The SEAFDEC/AQD Experience in Stock Enhancement

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Introduction

Populations of some aquatic animals such as sea turtles, humphead wrasse *(Cheilinus undulates)*, seahorses *(Hippocampus* spp.*)* sharks and rays, sea cucumbers (*Holothuria* spp.), giant clams (particularly the true giant clam *Tridacna gigas*) and abalone (*Haliotis* spp.) which provide livelihood for local fisherfolk have become highly depleted or threatened due to destruction of habitat and/or overexploitation. Therefore we must seek sustainable management of natural stocks of these species. Stock enhancement is a well known method of replenishing depleted stocks.

The Aquaculture Department (AQD) of the Southeast Asian Fisheries Development Center (SEAFDEC) started stock enhancement activities in 2000 as part of the Coastal Fishery Management Project in Malalison Is., Culasi, Antique, Philippines (SEAFDEC/AQD 1998). This was the same year as the Bangkok Declaration and Strategy for Aquaculture Development (NACA/FAO 2000), which affirmed the potential of stock enhancement to increase fish supply. Since then, research on seed production, and release and monitoring strategies has been initiated on the abalone *Haliotis asinina*, seahorses *Hippocampus barbouri* and *H. kuda*, mud crabs *Scylla serrata*, *S. olivacea* and *S. tranquebarica*, top shell *Trochus niloticus*, and window-pane oyster *Placuna placenta*. Closing the life cycle and mass production of juveniles have been attained for most of these species, but actual releases have been conducted only for abalone and mud crabs. In this review article, we describe the present situation of stock enhancement of abalone, mud crab and seahorse at AQD.

Abalone

Research and development of abalone at AQD started in 1994. Around 10,000-47,000 juveniles have been produced yearly since 1998. A diet-based bluish-green shell band was used to mark later batches, and exposure to predators and natural food provided behavioral conditioning. Packing and transport techniques have been refined using polyethylene vinyl chloride (PVC) pipes in styrofoam boxes. Releases in 2002-2004 in the Sagay Marine Reserve (SMR), Negros Occidental show recapture rates of 4.2% after one mo, and up to 6.9% after 6 mo (Gallardo et al unpub. manuscript).

Stock assessment and marking of release site

Existing abalone stocks in the release site were assessed by counting abalones found along a 1-m band on both sides of a 25-m transect line (belt-transect method) and measuring their sizes. Species composition and abundance of seaweeds, crabs, mollusks and fishes particularly carnivorous species were determined using transects and quadrats (Gallardo et al unpub. manuscript). This assessment revealed that the intertidal Carbin Reef in SMR is a natural abalone habitat suitable for release; it is exposed during the lowest tides. Points for deployment of release modules were marked using PVC plates and numbered PVC pipes along a 25-m nylon rope.

Seed production

Juvenile abalone of 10-12 mm shell length (SL) were fed a SEAFDEC/AQD–formulated diet for 2-3 weeks to produce a small bluish-green shell band which served as permanent marker (Gallardo et al 2003). They were subsequently fed the brown seaweed *Gracilariopsis bailinae* to produce the natural brownish shell after formation of a bluish-green band 6 mm wide. The colored band served as natural marker.

Three to four days before release, abalones were trained to forage for food among naturally growing seaweeds or an added supply of the seaweed *G. bailinae*, and to escape predators by stocking them with the crab *Metopograpsus latifrons*, fish *Epinephelus coioides*, and gastropod *Pleuroploca trapezium*, and with shelters (Schiel and Welden 1987, Brown and Day 2002, Buen 2005) either in flow-through outdoor tanks or in indoor tanks.

