Giant Clam Hatchery, Ocean Nursery and Stock Enhancement

S. Suzanne Mingoa-Licuanan
Edgardo D. Gomez

SEAFDEC

Aquaculture Department
Southeast Asian Fisheries Development Center

Government of Japan Trust Fund

University of the Philippines
Marine Science Institute

MSI

Department of Agriculture
Bureau of Agricultural Research

Giant Clam Hatchery, Ocean Nursery and Stock Enhancement

S. Suzanne Mingoa-Licuanan
Edgardo D. Gomez

Giant Clam Hatchery, Ocean Nursery
and Stock Enhancement

FOREWORD

The program of SEAFDEC on stock enhancement is regional in nature, covering ASEAN member-countries. And with SEAFDEC Aquaculture Department (AQD) in the Philippines tasked to take the lead, the program was launched during the *ASEAN-SEAFDEC Conference on Sustainable Fisheries for the New Millennium* in November 2001 in Bangkok, Thailand.

AQD immediately incorporated stock enhancement in its R&D program in 2001, starting with refinement of seed production techniques for abalone (*Haliotis asinina*), seahorses, and top shell (*Trochus niloticus*). Two studies were supported by the International Foundation for Science (IFS) (on the impacts of marine protected areas and artificial reefs on coral reef fisheries) and ICLARM/WorldFish Centre (on co-management to determine a community's preparedness for resource management interventions such as stock enhancement).

In 2002, seahorse culture trials were expanded to seacages in preparation for stock enhancement while giant clam, abalone, and topshell (all hatchery-reared) were released and monitored in the wild to study release strategies. A technique for diet-tagging of abalone (which leaves a green mark on the shell) was by then developed; this is a convenient method to identify enhanced stock vis-à-vis wild ones. AQD also published a flyer on "Protecting livelihood through stock enhancement" which is downloadable from www.seafdec.org.ph/

In 2003, potential sites for release and stock enhancement of abalone, top shell and seahorse were assessed. AQD also conducted a training program on stock enhancement for BFAR's fisheries resource management project personnel. Meanwhile, IFS granted another study on the release strategies for stock enhancement of abalone in Carbin Reef at the Sagay Marine Reserve (SMR), Negros Occidental, west central Philippines.

Finally in 2005, a new program prioritizes and consolidates stock enhancement-related studies at AQD. SMR is now one of the project sites of this GOJ Trust-funded program *Stock enhancement for threatened species under international concern*. The program is managed by AQD's Deputy Chief, firstly by Dr. Koichi Okuzawa (2005-2007) and secondly by Dr. Hiroshi Ogata (2007-present). The program has had prioritized giant clam, abalone, and seahorse for stock enhancement; it later added angel wing (or diwal, *Pholas*), nylon shell and Napoleon wrasse which are endangered in Philippine waters.

With regard to giant clam, AQD is proud to have world renowned experts of MSI or the U.P. Marine Science Institute collate their decades of experience into this manual on hatchery, ocean nursery and stock enhancement. As is often said, there's no need to re-invent the wheel; thus, we hope to build on their knowledge and contribute significantly to resource enhancement in the Southeast Asian region.

JOEBERT D. TOLEDO, D Agr
Chief, SEAFDEC Aquaculture Department

CONTENTS

ASEAN-SEAFDEC
10-point recommendations with regards to resource enhancement

1 Make measures to restore critical inshore habitats, which have been extensively degraded by various human activities

2 Assess the feasibility and environmental impact of artificial reefs and other man-made structures in inshore waters with respect to resource enhancement and coastal zone management objectives

3 Promote re-stocking activities (seed release programs) from hatchery-produced stocks and/or wild collected sources in areas where they are considered to be feasible particularly localities operating within the regime of rights-based fisheries

4 Further encourage a culture-based fisheries program in inland waters where favorable exploitation patterns and traditional management mechanisms prevail

5 Enhance marine engineering capabilities to address the physical constraints in the construction, installation and placement of resource enhancement structures

6 Note that the implementation of rights-based fisheries, more specifically exclusive fishing rights, and the enhancement of inshore habitats by expanded artificial reefs are prerequisite for the successful implementation of a restocking program

7 Conduct research on the release species' potential recapture rate and impact on the ecosystem

8 Ensure optimal recapture of the released stock through effective management measures, including predator control

9 Develop marine parks in limited areas such as coral reefs to protect fragile coastal ecosystems, given that the establishment of marine protected areas is not feasible in the region due to their negative social impacts and enforcement problems

10 Promote seasonal closure of specific areas to protect spawners and juveniles of certain commercial-valued species under rights-based fisheries management, as an alternative measure to marine protected areas

PREFACE

This manual is meant to serve as a guide to the culture of giant clams (Bivalvia, Subfamily Tridacninae). For more than 50 years, giant clam mariculture has been demonstrated by different researchers and mariculturists. In the Philippines, University Prof. *Emeritus* Edgardo D. Gomez pioneered research on giant clams, establishing an early collaboration with Prof. Angel C. Alcala (Silliman University). For more than twenty years, E.D. Gomez's unwavering vision to return giant clams back to Philippine waters was the steady beam of light that guided giant clam mariculture and restocking program in the country.

We have culled the relevant information borne out of our more than 20 years of experience in making giant clam culture work in a developing country. The minimum requirements of setting up a hatchery and associated laboratory, as well as an ocean nursery, are described here. The methodologies may further be refined with better equipment, granted the availability of high-end technology.

The interest from various sectors in giant clam culture has led us to write this manual in a form that answers the needs of technical and lay people. Those with basic biology/ chemistry background, or some laboratory experience should be able to follow this manual. We begin this manual with an introductory discussion on species identification; a dichotomous key for all nine reported species based on shell characteristics; their global distribution; a description of the species' natural habitats; and notes on the clam-alga symbiosis.

The first part focuses on hatchery methods. The first four Sections are discussions on giant clam broodstock; their maintenance in the ocean nursery; handling of broodstock and techniques to induce spawning in the landbased *(ex situ)* and in the ocean nursery *(in situ)*; protocols to rear clam larvae, microalgae and zooxanthellae; and husbandry of juveniles. It is noted here that in situ spawning induction followed by ex situ larval culture is a new feature, not having been addressed in other giant clam culture manuals (see Heslinga et al. 1990; Braley 1992). Next is a general discussion on population genetics, and invasives. The last sections deals, with data management and general housekeeping.

The second part is about the ocean nursery. The first two sections refer to selection and setting the ocean nursery site. Clam transport is introduced next although this is further tackled in the last part of this manual. The next five sections relate to husbandry in the ocean nursery. The last is on record keeping pertaining to ocean nursery data.

Finally, the third and last part discusses the purposes of stock enhancement, survey methods for stock assessment, protocols for quarantine and transport, monitoring, and record keeping.

S. Suzanne Mingoa-Licuanan

Acknowledgments

INTRODUCTION

Species identification

There are nine (9) extant species of giant clams, belonging to two (2) genera, *Tridacna* (*T. gigas, T. mbalavuana, T. derasa, T. squamosa, T. maxima, T. rosewateri, T. crocea*) and *Hippopus* (*H. hippopus, H. porcellanus*). The seven (7) Philippine species are shown in Figure 1.

Tridacna gigas is more commonly known as the true giant clam, because adults have such immense shells. In the Philippines, *T. gigas* is called *taklobo*, which applies to most giant clam species. Specifically, *T. gigas* is locally known as *buka*, with reference to the clam's gaping habit – the mantle of *T. gigas* is so fleshy that the clam can no longer fully close its valves upon adduction. The largest shell of *T. gigas* on record is 1368.7 mm, about 1.4 meters (or about 4 feet and 7 inches), and weighs as much as 507 pounds, about 230 kilos (see Dillwyn 1817, cited in Rosewater 1965). But the heaviest pair of valves on record weighs 579.5 pounds, about 263 kilos (see Miner 1938, cited in Rosewater 1965). The highest egg production recorded by the Marine Science Institute was 105 million eggs from a 61-cm clam. In *T. gigas*, the shells' dorsal edge exhibits interdigitating processes that are distinctively elongate and triangular. The mantle may be olive green to brown in color, sometimes bearing a dark-green to violet band along the mantle edge. The mantle bears small iridescent blue and green circles associated with hyaline organs (Norton & Jones 1992).

Tridacna mbalavuana is known in Fiji as the *tevoro,* or devil clam. Its record size is 560 mm, about 22 inches. Moderate-sized shells may display strawberry-colored patches, appearing as stripes on the shell ribbing near the umbo. In contrast to *T. derasa* which it otherwise resembles, its mantle surface is rugose, and its incurrent aperture bears prominent tentacles. Mantle colors are subdued brown, gray-brown, or green-brown. Unlike the other *Tridacna* spp., the mantle does not extend beyond the shell margins (Lucas et al. 1991), similar to the *Hippopus*.

Tridacna derasa is known as the southern, or smooth giant clam. The largest size reported for *T. derasa* is 513 mm, about 20.2 inches (Rosewater 1965). Its shells are heavy and plain. The mantle is brilliant and may display shades of blue and green, usually in linear patterns. The mantle's incurrent aperture bears relatively inconspicuous guard tentacles (Lucas et al. 1991).

Tridacna squamosa is called scaly or fluted clam. In the Philippines, it is called *hagdan-hagdan*, referring to the step-like distribution of its scales on the shell. Its record size is 429 mm, about 16.9 inches (Hutsell et al. 1997). The mantle displays mottled patterns of mixed colors of yellow, green, and brown. The incurrent aperture

Tridacna gigas

Tridacna crocea

Tridacna derasa

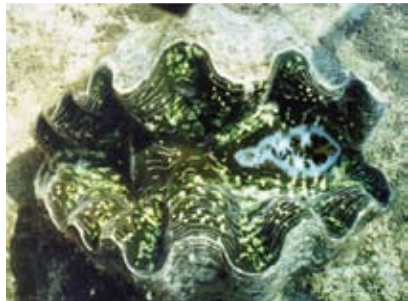*Tridacna squamosa*

Tridacna maxima

Hippopus hippopus

Hippopus porcellanus

Fig. 1. Seven giant clam species of the Philippines (Photos courtesy of Wilfredo Licuanan; except *T. squamosa* by Ivan Lim and *H. porcellanus* by Richard Braley)

bears tentacles. Shells of *T. squamosa* are often colored (yellow, orange-pink), thus highly prized by shell collectors.

Tridacna maxima is known as the elongated giant clam. In the Philippines, it is called *manlet,* or *manlot*. The largest recorded size for the shell is 417 mm, about 16.4 inches (Stasek 1965, cited in Newman & Gomez 2000). It is fully mature between 60-80 mm shell length (Munro 1993). The shells bear low scales in closely spaced rows. *T. maxima* is one of two tridacnid boring species, although it is only partially imbedded in the substrate. From juvenile stage, it begins to burrow into the substrate, usually of limestone, by mechanically abrading and/or chemically dissolving the limestone. As the clam grows, the dorsal portion of the shells remains exposed, while the rest of the clam becomes concealed in its burrow. The burrow may be relatively shallow, but it is tight around the clam's shells. The tendency to byssally attach is a persistent characteristic of boring tridacnids; thus, *T. maxima* remains attached to its burrow. Older individuals may outgrow their burrow, which makes it easy to extract the clam from its burrow (Thomas 2001). The mantle is brightly-colored from blue to brown, and may have a variety of patterns. The mantle's incurrent aperture bears tentacles (Lucas et al. 1991).

Tridacna rosewateri. The largest recorded shell measures 191 mm, about 7.5 inches. Shells are moderately thin with well-developed sparse scales. The dorsal shell margins have triangular interdigitating processes. Shell color is usually white, sometimes tinged with yellow (Sirenko & Scarlato 1991).

Tridacna crocea is called the boring, or crocus, or saffron-colored giant clam. In the Philippines, it is called *let-let*. Of all tridacnids, *T. crocea* is the smallest, with a record size of 150 mm, about 5.9 inches (Rosewater 1965). The scales are poorly developed, and appear eroded. Like *T. maxima*, it is a rock-borer. *T. crocea*'s burrow is as deep as its shell, so that the clam is completely imbedded in the hole, with only the mantle extending out of the burrow. *T. crocea* is byssally attached to its burrow. The mantle is brightly colored, displaying several shades of green, blue, purple, and brown. The mantle's incurrent aperture bears tentacles (Lucas et al. 1991). The external and internal shells may be tinged with yellow or orange.

Hippopus hippopus goes by several common names, for example, strawberry clam or bear paw clam. In the Philippines, it is known as *kukong kabayo*, referring to the shell's close resemblance to the horse's hoof, whence its scientific name. The record size of *H. hippopus* in published literature is 423 mm, about 16.7 inches (Hutsell et al. 1997). However, the Marine Science Institute had a live animal whose shell length measures 500 mm. The shell is at the Bolinao Marine Laboratory. *H. hippopus* is mature from about 140 mm shell length, or when no longer attached to coral rubble (Alcala et al. 1986, cited in Thomas 2001). The shells are thick and characteristically display strawberry-colored marks in irregular bands crossing the prominent radial ribs. The shells have a distinct ventral region, which is heart-shaped and concave in large specimens (Lucas et al. 1991). In particular, this genus has a very narrow

byssal gape, with tightly-fitting teeth (Rosewater 1965). The mantle may display yellow-brown, green or gray mottled patterns. The mantle does not extend beyond the shell edge.

Hippopus porcellanus is commonly called the china or porcelain clam. In the Philippines, locals refer to *H. porcellanus* also as *kukong kabayo*. Its record size is 411 mm, about 16.2 inches (Hutsell et al. 1997). The shells are thin and smooth, and have a distinct ventral region that is heart-shaped and concave at larger sizes. The mantle color is light gray, or light brown. Its incurrent aperture distinctly bears frilled tentacles (Rosewater 1982).

Key to the Subfamily Tridacninae

Species identification of live specimens is commonly based on mantle and shell characteristics. When only shells are available, characteristics of the shell (Figure 2) alone may be sufficient.

The key presented here is based on Rosewater (1965, 1982), Lucas et al. (1991), Sirenko & Scarlato (1991), Norton & Jones (1992), Schneider & Foighil (1999), & Newman & Gomez (2000). It includes two additional species *T. mbalavuana* and *T. rosewateri*, not included in previous keys by Rosewater (1965) and Norton & Jones (1992).

1 Byssal gape lined with interlocking teeth; ventral region distinctly outlined by paired prominent radial ribs; mantle extension limited to shell margin – *Hippopus* (2)

Byssal gape without interlocking teeth; ventral region not bordered by prominent radial ribs; mantle extension usually exceeding shell margin – *Tridacna* (3)

2 Shell thick and strongly ribbed; shell shape triangular to sub-rhomboidal; shell often bearing strawberry-red irregular bands on the ribs, and pinkish blotches between shell ribs; incurrent siphon aperture without guard tentacles – *Hippopus hippopus*

Shell thin, with shallow ribs; shell shape semicircular; shell occasionally bearing pinkish blotches; incurrent siphon aperture with guard tentacles – *Hippopus porcellanus*

3 Umbo central; interdigitating processes elongate-triangular; radiating sculpture deeply folded – *Tridacna gigas*

Umbo other than central; interdigitating processes bluntly triangular or rounded; radiating sculpture broad and low (4)

4 Upper region of large shells, plain without scutes or strong ribs (5)
Upper region of large shells with scutes or eroded scutes (6)

DORSAL

excurrent siphon aperture   *incurrent siphon aperture*

mantle

ANTERIOR   **POSTERIOR**

rib

VENTRAL

adductor muscle scar

pedal retractor muscle scar

pallial line

teeth

hinge   *umbo*   *byssal gape*
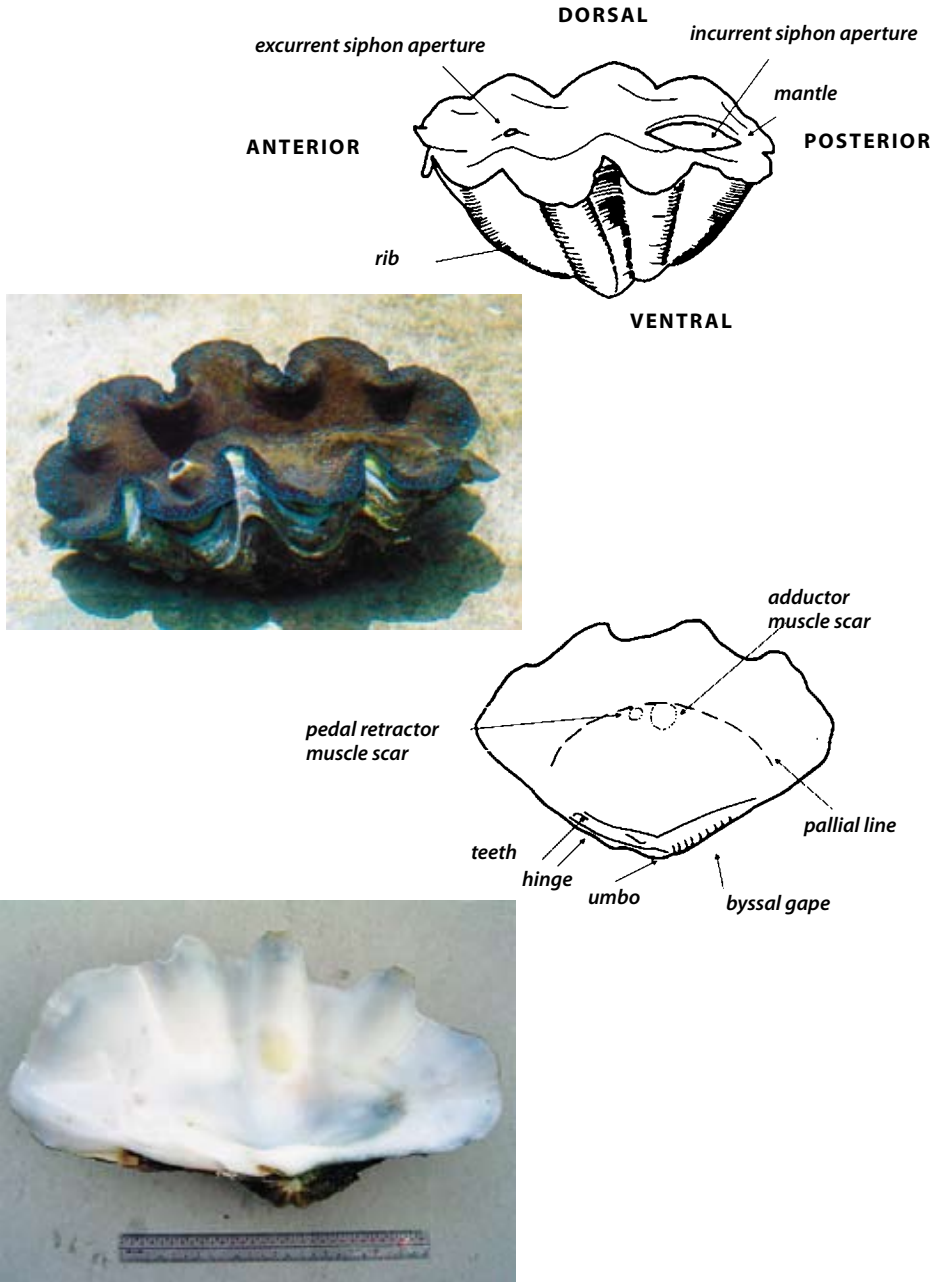
Fig. 2. Gross morphology of whole clam (top), and parts of the internal shell (bottom)

5 Rib-like radial folds on shell without colored patches; mantle without protuberances; incurrent mantle aperture with inconspicuous guard tentacles - *Tridacna derasa*

Rib-like radial folds on shell usually striped with colored patches near umbo; mantle with protuberances; incurrent mantle aperture with conspicuous guard tentacles – *Tridacna mbalavuana*

6 Concentric sculpture projecting, bladelike; valves equilateral to nearly equilateral; shell shape semicircular – *Tridacna squamosa*

Concentric sculpture appressed and low; valves inequilateral; shell shape triangular to elongate (7)

7 Umbo posterior; scutes large and sparse; dorsal margin with well-developed interdigitating processes; hinge line longer than half the shell length – *Tridacna rosewateri*

Umbo anterior; scutes low or closely spaced near dorsal margin; interdigitating processes of dorsal margin, moderately or slightly developed; hinge line less than half the shell length (8)

8 Shell strongly asymmetrical about umbo in lateral view; byssal gape moderately wide to wide; scutes present in upper shell region – *Tridacna maxima*

Shell not strongly asymmetrical about umbo in lateral view; byssal gape wide; scutes appear eroded except those close to shell margin – *Tridacna crocea*

Present distribution and species habitat requirements

Giant clams are known only from the Indo-West Pacific. Rosewater (1965) provides the initial report on the distribution of natural populations of giant clams. Over the years, various scientists have reported:

- the existence of at least three new species: *H. porcellanus* (Rosewater 1982), *T. mbalavuana* (Lucas et al. 1991, Newman & Gomez 2000), and *T. rosewateri* (Sirenko & Scarlato 1991)

- the extirpation of at least four species: *T. gigas, T. derasa, H. hippopus*, and *H. porcellanus*, in various countries (Copland & Lucas 1988)

- the re-introduction of cultured stocks of at least three species: *T. gigas* (Gomez & Belda 1988), *T. derasa* (Gomez & Belda 1988; Price & Fagolimul 1988, Zann & Ayling 1988), and *T. squamosa, T. maxima, T. crocea,* and *H. hippopus* (Gomez & Mingoa-Licuanan 2006), in several countries

- the introduction of one species (cultured): *T. derasa*, in the Cook Islands (Sims & Howard 1988)

The present distribution of giant clams is summarized below in Figure 3. Details of geographical limitations per species follow. Species habitat requirements are also briefly described below.

