agency, Kanagawa, Japan
[b] Fish Ecophysiology Laboratory, Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida, USA
[c] Aquaculture Department, Southeast Asian Fisheries Development Center, Iloilo, Philippines
[d] Aquaculture and Aquatic Resources Management, School of Environment, Resources and Development, Asian Institute of Technology, Pathumthani, Thailand
[e] Institute of Biology, College of Science, University of the Philippines, Quezon City, Philippines

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Stock Enhancement of Threatened Species in Southeast Asia

KOICHI OKUZAWA,[5] RONALD J. MALIAO,[2] EMILIA T. QUINITIO,[1] SHELAH MAE A. BUEN-URSUA,[1] MA. JUNEMIE HAZEL L. LEBATA,[1] WENRESTI G. GALLARDO,[3] LUIS M. B. GARCIA,[4] and JURGENNE H. PRIMAVERA[1]

[1]Aquaculture Department, Southeast Asian Fisheries Development Center, Iloilo, Philippines
[2]Fish Ecophysiology Laboratory, Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida, USA
[3]Aquaculture and Aquatic Resources Management, School of Environment, Resources and Development, Asian Institute of Technology, Pathumthani, Thailand
[4]Institute of Biology, College of Science, University of the Philippines, Quezon City, Philippines
[5]Fisheries Research Agency, Kanagawa, Japan

Natural populations of global inshore fisheries are coming under heavy pressure, primarily due to overexploitation and habitat degradation. Stock enhancement of hatchery-reared seeds is perceived as an alternative strategy to enhance the regeneration process. The Aquaculture Department of the Southeast Asian Fisheries Development Center in the Philippines has been implementing activities related to stock enhancement of donkey's ear abalone (Haliotis asinina)*, mud crabs* (Scylla *spp.), giant clam* (Tridacna gigas)*, and seahorses* (Hippocampus *spp.). Seed production techniques for abalone including a diet tagging method were established, and juvenile abalone were released and monitored in a marine protected area. Mud crabs conditioned before release had higher recapture rates compared to the non-conditioned crabs, which can be translated to higher survivorship. Giant clams stocked at 8–10 cm shell length have high survival 4 mo after stocking (90%), with initial mortalities occurring within the first few days due to transportation stress. Seed production trials for seahorse have begun.*

Keywords SEAFDEC-AQD, restocking, mud crab, abalone, giant clam, seahorse

INTRODUCTION

Natural populations of global inshore fishes, particularly invertebrates, are being overexploited due to increasing demand and prices (Pauly et al., 2002). This demand arises historically from food but is now amplified by the marine ornamental fish trade (Job, 2005; Pomeroy et al., 2006). This is further exacerbated by the degradation of their natural habitats (Brown and Day, 2002). Thus, fishery managers are struggling with the paradox of conservation while also meeting the increasing demand for marine resources. Marine Protected Areas (MPAs) are becoming a prominent tool to restore depressed natural stocks (Bohnsack, 2000; Rogers-Bennett and Pearse, 2001; Maliao et al., 2004) but may not suffice where recruitment overfishing occurs (Wallace, 1999). Stock enhancement of hatchery-reared seeds is perceived as an alternative strategy to enhance the regeneration process (Burton and Tegner, 2000), particularly for the conservation of threatened species (Flagg et al., 1995). Such a strategy is made possible by the development of technologies to mass produce juveniles in hatcheries (Bell and Gervis, 1999; Howell et al., 1999). There have been several attempts worldwide to bolster natural stocks through stock enhancement (Bartley, 1999; Fushimi, 2001; Brown and Day, 2002).

The importance of stock enhancement, particularly in Southeast Asia, was recognized by the 2000 Bangkok Declaration and Strategy (Network of Aquaculture Centres in Asia and the Pacific (NACA)/Food and Agriculture Organization of the United Nations (FAO), 2000). Similarly, the Conference on Sustainable Fisheries for Food Security in the New Millennium: "Fish for the People" organized by the Association of the Southeast Asian Nations (ASEAN) and the Southeast Asian Fisheries Development Center (SEAFDEC) in 2001 produced