Packing and transport

Prior to transport, juveniles were tagged individually with numbered, color-coded (by size group) Dymo tape glued to the shell for monitoring. Shell length, body weight and gonadal stage were recorded. Samples of the epipodia of foot muscle were taken from each released abalone and preserved for DNA analysis to determine if future juveniles in the release sites are their offspring. After tagging and sampling, 20 abalones per size group were placed in the numbered release modules stocked in flow-through tanks. The modules were made of 5.1 or 7.6 cm diameter x 15.2 cm long PVC pipes covered on both ends with mesh net. The PVC pipes containing abalones were placed inside double-layered plastic bags without seawater but provided with oxygen and placed in styrofoam boxes with small packs of seawater ice to maintain low inside temperature. Abalone survival within 8 h of transport was 100% as in the June 2002, October 2002 and March 2004 batches. When transport time exceeded 8 h, mortalities occurred, e.g., the April 2003 batch had 3.5% mortality after 9 h, maybe due to oxygen depletion and temperature increase (Gallardo et al unpub. manuscript).

Release and monitoring

In June 2002, a total of 1,800 diet-tagged abalones of four sizes (SL 1.5, 2.5, 3.5-4 and 4.5-5 cm), at 450 abalones per size group were released in the SMR Carbin Reef during neap tide. Upon arrival, the PVC pipes were sprinkled with seawater from the release site to acclimate the abalones. For protection from predators, the pipes containing all abalone sizes were placed inside net bags with bottom holes to allow exit of abalones (McCormick et al 1994). Each release module (comprising 5 pipes per net bag) was tied to the corresponding number of PVC pipe along the nylon rope within the plot. The nets covering both ends of the pipe were removed to release the abalones. The net bags containing pipes prevented predators from directly entering the pipes thereby protecting the abalones. Monitoring was carried out immediately after module deployment and twice daily for 3 days after release. The distance from release point to where the abalones were sighted was measured using a tape; dead abalones and empty shells were collected and recorded. Based on higher survival of medium-sized abalones in the June 2002 release, 3-cm SL juveniles were used for releases in October 2002, April 2003 and March 2004 in different sites of Carbin Reef with 2,500 pieces per release.

Exit from release modules, dispersal and survival after release

The released abalones were monitored from the release point without destroying or turning over corals and rocks. In June 2002, the abalones left the release modules within the first 2 h. Only 2.3% remained in the modules by the third day. Dispersal from the release point was within 1 m, except for some large abalones found 4 m from the

Table 1. Dispersal distance (means and ranges) of abalone *Haliotis asinina* released in Carbin Reef, Sagay Marine Reserve, Negros Occ., Philippines in June 2002.

Size group (shell length)	**Distance from release point (cm)**		
	Day 1	Day 2	Day 3
Small (2.5-3.0 cm)	63.7 (23-143)	29.7 (16-51)	54.6 (16-120)
Medium (3.5-4.0 cm)	61.2 (5-400)	64.0 (5-400)	92.7 (10-234)
Large (4.5-5.0 cm)	72.6 (25-400)	40.0 (21-100)	131.9 (8-400)

release point (Table 1). In the October 2002 batch, almost all of the abalones left the modules by day 3 but remained within 1 m from the release point. In the April 2003 and March 2004 batches, small abalones left earlier but did not disperse as widely as the medium and large ones (Table 2).

In the June 2002 batch, 27 pieces of whole and broken shells (1.5% of total released [27/1800=0.015]) were found (Table 3). Whole shells probably came from abalones that died from handling stress and/or predation by carnivorous fishes and gastropods, whereas broken shells were due to reef crabs which prey on abalone (Tegner and Butler 1985). Crab predation can be recognized by the chipped margin of abalone shells (Shepherd and Breen 1992). After one month, a total of 52 empty shells were found of which 53.8% were large (4.5-5.0 cm SL), perhaps because the bigger ones have greater exposure to predators being more visible with a tendency to move farther away. The smaller abalones have higher survival because they are more cryptic and do not move far from the release point. This is confirmed by results of the October 2002

Table 2. Dispersal distance (means and ranges) of abalone *Haliotis asinina* released in Carbin Reef, Sagay Marine Reserve, Negros Occ., Philippines in April 2003 and March 2004.