Tridacna gigas. Sumatra to southernmost Ryukyus (Watanabe et al. 2004) and northern Marshall Islands but not Mariana Island or Taiwan, south to New Caledonia and east to Fiji (Newman & Gomez 2000), none in Tonga (Langi & Aloua 1988). Found on sandy substrate in coral reefs, from water depths of a few feet to several fathoms, depending on light attenuation underwater (Rosewater 1965). Found on reefs to depths of 15 m (Newman & Gomez 2000).

Tridacna derasa. From southern Sumatra, but extinct in western Indonesia (Pasaribu 1988); Cocos-Keeling, Solomon Islands (Govan et al. 1988), Vanuatu (formerly New Hebrides) (reintroduced, Zann & Ayling 1988), southern Japan, south to Australia and Lord Howe Island, and east to Tonga (Langi & Aloua 1988), extinct in Taiwan (Newman & Gomez 2000); introduced to Cook Islands in 1986 (Sims & Howard 1988). Lives on outer edges of barrier reefs and in coral atoll lagoons (Rosewater 1965) at depths of up to 20 m (Newman & Gomez 2000). Juveniles attach to corals, while adults live freely on the bottom.

Tridacna mbalavuana. Eastern Lau Islands of Fiji, particularly Cakai Tabu, Komo Island, Namuka Island, Vatoa Island and Vuata Vatoa (Lewis & Ledua 1988), and northern Vava'u Ha'apai island groups, Tonga (Lucas et al. 1991), and Holmes Reef (120 nautical miles east of Cairns, Australia) (Newman & Gomez 2000). Found along
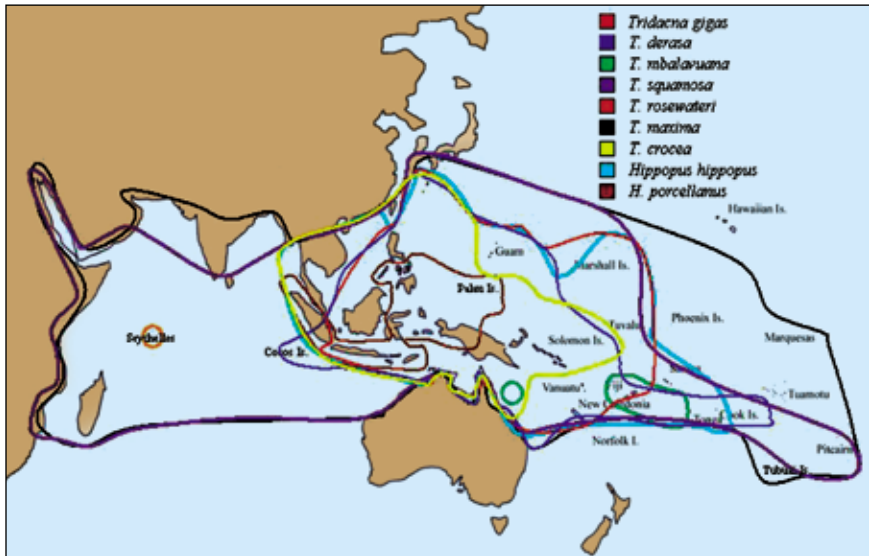
Fig. 3. Present distribution of giant clams in Indo-Pacific region

outer slopes of leeward reefs, in very clear, oceanic water at 20-30 m. They are intolerant of shallow habitats (Lucas et al. 1991).

Tridacna squamosa. Red Sea, south nearly to Durban in South Africa, east to Taiwan and southern Japan (specifically Kaiki Sima), Guam and the Marshall Islands, Australia, New Caledonia and Cook Islands (Paulay 1987, cited in Newman & Gomez 2000), the Pitcairn Island (Paulay 1989, cited in Newman & Gomez 2000), Kingman Reef, Line Islands (Newman & Gomez 2000). Lives on the surface of corals, usually in somewhat protected localities; in reef moats (Stephenson et al. 1931, cited in Rosewater 1965), up to 18 m depth, but deeper on outer reef slopes (Newman & Gomez 2000).

Tridacna rosewateri. Saya de Malha Bank, Indian Ocean. This species has been found among sparse madrepore corals densely covered by sea grass *Thalassodendron ciliatum*. Found from 12-13 m depth (Sirenko & Scarlato 1991).

Tridacna maxima. From the Gulfs of Suez, Aqaba, and Arabia south to Durban, east to southern Japan, Australia and Lord Howe Island, and further east to Pitcairn Island (Paulay 1989, cited in Newman & Gomez 2000, Blake 1995), but not reaching Easter, Johnston, or the Hawaiian Islands. Found in shallow areas in seaward reefs, in lagoons, to depths of about 10 m (Newman & Gomez 2000). Often found embedded into coral, sometimes into rubble and sandy substrates.

Tridacna crocea. Sumatra to Taiwan and the Ryukyus and the southern Great Barrier Reef, as far east as Palau and Yap north of the equator and the Solomon Islands south of the equator (Newman & Gomez 2000), none in Vanuatu and Fiji (Lewis et al. 1988). Lives on reef flats, fully embedded in coral heads, often in shallow water. In some very shallow localities, *T. crocea* may be found emersed on coral heads during low tides.

Hippopus hippopus. Sumatra to Pratas Island, none in Taiwan; Ryukyus, Caroline and Marshall Islands, extinct in the Marianas; south from Australia, New Caledonia, east to Tonga. Often found in sandy areas in coral reefs, harbors, in depths up to 10 m (Newman & Gomez 2000). May also be found in shallow, nearshore patches, and may experience complete exposure during spring low tides (Thomas 2001). Juveniles byssally attach coral rubble to plug their byssal aperture, or may attach to coral heads. Adults lose the tendency to attach, as the byssal gland becomes vestigial and the byssal gape becomes very narrow with age (Rosewater 1965).

Hippopus porcellanus. Virtually extinct in the Philippines, found in the Sulu Archipelago, southern Sulu Sea, Masbate Island (Rosewater 1965), Palau (Heslinga 1993), Celebes and West Irian, New Guinea (Lucas 1988), Indonesia (Romimohtarto et al. 1987, cited in Pasaribu 1988). Lives on coral rock and sand, not attached as adults (Newman & Gomez 2000).

Clam symbiosis

Giant clams are symbiotic with zooxanthellae, particularly *Symbiodinium micro-adriaticum*. Zooxanthellae are dinoflagellates that live within the clam's mantle, where they are able to photosynthesize, reproduce, and grow in large numbers. Free zooxanthellae may also be found in the seawater column, but not in the same numbers as those in the clam. Free zooxanthellae exhibit attraction to ammonia, a common excretory product among non-symbiotic marine organisms (Fitt 1984), which may explain the affinity observed between clam larvae and zooxanthellae during larval culture. The clam-alga symbiosis is established after metamorphosis of pediveliger to small juvenile, about 1-2 weeks post-fertilization. Ammonia levels are hardly detectable in culture water because of its quick uptake by clam zooxanthellae (Mingoa-Licuanan 1993).

The zooxanthellae's need to photosynthesize has naturally restricted giant clams to sunlit environments. Photosynthesizing zooxanthellae provide as much as 99% of the clam's metabolic requirements, particularly among juveniles (Fisher et al. 1985). Juveniles reared in the dark are photosynthesis-starved, and die in less than a week (Mingoa 1988). However, adequate juvenile nutrition still strikes a balance between phototrophy and heterotrophy, while the nutritional requirements of large clams may be sufficiently met with phototrophy (Griffiths & Klumpp 1996).

Molecular studies of zooxanthellae clones in culture have shown that they may be grouped into three clades, A, C and D (Ishikura et al. 2004). Each clade exhibits unique physiological attributes. Clade A zooxanthellae are adapted to conditions of high irradiance conditions, while Clade C zooxanthellae are adapted to conditions of low irradiance.

HATCHERY

Broodstock selection and maintenance

Tridacnids are generally protandric hermaphrodites (Wada 1942). Broodstock that exhibit female reproductive maturity are used for spawning. Selective breeding based on genetic considerations are briefly discussed in the section on population genetics.

1 **Choose mature clams from the wild or from culture based on clam size or shell length.** Table 1 shows size data mostly used by MSI. No data are available for *T. mbalavuana* and *T. rosewateri*.

The sizes at male and female maturity may vary with location. For instance, in Palau, cultured *T. gigas* has been reported as male at 18.7 cm, and *T. derasa* at 15.5 cm (see Heslinga et al. 1984).

Other conservative methods to assess the broodstock maturity are (1) ocular inspection of the gonads via the excurrent aperture, and (2) periodic spawning induction. The gonads are located in the viscero-gonadal mass at the clam's anterior portion (Fig. 4).

Species	Male	Female
T. gigas	42	53
T. derasa	29	30
T. squamosa	18	19
T. maxima	13	14
T. crocea	5	7
H. hippopus	24	24
H. porcellanus	20	35

Table 1. Sizes of wild and cultured clams (cm, shell length) exhibiting maturity

No data available for *T. mbalavuana* and *T. rosewateri*
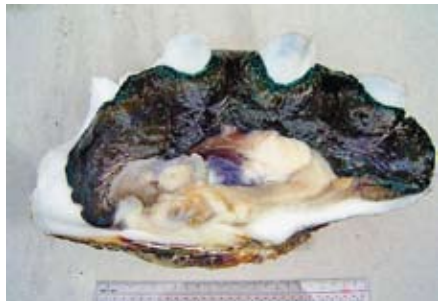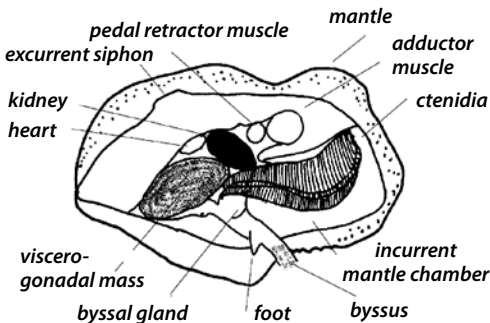
Fig. 4. Internal anatomy of the giant clam
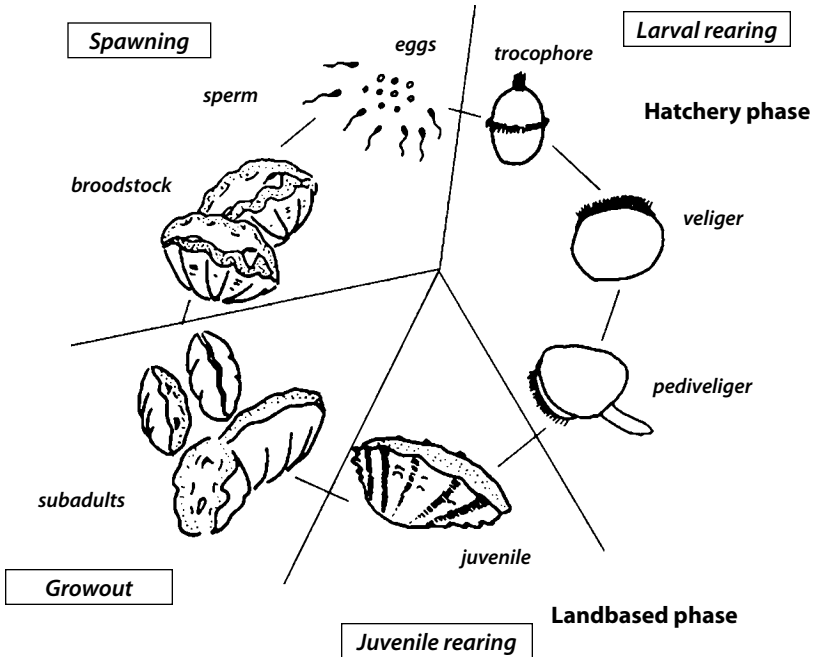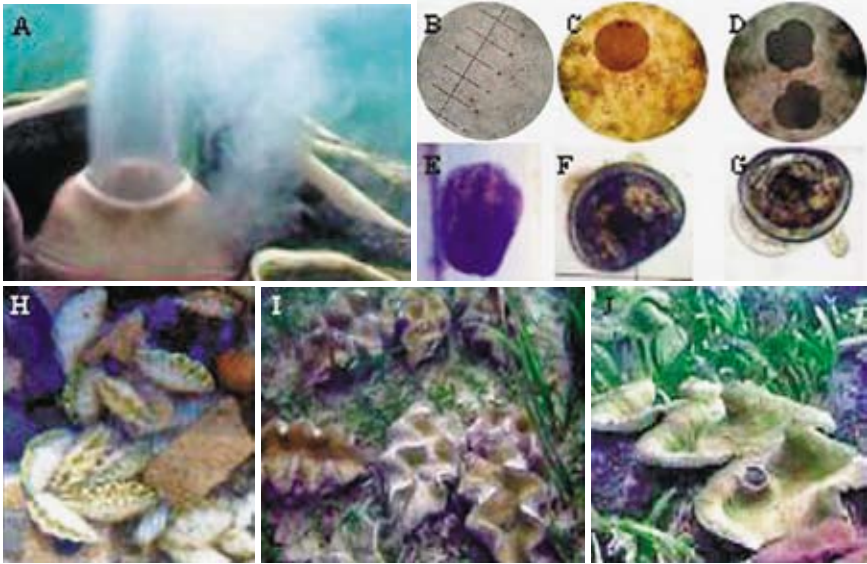
Fig. 5. **Life and culture cycles of the giant clam** (adapted from Braley 1992).
Photos above show (A) clam spawning, (B) sperm, (C) egg, (D) fertilized egg,
(E) trocophore, (F) 6-day veliger, (G) pediveliger, (H) juveniles, (I) subadults, and
(J) broodstock

2 **Record all observations on gonad condition of the broodstock** on *Data sheet A - Spawning* (Appendix 1). Ocular inspection of gonads may be possible only for large broodstock. As viewed through the clam's excurrent aperture, ripe gonads in *T. gigas* appear full, and have cream to orange coloration.

Periodic spawning induction is useful if there are many cohorts of broodstock to spawn. In addition to knowing more about the reproductive cycle of the broodstock, clam production is increased with each successful spawning. However, broodstock that have recently been spawned, or induced to spawn should be allowed to rest before the next spawning activity. The tridacnid reproductive cycle (Fig. 5) has been linked to the lunar cycle (Braley 1992, Heslinga et al. 1984). At Bolinao, Pangasinan, spawning success seems to be more frequent during the new moon phase. Giant clams generally exhibit a diel rhythm when spawning, which is often observed from midday to late afternoon.

The hatchery culture of giant clams begins from maintenance of broodstock to harvest of small juveniles. Figure 6 is a diagram showing inputs of algal culture methodologies to giant clam culture.

3 **Tag the clam broodstock.** Clam tagging is done only for clams that are large enough to accommodate the tag. The minimum recommended clam size is 7 cm (or 3 inches). Tagging is often done on land, or on the boat. Clams should be tagged within 30 minutes after emersion. Out of water, they should be protected from the sun, heat, rain, and wind.

Tag the clam externally with a dymo tape strip bearing unique combinations of characters and number. Prepare the dymo tape tags by consecutively imprinting the identification tag with a dymo gun. The identification tag should contain the following information:

Locality of seeding – 1 to 2 characters
Cohort year – 2 number digits
Source of broodstock, or place of origin of the mother clam – 1 to 2
    characters
Number – as needed

Example: HI95S1
    HI refers to Hundred Islands where clams were seeded
    95 refers to 1995, which is the clam's birth year
    S refers to Solomon Islands from where the mother clam originated
    1 refers to the clam number

Attach each dymo tape tag to the clam shell with marine epoxy. Marine epoxy consists of two components, adhesive and hardener. The desired ratio of these two components to make the epoxy putty is 1:1. Mix small batches of the epoxy putty as needed to avoid wastage.

Zooxanthellae culture (optional)

Maintenance culture, 100ml

mixed

or

single cell - clone

Microalgal culture

Maintenance culture 100ml

⇩

1L culture

⇩

10L culture

Giant clam culture

Broodstock preparation

⇩

Broodstock conditioning

⇩

Induced spawning

⇩

Larval rearing

⇩

Settlement

⇩

Juvenile rearing

⇩

Ocean nursery phase

Greenwater culture
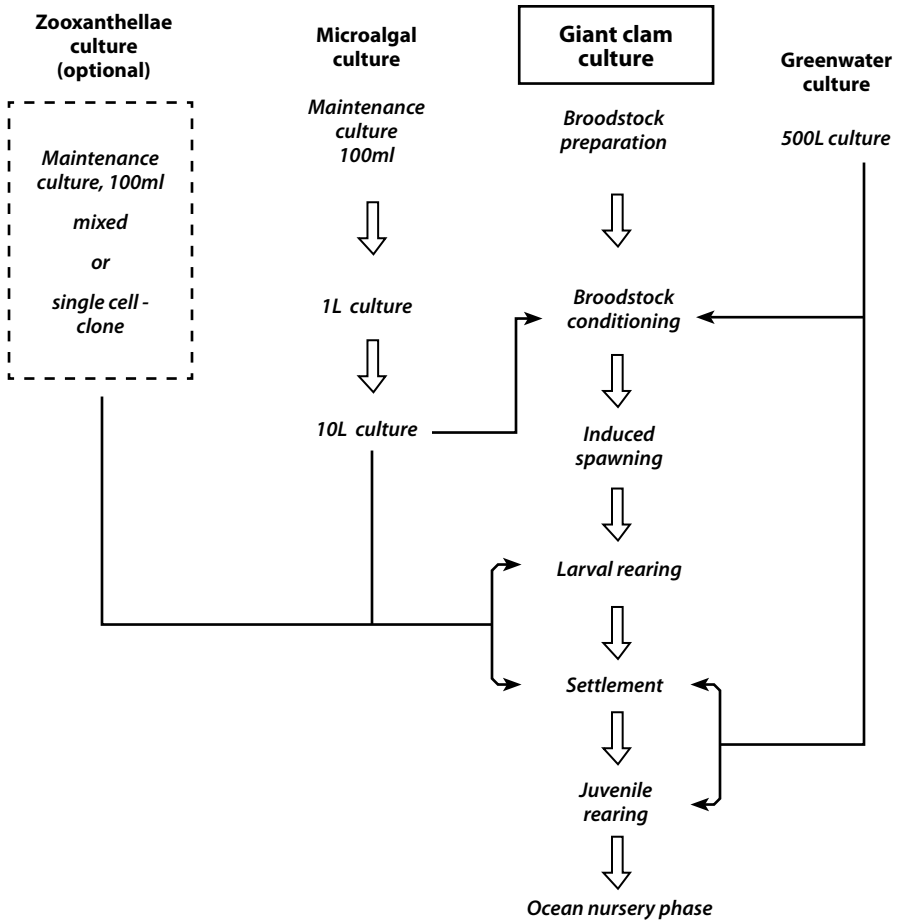
500L culture

Fig. 6. Diagram of algal culture methodologies showing inputs to giant clam culture

Place the tag consistently in a specific shell area, for example, 5 cm down the shell edge of the right valve, and between the second and third ridges of the shell. It will be easy to re-locate the tag during the next broodstock inventory. Brush this shell area free of epibionts, and wipe dry. Apply the epoxy putty onto the shell, and wait a few minutes. Remove the protective backing of the dymo tag, then press it onto the epoxy putty. The tag must be visible, aligned, and adheres securely to the putty. Allow the putty to completely set for about 10 minutes before returning the clam to the sea. If tagging is done in the landbased nursery, re-immerse the clam in a tank with running seawater. Tag the remaining clams consecutively. Within 24 hours from tagging, observe the clam for adverse reactions. Re-tag the clam when the tag is lost, or becomes difficult to locate.

Large clams > 22cm shell length may be permanently tagged using riveted steel tags. Cable ties bearing numbered tags may be used on small clams > 7cm shell length. This tagging method entails drilling the shell at pre-marked spots. Exercise care during drilling to avoid injury to the clam mantle. Also, consistency in tag placement is essential for its easy detection. It takes about a month for the clam to recover, i.e. deposit shell material over the tag. In addition, for the small clams, an inert glue like dental glue is used to keep the cable tag in place (Mingoa-Licuanan, in press).

Shell staining with alizarin red has also been used on clams < 10cm shell length. This process however takes 1-2 weeks immersion in the stain. The shell stain is manifested as a linear band at the shell edge, which may remain visible at the external side of the shell for a few months (Mingoa-Licuanan, in press).