Address correspondence to Ma. Junemie Hazel L. Lebata, Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo 5021, Philippines. E-mail: jlebata@aqd.seafdec.org.ph

a Plan of Action for Sustainable Fisheries for Food Security for the ASEAN region, in which stock enhancement is identified and adopted among the strategies for fisheries management (ASEAN/SEAFDEC, 2002). In 2005, SEAFDEC initiated a 5-year Program on Stock Enhancement for Threatened Species of International Concern (PSETSIC), under the Government of Japan Trust Fund to refine and/or develop aquaculture and release technologies for species identified as threatened. The Aquaculture Department (AQD) of SEAFDEC, based in Iloilo, Philippines, is tasked to implement the aquaculture-based stock enhancement component. Stock enhancement began at AQD in 1991 as part of the Community Fishery Management Project undertaken in Malalison Island, Culasi, Antique, west central Philippines (Amar et al., 1996). In July 2005, AQD convened participants from ASEAN-SEAFDEC member countries and SEAFDEC Departments in a Regional Technical Consultation in Iloilo City, Philippines, to identify suitable species for rehabilitation and to review existing technologies for stock enhancement. Among the species identified for stock enhancement were donkey's ear abalone (*Haliotis asinina*), giant clam (*Tridacna gigas*), sea cucumber (*Holothuria scabra*), and seahorses (*Hippocampus* spp.). PSETSIC will focus first on these threatened species with existing aquaculture technology but will include other species as the technology becomes available. In this paper, the output of stock enhancement activities of mud crabs, including the newly started PSETSIC of AQD on abalone and giant clam were reviewed. AQD's work in seahorse aquaculture is also reviewed.

SITE DESCRIPTION

AQD's stock enhancement projects are implemented in collaboration with local governments with experience in MPAs and sanctuaries. The initial release site for abalone and giant clam stock enhancement was the Sagay Marine Reserve (SMR). It is located at the northern tip of the island of Negros Occidental at 10°53′51′N lat. and 123°24′53′E long. (Figure 1). The SMR covers an area of 32,000 ha comprising coral reefs, seagrass beds, mangrove forests, sand cays, and islands. Presidential Proclamation No. 592 in 1995 made it part of the National Integrated Protected Area System of the Philippines and Republic Act No. 9106 of 2001 made protection of Sagay waters part of the law of the country. Protection of Carbin Reef as an MPA dates back to 1983 and strict round-the-clock monitoring by the sea patrol began in 1995 (Maliao et al., 2004; Webb et al., 2004). The specific