Size group (shell length)	**Distance from release point (cm)**							
	April 2003*				March 2004*			
	Mean (±SD)	Range	N	%	Mean (±S.D)	Range	N	%
Very small (1.5-2.0 cm)	23.6 (4.6)	20-35	11	12.94				
Small (2.5-3.0 cm)	42.8 (38.1)	0-13.5	18	21.18	29.9 (18.2)	10-55	8	18.18
Medium (3.5-4.0 cm)	92.0 (76.6)	5-300	36	42.35	83.6 (131.5)	0-500	12	27.27
Large (4.5-5.0 cm)	137.4 (105.1)	5-383	20	23.53	145.6 (159.0)	0-770	24	54.54
Total			85				44	

*2,500 abalones per release

Table 3. Post-release mortalities of abalone *Haliotis asinina* in Carbin Reef, Sagay Marine Reserve, Negros Occ., Philippines in June 2002, April 2003 and March 2004.

Size group (shell length)	June 2002*				April 2003**	March 2004*			
	Day				Day	Day			
	1	2	3	Total	3	1	2	3	Total
Extra small (1.5-2.0 cm)									
Whole shell					1				
Broken shell					0				
Small (2.5-3.0 cm)									
Whole shell	1	1	2	4	0	0	0	0	0
Broken shell	1	0	0	1	1	0	6	1	7
Medium (3.5-4.0 cm)									
Whole shell	2	2	2	6	6	0	0	2	2
Broken shell	2	0	1	3	0	0	1	5	6
Large (4.5-5.0 cm)									
Whole shell	2	2	4	8	14	0	0	5	5
Broken shell	3	0	2	5	0	0	2	2	4
Total	11	5	11	27	22	0	9	15	24

*Monitoring from Day 1 to Day 3
**Monitoring on Day 3 only

release using 3-cm SL juveniles and the April 2003 and March 2004 (Table 3) releases using different sizes.

Recapture rates

One month after release of the June 2002 batch, 77 juveniles (38 large, 25 medium, and 14 small) or 4.2% of total releases were seen within 5 m from the release point. The total number could be higher with more intensive search, i.e., by turning over corals and rocks. Subsequent monitoring of the June 2002 and October 2002 releases was limited to 5 m from each side of the release point to avoid destroying corals in the marine reserve. Recapture rates were 12.3% and 6.9% after 2 and 6 mo, respectively. These figures are lower than the reported 16-21% recapture rate after 6 mo for *Haliotis midae* in South Africa (Sweijd et al 1998) but higher than the <1% recapture rate after 6 mo for *H. rufescens* in Northern California (Rogers-Bennett and Pearse 1998). For the temperate *H. discus hannai* in Japan, the recapture rate is 5-10% after 2-3 yr upon reaching marketable size (Masuda and Tsukamoto 1998).

Mud Crabs

Mud crabs of the genus *Scylla* are highly prized as food, and therefore subject to heavy fishing pressure all over Asia and Australia. One of the three principal strategies to replenish