4 **Stock the tagged broodstock in an ocean nursery.** Broodstock are better held in an ocean nursery than in a raceway since they have access to more food and nutrients in the sea. In addition, maintenance is minimal. A suitable ocean nursery (Mingoa-Licuanan et al. 2000) would be:

- shallow and clean
- with good water movement
- protected from typhoons and strong wave action
- accessible to the hatchery staff
- not in the way of boat navigation
- far from freshwater runoff
- protected from clam poaching, dynamite fishing, and cyanide fishing
- far from pollution sources (chemicals, oil spills, thermal pollution, siltation, garbage)

5 **Prepare for spawning induction.** There are two approaches to broodstock spawning: (1) *ex situ* and (2) *in situ* spawning induction. Both approaches are followed by *ex situ* larval rearing.

For *ex situ* spawning (Figure 7), collect about 20 broodstock from the ocean nursery, up to two weeks before spawning, and transfer them to a spawning tank in the landbased nursery. Broodstock of smaller species, *T. maxima* and *T. crocea*, are collected with their substrates attached. Larger species, i.e. > 30cm SL may require at least 3 persons for collection.

To collect broodstock, prepare a rope (12-16 mm diameter) by forming a noose at both ends. A diver holds one noose underwater, while a boat companion holds the other noose. Underwater, the clam is snugly fitted through the noose. The diver then tugs at the rope to signal that the clam is ready. The boat companion/s either manually pull the rope, or use a winch to haul up the clam onto the boat (Braley 1992). If emersion time is < 2 hours, the clams may be transported without insulated boxes on the boat. Lay the clams on one valve to minimize stress to body tissues, and water loss during transport.

Fig. 7. *Ex situ* **spawning and larval rearing setups in giant clam culture**
A, broodstock collection; B, clams in spawning tank; C, greenwater culture to
condition broodstock; D, filtration set-up; E, larval tanks

Protect them from the elements (solar radiation and engine heat, wind, rain and freshwater) with some cover like a light canvas material. Douse the clams with clean seawater every 15 minutes to moisten and cool them down. If emersion time is > 2 hours, the packing and transport protocols (see page 69) should be employed. At the hatchery, carefully move the broodstock to the spawning tank.

On the Data sheet A-Spawning (page 76), record the broodstock tags and corresponding broodstock shell lengths. Re-tag broodstock as necessary.

In situ spawning of broodstock is a coordinated activity between spawning induction in the sea and preparation of larval tanks in the hatchery. This activity requires about 6 persons. The first 5 place themselves in the ocean nursery:

- a diver to inject broodstock with serotonin, and carry on fertilization work on the boat
- 2 divers to move materials between the spawning area and the boat
- 2 snorkellers to collect released gametes
- 1 boatman who also serves as assistant during the fertilization process
- The sixth person is in the hatchery to prepare the larval tanks

Arrangement of broodstock

Measurement of broodstock

Spawning induction by serotonin injection

Clam responding to serotonin

Clam releasing sperm

Clam releasing eggs

Collectors with bags containing gametes

Transfer of gametes to the boat
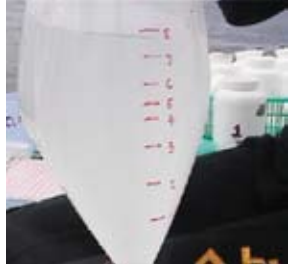
Eggs in calibrated bags

Fertilizing eggs with sperm

Filling egg bag with oxygen

Tying oxygenated egg bag for transport in styropore box

Fig. 8. *In situ* induced spawning of giant clams in the clam ocean nursery

Select and prepare about 20 broodstock (see page 10). Transfer and position the broodstock in two concentric circles with half the larger clams in the inner circle. The preferred spawning depth is 1.5 m, to facilitate movement of materials between the spawning area and the boat. Record the broodstock tags and their corresponding shell lengths. Re-tag broodstock as needed. Allow all clams to recover from the handling and movement for 3-4 hours before spawning (see Fig. 8).

6 **Clean and condition broodstock.** With a handbrush, scrub off epibiota from the shells of the broodstock. Record the tag numbers and shell lengths, and re-tag clams that have lost tags. If the broodstock are in the landbased nursery (*ex situ* spawning), rinse the clams with UV-treated 0.2μm-filtered seawater (UVFSW) after scrubbing. Place the clams in a sunlit holding or spawning tank filled with UVFSW. Regulate the seawater flowrate into the tank to provide 1-2 turnovers per day (Braley 1992). Maintain the clams in the holding tank for at most 2 weeks.

For *ex situ* spawning the broodstock's gametogenic development may be enhanced towards reproductive maturity by applying conditioning procedures for at most 2 weeks prior to spawning. These procedures may involve elevated tank water temperatures through greenhouse heating (Braley et al. 1992), daily supplemental feeding with microalgae (Braley 1992, Roa 1997), or daily addition of dissolved inorganic nitrogen (Heslinga et al. 1990, Roa 1997).

Spawning (*ex situ* and *in situ*)

1 **Prepare the materials needed for *ex situ* spawning.**
You will need:

> 2 raceways, as spawning tank and as holding tank for broodstock
> 1 x 500L plastic tanks, as spare spawning tank
> Seawater filtration setup (preferably 25μm, nearest the water source; then 10, 1, 0.2 μm)
> 250L plastic tank, as reserved UVFSW (UV-treated 0.2μm-filtered seawater)
> 2-3 x 60L plastic containers, for egg release
> 2-3 x 60L plastic containers, for collected eggs
> UV-treated 0.2μm-filtered seawater (UVFSW)
> Serotonin (5-hydroxytryptamine creatinine sulphate complex; $C_{14}H_{19}N_5O_2 \cdot H_2SO_4$), crystalline
> 10 spawning needles (=spinal needles, 25G x 89 mm)
> 20 unused large plastic bags, 20 cm x 30 cm, clear and heavy duty
> Waterproof writing slate
> Long caliper, 60cm to 1m length, to measure broodstock

Laboratory thermometer
10 x 1L containers or dippers (depends on number of persons collecting sperm)
3 x 1L jar, for sperm mixture
50ml beaker, for sperm
3 x 20L pails
Plunge-type mixer
Hoses, 2.5cm diameter
Glass slides with wells, and coverslips
10 Pasteur pipettes, and bulbs
250ml wash bottle with distilled water
250ml wash bottle with UVFSW
1000μl micropipette, and tips
10 tissue culture plates
2 hand tally counters
Compound microscope, calibrated (method described below)
Dissecting microscope
Pre-weighed streptomycin, wrapped in aluminum foil (see page 27)
Scientific calculator
Data sheets (Appendix 1)

2 Calibrate the microscope. To calibrate the microscope, replace the eyepiece with an ocular micrometer (OM), and place the stage micrometer (SM) on the microscope stage. Using the 10x objective, adjust the viewing field to align the scales of the ocular and stage micrometers at the zero line. Without additional adjustments, find two lines that are exactly superimposed upon each other, at best at the most extreme right. Count the divisions between the zero line and the superimposed lines, for the ocular and stage micrometers. Compute the calibration factor CF (μm per ocular unit) for the 10x objective as follows:

$$CF = \frac{\text{\#divisions SM in mm}}{\text{\#divisions OM}} \times 1000 \ \mu m/mm$$

Repeat the procedure for the microscope's other objectives, and for every new objective used with the ocular micrometer (Todd et al. 1979). Post the CF values per objective close to the microscope for easy access.

3 Prepare 2mM serotonin solution. Weigh 0.019g of serotonin on an analytical balance, and dissolve in 25ml UVFSW. Excess serotonin solution may be stored in the refrigerator (4ºC) for 1 month. Discard if the solution turns yellow. The crystalline serotonin is similarly stored in the refrigerator (Braley 1992).

Preparing other materials for *ex situ* spawning. Have ready several pre-weighed streptomycin sulfate wrapped in aluminum foil (see page 27).

Sterilize all fiberglass, plastic tanks, filter cartridges and filter bags using chlorine. This is done by first scrubbing the container inside and out with 3-10% chlorine solution. Give the container several rinses of freshwater before inverting to dry under the sun. (Chlorine sterilization is also applied to cement tanks and substrates.) Sterilize all glassware, tissue culture plates, and micropipette tips by acid wash (Appendix 2).

Calibrate all containers. This is done by pouring beakers of 1L freshwater into the container, and marking the container at every 1-liter addition of water for small containers, and 5 or 10 liters for large containers. Label each mark with the water volume accumulated at that level.

Mark tissue culture plates for counting eggs and larvae. Turn over the plate, and mark (by scratching) its underside following a straight edge guide. The scratch marks will be useful during counting.

As spawning tank, a raceway measuring 5 m x 2 m x 0.5 m (length, width, depth) is suitable for 20 large broodstock. Place the 250L tank and 60L containers in a shaded area but within the vicinity of the spawning tank. A spare tank will be needed to hold the broodstock if the spawning tank is being prepared for the spawning activity (see page 20). The 250L tank will serve as a reserve water tank. Fill the spawning, spare, and reserve water tanks with UVFSW. The water levels for the spawning and spare tanks may be just a few cm over the height of the clams, to allow projectile expulsions, and facilitate gamete collection. Water in the reserve tanks shall be used if any of the tanks or containers need to be filled during spawning. Fill the 500L hatching tanks with UVFSW.

Keep microscopy equipment dry and place in a shaded area.

4 **Prepare the materials for *in situ* spawning.** You will need to bring on-board the following:

Serotonin (5-hydroxytryptamine creatinine sulphate complex), crystalline
10 spawning needles (=spinal needles, 25G x 89 mm)
20 unused large plastic bags, 20 cm x 30 cm; clear, heavy duty and
    calibrated
Waterproof writing slate
Long caliper, 60cm to 1m length, to measure broodstock
3 x 1L dippers
3 x 1L jar, for sperm mixture
50ml beaker
1000µl micropipette and tips
Pre-weighed streptomycin, wrapped in aluminum foil (see page 27)

In the hatchery, make ready the following:
    1000µl micropipette and tips
    10 tissue culture plates
    2 hand tally counters
    Compound microscope, calibrated (method described above)
    Dissecting microscope, calibrated (method described above)
    Scientific calculator
    Small sieve, 63 µm
    Data sheets (Appendix 1)

For *in situ* spawning, the sperm and eggs are collected using large plastic bags. Ensure that bags for sperm are not used for eggs. To prepare the bags for gamete collection, they need to be calibrated first. The water levels must be marked as the bag is clutched within one's fist and lifted off the surface. Such calibration will serve as a guide to determine the sperm volume required for egg fertilization. For the spawning activity, air is released freely from the bag by passing it through one's fist. Still held by the fist, the bag's mouth is then flipped to form a skirt over the fist. The bag is held in this manner underwater, ready to receive gametes when released.

5 **Induce the clam to spawn.** Broodstock are induced to spawn by injecting the gonad with the recommended dose of serotonin solution (Braley 1986). Small species like *T. crocea* require a lower dose (0.5 ml x 2mM serotonin solution), whereas the larger species (*T. derasa, T. squamosa, T. maxima*, and *H. hippopus*) require the full dose of 2 ml x 2mM serotonin solution. Large *T. gigas* responds well to serotonin solution at 4ml x 2mM. Use a 5ml plastic syringe that can accommodate either of two kinds of needles: the hypodermic needle (25G x 25 mm) for broodstock <30cm SL, and the spinal needle (25G x 89 mm) for a clam with SL at 30 cm and greater.

To induce spawning in *Tridacna*, load the syringe with serotonin solution, and release any bubbles from the syringe by holding the needle up, slightly pressing the plunger. Wait for the clam to relax and gape normally. Then aim the syringe's needle in a vertical position (90º) over the mantle. Target the point about 2.5-5 cm (or 1-2 inches) anterior to the conical (=excurrent) siphon, as this approximates the position of the underlying gonads (see Fig. 2 for antero-posterior orientation; Fig. 4). Inject the mantle with a steady hand, until some resistance is felt. Press the syringe's plunger to release the serotonin into the gonad, emptying the syringe receptacle by 5-second counts.

To induce spawning in *Hippopus*, follow the same procedure except that a horizontal position is applied (see Shelley & Reid 1988). It is usual for this species to react to injection by closing its valves. Hence to retain some valve gape, hold a plastic pipe (~4cm diameter) firmly over the incurrent aperture area and between the valves, while serotonin is injected.

Inject only 10 out of 20 broodstock and leave the other half of the broodstock to respond to chemical cues in the water after injection. Record the details of spawning induction (broodstock tag, corresponding shell length, volume of serotonin used, time when serotonin was injected, time when sperm and eggs were first released), and other relevant information on Data sheet A-Spawning (Appendix 1).

6 **Use spawning needles only once.** The needles are disposable, and recommended for single use. Dispose accordingly. However, if spawning needles are not enough, they may be re-used provided they are sanitized first. After every use, wash the needle in denatured alcohol ((95%) ethyl alcohol and (5%) methyl alcohol), then air-dry. Before re-use, wash the needle in distilled water (Braley 1992).

7 **Temperature shock is an option.** When broodstock are subjected to increased temperature, they suffer from temperature shock and often respond by spawning. Serotonin injection in combination with temperature shock may also be used to induce clams to spawn. Two methods have been used to induce temperature shock in clams: (1) Take the clams out of water; lay them on one valve for 30-60 min. During this time, the clamshells may be brushed and briefly rinsed with UVFSW. When the clams are returned in the spawning tank for serotonin injection, they also experience temperature shock. (2) Hold the clams in another raceway, while preparing the water in the spawning tank. Increase the water temperature in the spawning tank up to 35°C, either through exposure to solar radiation, or by using portable heaters. Meanwhile, place broodstock in a holding tank at ambient water temperature. When water temperature in the spawning tank is sufficiently elevated (from ambient temperature but not higher than 35°C), transfer the broodstock being held in ambient water temperature. Record the details of spawning induction used on the corresponding record sheet (page 76).

A stimulated clam will intermittently expel jets of water through its excurrent siphon within 5 minutes from injection. Observe the "zipping up" behavior of the clam's incurrent siphon, and the retreat of the excurrent conical siphon into the mantle. In a few seconds, the excurrent siphon re-extends as it forcefully expels sperm-laden water (whitish flumes in appearance). The clam may eject sperm for about 30 minutes, and then eggs (tiny, white, and spherical). Record the times of first sperm and egg release and other related data on Data sheet A.

8 **Collect the gametes *ex situ*.** Hold a 1L plastic container or dipper near the clam's conical excurrent aperture as sperm-laden water is expelled. Keep good quality sperm (appears milky and homogeneous) to fertilize the eggs later. Discard sperm of inferior quality (appears diluted, or contains whitish agglutinated sperm). Label each sperm container with the broodstock number, and

set aside in an area away from heat, solar radiation, or freshwater. Continue collecting or removing sperm until the clam appears to have exhausted all its sperm.

It is important to minimize the amount of sperm in the spawning tank; hence the continuous collection of expelled sperm. However, when several brood-stock expel sperm simultaneously, and the collectors are not able to keep up, the sperm contaminates the tank water, makes the water too cloudy, and increases the chance of polyspermy upon the release of eggs. As a remedy, transfer the broodstock to a spare tank. This step needs to be done quickly and with least disturbance to the broodstock. Lift the broodstock, and rinse off any adhering sperm with UVFSW before transferring to the spare tank. Continue sperm collection. If no spare tank is available, water in the spawning tank may be drained to half the volume, and immediately replaced with fresh UVFSW. The timing of water change is critical, since this should be done before egg release commences. The end of sperm release is signalled by a brief lull in broodstock activity, followed by egg release (usually 30min from the first release of sperm) (Braley 1992).

Broodstock may either release eggs in copious amounts at the first instance, or initially as a thin stream of eggs (like very small grains) before it gains momentum. When the clam begins to release eggs, transfer the spawning clam to a 60L plastic tank filled with UVFSW enough to cover the clam. When the clam displays strong expulsions, collect the expelled water, using large plastic bags for the large species, and 1L dippers for the small species. Refill the tank (containing the spawning clam) with UVFSW of similar temperature. If the density of released eggs is appreciable, empty the bag into another 60L container (The egg tank must be situated within the vicinity of the spawning tank, but protected from solar radiation and freshwater.) Label the egg tank according to the broodstock number. Water temperature must be between 26 to 30°C. The egg tank is gently aerated, as egg collection continues. Continue egg collection until the clam's spawning subsides. If more than one broodstock releases eggs, have a separate egg tank for each spawning broodstock (Braley 1992).

9 **Measure egg size.** It is good practice to inspect the gametes (and resulting embryos and larvae) under the microscope, and to take size measurements. These data serve as a backstop to ensure that appropriate techniques are applied at every stage in the hatchery. To determine egg size, take a small sample of water in a 50ml beaker. Mount a few ml onto a depression slide, and view (use 40x or 100x objective) the sample under a compound microscope with an ocular micrometer. Select 50 eggs at random, and measure their diameters based on micrometer readings. Compute the mean micrometer reading (and its standard deviation). To determine the mean egg diameter, multiply the mean micrometer reading with the calibration factor of the objective used. Record data on Data sheet B (Appendix 1).

After measurements, discard the sample. If several samples need to be examined, always use clean materials (e.g. micropipette tips, pipettes, slides, tissue culture plates, etc.). Do not return to the tank any egg (or larval) samples that had been examined, nor excess samples, to avoid introducing bacterial or viral agents.

10 **Collect gametes *in situ*.** Assign divers to collect gametes from 2-3 induced clams. As the clam appears to be ready to expel gametes, position the mouth of the collection bag over the conical siphon, and open it up as soon as water is expelled. In so doing, only gamete-laden seawater is collected. Immediately after each release and collection of gametes, seal the bag and hand it to the surface swimmer who then takes the bag of gametes to the boat assistant.

11 **Fertilize eggs in the hatchery or on the boat.** In the **hatchery**, fertilize the eggs as soon as possible, or within 15 min of spawning, i.e. from first release of eggs. Use sperm from another clam that belongs to another cohort. It is not advisable to fertilize eggs with sperm from the same clam as this results in self-fertilization, nor with sperm from a sibling (same cohort) as this leads to inbreeding (Newkirk 1993). The sperm to egg volume ratio to be applied is 5ml:1L. Estimate the volume of water in the egg tank, and determine the volume of sperm to be added. Transfer sperm to a 50ml beaker, and slowly pour the sperm into the egg tank in a circular manner. The egg water may be mixed using a plunge-type mixer while pouring the sperm. Mixing is done with slow up and down motions, and without touching the walls of the tank (Braley 1992). Wash the mixer with UVFSW after every use. Add sperm in corresponding amounts as eggs are added to the egg tank.

Sperm from two or more clams may be combined to fertilize the eggs. Ensure that the sperm mixture to fertilize a particular egg collection is sourced from clams belonging to cohorts different from the egg source. Pour sperm from two or more clams in equal proportions into a 1L jar. Label the jar according to the source broodstock #s. Fertilize the eggs as described above.

In case the collected eggs cannot be fertilized with the available sperm (same clam source, or source is sibling clam), or there was no more chance to collect sperm (spontaneous spawning), new sperm may be obtained by inducing 1 or 2 clams to release sperm by serotonin injection. Such clams may be selected from those that did not respond during the previous spawning activity, or were pre-selected clams as males (belonging to a younger cohort, to ensure that no egg release will follow sperm release). Prepare the sperm for egg fertilization as described above, and apply the recommended sperm to egg volume ratio.

Determine the percentage of fertilization by sampling the fertilized eggs in the egg tanks at 2 hours post-fertilization. Mix the eggs well with a plunge-

type mixer, and get a small sample in a 50ml beaker. Mount a few ml of the sample on a depression slide, and view under a compound microscope. Count 100 random eggs, and tally those that are dividing (2- or 4-cell stage). The number of dividing eggs indicates the percentage of fertilization. Prepare two more mounts to tally the number of dividing eggs, and compute for the average percentage of fertilization based on three counts. A mean fertilization percentage of > 85% may indicate a fairly good batch of larvae, while a value < 50% may indicate a poor batch of larvae (Braley 1992). Record data on Data sheet B (Appendix 1).

On the **boat**, transfer the sperm from plastic bags into labeled 1L containers. Immediately fertilize the eggs in each bag using the same sperm to egg volume ratio of 5ml:1L. Pour the sperm slowly into the bag, while gently shaking the bag for uniform dispersal of sperm. Add the antibiotic streptomycin (page 27) to the fertilized eggs at a concentration of 10 ppm. Finally, inflate bag with oxygen. Seal bag with rubber bands, and transport in insulated containers or styropore boxes to the hatchery.

For both *ex* and *in situ* spawnings, record the clam # of the egg source, as well as the clam #s of the sperm source/s on Data sheet A (Appendix 1). Note that the countdown starts here, from the 1st to the 59th day (D 0-D 60), for monitoring larvae and metamorphosed juveniles.

12 **Estimate the number of eggs (post-fertilization).** In the **hatchery**, estimate the total number of eggs by adding up the numbers of eggs estimated per egg tank. Do this by, first, recording the egg water volume (converted to ml), which is read off the water level marked on the egg tank. If the egg water level is between level marks, estimate water volume. Or, to have a definite egg water volume, add UVFSW (of similar temperature) enough to reach the next higher water level mark of the egg tank, and record this volume as the egg water volume. Whether estimated or not, the number of eggs in this egg tank shall be based on this recorded volume. The egg water volume as recorded on Data sheet B must be identified by the egg tank # and the broodstock tag # (source of sperm and eggs).