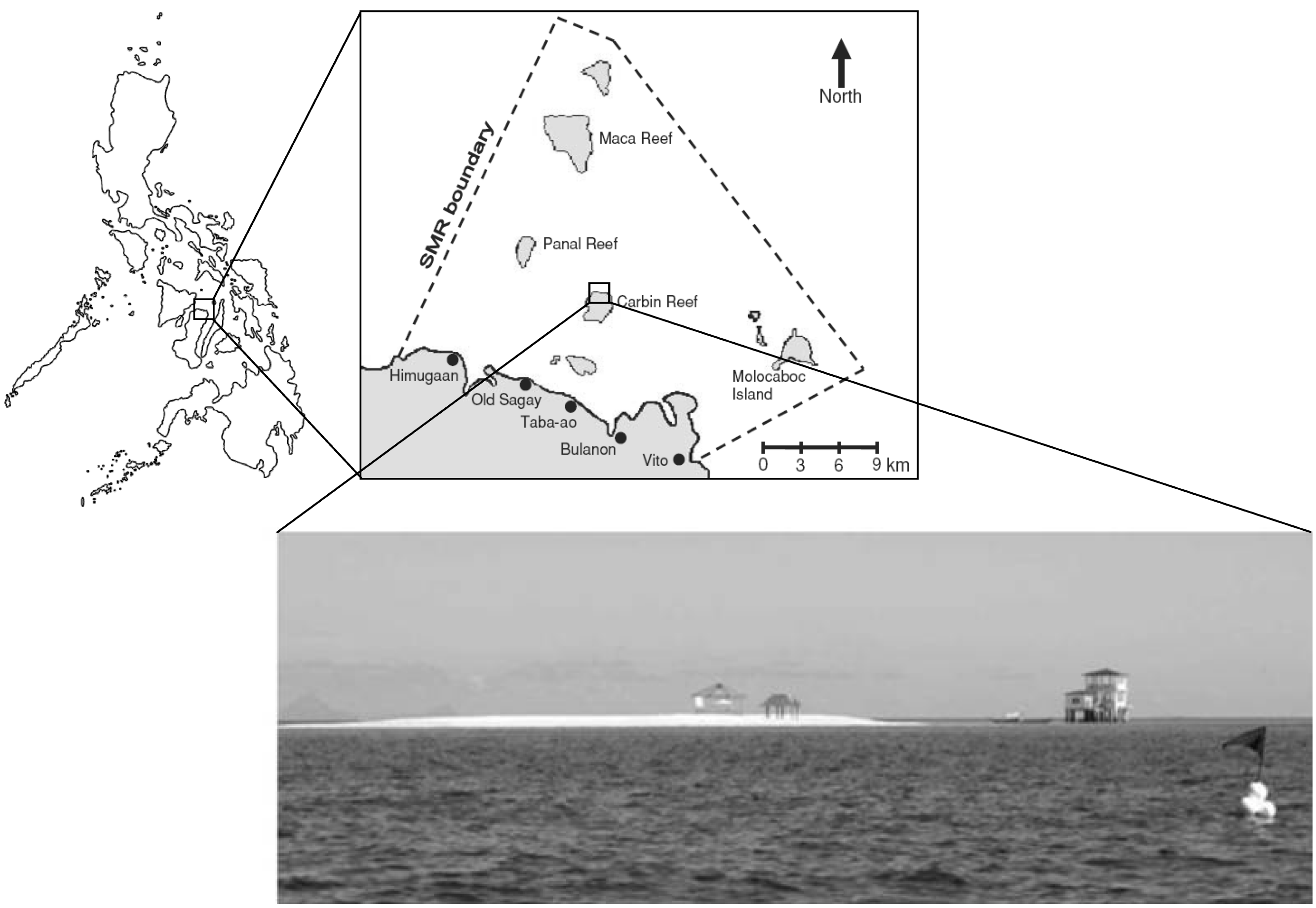

Figure 1 Map of the Philippines showing location of the Sagay Marine Reserve (SMR), Negros Occidental; inset is the Carbin Reef, the release site of both abalone and giant clam. Note the watchtower to the right and the white Styrofoam buoy with flag that serves as the boundary for the Carbin Reef sanctuary (modified from Maliao et al., 2004).

release site is the Carbin Reef, one of the four reefs in SMR and a no-take zone. It is about 200 ha and is a natural habitat of abalone (Maliao et al., 2004).

The mean density of abalone in SMR was 0.2 individuals/m^2 with size ranging from 2.0 to 8.0 cm shell length (SL) (Maliao et al., 2004). *H. ovina* was also present in Carbin Reef but at low density. Tridacnids *T. crocea* (mean density = 1 ind/m^2; mean SL = 10 cm; size range = 2–25 cm), *Hippopus hippopus* (0.4 individuals/m^2; 27 cm mean SL; 2–42 cm), *T. squamosa* (0.1 individuals/m^2; 24 cm mean SL; 6–45 cm), and *T. maxima* (only 1 found; 28 cm SL) were also found in Carbin Reef. *T. crocea* was the most abundant species which agreed with the previous results of Juinio et al. (1989).

The release site for *Scylla* spp. stock enhancement was the natural mangroves in the villages of Bugtong Bato and Naisud in Ibajay, Aklan province, Philippines. The study site is home to 27 of the 34 species of mangroves found in the Philippines (Primavera et al., 2004) and has been described in Lebata (2006).

ABALONE

Abalone (*Haliotis* spp.) are the most prized of the marine gastropods used for food (Bell et al., 2005). *H. asinina* has the potential to become an export earner for Australia and throughout Southeast Asia (Freeman, 2001). Several abalone fisheries worldwide have collapsed in recent years (Wallace, 1999; Shepherd and Rodda, 2001; Shepherd et al., 2001). Although total global abalone production has increased since 1994 and peaked in 2005 (31,296 mt), Philippine production has decreased annually (FAO, 2006). Records of abalone production in the Philippines date back to 1976 at 28 mt, peaked in 1995 at 483 mt (contributed 11% of Asian production) then experienced annual decreases until 2005 (146 mt) (FAO, 2006). The wild stocks of Philippine abalone are vulnerable to depletion primarily because the current fishery depends on them.

Abalone has some characteristics suitable for stock enhancement, such as high commercial value, high fecundity, relative ease in seed production and tagging, and semi-sedentary behavior. Furthermore, tropical abalone grows fast and can reach marketable size (5.5–6.5 cm SL) in a year compared to temperate species which take 3–4 years. Research and development of abalone at AQD started in 1993, completing the life cycle in 1997, and juveniles can now be mass produced for stock enhancement (Gallardo et al., 2003). All abalone seeded were the progeny of broodstock from the release site. Released abalone were previously fed with a specially formulated diet for 2–3 weeks to produce a 4 mm wide bluish-green shell band which serves as permanent marker (Figure 2). They were subsequently fed with brown seaweed (*Gracilariopsis heteroclada*) to produce the natural brownish shell (Gallardo et al., 2003).