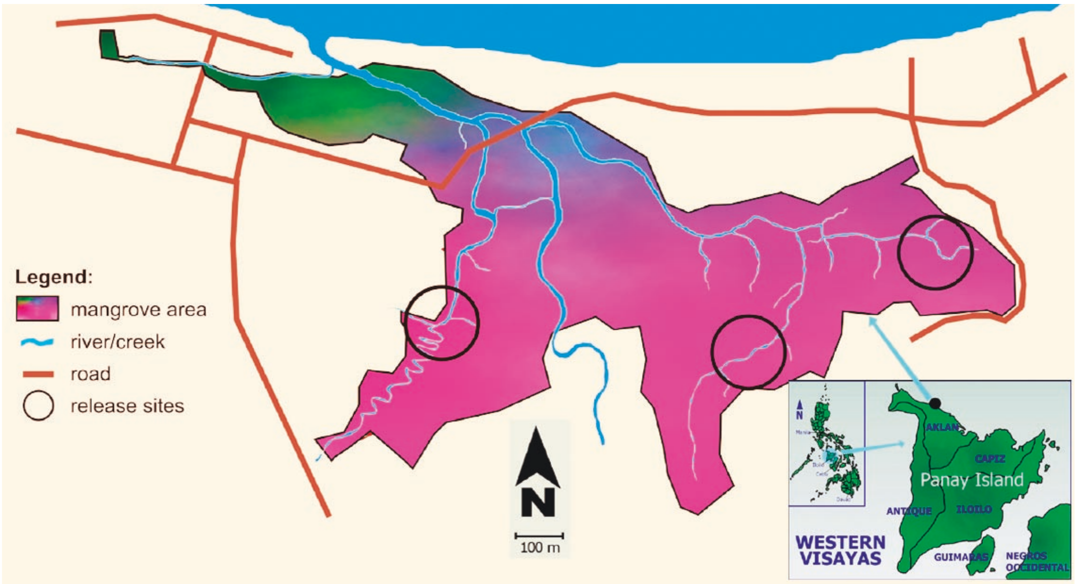

Fig. 1. Release sites of tagged crabs for stock enhancement trials in the mangroves of Naisud and Bugtong Bato, Ibajay, Aklan, central Philippines.

depleted stocks and manage fishery yield is to increase recruitment through propagation and release, that is, through stock enhancement (Blankenship and Leber 1995), hence this study was conducted.

Seed production

Zoeae were reared in 1-ton and 10-ton tanks at 60-80/L and fed rotifers and *Artemia* (Quinitio and Parado-Estepa 2003). Water exchange was 30-50% depending on the water quality. Megalopae were produced in 15-18 days and crab instar (C) in 21-23 days after hatching. Megalopae or crab instar were further grown to ≥2 cm carapace width (CW) in 10-ton concrete tanks or in net cages installed in earthen brackishwater ponds. Crabs were fed fish and mollusks. The crabs were then released directly in ponds for another 3-4 weeks or until they reached the desired size of 3-6 cm CW for stock enhancement.

Tagging and transport

One day before release, crabs were tagged with a coded microwire tag at the base of the left third walking leg or pereopod. Released crabs measured 30.0-79.9 mm CW, excluding mature females. They were transported in native palm bags to the release site; travel time was approximately one hour. First release was on 4 May 2004. Monitoring of tagged crabs started during the 18-21 June 2004 spring tide and every spring tide thereafter.

Hatchery–produced *S. serrata* and *S. tranquebarica* were obtained from the Crustacean Hatchery of SEAFDEC/AQD. Crabs were reared individually in perforated plastic containers altogether placed in concrete tanks provided with filtered seawater. Juvenile crabs were tagged for release at a minimum size of 30 mm CW. Crabs were measured, sorted and grouped into size classes from 30.0 to 79.9 mm CW at 4.9 mm intervals and placed in containers; mature females were excluded. Each crab was injected at the base of the third walking leg on the left side. Tagging was done in the hatchery a day before release. Tagged crabs were placed in containers and transported the following day to the release site, approximately 210 km from the hatchery.

Table 4. Mud crabs *Scylla olivacea, S. serrata* and *S. tranquebarica* caught in Ibajay, Akan, central Philippines in June 2004–April 2005.

Month	No. crabs caught	% crabs tagged (cumulative rate)
Jun 2004	651	11.52
Jul 2004	505	25.74
Aug 2004	364	27.47
Sep 2004	179	34.64
Oct 2004	252	21.83
Nov 2004	332	33.73
Dec 2004	403	38.96
Jan 2005	402	33.58
Feb 2005	599	32.89
Mar 2005	576	47.92
Apr 2005	427	59.25

Stock enhancement

Stock enhancement trials were conducted in a natural mangrove stand 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 (Agbayani et al 2000, Primavera et al 2004). Three release sites located at the end of each of the major branches of the Naisud river system were chosen for this activity (Fig. 1). Release of crabs was done during the spring tide of each month from May 2004 to April 2005, except in October 2004. Three species of crabs were released – *S. olivacea* from the replanted mangroves of New Buswang, Kalibo Aklan; and *S. serrata* and *S. tranquebarica* from the Crustacean Hatchery of SEAFDEC/AQD.