Second, mix the egg water evenly using a plunge-type mixer, with slow up and down motions, and without touching the walls of the egg tank (Braley 1992). While mixing, quickly get 10 x 1ml samples and transfer them to tissue culture plates at 1ml per well. Under the dissecting microscope, count the number of eggs per well, and record the counts on Data sheet B-Estimating Egg Numbers, and Stocking Eggs (Day 0). Calculate the average egg count, based on 10 samples. (If egg counts are based on 0.5ml samples, the average egg count must be multiplied by 2.) Compute the number of eggs in an egg tank by multiplying the average egg count with the recorded volume (Heslinga et al. 1990). Determine the total egg count for tanks combined, by adding up

the computed number of eggs per egg tank. If more than one broodstock releases eggs, egg counts must be kept separate per broodstock.

For *ex situ* **spawning**, the total egg production may be estimated based on egg counts at post-fertilization, if data collection for such is not possible prior to fertilization. The value obtained is a conservative estimate, not accounting for the number of eggs that are degenerating or have degenerated.

13 **Stock fertilized eggs in hatching tanks.** After the eggs are counted (per tank) and the total number determined, transfer the eggs to the hatching tanks for further development. The recommended egg stocking density is 20-25 eggs per ml, if hatching tanks are not limited. Otherwise, a relatively higher stocking density of 30-40 eggs/ml may be used. For example, a 500L hatching tank has a capacity of 15million eggs at 20 eggs/ml stocking density. If there are 8 such tanks, the total hatching tank capacity is 120 million eggs. If the total egg estimate is less than the total hatching tank capacity, say 105 million, then they can be stocked at 20 eggs/ml. However, if the total eggs estimate is greater than the total hatching tank capacity, say 125 million, then they must be stocked at 30-40 eggs/ml.

Therefore, calculate the volume of egg water to be transferred from a particular egg tank to a hatching tank, using the formula (record your computations on Data sheet B):

$D_1V_1 = D_2V_2$

where, D_1 = egg density in egg tank (eggs/ml);
V_1 = unknown; volume of egg water to be stocked in hatching tank (ml);
D_2 = egg stocking density (eggs/ml); and
V_2 = water volume in hatching tank (in ml)

Compute for the unknown (V_1, volume of egg water to be stocked) by transposing all other terms to the right side of the equation:

$V_1 = (D_2V_2)/D_1$

For example, the total egg estimate is 62 million. If the total tank capacity is 120 million eggs at 20 eggs/ml stocking density, then one can safely assume that all eggs can be accommodated at that stocking density. Computing for V_1,

if egg water volume = 60 L;
D_1 = 62 million eggs in 60L of egg water = 1,250 eggs/ml
D_2 = 20 eggs/ml; and
V_2 = 500,000 ml

then,
V_1=(20 eggs/ml * 500,000 ml)/1,250 eggs/ml = 9,677 ml

which means that about 9.6L of the egg water needs to be transferred to a hatching tank. Dividing the volume of egg water (=60L) by egg water volume for stocking of hatching tank (=9.6L) gives 6.2, which means that 6-7 x 500L hatching tanks will be needed for all 62 million eggs. Prior to egg transfer, put antibiotics as required for each hatching tank (see page 27).

If the total egg estimate is low, the hatching tank/s may not be used at full volume, and the egg stocking density may be lowered further. For example, the total egg estimate is 900,000 eggs in 30L egg water, to be stocked in 500L hatching tanks. Since the capacity of the hatching tank at full volume of 500 L at 20 eggs/ml stocking density is 15 million eggs, it is apparent that only one hatching tank is needed for all 900,000 eggs. Therefore, have the hatching tank at 250 L of UVFSW, put antibiotics required for the adjusted volume, and transfer all the egg water to the hatching tank. The egg stocking density, D_2, is then determined as follows

$D_2 = (D_1 V_1)/V_2$
Given, D_1 = 900,000 eggs in 30L of egg water = 30 eggs/ml
V_1 = 30 L
V_2 = 250,000 ml

then,
D_2 = (30 eggs/ml * 30,000 ml)/250,000 ml = 3.6 eggs/ml

This means that the effective egg stocking density applied is 3-4 eggs/ml.

Larval rearing

Materials for larval rearing are as follows:

500L hatching/larval tanks, fiberglass, with lids, and air supply
UVFSW (UV-treated 0.2μm-filtered seawater)
Waterproof writing slate
Data sheets (Appendix 1)
Thermometers, for ambient air and water temperatures
Dippers and overflow pails
Hoses (1cm diam. for sampling larvae; 2-2.5cm diam. for water changes)
53μm sieve
Underwater flashlight
Streptomycin
Dissecting microscope
Tissue culture plate, depression slide and cover slip
2 tally counters
Micropipette 1000μl, Pasteur pipettes
50ml beaker

Live microalgae, cultured
Zooxanthellae, fresh extracts

1 **Clean and disinfect all materials.** As with preparation of materials for spawning, sterilize all fiberglass and plastic tanks using chlorine; and all glassware, tissue culture plates, and micropipette tips by acid wash (Appendix 2). Calibrate and label the containers and tanks per 1, 5 or 10 liters, depending on their size.

The larval tank volume may be between 300-500L. The preferred tank shape is cylindrical, but with a short conical bottom equipped with a drainout and valve control. Preferred tank colors are black (within the tank) and white (external color).

2 **Prevent bacterial growth.** To improve larval survival, bacterial growth in larval tanks should be minimized if not eliminated. The antibiotic streptomycin sulfate is effective against gram-positive and gram-negative bacteria. Streptomycin sulfate is administered to make a final concentration of 10 ppm, or 10 g per 1000 L. When administered at 10 ppm, streptomycin sulfate is not harmful to humans (Braley 1992). In preparing streptomycin sulfate, first determine which tanks will be used for hatching larvae, and note their particular volumes (in liters). The amount of streptomycin sulfate to be weighed (in grams) will depend on tank volume. Use the formula:

x = (10g/1000L)*y

where x = unknown, weight of streptomycin sulfate to be weighed (in grams)
10g/1000L = refers to 10ppm concentration of streptomycin sulfate
y = particular larval tank volume (in liters)

For example, if y = 500L, then computing for x = 5g streptomycin sulfate.

On an analytical balance, weigh the required amount of streptomycin sulfate per tank. Store weighed streptomycin sulfate in small aluminum foil wrappers, and label with the corresponding tank #.

3 **Prepare larval tanks for hatching.** Keep the hatched larvae in motion by aerating the tank. Select airstones that produce fine bubbles (Braley 1992) so that there is a high surface area for dissolved oxygen exchange. Set the airstone about 15cm from the bottom to ensure larval motion, and still allow any dead larvae to settle at the bottom for routine draining. Apply only mild aeration, as strong aeration may cause larval mortality. Also, closely monitor water temperature. Record daily observations in the Summary Sheet (Appendix 1).

The eggs hatch into swimming trochophore larvae within 12-18 hours post-fertilization (Braley 1992). The black color of the inner surface of the tank facilitates viewing of the hatched larvae that appear as white specks against

a dark background. To further enhance viewing, shine a horizontal beam from an underwater flashlight a few cm below the water surface. Also, if the color within the tank is other than black, hatched larvae may be detected against a black disc (7-10cm diam.) held a few cm below the water surface (Heslinga 1990).

4 **Monitor larval development. Day 1 or 24 hours post-fertilization.** After 24 hours, all embryos should be in the swimming trochophore stage. Verify all larval stages under the microscope. Collect a sample of larvae by directly siphoning the tank water through a small 53 or 63μm sieve. Wash the trochophores off the sieve by squirting UVFSW down the sieve mesh while holding the sieve sideways. Catch the trochophores onto a petri dish, and view under a dissecting microscope. Inspect the larval tank for trochophores in the water column and tank bottom material. If the trochophores are fairly dispersed throughout the water column, and minimum powdery material (debris and dead embryos) is observed at the bottom, do not disturb the tank until Day 2.

However, if substantial white powdery particles are observed at the tank bottom, they should be removed by siphoning with a hose. First, turn off aeration for 1 hour to allow as much dead material to settle to the bottom. Then siphon off the settled material by holding one end of the hose over the tank bottom, and gliding the hose slowly to avoid stirring up the settled material. After all settled material have been removed, finger-plug the siphoning end of the hose while lifting the hose out of the tank. Note the remaining tank water level. If the water level is significantly reduced, refill with UVFSW (of similar temperature) to the original level, and add corresponding amount of antibiotics to retain the 10ppm concentration in the tank. Maintain moderate aeration in the tank.

Water changes. The hatching tanks may subsequently be used for rearing larvae, through a regimen of water changes. Water in the larval tanks needs to be changed on Day 2, 4, and 6. At 40 hours post-fertilization, the larvae may be in the veliger stage, although a mix of developmental stages from trochophore to trochophore-veliger to veliger stage may also be encountered. In any case, all these stages exhibit a swimming habit. Assess the stage of larval development prior to water changes.

You will need the following:
    Micropipette, 1000μl and (blue) tips
    24-well tissue culture plates
    Depression slides and coverslips
    Dissecting microscope
    Compound microscope with micrometer eyepiece
    Pasteur pipettes
    Data sheets (Appendix 1)

Determine the percentage of veligers (against non-veligers) using similar techniques to determine percentage of fertilization (page 33). Sample the larvae directly from the tank water with a micropipette. View the sample under a compound microscope, and tally the number of veligers against non-veligers for 100 larvae counted (see Braley 1992). If the percentage of veligers is high (>85%), proceed with Day 2 water change, following the procedure below. Otherwise, wait a few more hours (but not to exceed 6 hours) to allow larvae to further develop into veligers.

Prior to water change, determine the average veliger size, using techniques similar to determining egg size (see page 22). These data will determine the kind of sieve to be used for retrieving larvae for water changes. Record all data on the Data sheets (Appendix 1).

Retrieve larvae and change water. You will need the following to retrieve larvae and change water on Day 2, 4 and 6

> Thermometer
> Hoses, 2-2.5cm diameter
> Overflow pails
> 53µm sieves
> 75µm sieves
> 100µm sieves
> 200µm sieves
> Aeration setup for tanks
> 500L tanks with UVFSW (UV-treated, 0.2µm filtered seawater)
> 150L tank with UVFSW
> Dippers and 10L pails
> Plunge-type mixer
> Pre-weighed streptomycin, stored in aluminum foil wrappers (previous discussion)
> Data sheets (Appendix 1)

Turn off aeration. Use a 25mm diameter hose, with the distal end secured onto a series of large, fitted sieves, 75µm (top) and 53µm (bottom), within an overflow bucket. To ensure that siphoned larvae will not dry out on the top sieve, position the top sieve mesh below the water level in the overflow pail. Siphon tank water by maintaining the siphoning end of the hose beneath the water surface. Check the top sieve for larval concentration; larvae appear like white, fine sand grains. If larvae begin to collect as small piles on the top sieve, temporarily cease siphoning. Note if the top sieve also contains debris, which needs to be excluded from larval culture. Hold the 75µm sieve almost sideways over the 150L tank, and wash off the larvae by passing UVFSW across the mesh. Ensure that another sieve, say 200µm, is positioned beneath the 75µm sieve, to catch debris from falling into the 150L tank. Resume siphoning

of the remaining water in the larval tank, and transfer larvae from the sieve to the 150L tank as necessary. Cease siphoning upon reaching the tank's conical portion. Transfer also larvae collected from the 53μm sieve to the 150L tank, following the technique described above.

To retrieve larvae from the conical bottom portion of the tank, similarly siphon the remaining water through the 75/53μm sieve setup. Then, wash off larvae from the top 75μm sieve into a pail half-filled with UVFSW, and allow to stand unaerated for about 20 minutes. Note if the water contains any swimming larvae. Carefully decant the upper water layer of swimming veligers into the 150L tank, and discard the remaining bottom water containing dead material (see Braley 1992).

Restock veligers in larval tanks for water change. After all larvae have been transferred to the 150L tank, they must be restocked into the 500L larval tanks. First, determine the total number of larvae (use techniques similar to estimating number of eggs, page 24), to calculate the larval density in the 150L tank. Restock the larvae to the larval tanks at 3-4 veligers per ml (Braley 1992). To determine the volume of larval water for restocking into the larval tanks, perform calculations as discussed in stocking fertilized eggs in hatching tanks (page 25). If the total number of larvae is greater than the total larval tank capacity, use a higher larval stocking density, up to 10 veligers per ml (see Braley 1992). Perform similar calculations as above to determine the volume of larval water to be transferred to the larval tanks at a higher larval restocking density. Thereafter, put antibiotics in the larval tanks and restock larvae accordingly. Maintain low aeration in all larval tanks.

If all the larval tanks are already restocked with larvae at the highest recommended restocking density, and if there is still excess larvae in the 150L tank, stock these larvae in any available alternative tanks, after calculating the volumes of larval water for restocking. Follow procedures for administration of antibiotics and aeration.

On the other hand, if the total larval count is low, the larval tank/s may be used at half the volume, at lower larval restocking density. Put antibiotics required per tank for the adjusted volumes, and transfer the required volume of larval water per larval tank. Then, determine the effective larval restocking density (or D_2), following calculations similar to determining effective egg stocking density. Maintain low aeration in all larval tanks.

Record all data on Data sheet C (Appendix 1).

Retrieve larvae and change water on Day 4 and 6. Repeat procedures for retrieving larvae and water changes on Day 4 and Day 6, using appropriate sieves, (e.g. 100μm sieve for the top sieve, and 75μm sieve for the bottom sieve). Remember to measure the average size of larvae to ascertain pore sizes

of sieves to be used (page 22) as done on Day 2. Record all data on Data sheets D and E accordingly (Appendix 1).

5 **Feed the clam larvae.** Giant clam larvae, being planktotrophic, need microalgal food for development. The larva has only a small amount of yolk for embryological metamorphosis. Granting that culture water conditions are limiting in terms of nutritional sources, it is advantageous to feed giant clam larvae with cultured unicellular algae (Braley 1992, Fitt et al. 1984).

Monospecific cultures of microalgae should be prepared about a week prior to broodstock spawning, since they should be ready as food for clam larvae. Several species, belonging to the genera *Isochrysis, Pavlova*, and *Tetraselmis*, for example, lend themselves well to culture conditions (Kurosawa 1994). In feeding, single species or combination of two microalgal species may be used. Considerations for choice of microalgal species are (Villegas 1981):

- Its cell size is smaller than the mouth of the larva, and that cells have high digestibility.
- It is able to move or float, to allow the larvae to feed on it. Otherwise, the food sinks to the bottom and is lost to the larvae.
- Its nutritive value, fed to larvae alone or in combination, is suitable to the larvae.
- It exhibits coloration, to facilitate their monitoring in culture.
- Its production capacity supports the required feeding density for larvae.
- It grows well in large volumes.
- Production is economically feasible.

The following discussion focuses on the microalga *Isochrysis galbana* (Division Chromophyta, Class Haptophyceae, Order Isochrysidales), which is used as food for giant clam larvae. Its cell size measures 5-8μm. It grows at temperatures between 15 and 25ºC. In culture, it may reach peak algal densities of 8-12 x 10^6 cells per ml in 10 days. It grows well in large volumes (Kurosawa 1994).

Summary procedures for microalgal culture. Remember these four important details: (1) Sterilize all materials. (2) Always use aseptic methods. (3) Prepare Walne's medium (Tech 1981) (page 87), and inoculate culture media with starter algae. (4) Subculture weekly.

To count microalgae and determine microalgal density, you will need:

    60 ml beaker
    Pasteur pipette with bulb
    Hemocytometer with coverslip
    Compound microscope
    Tally counter
    Calculator
    Data sheet C (Appendix 1)

Take aliquot samples of the microalgae *I. galbana* from the 3-6 days old cultures. Take note of the volume of the container. In counting microalgal cells, lay the coverslip over the hemocytometer's grid areas. Note that the hemocytometer has two grid areas. Agitate the aliquot sample by pressing the Pasteur pipette bulb a few times, while the pipette tip is in the sample. Then carefully get a sample with the pipette, and position the pipette tip close to the edge of the coverslip. Press the bulb slightly just enough to form a drop, maintain the bulb pressure, while allowing part of the drop to seep under the coverslip. Still maintaining the bulb pressure, move the pipette tip to the other grid area, and allow the remaining droplet or part of it to seep under the coverslip. View the sample under a compound microscope, and count all cells found within one grid area aided with a tally counter. Then, similarly count all cells found within the other grid area. Calculate the average microalgal count, and multiply this average by 10,000 to determine the number of microalgal cells in the culture container. To compute for microalgal density, divide the computed number of cells by the recorded volume of microalgal culture. Record all data on the Data sheet C (Appendix 1).

Start feeding larvae with microalgae on Day 2 after water change. Use 10,000 to 15,000 microalgal cells per ml (Fitt et al. 1984). Compute for the volume of microalgal culture to feed clam larvae by performing similar calculations as stocking fertilized eggs. Continue feeding daily until Day 30 post-fertilization. Record data on Data Sheets (Appendix 1).

6 **Establish, culture, and feed zooxanthellae.** In nature, clam eggs and larvae do not inherit any zooxanthellae from their parents (Heslinga & Fitt 1987). Zooxanthellae are acquired by giant clam larvae from the water column, although the basis for establishing specific clam-alga affinities still needs to be determined.

In culture, larvae are given fresh zooxanthellae taken from mantle clippings (biopsy sampling: Benzie & Williams 1992), which are small pieces of mantle snipped from large clams. Zooxanthellae from the same clam species are commonly used, although zooxanthellae from other species may do (JL Munro, pers. comm.). You will need the following:

> Forceps
> Surgical scissors
> Beakers with UVFSW (UV-treated 0.2µm FSW)
> Food blender
> Series of sieves: 100, 63, 25µm sieves
> Graduated cylinder
> Centrifuge
> Centrifuge tubes
> Compound microscope

Pasteur pipettes with bulbs
Hemocytometer
Tally counter
Data sheets (Appendix 1)

Have 2-3 large clams (preferably not <30cm in shell length) in the raceway with running seawater. The clams may be selected at random, or may be based on predetermined selection criteria, like high clam growth rate (Heslinga et al. 1990, Molea & Munro 1994), desired mantle coloration (RD Braley, pers. comm.), etc. To clip the mantle, clamp the mantle with a steady hand about half-cm from the mantle edge with a pair of forceps. Then with a pair of scissors, quickly snip around the forceps tip to get a small piece of the mantle, about 1 cm² (Fig. 9). The pair of scissors must be sharp and sterile. Get 2-3 clippings per clam. Immerse the mantle pieces in a beaker of UVFSW during mantle clipping.

Wash the pieces of mantle with UVFSW. Put the mantle pieces in a blender with some amount of UVFSW; cut the tissues into very fine pieces. This process results in a brown suspension containing zooxanthellae. The suspension will have tissue debris as well which need to be removed. Pass the brown suspension through a series of sieves (100, 63, 25 μm), and discard tissue debris collected on the sieves. Purify the zooxanthellae in the brown filtrate by transferring the filtrate into centrifuge vials and spinning the suspension in a centrifuge. The zooxanthellae will form a brown pellet residue at the bottom of the centrifuge vials. Decant the supernatant, and add a few ml of UVFSW to the centrifuge vial, enough to resuspend the pellet. Agitate the sample by carefully pipetting and releasing the sample several times until the pellet has disintegrated, and a suspension is formed.

Fig. 9. The mantle of a broodstock being clipped for zooxanthellae extraction

Zooxanthellae in culture. Zooxanthellae may also be propagated in cultures (refer to Appendices 2 and 3). Unlike microalgal cultures where a monospecific culture refers to the culture of one species, clam zooxanthellal cultures are based on clones derived from a single cell. Zooxanthellal clonal cultures may be further classified as particular clades, depending on genetic affinities. The maintenance of zooxanthellal cultures is more rigorous than that for microalgae. Unless starter cultures are readily available elsewhere, establishing clonal cultures derived from single cells takes time, with a low percentage of success. In addition, even as all clam zooxanthellae are lumped into one species, *Symbiodinium microadriaticum*, there are numerous strains. Different clam species may harbor zooxanthellae populations characterized by one or several dominant strains, assemblages of which may change over time (Belda-Baillie et al. 1999). Attempting to maintain zooxanthellal cultures representative of any clam species is a big task that is labor intensive and expensive. However, the value of using cultured zooxanthellal clones for clam larvae is in selective clam breeding. If several zooxanthellal cultures have been established long enough that their genetic and physiological performance are known, selected clones may be used to improve clam breeding.

Count and determine zooxanthellal density. With zooxanthellae freshly isolated from mantle clippings, add UVFSW to the vial as needed, to have a known volume of zooxanthellae extract, as well as to dilute the suspension. (With zooxanthellae culture, note the volume of zooxanthellae culture per culture vial.) Record this known volume on Data sheet G (Appendix 1). Take aliquot samples and count zooxanthellae cells with a hemocytometer, following methods for counting microalgae. Compute the average number of zooxanthellae cells based on hemocytometer counts (x 10,000), and the zooxanthellae density (based on the recorded volume), as done for microalgae.