Modules made from PVC (∼7 cm dia, 15 cm long), covered on both ends with a 3-mm mesh size net were used to transport *H. asinina* to the release site at 20 abalone per module. Modules were placed inside double-layered plastic bags (without seawater but with oxygen) and placed in styrofoam boxes with small packs of seawater ice to stabilize the temperature inside the box during transport.

Three size classes (A: 2.5–3.0, B: 3.5–4.0, C: 4.5–5.0 cm SL) of diet-tagged abalone juveniles (1,800–2,500 individuals/release) have been released in the SMR Carbin Reef in June and October 2002, April 2003, March and June 2004, and July 2006. Abalone were released along permanent transect lines (25 m long) to facilitate monitoring. Mortalities in the first few days were attributed to transportation stress and predation, mainly by reef crabs, factors also identified in stock enhancement of some fish species (Howell, 1994; Lepage et al., 2000; Svåsand et al., 2000). The importance of behavioral conditioning (food search capability and predator avoidance) of hatchery-bred animals before release to the wild has been discussed in several papers, including abalone (Schiel and Welden, 1987). Results of conditioning experiments of *H. asinina* on forage and avoidance of predation suggest that cultured abalone, particularly younger and smaller sizes (1.5–2.0 cm SL) may acclimate quickly to new environments if previously exposed to predators, corals, and seaweeds found in their natural habitat (Buen, 2005). Higher mortality was observed in bigger sizes (4.5–5.0 cm SL) compared to smaller ones (1.5–3.0 cm SL), which contradicts the general belief that survival is proportional to size (Brown and Day, 2002). This is probably because larger abalone are more visible to predators and tend to move farther away, in contrast to smaller abalone which are more cryptic and do not move far from the release point.

Recapture rates of 12.3% and 6.9% after 2 and 6 months, respectively, are lower than the reported 16–21% recapture rate after 6 months for *H. midae* in South Africa (Sweijd et al., 1998) but higher than the <1% recapture rate after 6 months for *H. rufescens* in Northern California (Rogers-Bennett and Pearse, 1998). For the temperate species *H. discus hannai* in Japan, the recapture rate was 5–10% after 2–3 years upon reaching marketable size (Masuda and Tsukamoto, 1998). The donkey's ear abalone did not reach market size in 6 months after release in the wild, and no data are available for recapture rates of market sizes. Therefore, it is necessary to estimate the economic viability of stock enhancement or sea ranching, including break-even expenses.

MUD CRAB

Mud crabs or mangrove crabs under the genus *Scylla* are large, edible crustaceans associated with mangrove swamps throughout the Indo-West Pacific region and are valuable components of coastal fisheries in many Southeast Asian countries (Overton et al., 1997; Le Vay, 2001). *Scylla* has four known species, *S. serrata*, *S. tranquebarica*, *S. olivacea,* and *S. paramamosain,* based on the classification of Keenan et al. (1998). Worldwide mud crab production was 146,253 mt in 2005 (FAO,