A total of 5,161 *Scylla* spp. have been released, comprising 34.66% wild *S. olivacea*, 58.86% hatchery-reared *S. serrata*, and 6.47% hatchery-reared *S. tranquebarica*. Of the 3,040 *S. serrata*, 60% were released straight from the hatchery while 40% were conditioned for at least one month prior to stocking in nursery ponds of the Dumangas Brackishwater Station of SEAFDEC/AQD. Conditioning was done by stocking crabs in ponds allowing exposure to scavenging, competition, predation and variations in environmental conditions.

Scylla spp. landings showed increasing retrieval of tagged crabs from 11.5% in June 2004 to 59.3 in April 2005 (Table 4). From June 2004 to April 2005, one third of total catches were tagged crabs (1,552 of 4,690 total) of which 52.1% were *S. olivacea*, 40.7% *S. serrata* and 7.2% *S. tranquebarica* (Table 5). Recapture rate of tagged crabs was highest for

Table 5. Mud crabs *Scylla* spp. released and recaptured in Ibajay, Aklan, central Philippines.

Species/source	No. of crabs tagged, released (% of total)	No. (%) of tagged crabs recaptured	Recapture rate (%)
Scylla olivacea/Wild	1,789 (34.66)	809 (52.12)	45.22
Scylla serrata/Hatchery	3,038 (58.86)	631 (40.66)	20.77
Scylla tranquebarica/Hatchery	334 (6.47)	112 (7.22)	33.53
Total	5,161 (100.00)	1,552 (100.00)	

Tags have been read but not yet analyzed as to batch released.

S. olivacea (45.2%), followed by *S. tranquebarica* (33.5%) and lowest for *S. serrata* (20.8%). Preconditioned *S. serrata* had higher recapture rates (mean=37.1%) compared to non-conditioned crabs (mean=10.2%). Conditioning hatchery-reared crabs has been shown to improve survival in the wild. Hatchery-reared, conditioned crabs had higher recovery and growth rates compared to hatchery-reared, non-conditioned ones, and even comparable to wild-released crabs in the case of *S. olivacea*. Crabs conditioned in the pond had been exposed to competition, cannibalism, temperature and salinity fluctuations, and had experienced foraging for food. There is evidence that performance and survival of hatchery-reared animals can be improved to the same level as their wild counterparts by conditioning steps in the hatchery prior to release in natural habitats (Davis et al 2005). Conditioning has likewise proven to be effective in increasing fitness of hatchery-reared *Homarus gammarus* (Wickens 1986, van der Meeren 2001) and *Callinectes sapidus* (Davis et al 2005). The results of this study show the importance of conditioning in improving success and efficiency of stock enhancement by compensating for deficiencies in hatchery-reared organisms.

Growth was also compared between species and between sources of juveniles to identify suitable species for a given area and suitable source of stock for future resource management and stock enhancement programs. Regardless of source, *S. olivacea* showed the fastest growth rate and *S. serrata* the slowest; *S. tranquebarica* had an intermediate rate. The observed growth rates of wild-released *S. olivacea* are comparable to those for the same species cultured in ponds (0.12-0.25 mm/d) (Fortes 1999). However, growth rates obtained for all species regardless of source were lower compared to the growth rates obtained by Triño et al (1999) for pond culture of mixed *S. serrata* and *S. tranquebarica* (1.1 mm/d) and Walton et al (2006) for wild *S. paramamosain* (0.67 mm/d).

Seahorses

Fisheries

Seahorses are one of the most heavily exploited groups in the Philippines. The seahorse fishery (primarily *Hippocampus comes*) in northwestern Bohol has declined to 70% since 1985 (A. Vincent, unpub. data as cited by Perante et al 2002, Vincent and Koldeway, this volume). Other existing seahorse fishery grounds in Palawan, Panay and parts of Mindanao remain unassessed.

Seahorses are caught using small nets or collected as by-catch in trawls and seine nets. They are traded in dried form for traditional Chinese medicine, tonic food and curiosities; and as live animals for the ornamental fish industry (Lourie et al 1999). Seahorses are exported to North America, Europe and Japan (Vincent 1996). Due to increased fishing effort, fishers claim that seahorse catches have declined significantly since 1985 (Vincent 1996). Many species of seahorses are listed as Vulnerable in the 2000 Red List of Threatened Species of the World Conservation Union (Hilton-Taylor 2000), and all species of genus *Hippocampus* are listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora in 2002.