Feed clam larvae with zooxanthellae. The use of zooxanthellae as food for clam larvae needs to be coupled with larval development, particularly of tissues and organs for zooxanthellae uptake, ingestion and establishment of symbiosis. Larvae may be inspected daily during the early developmental stages, with minimum disturbance to the larvae, or in time with larval retrieval during water changes. To feed larvae, apply a density of zooxanthellae at 100 cells per ml of larval culture. A feeding density of up to 400 zooxanthellae cells/ml may be used (Heslinga et al. 1990).

After estimating the number of zooxanthellae in a particular volume of suspension (or culture), determine the volume of zooxanthellae suspension (or culture) needed per larval tank. Record data in Data sheet G (Appendix 1).

Cultured larvae may be fed with zooxanthellae as early as Day 3 (Braley 1992). The addition of zooxanthellae to developing larvae has its greatest impact

on the onset of metamorphosis, while larvae are still at swimming stage. (At metamorphosis, swimming veligers settle as pediveligers.) During this time, larval uptake and ingestion of zooxanthellae occur very rapidly (Heslinga et al. 1990). (At MSI, zooxanthellae feeding starts on Day 5.) Up to Day 7, alternate zooxanthellae feeding (i.e. Day 5, 7) with water changes (i.e. Day 2, 4, 6). By Day 8, the swimming larvae have metamorphosed and settled as pediveligers. Continue feeding the pediveligers with zooxanthellae until Day 9 and 13 (see Fitt et al. 1984).

7 **Prepare for larval settlement.** By Day 7 or 8 post-fertilization, most swimming veligers have metamorphosed and settled as pediveligers. During settlement, the clam larva's velum disintegrates, and a protrusible foot develops. The foot signals the start of the benthic habit, and aids the clam larva to identify the suitability of substratum for settlement. (A small percentage may still be at the veliger-pediveliger transition stage, i.e. displaying combinations of swimming, crawling, or probing behavior in the tank bottom.)

You will need:
    Settlement/juvenile rearing tanks, up to 5,000L volume, fiberglass or
        cement, filled with 1µm FSW (filtered seawater), with aeration
    1µm filter bags, for the settlement tanks
    Mosquito net material
    Thermometer
    Hoses, 2-2.5cm diameter
    Overflow pails
    75µm sieves
    100µm sieves
    200µm sieves
    Several 60L rectangular containers, fiberglass or plastic, with 1µm FSW,
        with aeration
    Dippers and 10L pails
    Plunge-type mixer
    Micropipette, 1000 µl, with (blue) tips
    Pasteur pipettes, with bulbs
    Dissecting microscope
    Compound microscope, with micrometer eyepiece
    Tissue culture plates
    Depression slides, with cover slips
    2 hand tally counters
    Data sheets (Appendix 1)

Prepare the settlement tanks. Ensure that the temperature of seawater in the settlement tanks does not go up while retrieving the pediveligers. Cover the water inlet with a 1µm filter bag, and have the seawater at slow flowrate while awaiting retrieval of pediveligers to finish. Cover the settlement tanks

with mosquito net material so that insects and leaves do not fall in the tanks. Provide moderate aeration. Keep the FSW running even at a slow flow rate just to keep the water from heating up while the larvae are still being harvested. Cover the standpipe of the settlement tank with a 100μm mesh, to prevent late developing swimming veligers from going down the drain. When the pediveligers are ready for transfer, turn off the seawater intake (Braley 1992). If there is more than one clam cohort being reared in the vicinity of the settlement tanks, label the tanks indicating source of larvae to avoid confusion.

Ascertain the sieve pore size for use in retrieving pediveligers, by determining the average size of pediveligers. Get samples directly from the larval tank with a Pasteur pipette, and use techniques similar to measuring egg size.

Harvest larvae from the rearing tanks. Retrieve all pediveligers by carefully draining the larval tank through a sieve series of 100μm sieve on top and 75μm sieve at the bottom (See Braley 1992). Pediveligers are washed off from the sieves, and transferred to 60L containers for counting. Simultaneously remove any observed debris from the sieve containing pediveligers, by holding a 200μm sieve beneath the 100μm (and 75μm) sieve, as pediveligers are washed off.

Estimate the number of pediveligers in the 60L containers, following techniques of estimating number of eggs. Mix the pediveliger water with a plunge-type mixer. Mixing should be relatively fast. Pediveligers are no longer swimmers, and they are heavier than younger larvae. Having lost their velum, they will now more likely sink to the bottom than stay in the water column. At all instances, the pediveligers should not come in contact with the hard surfaces while mixing (or transferring pediveligers), as they may get crushed. Maintain the mixer at a constant mixing rate and motion, so that during sampling (of 1ml aliquots) for counting, the pediveligers in the 60L container/s are fairly mixed (Braley 1992).

Restocking of pediveligers in settlement tanks. After all pediveliger containers have been sampled and pediveligers counted, compute for the densities of pediveligers per container. The recommended restocking density is 1-5 larvae per cm^2. Determine the water volume of settlement tank to be restocked. Record all data on Data sheet F (Appendix 1). After all pediveligers have been transferred to the settlement tanks, resume seawater inflow to the tanks at very slow flowrate (see Braley 1992).

8 **Feed the developing juveniles.** During metamorphosis, feed the developing juveniles with microalgae and zooxanthellae. Microalgae (from large cultures, see page 45, on supplemental nutrition) are given daily from Day 8 to Day 30. Compute for the volume of microalgae required for a feeding density of 10,000 – 15,000 cells per ml of tank water. Microalgal food contains both microalgae and nutrients, so that if given during daytime, the nutrients may be utilized

by the zooxanthellae for photosynthesis as well (Braley 1992). Ensure that the microalgae is poured throughout the tank.

Zooxanthellae (fresh extracts, see page 32; or from culture, Appendix 2) are given during daytime on Days 9 and 13, at a feeding density of 100 cells/ml tank water. Feeding the developing juveniles at postfertilization is done to assure that all developing juveniles have taken in zooxanthellae (see Braley 1992).

9 **Perform daily maintenance tasks.** In general, disturbance of settlement tanks must be kept to a minimum, as pediveligers are sensitive while meta-morphosing into small shelled juveniles (Braley 1992). Daily maintenance (from Day 8) of the settlement tanks focus on seawater filtration and aeration. Maintain seawater inflow at a very slow rate (see Braley 1992). Seawater entering all settlement tanks must be filtered through 1µm filter bags until Day 30 (Heslinga et al. 1990), with daily changes. When changing filter bags, close the water valve first before putting on the new filter bag. Clear and remove debris from the 100µm mesh covering the standpipe. Wash off anything adhering, most probably pediveligers, on the mesh back into the tank, by simply dipping the mesh several times into the water. Used filter bags must be chlorine-sterilized (page 19), and ready for next use. All tanks must be moderately aerated.

Use of shade material during metamorphosis. If larval rearing coincides with extended periods of high solar radiation, say warm months or summer time, shade the tanks up to 50% of surface area. This reduces exposure as well as controls the growth of filamentous and macroalgae thriving from nutrients added to the tank during microalgal feeding. Note that the mosquito net already in place over tanks provides shading of 5-10%. Shading may be up to Day 60, afterwhich the small juveniles will need sunlight to grow well (Braley 1992).

Countermeasures against heavy rains during metamorphosis. If larval rearing coincides with a period when heavy rains occur, remove the shading material, and instead use laminated sack or waterproof material to make a detachable roof. This will protect the developing juveniles from rainwater. Such roof material may be used until Day 60, afterwhich the small juveniles will need sunlight to grow well (Braley 1992). Alternatively, close seawater and aeration valves to allow rainwater to drain out with a little mixing with tank water. This will allow surface rainwater in the tank to drain over the pipe, minimizing salinity fluctuations.

Transition. By Day 13 or 14 postfertilization, metamorphosis into juveniles is almost complete. Increase the flowrate a bit from very slow (on Day 8) to moderately slow. Remove the 100µm mesh over the standpipe, and instead place a pipe sleeve to improve water circulation (Braley 1992). The pipe sleeve must be removed during heavy rains.

By Day 20, the seawater flowrate is increased but still slightly below normal levels as generally used for land-based nursery tanks. After Day 30, seawater inflow is maintained at normal flowrate. Seawater is filtered through 10μm mesh to allow clams to have access to more nutrients. Use of 10μm filter bags until Day 90.

Juvenile rearing in land-based nursery

At Day 30, clams are considered juveniles.

1 **Monitor and estimate survival of juveniles.**
You will need the following:

> 10cm x 10cm square, bottomless, Plexiglas template
> Hose, clear, 2.5cm diameter
> 200μm sieve
> Wash bottle, with 1μm FSW
> Beaker, 500 ml
> Petri dishes, glass
> Dissecting microscope
> Ocular micrometer eyepiece
> Pasteur pipettes, with bulbs
> 10μm filter bags
> Data sheet (Appendix 1)

Assess survival of settled juveniles monthly or at Day 30, 60 and 90. With several tanks to sample, always use clean equipment and materials, so that disease agents are not transferred between tanks. Sample each settlement tank using a square bottomless template placed randomly on the tank bottom, and then siphoning the water within the template (Fig. 10).

Make sure that one end of the hose is close to the template, and the other end is secured to a 200μm sieve. Take 3 samples per tank. Inspect the sample collected on the sieve. If it looks like a clean sample (with little siphoned algae), squirt FSW down the sieve, as the sample is transferred onto a petri dish. Count the number of live juveniles per sample, and calculate the average. Record all data on the Summary Sheet (Appendix 1).

To estimate the total number of juveniles:

Total # juveniles in tank = (#juvs/100 m^2) * base area (m^2) of tank

With time, the amount of fouling algae sampled along with the small juveniles may increase. If the amount of algae makes inspection of the juveniles difficult, separate the algae first by transferring the sieved materials to a

sieves

Sampling metamorphosed juveniles using plexiglass template

Siphoning metamorphosed juveniles through a sieve
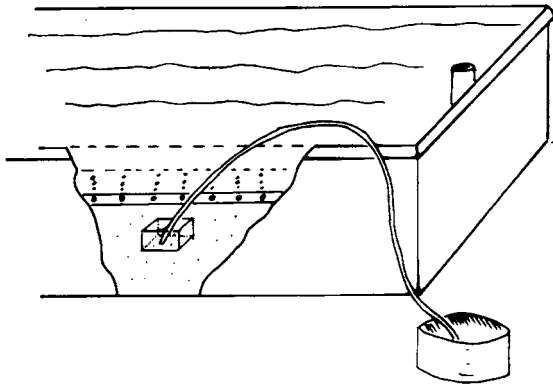
Fig. 10. Sampling of juveniles using 10cm x 10cm square bottomless template and sieve

beaker. For about a minute, allow the sample to settle, then slowly decant the upper layer of floating algae, leaving the bottom layer containing the small juveniles. Transfer the remaining contents to a petri dish, for viewing under the dissecting microscope.

Measure size of small juveniles. Measure the shell length of 50 juveniles under a dissecting microscope, using an ocular micrometer. Multiply the average value by the Calibration Factor to determine the average shell length. Record all data on the Summary Sheet (Appendix 1). Return live juvenile samples to their respective rearing tanks.

2 **Introduce grazers on Day 60.** Raceways are not to be disturbed till harvest time to allow complete metamorphosis of pediveligers into small juveniles. However algae will eventually take residence in the tanks and cause fouling. As early as Day 60, local grazers are then used to control algal growth in the tanks. Before using a new grazer, identify the grazer at least to family level, but ideally to species level. From the literature, determine their food requirements. Conduct short-term (about 2 weeks) feeding observations on the new grazer in a small tank containing algae (similar to that found in the tanks) and a few clams. Consider the following when choosing a suitable grazer:

- Must be an herbivore at all life stages
- Does not produce undesirable metabolic products or wastes that are harmful to clam
- Must exhibit clam avoidance when grazing
- Requires minimal maintenance work

Continue to observe the feeding behavior of the grazer while it is being used in the raceways, for any dubious clam mortalities. Cerithiids and small herbivorous hermit crabs (without enlarged chelipeds) have been successfully used by MSI as tank grazers.

3 **Harvest juvenile clams after Day 90.** Harvest juveniles when they reach 10-15mm shell length. The time of first harvest varies between 3 to 5 months from settlement, depending on the clam species. Fast growing species like *T. gigas* may be harvested earlier. However, some cohorts of other species have exhibited seemingly unique initial growth rates, attaining harvestable size earlier than expected. Refer to Griffiths & Klumpp (1996) for reported growth rates of different giant clam species.

To harvest, you will need the following:
    Scalpel
    Mask and snorkel
    Hose, 25mm diameter
    500µm sieve
    Deep pails
    Chlorine-sterile raceways
    Graduated cylinder, 500ml, 1000ml
    Cheesecloth
    Underwater slate
    15cm vernier caliper
    Data Sheet – Summary (Appendix 1)

First, chlorine-sterilize raceways where juvenile clams will be transferred after harvest.

Avoid stepping into the raceway to be harvested, since clams may get crushed. If the tank is large enough, don a mask and snorkel, and float at the water surface while harvesting clams. If the tank is small, reach out from the side of the tank while harvesting.

To harvest, detach juveniles from the substrate with a scalpel by pressing the blade close to the substrate's surface, to cleanly cut the clam's byssal threads. Leave the detached juveniles on the tank bottom for siphoning later. Prepare the sieve for siphoning by securing a 500µm sieve onto a deep pail. One end of the hose is held inside the tank, while the other end is clamped onto the sieve. Position the sieve/pail series lower than the water level in the tank to facilitate

siphoning. Hold the hose close to the tank bottom, and begin to siphon the detached clams. Inspect the sieve and pail periodically to ensure that small clams are not lost. Inspect any algal clumps collecting on the sieve for small juveniles; separate clams from the algae as necessary (see page 44).

Estimate the number of small clams. The number of small clams harvested is determined by volumetric estimation, which is based on water displacement. This is especially useful for clams not >10mm shell length and for very large harvests.

First, establish the average number of clams per volume (ml) of water displaced by taking a random handful of clams from any pile of clean clams. Place these clams on a slate, and count 5-10 groups of 100 clams. Briefly blot each group of 100 clams on cheesecloth.

Then, pour a known volume of 1μm FSW in a 500ml graduated cylinder. Note this initial water volume as V_0.

Add the first group of 100 clams to the graduated cylinder. Compute and record the volume of water displaced per group of clams as V_1. Repeat for the remaining 9 groups of clams.

Compute the average water volume displaced per 100 clams, and record as V_2. For example,

$V_0 = 100$ ml
$V_1 = V_0 -$ water volume displaced
For 5 groups of clams $= 30, 28, 29, 30, 31$ ml
$V_2 = (30+28+29+30+31)$ml$/5 = 29.6$ ml

This means that for every 1,000 clams, the volume of water that needs to be displaced is 296 ml.

4 **Stock juveniles in tanks.** The number of juvenile clams to be stocked in a raceway will be based on clam size and the bottom area (=length x width) of the raceway. One may strictly adhere to values obtained from straightforward calculations for number of clams required, given a particular tank area, at a particular stocking density. However, for practical purposes, clams are given enough space for growth at any given time, so that allowances need to be considered. Furthermore, the use of rounded off values, instead of exact figures, facilitates easy recall and notation.

The recommended stocking density for clams ~10mm in shell length is 1000 clams/m^2 (Table 2). To employ this stocking density, the number of harvested clams in a tank may be estimated in parts. The required number of clams for a given stocking density in a raceway may be similarly measured out volumetrically. Continuing from the above example on estimating the number of small clams –

Table 2. Recommended stocking densities of clams at different sizes for landbased nursery

Clam size range (mm)	Recommended stocking density (clam per m²)	Number of clams needed if tank bottom area is 10 m²
10-15	1000	10000
16-20	600	6000
21-25	400	4000
26-30	300	3000

If 296 ml of FSW needs to be displaced to get 1000 clams, fill a 1000ml graduated cylinder with FSW up to 704 ml. Put enough clams to displace the water level to 1000 ml. For every 1000ml level of the cylinder reached, make a record of 1000 clams harvested. Transfer the sampled clams to the intended raceway. This should be done 10 times to get a total of 10,000 clams for the raceway.

As clams grow, differences in growth rate may be apparent between species, cohorts, and even within the same cohort. Cull the larger clams and transfer to a chlorine-sterile raceway. With time, the stocking densities in the raceways will vary, decreasing as the clams grow in size. As a rule, stock juveniles at a low stocking density for better growth. The reasons for this are: (1) competition for space to grow in is reduced; (2) more food and nutrients are available per clam; (3) waste inputs to the water are less; (4) disease is less likely to occur; (5) predators that find their way into the tanks are easier to detect; and (6) parasitic infestations are easier to control. See the Table 2 for recommended stocking densities for juveniles in landbased nursery.

Attachment substrates. At first harvest, about 3 months from settlement, the small juveniles will already attach to portable substrate modules (Figs. 11, 15). Substrates of cement material are generally suitable, especially if they have rough surfaces. Preferably, substrates should be in the form of slabs, have uniform sizes, and about 15 mm thick. Plastic net material (same size as the
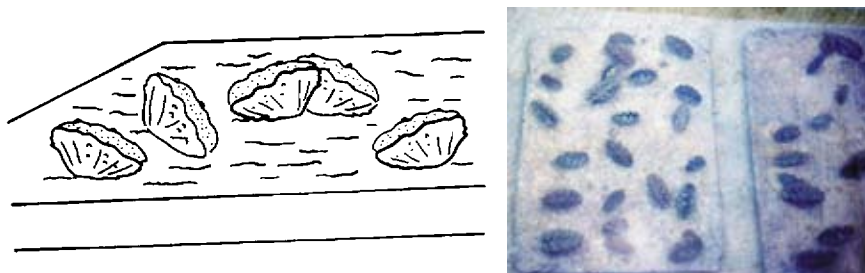
Fig. 11. Juvenile giant clams attached to a cement substrate slab

slab) is used to provide internal support in the construction or fabrication of the slab. Have a lead time of 1-2 weeks to cure the cement or concrete substrates in seawater before use. The substrate may be used as basis for stocking density (compare with use of tank area in previous discussion). It also facilitates the estimation of clam numbers during inventory.

Determine the number of substrates needed in the raceway. Closely align the substrates at the bottom of the raceway. Determine the stocking density, and the number of clams, given the bottom area of the raceway. Divide the number of clams by the number of substrates. Distribute the clams evenly, possibly in an upright position. To continue from the previous example, study the following computations based on substrate area:

Given small clams at shell length of ~10mm
Area of cement substrate, 45cm length, 30cm width
    $= 45$ cm x 30 cm
    $= 1350$ cm^2 $= 0.135$ m^2
Number of clams per substrate $= 1000$ clams/m^2 * 0.135 m^2
                                    $= 135$ clams/substrate
Number of substrates per 10sqm raceway = 60 substrates
#clams, 10m^2 raceway = 135 clams/substrate * 60 substrates = 8,100 clams

Water distribution and turnover rate. It is best for all the clams to have equal access to fresh seawater, as it brings food and nutrients. Tanks must have good water circulation. Given any tank design, seawater flow may be improved in different ways: (1) increase the number of water intake, (2) install water jets at the tank bottom, (3) maintain uniform aeration from the tank bottom, (4) install water baffles, or (5) install a sleeve over the stand pipe to drain water at the bottom. Allow a turnover rate of 1-2 times per day per tank.

Even if seawater is filtered, planktonic larvae may still be able to enter the raceway. If not detected early, these organisms develop into adults. Some of these organisms have good (if they are grazers) or bad (if they are predators) effects on clam culture. Regular inspection of the raceways makes evaluation of clam mortalities less difficult, as prior knowledge of occurrence of other organisms deemed harmful to clams leads to proactive responses, rather than reactive. Remove known predators immediately. Collect organisms of unknown effects on clams for study. If such organisms are found to have a positive role in clam culture, then they are allowed to take residency in the tanks (e.g. grazers). Their potential for polyculture (if such organisms are edible) may be investigated later.

All tanks should be vigorously aerated, either through airstones or through perforated pipes.

Control of algal fouling by manual cleaning. Manually clean settlement tanks beginning Day 60, or, after the juveniles are 2 months old. Cleaning the tank by siphoning earlier than the prescribed time may damage the byssal organs of small juveniles, and lead to mortality. Reduce algal fouling by using shade materials (see page 37). If, for some reason, extensive algal fouling occurs before Day 60, some method of cleaning may be improvised, as long as the juveniles are not lost or damaged. Check the effectivity of the 10µm filter bags; new ones may need to be purchased. Remember that 10µm filter bags are used for land nursery tanks after Day 30 until Day 90. Beyond Day 90, 25µm filter bags are used.

Before cleaning off fouling algae, inspect the algae first under the microscope to check if small juveniles are attached. If so, devise methods that will remove the algae and keep the small juveniles. If not, use methods that render least disturbance to the clam. For instance, juvenile-free floating algae may be gently induced to drift towards one corner of the tank by hand movements on the water surface, and thereafter picked out by hand.