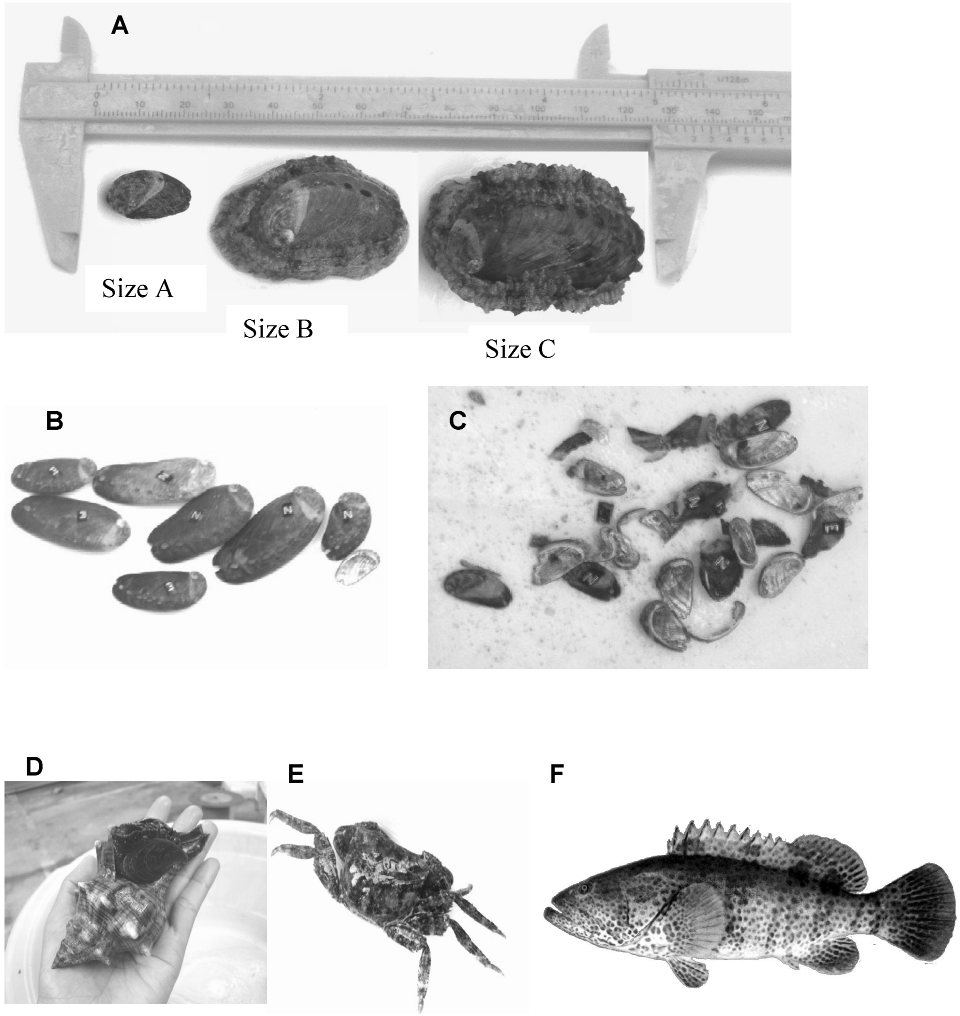

Figure 2 Three size classes of abalone: (A) size A (1.5–2.0 cm); size B (3.0–3.5 cm), and size C (4.5–5.0 cm). Note the bluish green band produced by feeding the abalone with artificial feed that provides a natural tag; tagged abalone (B); broken shells (C), and the predators used, namely *Pleuroploca trapezium* (D), *Metopograpsus latifrons* (E), and *Epinephelus coioides* (F).

2006). From 1950 to 1973, mud crabs came solely from Asia, and Asia provided 90–99% of total mud crab production from 1974 to 2005. Contribution of the Philippines to capture fisheries started in 1970 and peaked in 1992, with the Philippines contributing 41% of total mud crab production. However, a drastic drop was experienced from 1996 to 1997 (FAO, 2006). Mud crabs have been exploited traditionally in artisan fisheries targeting all size classes of crabs in areas where fishing is unregulated (Overton et al., 1997; Dat, 1999; Le Vay et al., 2001). The increasing demand coupled with rising prices subjects them to heavy fishing pressure (Fielder and Heasman, 1978). Moreover, the expanding export of mud crab as an alternative to shrimp has led to intensified harvesting, thus further threatening wild stocks (Quinitio et al., 2002). Important export markets of mud crabs are Malaysia, Singapore, Hong Kong, Taiwan, Japan, and the USA (Millamena et al., 2001).

Mud crab aquaculture has been practiced for many years in Southeast Asia based primarily on farming juveniles and fattening lean crabs. Due to the difficulty in obtaining wild seed for farming and the concerns of further exploitation, research to develop hatchery techniques began in the mid-1990s. Recent advances in *Scylla* spp. culture include the development of broodstock technology (Millamena and Quinitio, 2000), mass seed production (Quinitio et al., 2001), development of nursery technology (Ut et al., 2007), and the closure of the life cycle of *S. serrata* within 9 months (Quinitio et al., 2001). Hatchery technology has focused on *S. serrata* and *S. paramamosain*, with the Philippines and Vietnam producing hatchery crabs in commercial quantities for farming at present (Quinitio and Parado-Estepa, 2003; Lu, 2006).