Resource management

Established in 1996, Project Seahorse is an integrated program of conservation and management initiatives. It includes community-based conservation in fishing villages, e.g., establishment of no-take marine sanctuaries, fisheries modification and management, enforcement of legislation, education programs and development of alternative livelihoods for seahorse fishers (Lourie et al 1999).

Breeding and seed production

The SEAFDEC/AQD initiated seahorse breeding and seed production studies in 1995. By 2000, the F_3 and F_2 generation had been attained for *H. barbouri* and *H. kuda*, respectively.

Mating pairs of *H. kuda* continually reproduced throughout the year, but parturition frequency appeared to peak during February to April when seawater temperature and photoperiod started to increase, and decreased during low seawater temperature and short photoperiod in November to January. *H. kuda* produced broods of up to 1,751 juveniles and a parturition interval of 12 d (Hilomen-Garcia and Garcia 2004). Survival rate of a brood could reach 99% in 10 days, demonstrating a high potential for seed production. Mating pairs of *H. kuda* and *H. barbouri* offered either DHA Selco-enriched *Artemia* adults, mysids, or their combination had comparable frequency of parturition and produced similar brood size and stretched height of newly born juveniles. Likewise, survival rates on day 10 were comparable (SEAFDEC/AQD 2000).

Newly-born *H. kuda* offered a variety of food organisms preferred copepods (mostly *Pseudodiaptomus annandalei* and *Acartia tsuensis*) to rotifers (*Brachionus rotundiformis*)(Celino 2000). Size and amount of food ingested increased as seahorses grew. Increase in body weight was highest (5% per day) and mortality rate lowest (9% on day 10) in seahorses fed a combination of copepods and rotifers. Seahorses fed rotifers alone had the slowest growth rate (0.3% per day) and highest mortality rate (60% on day 7). The results indicate that copepods are suitable food organisms for seahorse juveniles but availability of a diversity of food organisms in the tank improves survival and growth of *H. kuda* in captivity.

Nine week-old hatchery-reared *H. kuda* transferred from ambient seawater (32-33 ppt) to salinities ranging from freshwater to 85 ppt were able to survive at 15 ppt for at least 18 days without affecting growth and survival (Hilomen-Garcia et al 2003). Mortality occurred within 4-24 h in seahorses abruptly transferred to freshwater, while survival of 5 ppt-reared seahorses decreased to about 65% in 18 days; and the upper limit of salinity tolerance was 50 ppt. The best stocking density for newly born *H. kuda* in tanks was noted to be ≥5 seahorse/L and one seahorse/L on day 20 (SEAFDEC/AQD 1999).

Nursery and grow-out

To continue the existing hatchery protocols for mass production of *H. kuda* for trade and possible stock enhancement, *H. kuda* juveniles were reared in illuminated marine cages (Garcia and Hilomen-Garcia unpub. manuscript). One group each of juvenile seahorses was fed *Acetes* in lighted and unlighted marine cages while a third group in lighted cages was not fed. After 10-12 weeks, mean body weight and stretch height increased in all treatment groups, with juveniles fed *Acetes* in lighted cages showing greater sizes than the two other treatment groups. The results indicate that *H. kuda* juveniles may be grown in lighted cages with *Acetes* to supplement the natural diet of zooplankton (attracted by light).

Conclusions

The basic technology for seed production of abalone, mud crab and seahorse is already established at AQD. However, some techniques need further development. Survival rates of juveniles are low due to cannibalism and lack of suitable feeds for mud crab and seahorse, respectively. In addition, more intensive studies on release strategies such as tagging and monitoring are needed. Social preparation of local communities that are the actual managers in the release sites is also required. To solve these problems, the SEAFDEC/AQD is implementing the project on Stock Enhancement of Threatened Species funded by the Government of Japan.

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