After 2 months, the tank bottom may be siphoned regularly, every 7-14 days, depending on the level of fouling. As a precaution, secure the loose end of the hose to a large 200µm sieve with overflow bucket, in order to catch any detached juveniles. To start cleaning, first turn off water and air supply to the tank. Then move your hand in a waving motion over the tank bottom to loosen up any algal clumps. Some algal clumps may float; these may be siphoned or removed with a hand net. Inspect the algal clumps if they contain any juveniles; retrieve the juveniles and hold them temporarily in a pail with aerated FSW. Keep this pail away from solar heat. Siphon any loose algae from the entire tank bottom. Inspect the sieve periodically, and transfer as needed the collected algal-juvenile clumps to a tall pail. With your hand, gently break up any algal-juvenile clump. Fill the pail with enough FSW, and slowly decant the upper algal layer, leaving the bottom layer of small juveniles. Repeat this procedure until the water is fairly clear and juveniles are visible on the bottom. Return the juveniles to the tank in a staggered fashion.

5 **Supplemental nutrition.** Juvenile clams may receive supplemental nutrition to boost their health and condition. The need for such nutrition depends on the condition of the batch of clams, and prevailing weather conditions. Small clams that exhibit mantle paling, or bleaching need supplemental food to augment poor nutrition derived from zooxanthellae (Braley 1992). Improved mantle coloration in the clam indicates that its zooxanthellae population is healthy and can provide sufficient nutrition to the clam from photosynthesis.

Supplemental nutrition provide food to clams under temporary but limiting culture conditions. In the tropics, prevailing weather conditions, such as monsoon rains and typhoons, result in increased cloudy conditions, fresh-water input, and seawater turbidity in the landbased nursery. When rainy, the

tanks may be covered with translucent material, but remember that even such materials may restrict available light for zooxanthellar photosynthesis. Tank operations are also hampered by heavy rains, restricting the use of aeration and running seawater in the tank.

The most common supplemental food for giant clams is cultured microalgae, and fertilizer (see Braley 1992). *Isochrysis* or *Tetraselmis* may be given during daytime while briefly turning off the seawater supply. Note that algal culture supplements serve two purposes: the microalgae as food for clams, and the algal culture medium as nutrients (nitrates and phosphates) for clam zooxanthellae.

Greenwater culture. When large volumes of microalgae are needed as supplementary food to juveniles and broodstock, large culture volumes of naturally-occurring mixed populations of microalgae are set up. Such microalgae may be propagated in large scale by direct fertilization of seawater. You will need:

> 500L fiberglass tank, with 10μm or 25μm filtered seawater (FSW)
> 15L plastic pail, with lid, for fertilizer stock solution
> Ammonium sulfate, 520 g
> Superphosphate, 200 g
> Vitamin B_1, 3.72 g
> Mortar and pestle
> 10L warm tap water
> Glass stirrer
> Micropipette, 1000 μl, with (blue) tips
> Compound microscope, with micrometer eyepiece
> Hemocytometer
> Hand tally counter

Grind the ammonium sulfate and superphosphate to powder, then dissolve in 10L warm tap water, and then add the vitamin. The solution must be stirred well, then covered for storage. Dispense 1 ml per liter directly to a tank with FSW. After a few weeks, a green-colored algal bloom will develop, hence it is called greenwater culture. Assess the culture sample under a compound microscope – a mixed microalgal culture containing flagellates between 2-10μm diameter is suitable as food for juveniles or adults. Discard the culture if it contains ciliated protozoans, fungus or dense bacteria, or if it emits a strong odor implying that algae are dying. To maintain the greenwater culture, subculture every 7-10 days (see Braley 1992, see Tech 1981).

***Supplemental nutrients for clam zooxanthellae* in vivo.** Commercial NPK fertilizer may also be used. Generally, nitrogen is in the form of ammonium sulfate, and phosphorus as superphosphate. Grind the fertilizer granules, then dissolve in equivalent UVFSW required for application. While turning off the seawater supply to the tank, pour the fertilizer solution over the tank water in

daylight (see Braley 1992). Nitrogen as nitrates may also be provided, although ammonium sulfate appears to be more readily absorbed by the clam zooxanthellae. The addition of nitrogen and phosphorus into the tank also enhances the growth of other algae (macro- and filamentous algae), hence fouling algae may need to be removed frequently.

6 **Watch out for diseases and parasites.** Periodically check the raceway for sick clams, so that infection may not spread and promp quarantine measures taken. Braley (1992) provides discussions and references on diseases/infections in larvae, juveniles and broodstock. These include:

> Bacterial disease caused by *Vibrio, Rickettsiales*-like organism
> Protozoan infection caused by *Perkinsus, Marteilia*-like protozoa
> Winter mortality; chilling
> Heat stress; gas bubble disease
> Bleaching; nitrogen deficiency
> Salinity stress

Tissue samples for histological or pathological study are to be handled as follows (Braley 1992, Calumpong 1992):

- Preserve small pieces of the clam tissue in 3-5% seawater-buffered formalin solution.
- Retrieve the fixed tissues from the preservative after 2-3 days, wrap each piece in paper towel pre-soaked in the same fixative.
- Store in small, sealed plastic bags.
- Send the samples to a suitable laboratory (which prescribes the appropriate packing material), e.g. the Oonoonba Veterinary Laboratory in Australia, as soon as possible, accompanied by relevant details.

Pyramidellids (Figure 12), particularly the genera *Turbonilla* and *Tathrella* (Govan 1992), are known ectoparasites of tridacnids, and may establish local infestations in the raceways (Cumming 1988). These parasitic snails are cryptic, nocturnal, and nighttime feeders. The easiest way to detect them is to observe the clams at night by directing a flashlight over the clams. These snails position themselves along the edge of the shell to feed on the clam's body fluids. Pyramidellids may decimate juvenile clams in raceways, but not so with adult and subadult clams. Such infestations may be controlled by using 1μm filtered seawater in the tank, and maintaining good husbandry (e.g. apply proper clam stocking density) and tank hygiene (e.g. control algal fouling, siphon off bottom sediments, drain and replace seawater in nursery tank, chlorine-sterilize the tank every month, and allocate cleaning equipment and materials per tank to avoid between-tank contamination).

Also, to manually eliminate pyramidellids, gently brush them off the clams, rinse the clams thoroughly with 1μm FSW, and return to a chlorine-sterile tank with

Fig. 12. Pyramidellids on juvenile clam

fresh seawater. Collect the pyramidellids and discard them by drying under the sun. Drain the tank every 7 days (with immediate seawater replacement), as this action may break larval settlement of specific pyramidellid species (see Braley 1992).

Population genetics and invasives

Although giant clams are very highly heterozygous (Benzie 1993), mariculture tends to restrict the possibilities for genetic heterogeneity, because broodstock are limited, and/or selected. One should make appropriate managerial decisions on the purpose of culture, whether to improve stock by selecting traits, or to employ the most random selection of broodstock and largest number of broodstock to minimize reduction of genetic variability. Stock transfers between oceanic regions may affect local populations, hence such movements should be carefully considered. Inbreeding between siblings, and close family lines is not recommended.

Recordkeeping (data collection and management)

Keep records of all hatchery activities ~ spawning outputs, seed stock inventories, laboratory stock inventory, landbased nursery stock inventory, suppliers, expenses, revenues, and forms. For the volume of records to be handled, the value of comput-

erization of records cannot be overemphasized. However, file or store properly the original documents, their photocopies, and backup electronic files regularly. Also keep notebooks and logbooks for reference.

The record of hatchery activities serves as a good monitor of daily tasks. In addition, observations may be recorded by hatchery staff and these may have relevance later. File the daily record every month, and from these, the handling history and performance of each spawned cohort may be obtained.

Record and file separately the spawning events and sort according to the species spawned and its spawning date. Each record contains a set of Data Sheets (Appendix 1):

> Spawning
> Day 0 - estimated number of eggs and stocked eggs
> Day 2
> Day 4   estimated number of larvae,
> Day 6   restocked larvae, larval size, and feeding
> Day 8
>
> Zooxanthellae
> Day 30, 60, and 90 – estimated number of juveniles, restocked juveniles, juvenile size summary (spawning to Day 90)

Broodstock and seed stock inventories (Appendix 4) show the numbers of broodstock and juveniles per cohort, respectively. Maintain an additional log of broodstock tag numbers and corresponding clam sizes. For juveniles, determine when the cohort is harvested and the total number of small juveniles per cohort. The duration of rearing time to harvest may vary between species, cohorts, and batches. After harvest, seed stock in the landbased nursery is inventoried at least every 6 months. Coincident with harvest time and inventory, measure and get the average clam sizes (=shell length) of 30 to 50 clams (±S.D.).

The laboratory and the landbased nursery stock inventories show the status of materials, chemicals, and equipment in the laboratory and landbased nursery. Do this at least once a year.

General housekeeping

Hatchery and landbased nursery operations are largely dependent on the seawater facility. Seawater should meet the requirements for culture. Ideally, the seawater facility should provide data on seawater quality. Technology makes available several types of monitoring systems that may be remotely controlled, portable or handheld. Equipment that record continuous data (e.g. temperature, salinity, pH,

turbidity, and dissolved oxygen) either as a single parameter or as multi-parameters may be an important reference source when monitoring the growth of organisms in culture. Discontinuous but periodic data may also be collected for reference.

Temperature. A recording thermograph for seawater use may be installed to measure water temperature. Such an instrument gives temperature readings at a glance, hence may be convenient and practical for mariculture.

Another instrument that measures temperature is a thermistor, technically called a 'bit', made of special material that is highly sensitive to temperature. The thermistor is programmable, easy to deploy, and data are electronically downloaded to a computer. However, unlike the recording thermograph from which instantaneous temperature may be read, the thermistor provides temperature data periodically, or at every retrieval.

Salinity. Salinity is easily determined with a handheld refractometer, which determines the amount of salt dissolved in the water through light refraction. When a drop of seawater is placed on the sample window, light passing through the drop bends as it passes through the optical prism. The salinity reading is then viewed through the eyepiece of the refractometer, as it is oriented in the direction of a light source. The bent light casts a shadow on the salinity scale, hence is read off directly. A salinity range of 32-36 ppt is acceptable for rearing clams (Huguenin & Colt 1989, cited in Braley 1992).

pH. pH is measured with a pH meter. The pH of seawater is naturally buffered by the various seawater elements. Seawater pH ranges acceptable for clam culture are from <7.9 to 8.2 (Huguenin & Colt 1989, cited in Braley 1992).

Dissolved oxygen. Most marine organisms need oxygen for respiration, hence it is important that there is sufficient oxygen dissolved in seawater. Dissolved oxygen, or D.O., may be measured with a D.O. meter. Since giant clams are photosynthetic organisms, they contribute to dissolved oxygen in the seawater in the presence of light. In suboptimal light conditions, or absence of light, giant clams may deplete the D.O. in seawater. Aeration of seawater increases the D.O. level.

Turbidity. In the raceways where the water is shallow, turbidity is measured with an electronic turbiditimeter. Relative measures of turbidity may be obtained by comparing water samples from the surface and bottom. A high turbidity reading implies a high level of suspended particles in the water, reducing the availability of light for photosynthesis.

As with all equipment and materials, prior to use, always refer to the manufacturer's specifications on maintenance, calibration, and operation.

OCEAN NURSERY

Selection of the site

Select the ocean nursery site for giant clams before transporting the clams. The criteria for site selection are initially discussed on page 14.

- The site must be shallow (up to 3 meters) and clean. Since the giant clam is symbiotic with photosynthetic zooxanthellae, the clam must be placed in a site where there is appreciable penetration of sunlight.

- The site must have good water movement. The giant clam siphons water for oxygen. It also expels particulate food rejects from its gills and excretes wastes. Good water flushing will provide new sources of dissolved oxygen, dissolved nutrients and particulate food. It will also ensure that wastes do not accumulate and decompose in the immediate vicinity of the clam.

- The site must be protected from typhoons. A cove or embayment will most likely be protected or least affected by strong water current movement during typhoons. Although very large giant clams may be able to weather typhoons and strong wave action, most clams are defenseless when they are covered by debris like seaweeds, seagrass, broken coral, sand, etc. brought about by strong wave action.

- The site must be accessible to the steward who will visit and monitor the site often.

- The site must not be in the way of boat navigation. It is a common practice for a fisher reaching shallow water to move his boat using a long bamboo pole as leverage. The fisher may unknowingly hit and kill the clams on the seabed with his bamboo pole.

- The site must be far from freshwater runoff. Exposure of clams to reduced seawater salinities will reduce clam growth rates. Clams exposed to freshwater will die.

- The site must be protected from clam poaching, dynamite fishing, and cyanide fishing. The nursery must be in a protected area (sanctuary or reserve) by law and/or stewardship. People's organizations, such as the *Bantay Dagat* (Sea Guardians) and fishers/farmers cooperatives, and local government units should be involved in protecting the giant clam ocean nursery. These safeguard the clams from poaching, and the effects of illegal methods like the use of cyanide and dynamite. Dynamite and cyanide fishing kill marine organisms and destroy habitable substrata. When substrata are destroyed, marine larvae are not able to settle and recruit.

- The site must be far from pollution sources (chemicals, oil spills, thermal pollution, siltation, and garbage). Pollution will either kill the clams or reduce their growth and reproductive rates, thus the clams' ability to contribute to natural recruitment is reduced.

As soon as the site has been selected, determine the specific areas for the installation of the cages or lines, and for clam growout.

Setting up the ocean nursery

A boat or raft is needed to set up the ocean nursery. The steward shall need either goggles and fins (snorkeling gear), or SCUBA gear, to work underwater. These are also needed:

Cage
Line
Cement slab substrates (10 per cage)
Boat or raft
Submerged or floating markers
Fiberglass measuring tape
Handbrush

1 **Mark the boundaries of the site underwater** by driving wooden pegs into the substratum, and/or above water by float markers. The site may be regular or irregular in shape. Determine the area of the site for planning purposes and its exact boundaries by taking GPS readings (from which area may also be determined). Otherwise, calculate the area using traditional methods and situate the site on a map. To estimate the area, get the length, width, or diameter of the site underwater using a fiberglass measuring tape. In the absence of a fiberglass measuring tape, a long piece of rope may be laid underwater along the boundaries of the site, the length paid out marked, and estimated above water using a meter stick. Some useful formulas for site area are:

Area of a rectangular (or square) site = length x width
Area of circular site = 3.1416 x (½diameter)^2

The cage (Fig. 13) is made of ¾" polyethylene (PE) screen and consists of two units, the rectangular box which serves as the base, and the lid, both supported by ¾" diameter PVC-pipe frames. The cage dimensions are 1.6m length, 1m width, and 230cm high The PE screen is sewn to the frame with 45-lbs monofilament nylon, or cable ties, if preferred. Single-stranded insulated wire or 5mm thick evelon cord (nylon rope) is used to join together the base and the lid when cage is installed. It is raised from the sea bottom by about 1/2m, propped and secured onto angle bars that have been driven deep into the sandy substratum.

The cage is used to rear clams between 3 to 15cm shell length (SL). Below 15cm SL, clams are vulnerable to predators. The lid has a short skirt of PE mesh that covers any gap between the lid and the base. With a nice fit between the lid and the base, the cage excludes clam predators from entering the cage. The skirt on the lid adds security to the cage against most crawling and swimming predators. Generally, as clams reach 15cm SL, they become less vulnerable to predation, and over time as they grow larger, predation-related mortality becomes even less. Past 15 cm, clams are ready for growout, i.e. planting large clams directly onto the seabed or onto raised platforms.

If the sea bottom is relatively flat, comprising of coral rubble and sand, with minimal plant growth, the line (Fig. 14) may be used for rearing clams <15cm SL. The line consists of units of PE screen, formed as a box without the lid, and installed upside down over the clams; the units are connected lengthwise to form a long line of cages. The line is installed directly on the sea bottom, hooked every half meter to the seabed by corrugated bars driven deep into the substratum. In addition, for each unit, a corrugated bar, with one end formed as a hook, is driven into the center of each unit under the polyethylene mesh, but leaving about 0.25 meter (or about 9-10 inches). This keeps the unit from sagging at the center. The height of the line unit must be at least 0.25 m.

Fig. 13. Raised cage for juveniles in the ocean nursery

Polyethylene screen

steel bar
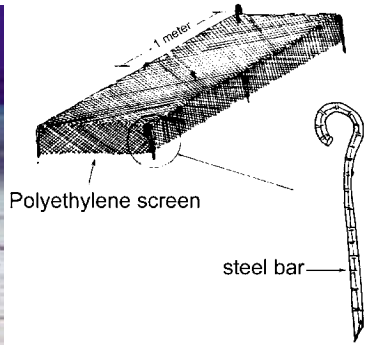
Fig. 14. Clam line in the ocean nursery
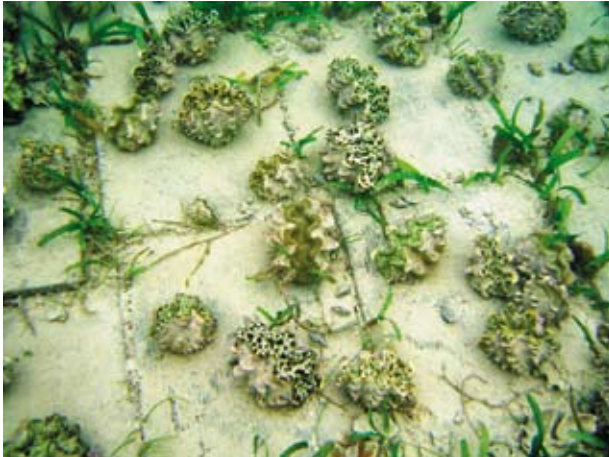
Fig.15. Clams attached to two kinds of cement substrates: rectangular slab (top), and circular with central pit (bottom)

Cement slabs (Fig. 15) are used as attachment substrates, lining the bottom of either cage or line. For the boring species like *Tridacna maxima* and *T. crocea*, circular cement substrates with shallow pits at the center (following the design by the Coastal Aquaculture Center, Solomon Islands) are used. The clam normally covers its byssal gape with, say coral rubble, or attaches to a firm substrate such as a coral rock. This behavior deters small predators from attacking the clam through its byssal gap. It is for this purpose that the clam attaches to the cement substrate.

2 **Determine clam stocking density.** Calculate or estimate the stocking density of the clam for a given area and clam size. Refer to Table 3 for recommended stocking densities.

Stocking density = total area of bottom / (shell length per clam)^2

Examples:

a) To determine stocking density for a cage lined with 10 cement substrates:
   Area per substrate = 1,350 cm^2
   Average clam shell length = 3 cm
   Clam stocking density = (10 x 1,350 m^2) / 3^2 m^2 per clam = 1,500 clams per cage

b) To determine stocking density of large clams ≥ 30 cm (*T. gigas*, for example):
   Area of seabed for growout = 900 m^2
   Clam shell length = 30 cm
   Clam area = 1 m x 1 m = 1 m^2 per clam
   Stocking density for large clams = 900 m^2 / 1 m^2 per clam = 900 clams

Table 3. Recommended stocking densities of clams at different sizes for ocean nursery

Clam shell length (cm)	Recommended stocking density*
3	1,500 clams per cage
5	540 clams per cage
10	135 clams per cage
12	94 clams per cage
20	10,000 clams per ON site
≥ 30	900 clams per ON site

*Assumes that the dimensions of the culture unit are as follows:
   Cage = 1.6m length, 1.0m width
   Ocean nursery (ON) site = 30m length, 30m width

As clams grow, they increase in weight exponentially. By the time a clam reaches 30cm size, it may weigh about 5 kg. Because of their weight, large clams are difficult to handle, hence fewer clams are deployed in the same area, than if 20cm clams were used.

Transporting and placing giant clams in the ocean nursery

Before clams are transported from one place to another, they should be quarantined (see page 69) to ensure that the batch is healthy and does not carry disease agents, parasites, or pests (see page 46). Prior to quarantine, tag the clams (see page 12) for identification and monitoring purposes. Continue the quarantine until such time when the clams are prepared for packing (see page 73).

Pack clams in styropore boxes as per approved protocol (page 73). Do not open the box until the seeding site is reached. Whenever possible, transport clams via the quickest, smoothest route, so that clam survival is not detrimentally influenced by transport-related stresses. For long hauls >10 hours, re-immerse the clams in water during travel stops (see page 73).

Upon reaching the seeding site, acclimatize the clams first. Then, have a diver bring down clams (using the sack for ease of handling) which he distributes to cage, line, or seabed. Apply the recommended clam stocking densities. To facilitate monitoring of clams, group together the same cohorts in a plot.

Monitoring growth and survival

Inspect the ocean nursery at least once a week. Depending on the number of clams and water depth, either SCUBA or snorkel may be used. Brush or scrape off organisms (such as seaweeds, sponges, soft corals) adhering to the culture unit. In addition, open the culture unit to inspect the clams and the cement substrates, and remove all other organisms that have gained entry into the culture unit. Grazers or herbivores may be allowed as biological control agents against algal fouling within the culture unit.