Among the four species, *S. serrata* has the highest growth, rate attaining a maximum size of 25–28 cm carapace width (CW) (2–3 kg) in males compared with 20 cm CW for *S. tranquebarica* and *S. paramamosain* and 18.0 cm CW for *S. olivacea* (Carpenter and Niem, 1998). *S. serrata* (3.2–16.0 g body weight; 2.6–4.5 cm CW) grows to marketable size of $\geq$500 g in

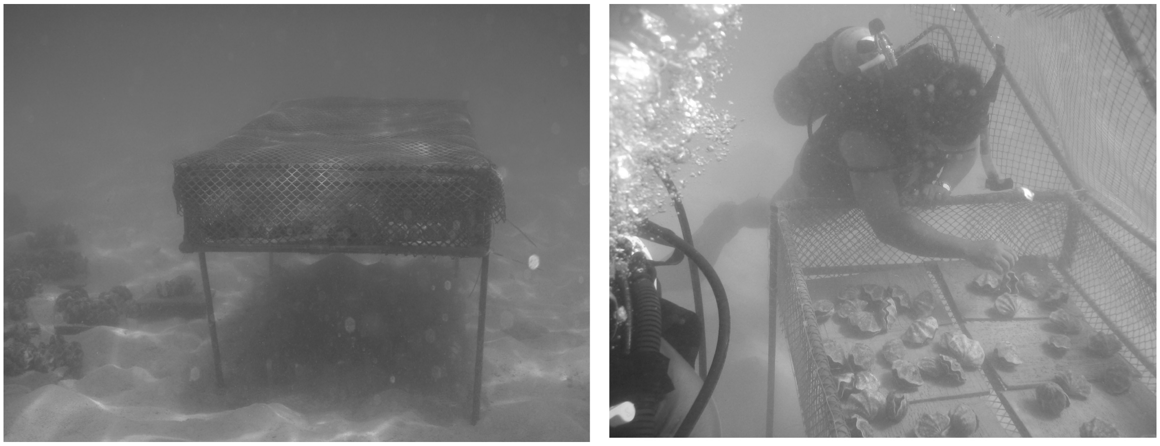

Figure 3 Underwater photographs showing the design of the giant clam (*Tridacna gigas*) cage (2.0 × 1.0 × 0.5 m) placed in Carbin Reef, Sagay Marine Reserve, Philippines. One hundred clams were stocked in each cage.

5–6 months. *S. tranquebarica* and *S. olivacea* of about the same size as *S. serrata* at stocking grow to marketable size of 250–350 g in 4–5 months.

Williams and Primavera (2001) noted the importance of stock enhancement in rehabilitating depleted stocks of the tropical portunid crabs including *Scylla* spp. Funded by the European Union through the European Commission, AQD became part of an international collaboration on the culture and management of *Scylla* species from 2001–2005. An integral part of this project was the stock enhancement of *Scylla* spp. with several releases of tagged *Scylla* spp. in the mangroves of Naisud and Bugtong Bato, Ibajay, Aklan, Philippines (Okuzawa et al., 2006).

S. olivacea, *S. serrata*, and *S. tranquebarica* used for stock enhancement trials were produced by the SEAFDEC/AQD Hatchery. Upon reaching a minimum size of 2 cm CW, crabs were tagged with coded wire tags and released in the mangroves. A total of 1,522 crabs have been released. Recapture of released crabs were monitored from the monthly catches of fishermen. Mud crabs were collected twice a month during spring tides for 4–5 days using baited traps. Crab landings of all crab fishers were monitored during each collection period. All crabs were measured for length and weight and checked for the presence of tags (Lebata, 2006). Of the crabs released, 45% of *S. olivacea*, 37% of *S. serrata*, and 34% of *S. tranquebarica* were recaptured. From June 2004 to April 2005, the percentage of recovered *Scylla* spp. in total monthly catches ranged from 11.9% to 59.2% (Okuzawa et al., 2006). Among these recaptures, crabs conditioned in the ponds before release had higher recapture rates than those released directly from the hatchery, suggesting the importance of conditioning hatchery-reared crabs before release (Lebata, 2006).

GIANT CLAMS

T. gigas is the most heavily exploited of all tridacnids and is virtually extinct in Philippine waters (Gomez and Mingoa-Licuanan, 2006) and in many parts of its entire distribution range in the Indo-Pacific (Bell, 1999a), placing it on the U.N. list of endangered species. Decline of giant clam populations is mainly due to overfishing for their highly prized abductor muscles, but subsistence harvesting is also considerable in the Indo-Pacific (Lewis et al., 1988; Mingoa-Licuanan and Gomez, 2002). Giant clam shells are also an important item for shell craft industry and for the aquarium trade, where they are highly valued, e.g., US$25–45 per *T. gigas* juvenile (Mingoa-Licuanan and Gomez, 2002).

The Philippines, primarily through the University of the Philippines Marine Science Institute (UPMSI), has the longest experience in giant clam restocking in Asia-Pacific that started in 1987 (Gomez and Alcala, 1988). With the identification of *T. gigas* among the species for stock enhancement by PSETSIC, AQD collaborated with UPMSI in 2005. In 2006, AQD stocked *T. gigas* juveniles in the SMR (600 juveniles of two size groups; 8 cm SL (300 individuals) and 10 cm SL (300 individuals) and Malalison Island sanctuary (500 individuals). Malalison Island is a small island of 65 ha at 11°25′N lat. and 122°01′E long., west central Philippines. The main objective of this ongoing study is to determine optimum size, season, and site of release.

The clams were transported from Bolinao to SMR via Manila, Philippines, by land from Bolinao to Manila (∼5 hr), by plane from Manila to Bacolod (1 hr), and by land from Bacolod to Sagay (∼3 hr). Total travel time was ∼15 hr including waiting at the airport. The clams were stocked in pre-established 2.0 × 1 × 0.5-m cages elevated 0.5 m from the bottom (Figure 3, Mingoa-Licuanan et al., 2000). Stocking density was 100 clams per cage to be thinned regularly (every 4 months). Each cage was set at no more than 5 m water depth to ensure high light incidence and good water circulation which affect clam survival and growth (Foyle et al., 1997; Hart et al., 1999).

Average monthly incremental growth of 8 cm and 10 cm clams was 0.80 cm and 0.85 cm, respectively (Figure 4). Growth rate was not statistically different between the two sizes (t-test; $p > 0.05$). Highest mortality occurred within the first 5 days post stocking which accounted for half of total mortality (10%) 4 month post stocking (Figure 4). The initial mortality was

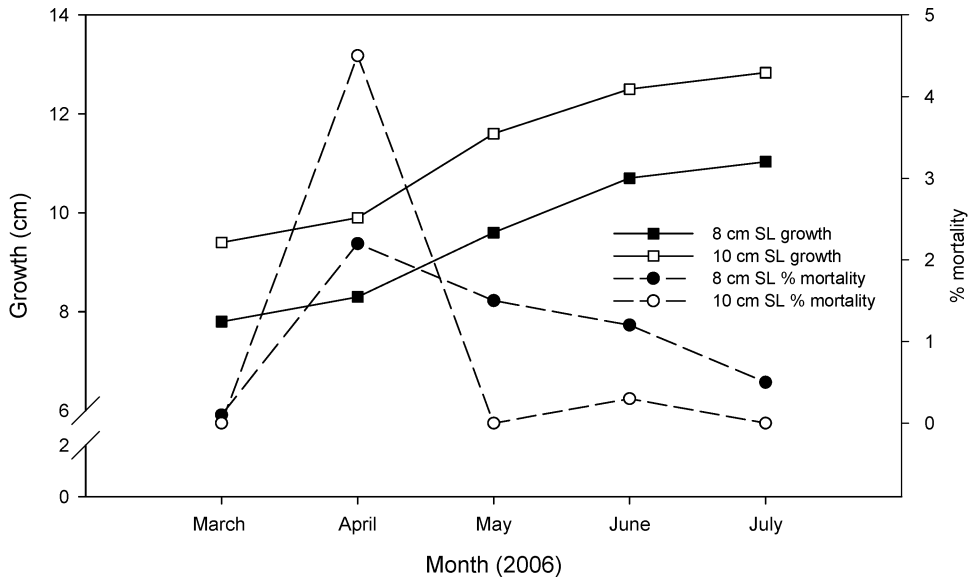

Figure 4 Monthly growth and mortality of giant clam, *Tridacna gigas*, stocked in the underwater cages (see Figure 3) placed in Carbin Reef, Sagay Marine Reserve, Philippines. Squares and circles indicate mean growth and mortality, respectively. Open squares and circles represent bigger clams (initial stocking shell length (SL) was 10 cm), and closed squares and circles represent smaller clams (initial stocking shell length (SL) was 8 cm).

due to transport stress as transport time exceeded 10 hr. Gomez and Mingoa-Licuanan (2006) reported that 99% survival can be achieved if transport time does not exceed 10 hr. The minimal subsequent mortalities were probably due to predatory attacks of octopus and eels. In Sagay, no ecto-parasitic snails were found inside the cages, although pyramedellid and ranellid snails were found in the reef. Ranellid (genus *Cymatium*) snails, which lodge between the shell and the mantle and consume the clam, are the most destructive predators of cultured giant clams (Govan et al., 1993). Calumpong (1992) reported that one adult/sub-adult *Cymatium* can kill 10 giant clam juveniles per week. All clams <17 cm SL are considered susceptible to *Cymatium* attack, but raising the cage from the bottom could reduce such attacks by 50% (Govan et al., 1993).