Record clam growth and survival data periodically. Two measures may be used to determine growth in giant clams: shell length (SL) with a caliper, and total clam weight with a spring balance. Clams <30cm SL are measured every month. Clams >30cm SL are measured every quarter (every 4 months). Clams >50cm SL are measured annually (every 12 months). Depending on the ease of handling the clams, length data may be collected either underwater or on the boat. Measurements taken either underwater or on the boat are recorded on a waterproof slate (Appendix 4). If clams will be taken up to the boat for measurements, ensure their protection from sun, heat, and freshwater. Thirty clams per cohort are measured for SL measurements. Emersion time should not exceed 1 hour; re-immerse the clams as soon as possible after measurement.

To obtain weight data, clams are taken up to the boat. Depending on the ease of handling the clams, 10-30 clams per cohort may be weighed. Turn the clam upside down for 5-10 min to drain water from its mantle cavity. If the clams bear tag numbers, record data corresponding to the clam tag. Similar precautions for

emersed clams as discussed above must be taken. Upon reaching homebase, transcribe data to a logbook. For each data set, calculate and record the average value as either average SL or average total weight, as the case may be.

Clam survival is determined by conducting at least a monthly inventory of all clams. Depending on the number of clams and depth, monitoring survival may be done by using either SCUBA or snorkeling gear. To inventory, count the number of clams per species, and per cohort. Record the data on a waterproof slate. All empty shells (dead clams) must be collected according to cohorts, their tags noted. At the homebase, transcribe the data from the waterproof slate to the logbook. Measure the shell lengths of the empty shells, and record similarly. All growth and survival data may be stored electronically in a computer database.

Husbandry

1 **Thin out giant clams in cages.** Since giant clams grow fast in the ocean nursery, they should be thinned out every month to prevent crowding. Crowding reduces clam growth, causes abnormal shell formation and, if unchecked, clam mortality. With every monthly inventory, apply the recommended stocking density (see Table 3, page 55). As a rule, the number of clams for any given area should ensure that clams do not "touch" each other.

To thin out or reduce stocking density in a crowded culture unit, remove a few clams from within a cluster, and place them in another culture unit that is empty or not crowded (but the same cohort). To detach clams for transfer, press the sharp edge of a blade onto the cement substrate, just beneath the clam's byssal gape, then scrape the byssal threads from the surface of the substrate (see Fig. 16). Refrain from pulling or tugging the clam off the substrate. Pulling may injure or detach the foot/byssal gland altogether, and cause clam mortality.

Fig. 16. Harvesting juveniles with scalpel aimed at the byssus threads

Periodic clam thinning improves clam growth and survival. It also aids in reducing algal fouling that may occur within the culture unit, since it is easier to clean cement substrates with fewer clams than with more. In addition, predators or parasites are easily detected and removed.

2 **Control fouling in cages.** Fouling is caused by growth of sedentary organisms (e.g. seaweeds, tunicates, sponges, soft and hard corals) on the cage or the line, or by siltation. These organisms settle as spores or larvae on and in the culture unit. Weekly removal will control further growth especially if scraped or brushed off at the base. Pulling is ineffective, as some of these organisms are colonial and/or have the ability to regenerate from the basal attachment.

In places where siltation or sedimentation is seasonal, a thick layer of fine silt may accumulate on the cement substrate and corners of the cage. Small clams <5cm shell length may suffocate if the silt layer is thick enough to cover them. Large clams may not be directly affected by the silt, since such clams are able to clean silt off their mantles using water jet expulsion from their excurrent siphon. Such ability becomes more effective with clam size. Siltation becomes a problem too if the silt becomes a haven for predatory organisms to breed or hide in.

To clean off the silt from the cage, remove each cement substrate, and while holding it vertically (away from the cage), fan off the silt by waving your hand. Assuming that the clams are well attached to the substrate, the fanning motion unsettles the accumulated silt/sediment and falls off the cement substrate. To clean accumulated silt/sediment at the cage corners, first remove all substrates, then similarly fan off the sediment. Return all substrates and clams to the cage after cleaning.

3 **Remove predators and parasites.** In the ocean nursery, there are many natural predators of giant clams. Predators of giant clams are site specific, i.e. particular predator species are found in a site but not in others, or may predominate in a particular site. Frequent visits to the ocean nursery will enable one to be familiar with actual and potential predators.

Juveniles (not > 15cm shell length) that are vulnerable to predators need to be reared in protective cages or lines. Organisms that have been reported to prey on clams include flatworms (Govan 1994), gastropod snails (Perron et al. 1985, de Perio & Belda 1988, Govan 1995), various crabs including hermit crabs (Govan et al. 1993), octopus, stingrays, and other fish (Govan 1992) (Fig. 17). The culture unit (whether cage or line) is designed to exclude predators whose size is bigger than the mesh used. It cannot bar the entry of predators in its larval or juvenile stages, as in the case of ranellid snails. Some predators like the flatworm, crab, puffer fish and moray eel are able to pass through the mesh because of their flexible body size/shape. Upon inspection, identify and determine undesirable animals to its species name and assign functional status (whether predator or grazer).
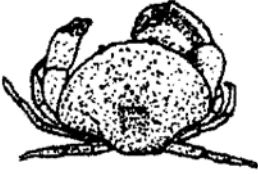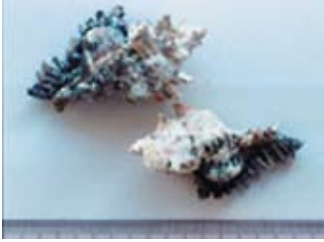
Carpilius sp. *Chicoreus brunneus* *Chicoreus ramosus*

Cymatium pileare *Cymatium muricinum* *Cymatium aquatile* *Cymatium nicobaricum*
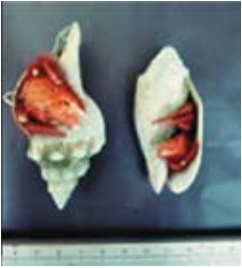
Dardanos megistos *Octopus* sp.
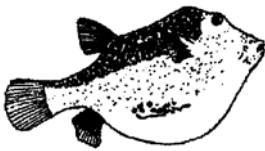
Puffer fish *Trigger fish*

Fig. 17. Some predators of giant clams

Parasites of giant clams may be internal (endoparasite) and/or external (ecto-parasite). Endoparasites are difficult to detect unless some body aberration is manifested (Shelley et al. 1988). Ectoparasitic pyramidellids (page 46) may also occur naturally in the ocean nursery, as they have been reported from oysters and mussels. Usually, small clams reared in culture units and infested with pyramidellids are not able to replace lost body fluids soon enough, and they die. In addition, natural predators of pyramidellids are excluded by the culture unit, allowing pyramidellids to establish breeding populations. But clams in the growout phase may be less affected by pyramidellids because they are able to recover lost body fluids, and in grow-out, natural predators of pyramidellids are present (see Cumming 1988).

Monitor and record the occurrence of predators and parasites, since such data can serve as basis to conduct intensified monitoring efforts.

Manually collect the predator or parasite weekly. When removing predators or parasites from the culture unit, refrain from discarding them in the ocean nursery, as they will find their way back to the culture unit. Predators may be immediately killed on site, or manually picked and taken up to the boat. Dry predators like gastropod snails under the sun, and store in a plastic bag, while preserve/store fish predators in ethanol in a glass bottle. Stored samples may serve as reference material, hence must be labeled with collection date, site, collector, and identification. On the other hand, brush pyramidellids off clamshells and cement substrates into a fine collecting mesh. The mesh may be an improvised bag that opens up, or with a wide enough mouth, or may be attached to a weighted frame. Take the mesh up to the boat, dry the contents on land and discard. Weekly removal of predators and pyramidellids from the culture units may reduce their natural population at the site, such that they are no longer considered a threat to the clams.

Monitoring disease occurences

Diseases evaluation is best conducted in a pathology laboratory. Whole speci-mens, organs, or samples of diseased tissues are preserved for histological study. Manifestation of disease is evident mostly when the clam has already succumbed, rendering reactive rather than proactive action.

If the services of a pathology laboratory are lacking, frequent visits to the ocean nursery to monitor the clams become more important. Disease in giant clams may be detected by ocular inspection and comparison with healthy-looking clams. Early detection and control prevent the spread of disease.

A healthy clam is able to extend its mantle over the shell edge (for *T. gigas, T. derasa, T. squamosa, T. maxima*, and *T. crocea*), or up to the inner shell margin (for *T. mbalavuana, H. hippopus*, and *H. porcellanus*). The mantle is actively passing water

through its apertures, and immediately responds to external stimulus (passing shadow created by a hand). The inner shell margin is white and smooth. Shell growth as seen from the outer shell is typically continuous, and without growth check. The valves appear solid, without perforations.

If clams appear to be in poor condition, they may not be necessarily diseased. However, if the clam condition is not properly and immediately addressed, disease may set in. Poor condition may reflect poor husbandry, unsuitable light and water conditions. Often, a growth check appears, i.e. a marked shell deposition. Poor deposition may indicate reduced calcification rates, in response to suboptimal environmental conditions (such as low or high temperature, very high irradiance levels, nitrogen deficiency, low or very high salinity), persistent irritants (like parasitization), among others. These conditions may lower the general health of the clam, making it susceptible to bacterial or fungal infection (see Braley 1992; Calumpong 1992).

Table 4. Some indicators of poor clam condition are as follows.

Poor mantle condition	Poor shell condition
Retracted	Improper valve closure
Flaccid	Presence of growth check
Pale	Presence of perforations
Presence of white spots; white blotches (bleaching)	Presence of brown/green line on inner shell
Presence of lesions	Abnormal shell shape
Presence of translucent air blisters	Thickening of shell margin; stunting
Presence of inflammations	
Mantle edge detached from inner shell	

Probable cause of poor condition may be determined with further observations. This applies to mantle retraction, the cause of which may be difficult to initially determine.

Clams that are suspected to be diseased may be separated from the others to prevent the spread of infection. Mantle discoloration may be related to nitrogen deficiency, or to high temperature anomalies. High temperatures and high irradiance may cause bleaching in clams. High temperatures also influence the dissolved oxygen saturation levels in seawater, and may lead to blister formation in the mantle (Braley 1992). Under such conditions, affected clams should be transferred to waters 1-2 degrees lower than the prevailing temperature levels. Clams bearing lesions may harbor bacteria or fungi in the infected area. Presently, no antimicrobial treatment is available.

Improper valve closure may indicate a cracked hinge. This may be observed in large clams that have been dropped or mishandled. Improper valve closure makes the clam vulnerable to all kinds of predators. Furthermore, secondary microbial infection may set in at the hinge. Such clams require a flat surface, like a cement slab, to encourage proper valve closure. However, it should be noted that large *T. gigas* do not have the ability to fully close their valves, even if healthy. For *T. gigas*, the other measures of clam condition need to be assessed to determine poor condition.

Deviations from normal shell growth like growth check, abnormal shell shape, and stunting in clams all indicate that the clams are already overcrowded, and need to be thinned out. If the stocking density is high, there is also a greater chance for disease to spread, since clams are in close proximity, than if the stocking density is low.

Similarly, clams afflicted with shell-boring sponges (e.g. *Cliona* sp.) should be moved apart from other clams. Shell-boring sponges similarly harbor zooxanthellae in their tissues, and hence may prefer lateral extension (that will allow sponge zooxanthellae access to sunlight) in the clam shell rather than depth penetration into the shell (see Schonberg 2002). However, infected shells of Philippine clams reared in the ocean nursery show that sometimes the shell-boring sponge may completely penetrate through the shell. Chemical treatment of the shells externally with formalin (say 1%) has been recommended but must be applied with caution (Govan 1992).

Mantle retraction and growth of a green or brown band of filamentous algae on the inner shell edge are often observed simultaneously. Protracted mantle retraction exposes the inner shell edge to the settlement of algae. Although the cause of mantle retraction may remain undetermined, the algal band may be removed by carefully scraping the inner shell edge with a sharp blade.

Record keeping

The need to keep good records cannot be overemphasized. Maintain a log of work done for every trip made to the ocean nursery. This will serve as a reference for work that have been accomplished or for planning future activities. Keep records of clam stock inventories, field supplies and equipment inventory, suppliers, expenses, revenues, and forms. To ease information retrieval, it is wise to computerize all records, and backups of electronic files be made regularly. The electronic storage of data should always be supported by storage of original documents, including logbooks or their photocopies, which should be filed and stored properly.

The regular clam stock inventory shows the numbers of clams per cohort per species, and in chronological order. A good inventory starts from the time the clams were transferred from the landbased nursery phase to the cages in the ocean nursery,

and to the growout phase. The inventories of juveniles, subadults, and broodstock may be kept separate for easy retrieval of information.

The inventory of field materials and equipment shows their status, which may be evaluated regularly, but at least every 6 months. Separate files should be kept for particular equipment that need periodic maintenance, e.g. boat, outboard engine, scuba tanks, compressor, etc. A list of suppliers and contact information are compiled for specific materials, and equipment, together with their related brochures and information sheets.

Similar procedures (such as record of expenses and income) and other forms used for the hatchery may be used for ocean nursery.

STOCK ENHANCEMENT

Purposes of stock enhancement

Giant clams are commercially-important species. In many places in the Indo-Pacific region giant clams have been subjected to unregulated harvesting, leading to their decimation (Lucas 1994). All giant clam species are presently listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 1979). In effect, their movement to and from CITES signatory countries is regulated.

In addition to fishing pressure, natural and anthropogenic disturbances continue to affect clam stocks. Natural disturbances include the El Nino phenomenon (Gomez & Mingoa-Licuanan 1998), strong typhoons (see Gomez & Mingoa-Licuanan 2006), and disease (see Humphrey 1988). Mass extinctions that have been registered in the fossil record (Valentine et al. 1973) and previous mass mortalities still need further investigation (Alder & Braley 1988). Anthropogenic disturbances, on the other hand, include pollution (Blidberg et al. 2004), and habitat destruction (Caddy 1993). In order to reverse current trends that may lead to extinction, management options such as mariculture, stock enhancement, and restocking need to be seriously considered.

Stock enhancement (Caddy & Defeo 2003, Bell et al. 2006) is a process that is applied to a natural resource to obtain improved yields. This process usually involves the addition of cultured stock by various stakeholders such as private and/or government sectors, cooperatives, and local communities. Often, such stocks are periodically harvested. In order to sustain harvesting, addition of cultured stock is done regularly. The endpoint of stock enhancement is the presence of reproductive individuals that ably contribute to recruitment (Sims 1993). Restocking is a similar process involving the addition of cultured stocks. However, such additions are done with the intention to increase the reproductive potential of a depleted population (Bell et al. 2006).

For both stock enhancement and restocking processes, a stepwise approach may be conducted (see Bell et al. 2006). Such an approach requires the following:

- a biological understanding of the resource (including genetic impact of cultured on wild stocks (see Newkirk 1993)
- stock assessment
- simulation models of benefits
- release of cultured stock (with quarantine measures)
- internalization by the stakeholders of the effected process.

The methods that have been used to survey giant clam stocks are: belt transect (e.g. Hester & Jones 1974, Mingoa & Menez 1988), circular plot (Hester & Jones 1974), areal survey (Bryan & McConnell 1976), flowmeter technique (Pearson 1982, Mingoa & Menez 1988), modified manta-tow (Pearson 1982), tow (Munro 1986, Braley 1989), and reef flat transect (Braley 1989).

Remaining giant clam populations now generally lack the critical mass needed for reproductive success. Given that giant clams have a short larval phase, larval replenishment possibly occurs within local populations, or within short distances from single or several sources (see Allison et al. 1998, cited in Caddy & Defeo 2003). Reductions in populations have rendered them more remote and disconnected from other clam populations, thereby cutting larval exchanges. For these reasons, additional initiatives like clam restocking are needed to complement CITES protection. The giant clam's intrinsic characteristics, such as short larval phase in culture, self-feeding, and sedentary adult habit, render them suitable species for restocking (Bell 1999). The determination of possible connectivities (source and sink populations), or lack thereof, between clam populations, as well as measures of larval dispersal (of which little is known), especially for extensive reef areas, may be a good basis for a stock enhancement program that seeks to reestablish source populations and refuges (Carr & Reed 1993, Caddy & Defeo 2003).

The giant clam restocking program in the Philippines is unique in that *Tridacna gigas* has become virtually extinct from overfishing. Clams for restocking are now obtained from extensive cultures. In addition, clam restocking activities have been rendered socially acceptable by consciously steering the development of clam mariculture into a simple technology, including low cost components, and utilizing extensive culture methods (Tisdell & Menz 1992). The restocking activities involving stocks cultured by the Marine Science Institute (MSI) have the support of various stakeholders, who are willing to shoulder the expenses for restocking, for training on husbandry of giant clams and for security and monitoring of the clams (Gomez & Mingoa-Licuanan 2006).

MSI's two decades of experience in clam restocking in the Philippines (see Gomez et al. 2005, Gomez & Mingoa-Licuanan 2006) have shown the importance of the following:

1 **Security of clams at the selected site** – Many a clam have been lost to poaching at sites where security had been lacking or limited. The establishment of security at the site may be part of the process of coastal environment protection or community-based resources management, often involving stakeholders, for example, the local government unit, a government agency, people's organization, non-government organization, academe, environmentalists, resort owners, private companies, and volunteer groups (page 50).

2 **Training** – Education through training has been a stepping stone to encourage the local stakeholders to participate in giant clam restocking. Stakeholders

may take a formal short-term training on giant clam ocean nursery methods, afterwhich the trainee serves as the steward for the giant clam ocean nursery. Additionally, he may serve as the local trainor in his community. Also, stakeholders may acquire information on giant clams and ocean nursery methods from published materials, such as giant clam manuals, related scientific papers and non-technical articles. Stakeholders may also discuss monitoring concerns with MSI giant clam experts. Training ensures that the stewards are equipped to take on the monitoring tasks. Although training is time-limited, MSI maintains open consultation with the stakeholders/stewards as needed.

3   **Proper methods for transporting clams** – As the inadequacies of the early protocols and/or implementation were discovered with every restocking activity, remedial measures were made. This reduced transported-related clam mortality to an acceptable maximum level of 15% (Gomez & Mingoa-Licuanan 2006). In moving cultured stocks from the MSI Giant Clam Hatchery at the Bolinao Marine Laboratory, the protocol for quarantine (page 69) is being employed to eliminate disease and parasitic agents. The packing protocol (page 73) has evolved from the methods in current use at the time and out of the exigencies of moving clams under a Philippine setting. The mode of transportation and the route to be taken to the restocking site are seriously considered, with preference for the options that will maximize clam survival after transport.

Table 5 shows the numbers of clams restocked by MSI in the Philippines from 1990 to 2007, updating the data presented in Gomez & Mingoa-Licuanan (2006). The restocking activities are largely in cooperation with the stakeholders through a 'users pay' approach, wherein the expenses for restocking were shouldered by the recipients (Gomez & Mingoa-Licuanan 2006). The restocking effort had virtually no support from government fishery agencies.

Initial restocking activities have led to the formulation of criteria for site selection (page 50). Clams were placed in coral reefs or seagrass habitats, at water depths up to 3 meters. A few sites were ~6m deep, such preference for greater depth serving as a precautionary measure against fishers. Water clarity ranged from clear to slightly turbid, varying periodically with monsoonal changes. Substrata were relatively stable or hard, with sand and/or coral rubble. Mangrove and seaweed (such as *Sargassum*) habitats were avoided.

More than 90,000 clams (all species) consisting of juveniles, subadults, and adults were deployed in 59 sites (but see below) (Table 5, Fig. 18). *T. gigas* comprised 68% of all clams restocked, while 32% consisted of other species such as *T. derasa*, *T. squamosa*, and *Hippopus hippopus*. Of the restocked *T. gigas*, juveniles (3-15cm shell length, SL) comprised ~81%, and adults / subadults, ~19%. Giant clam restocking involved more juveniles than adults / subadults because more juvenile clams can be transported for the same volume (Gomez et al. 2005), and the use of juvenile clams for restocking maximized the transport costs.