Restocking of hatchery-produced giant clams in the Philippines has waned, probably because husbandry is needed for around 3 yr before the clams attain ~25 cm SL and are no longer vulnerable to predators (Bell, 1999b). In most cases, the purpose of giant clam restocking is to rebuild locally extinct populations, not for fisheries but for conservation and ecotourism. Therefore, the ultimate criteria of restocking success is whether the released animals contribute to the genetic pool of the local population and whether their offspring are able to survive and reproduce, which may take decades because they require up to 9 yr to reach reproductive maturity. Nevertheless, cost-benefit analyses are needed to evaluate the economic feasibility of restocking.

SEAHORSES

The Indo-Pacific waters have around 70% of the total known species of seahorses worldwide (Lourie et al., 1999), many of whose wild populations are severely threatened by over-fishing (Perante et al., 2002). Vincent (1996) reported that Asian seahorse landings have decreased by 15–50% since 1990 and, in particular, the *Hippocampus comes* fishery in Bohol, Philippines, decreased by ~70% since 1985 (Perante et al., 2002). To regulate seahorse trade worldwide and reduce pressure on wild populations, seahorses are now listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Martin-Smith et al., 2004). Also, a minimum height limit of 10 cm for all fished seahorses is being proposed (Foster and Vincent, 2005).

AQD initiated seahorse breeding studies in 1996. By 1998, F_3 and F_1 generations were produced for *H. barbouri* and *H. kuda*, respectively, and in 2003, F_6 generation was obtained for *H. kuda* (Figure 5). Mating pairs of *H. kuda* breed year-round. Parturition frequency appeared to peak from February to April, when seawater temperature and daylight period increased, but declined from November to January when seawater temperature and daylight period decreased. *H. kuda* produced broods of up to 1,751 juveniles, with mean brood size of 660 juveniles ($n = 210$ parturition events) and a parturition interval of 12 days. Brood size of *H. kuda* was the highest documented, while the size at birth falls in the lower range compared to *H. abdominalis*, *H. barbouri*, and *H. guttulatus*, suggesting a trade-off between high fertility and large-sized young. Survival of juveniles, however, was highly variable (0–99% up to 3 months), perhaps due to brood quality since maternal diet influences egg quality and subsequent juvenile survivorship (Chambers et al., 1989; Wootton, 1998). Nevertheless, the study has demonstrated the year-round viability of captive propagation of these threatened species.

After parturition, juvenile seahorses stay in the water column until day 7 of rearing. Newly-born *H. kuda* preferred copepods (mostly *Pseudodiaptomus annandalei* and *Acartia tsuensis*) over

A. *H. barbouri* B. *H. comes* C. *H. kuda*

Figure 5 Three species of seahorse cultured at SEAFDEC/AQD: (A) *Hippocampus barbouri*, (B) *H. comes,* and (C) *H. kuda*.

rotifers (*Brachionus rotundiformis*). An increase in body weight was highest (5% per day) and mortality rate lowest (9% at day 10) in seahorses fed with a combination of copepods and rotifers. Seahorses fed with rotifers alone had the slowest growth (0.3% per day) and highest mortality (60% at day 7), indicating that copepods are more suitable food for seahorse juveniles and that the availability of diverse food organisms in the tank improves survival and growth of captive *H. kuda*. Similarly, newly born *H. sublongatus* reared on cultured copepod nauplii had greater growth and survival than those reared on brine shrimp nauplii (Payne and Rippingle, 2000). Moreover, fluctuating salinities characterize the habitat of most seahorses. The optimum salinity for survival and growth of *H. kuda* juveniles was 15–30 ppt (Hilomen-Garcia et al., 2003).

CONCLUSIONS

The basic technology for seed production of abalone, mud crab, and seahorse is already established at AQD. The technology of giant clam hatchery aquaculture for mass production is also available. However, some techniques need further refinement. Survival rates of juveniles are low due to cannibalism and lack of suitable feeds for mud crab and seahorse, respectively. Studies are needed to address the behavioral, physical, and physiological deficits of hatchery-bred organisms and maximize post-release survival. This includes culture schemes that best mimic the natural environment of the animals and promote food search capability and predator avoidance responses. As the seeded animals need protection before they can reproduce, the best strategy is to couple stock enhancement programs with established MPAs that have strong community support.

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