Table 5. Cultured giant clams restocked in the Philippines by MSI, from 1990 to Feb 2007. (Highlighted in blue = total mortality; * = some mortalities reported among *T. gigas*)

Sites			No. *Tridacna gigas* restocked		No. other species restocked
Northern Philippines:			As adults/ subadults	As juveniles	
Bataan	Olongapo City	Grande Is.	140		
Batangas	Batangas City	Ilijan	125		
		Pagkilatan	45		33
	Bauan		78	200	
	Mabini*		631	6,750	154
	Nasugbu	Fortune Is.	15		
		Pta. Fuego	50		
		Tali	20		
Cavite	Cavite City	Corregidor Is.	4	400	
	Ternate		72		
Ilocos Norte –	Batac			100	100
	Currimao & Pasukin			121	100
Mindoro Oriental	Pto. Galera		395	25	116
Pangasinan	Anda	Cangaluyan Is.	32		
		Tondol	225	1,000	
	Bolinao	Agno	35		
		Balingasay	12		
		Lucero	80		
		Pisalayan	2,500		381
	Alaminos City	HINP - Children Is.	37		
		HINP - Clave Is.	423	100	
		HINP - Governor Is.	1,097	3,310	
		HINP - Quezon Is.	2,781	4,289	1,901
		Telbang			12
Quezon	Alabat		75	1,000	
	Infanta		75		
	Lucena		50		
	Mauban		217		4
Zambales	Masinloc		225	1,000	
		San Salvador Is.	164		97
	Sta. Cruz		51	1,000	25
		Hermana Mayor Is.	112		

(continued)

Table 5 (continued)

Sites			No. *Tridacna gigas* restocked		No. other species restocked
Central Philippines:			As adults/subadults	As juveniles	
Antique	Culasi		500		
	Caluya	Semirara Is.	25	125	
Bohol	Calape		105	70	
Cebu	Alcoy		75		
	Camotes		150		
	Lapu-lapu City	Olango Is.	200	2,748	20
	Madridejos		12		
	Malabuyoc		20		
	Sogod		4		17
Guimaras Is.				800	
Negros Occidental	Cauayan	Danjugan Is.		500	
	Sagay City	Carbin Reef		600	
	Dumaguete City*		81	5,300	2,520
Samar	Catbalogan			2,000	
Samar, Eastern	Guiuan		109	10,335	70
	Lawaan		75	2,350	
Samar, Western	Marabut*		4	376	
Southern Philippines:					
Camiguin	Guinsiliban		4	202	200
Davao del Norte	Samal Is.			1,066	22,090
Misamis Occidental	Clarin				1,000
	Sinicaban		25		
	Cagayan de Oro		20	500	30
Palawan	El Nido	Miniloc Is.	15		
	Pag-asa Is.		271	2,314	
	Pto. Princesa		18		
Tawi Tawi	Bongao		300	100	
Zamboanga del Norte	Dapitan City	Aligbay Is.	24	800	
No. clams			11,803	49,481	28,870
Total no. *T. gigas* restocked				61,284	
Total no. clams restocked					90,154

Note: Additionally, *T. derasa* and *Hippopus hippopus* (total no. of clams = 50) were transplanted at Scarborough Shoals off western Philippines in 1991
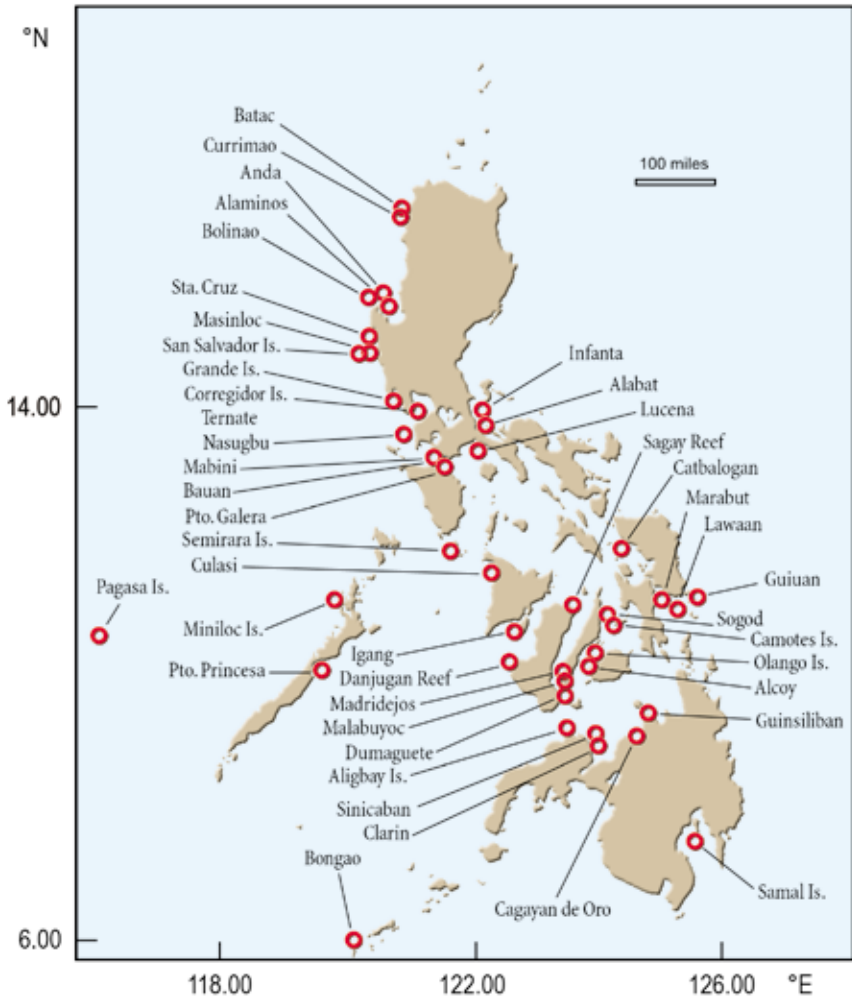
Fig. 18. Giant clam restocking sites in the Philippines by MSI, from 1990-2007. Clams were also transplanted in Scarborough Shoals (not shown in map, but see text)

Feedback on the restocked *T. gigas* clams was received by MSI from about half the number of sites, and usually given by the collaborators after clam mortalities occurred. Survival / mortality was reported in ~77% of those that gave feedback, while such data were lacking or insufficient in others. No feedback was received for the other species. Regular feedback to MSI was encouraged although considered not mandatory, depending on the availability of resources of the collaborator.

Presently, there are 54 sites remaining, after feedback from five sites indicated total clam mortality (either from poaching, microalgal blooms, or failure to locate the site). Site distribution are as follows: ~54% in northern Philippines, where the MSI Giant Clam Hatchery is located; ~29% in central Philippines; and ~17% in southern Philippines. Additionally, it is noted that a total of 50 clams of *T. derasa* and *H. hippopus* were transplanted in Scarborough Shoals in 1991.

For *T. gigas*, the largest size at which clams were restocked was ~44cm SL, transported ~10 hrs from the town of Bolinao (Pangasinan Province) to the site Mauban (Quezon Province). This cohort is now 11 yrs and 8 months old, and female-mature. [In culture, *T. gigas* reaches female maturity at ~9 yrs old (Gomez & Mingoa-Licuanan 2006).] On the other hand, the smallest size at which *T. gigas* was restocked was ~3 cm SL, transported ~12 hrs from Bolinao to its site in Batac (Ilocos Norte Province). This cohort is 5 yrs and 11 mos old, at the subadult stage at ~35cm SL.

Presently, the youngest cohort of *T. gigas* being used for restocking was spawned in October 2004. Several groups of this cohort were transported to five sites: Talima (Cebu Province) when cohort size was ~3cm SL; and later as larger juveniles between 8-12.5cm SL, as follows: Guimaras Is.; Culasi and Semirara Is. (both in Antique Province); and Sagay Is. (Negros Occidental Province).

Clam restocking is possible at most times of the year, for as long as precautions are taken in times of strong typhoons (Gomez & Mingoa-Licuanan 2006). At least ten (10) demonstration sites of clam restocking have been established for other communities to follow, specifically at: Anda (Pangasinan Province); Masinloc (Zambales Province); Infanta and Alabat (Quezon Province); Alcoy, Camotes, and Olango Is. (all in Cebu Province); Calape (Bohol Province); Lawaan (Eastern Samar Province); and Samal Is. (Davao del Norte, Province) (Gomez et al. 2005).

Quarantine and transport protocols

Stock enhancement and restocking initiatives must include a program on quarantine measures that minimize the spread of disease agents. A quarantine protocol is required so that there is a level of accountability for movement of clams between locations (within and between the countries). The main purpose of quarantine is to isolate potential disease agents, so that the dispersal of such agents is controlled or minimized.

Quarantine guidelines. A good quarantine protocol will minimize the risk of introducing pathogens or parasites. It is based on good sanitary principles, minimal clam stress, and regular monitoring of clams. This set of guidelines is adapted from Braley (1992). Clams are quarantined for at least two weeks to observe their health and condition, before they are transported from one place to another. After successful quarantine, the culture facility should issue a quarantine clearance.

Quarantine may be conducted in a raceway with access to sunlight and running seawater (1 µm filtered and aerated). The raceway (=quarantine tank) should first be chlorine-sterilized (page 19) for one hour, rinsed thoroughly with freshwater, and sun-dried.

The shells of the clams are carefully brushed free of epibionts before and after quarantine. Rinse the scrubbed clams in 1µm FSW. Repeat shell scrubbing after 7 days, or as needed. Tagging of clams is optional. As part of stock identification data, measure shell lengths of up to 30 clams belonging to the same cohort, and compute the cohort's average shell length. Observe the clams daily for abnormalities or mortalities. Eliminate sources of eco-physiological stress (e.g. heat, freshwater, siltation, predation, algal fouling, etc.) that may affect the clams.

Observe the quarantine system daily for water clarity, flowrate, temperature, and salinity. Replace the tank filter (1 µm) daily. The used filter should be chlorine-sterilized for one hour, pressure-rinsed thoroughly with freshwater, and sun-dried. To eliminate pyramidellid infestation in the quarantine tank, completely drain tank and immediately refill with fresh 1µm filtered seawater. Do this weekly or every 7 days. Make extra precaution against contaminating the quarantine tank with water from non-quarantine tanks.

If mortalities occur during quarantine, determine and control the cause, otherwise entirely replace the batch. At MSI, if total mortality reaches 5% at the end quarantine, further observation is made on the batch for another two weeks.

Furthermore, when landbased quarantine is not possible, clams may be observed for overall condition at sea. Segregate clams that need to be observed closely in a designated area. Scrub the shells, and every 7 days thereafter, or as needed. Tagging is optional, but get shell length data. Observe the clams every 1-2 days for abnormalities or mortalities. Similar actions as in landbased quarantine are taken for observed mortalities.

Transport only healthy batches of clams. Reject any batch showing signs of disease; symptoms include lesion or blister on the mantle and other soft parts (Braley 1992). Isolate these clams for further observation, or possible recovery.

Imported clams as well as those for export need to be quarantined for at least two weeks. If imported clams appear stressed and fail to manifest early recovery from transport, give antibiotics (10 ppm streptomycin) as prophylaxis, simultaneously with seawater changes every 2 days. If no mortality occurs after a few days, discontinue administration of antibiotics.

Exported and imported clams need proper CITES and country documentations (Braley 1992). These include a *Certificate of Origin* and an *Auxiliary Invoice* or *Municipal Clearance*. For CITES signatory countries, the exporting country also needs to secure a CITES export permit.

The *Certificate of Origin* is a 1-page document issued by the head of the giant clam culture facility where the clams were bred and reared. It contains information on the clams being shipped, indicates the location of the giant clam culture facility, the consignee's name and address, and the destination locality where the clams will be placed. The *Declaration of Quarantine and Health* may also be included in this certificate. Most quarantine procedures are specifically tailored to particular organisms. As such, it is difficult for the government to centralize quarantine, and it is almost the duty of the exporting facility to ensure that proper quarantine methods have been applied. Cognizance and approval by a regulatory agency, like the Quarantine Division of Bureau of Fisheries and Aquatic Resources (BFAR), a line agency of the Department of Agriculture (DA), of the quarantine capabilities of a particular facility are therefore important.

In the Philippines, the *Auxiliary Invoice* for aquatic organisms is issued by the BFAR. This invoice approves the movement of specific aquatic organisms, in this case, giant clams, from one location to another.

In lieu of the *Auxiliary Invoice*, a *Municipal Clearance* from the local government unit may be obtained to support the clam shipment during transport. This clearance is requested by the head of the giant clam culture facility, and issued by the municipal agricultural officer of the LGU where the said culture facility is located.

CITES and relevant Philippine laws. Since giant clams are included in the CITES Appendix 2, their involvement in international commerce and trade is regulated. All Philippine species are protected under DA-BFAR Fisheries Administrative Order No. 168 (BFAR 1990) and Republic Act No. 8550 (or The Philippine Fisheries Code of 1998).

In the Philippines, cultured clams may be exported under government regulation, and in limited quantities. (Export of giant clams for commercial trade is presently not allowed by DA-BFAR.) The CITES export permit that is secured from the DA-BFAR shall be presented and declared at the Bureau of Customs. The BFAR Quarantine office also needs to clear the shipment. The CITES permit accompanies the shipment to its destination.

Summary of documents for local transport, export and import

To transport giant clams and their derivatives (as applicable) within the Philippines, you will need:

- Certificate of Origin

- Certificate of culture

- Declaration of Quarantine and Health Certificate (for live clams)

- Certificate of origin of giant clam shells

- Letter of request for municipality clearance

- Municipality clearance, issued by the local government unit nearest to the place of culture

- Auxiliary Invoice, issued by BFAR if clams originate from Metro-Manila

To export clams for research purposes from the Philippines, these additional documents are needed:

- Letter of request to BFAR Director to export giant clams for research

- Memorandum from BFAR Director to BFAR One-Stop Export Documentation Center (OSEDC)

- CITES Permit, issued by BFAR OSEDC

- BFAR Commodity Clearance, issued by BFAR OSEDC, together with CITES Permit

- Proforma Invoice, submitted to Customs Office, either at Ninoy Aquino International Airport (NAIA) or at the OSEDC

- Export Declaration, issued by Customs Office at NAIA or at the OSEDC

- Approval of quarantine, issued by BFAR at the NAIA departure area, or departure cargo area

An import permit, issued by the receiving country, is also required prior to clam shipment.

Methods to transport giant clams

Materials needed for broodstock, sub-adults and juveniles, and larvae:

Clean flour sacks or cheesecloth
Plastic bags, unused, clear/translucent, 30 cm x 20 cm (length x width)
Rubber bands
Oxygen tank (medical)
Foam, 5cm thick
Corrugated cardboard
Seawater ice bags
Insulated box (styropore), 80cm x 40 cm x 60 cm (length x width x height)

After quarantine, give the clams the final shell scrub at least 6 hours before packing. Rinse the scrubbed clams in 1µm FSW, and re-immerse in the quarantine tank, refilled with fresh 1µm FSW.

When clams are to be packed, rinse them first with UV-treated 0.2µm FSW. Clams ≥ 30 cm shell length are packed singly. Clams 20 to <30 cm shell length are packed in twos or threes. For <20 cm clams, the number of clams that can be packed increases with smaller clam sizes. Transport clams in an insulated or styropore box (length, width, height: 80 cm x 40 cm x 60 cm) to minimize stress to the clams resulting from temperature fluctuations. Line the box at the base with about 5cm thickness (or 2 inches) foam to cushion the clams.

Soak flour sacks or cheesecloth in UV-treated 0.2µm FSW. Lay the clams on their side. Loosely wrap the clam/s with the sack or cheesecloth, and place in the plastic bag. Clams that will be packed in groups are laid on the sack in a row, ensuring some space between clams. Align the rows of clams to make one layer; up to two layers of clams may be placed in a bag. Holding the bag's neck with one hand, add enough medical oxygen (Heslinga et al. 1990) to inflate the plastic bag to at least twice the volume of the clams. Twist the bag at the neck to make a tight bulge, and secure with rubber bands. Place this sealed plastic bag in another plastic bag; similarly secure tightly with rubber bands. Lay each plastic bag of clam/s horizontally in an insulated (or styropore) box, large clams at the bottom, and small clams on top. About 5-6 bags of clams may fit in the styropore box. Between plastic bags, place a cardboard insert fitted to the box, to serve as additional cushion. Previously prepared small seawater ice bags are also placed at the corners of the box to decrease in-box temperature, thence lowering the clam metabolic rate. Secure the lid over the styropore box until the clams reach their destination.

The use of a styropore box of a size different from the one prescribed here requires modifications in the size of the plastic bag and the number of seawater ice bag used.

Transport of small juvenile clams ≤2cm shell length may need antibiotics (see pages 27 and 71).

A transport time (=begins from the time clams are packed and ends by the time clams are re-immersed in seawater) which is shortest is usually recommended for clams for transport. This is to ensure that the clams will arrive at its destination in still excellent condition. A person coordinating the requirements of long-distance transport must be mindful of the clams' physiological requirements as well. Even as giant clams are able to breathe air for short periods (Mingoa-Licuanan & Lucas 1995), they are only able to endure long periods out of water during transport (for up to 24 hours) if they are properly packed (in moist conditions and with oxygen). If such long-distance transport is done through a combination of land and/or boat transfers, it is advised to re-immerse the clams in fresh seawater, say every 6 hours for at least 1 hour. Re-immersion will allow the clams to restore oxygen resources in the body, derived from the fresh seawater. In addition, the clams are able to excrete into the water stored ammonia wastes, which may be toxic to the clam if retained in the body at abnormally high levels. However, if the clams' total travel time is between 8-10 hours, it is more practical to transport directly to destination site.

During travel, treat clams as fragile cargo and ensure careful handling. Clam survival may be affected by travel conditions, such as poor road conditions, exposure to warm vents, mishandling of cargo (throwing or dropping the box of clams), and temperature shock.

At the clams' destination, first acclimatize the clams, then stock.

Transporting embryos and larvae. Drain or siphon the larvae (preferably before pediveliger stage) from the hatchery tank into double plastic bags (calibrated) (see page 20), to about half the bag's volume (with known water level mark). Take three samples using a Pasteur pipette and transfer the samples onto a depression slide. Estimate the number of pediveligers, using methods and calculations similar to estimating larvae (see page 24 and 30). Add medical oxygen, and seal the plastic bag with rubber bands. Add antibiotics. Place the bags upright in the insulated or styropore box.

Embryos may be transported soon after fertilization of eggs on the boat. Taking the precautions concerning packing embryos, MSI has successfully transported such larvae for about 10 hours (just before the embryos become trochophores) over 600 km distance by water, plane, and then land.

Monitoring and record keeping for restocked clams

The giant clam culture facility must keep records of all of their cultured clams that were used in giant clam restocking, as part of the database on giant clam stock enhancement. After clam restocking, as per prior agreements, the local collaborators are tasked to monitor the clams at the site. Agreements may or may not be formalized and supported by proper documentation (e.g. stewardship agreement). In any case, the collaboration is based on trust, granting that the local collaborators

are trained on giant clam monitoring, and that protective measures are in place to ensure that the restocked clams will attain reproductive maturity and spawn on site.

Communication between the giant clam culture facility (source of clams) and the local collaborators must be kept open, in order to quickly address any emergencies (e.g. threat of oil spill) that may arise and affect the restocked clams. Formal agreements over custody of the clams may need to be renewed, as needed.

The local collaborator or steward employs clam monitoring methods, and is expected to keep records of all relevant information (refer to page 72 and Appendix 4), including monitoring of growth performance and survival. Clam spawning events that occur need to be documented, as well as occurrence of recruitment in the long-term. Stewardship over the clams need not be vested in one person, so that in case of changes in staff management, monitoring of clams remains a continuing effort.

APPENDIX 1

Data sheets for hatchery use

DATA SHEET A
SPAWNING

Date: _____   Tide: _____ ; _____
Species: _____   _____ ; _____
Source: _____ ; _____   _____ ; _____
Site: _____ ; _____   _____ ; _____
Type: _____   Recorder: _____ ; _____
Lunar Phase: _____

Birthyear/Tag	Shell Length	Injected (∅; and volume)	Time	Sperm(∅)	Time	Egg(∅)	Time

Fertilization time: _____
Remarks:

DATA SHEET B
ESTIMATING NUMBER OF EGGS, AND STOCKING EGGS (DAY 0)

Species: _____   Time: _____
Date: _____   By: _____

I. Estimating Number of Eggs

Egg tank(ET) #	1	2	3	4
Volume (ml):				
Parents:				
sample #				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
mean (D_1; eggs.ml^{-1}):				
SD ±:				
D_1 x bin volume (ml)				

TOT. FERTILIZED EGGS: _____
Eggs.clam^{-1}: _____ ; _____
_____ ; _____
_____ ; _____
_____ ; _____

III. Size Measurement
Eyepiece micrometer readings:

Calibration factor: _____ (check CF of microscope)
Average egg size: _____ ± _____ μm

Larval tank (LT) assignments:

LT #	Eggs. tank^{-1}	Parents (birthyear / tag #)	
		Male gametes	Female gametes
1			
2			
3			
4			
5			
6			
7			
8			

II. Stocking 30-40 eggs.ml^{-1} in larval tanks:

$$\boxed{D_1V_1=D_2V_2}$$

Where: D_1= egg density in bin (eggs.ml^{-1})
V_1= unknown volume of eggs to be stocked (ml)
D_2= stocking density (30-40 eggs.ml^{-1})
V_2= volume of larval tank (e.g. 350,000 ml)

Given: D_1= _____ eggs.ml^{-1}
V_1= unknown volume of eggs to be stocked (ml)
D_2= 30-40 eggs.ml^{-1}
V_2= 350,000 ml

for ET # 1; $V_1 = \dfrac{(30\text{-}40 \text{ eggs.ml}^{-1})(350,000 \text{ ml})}{_____ \text{ eggs.ml}^{-1}}$

for ET # 2; $V_1 = \dfrac{(30\text{-}40 \text{ eggs.ml}^{-1})(350,000 \text{ ml})}{_____ \text{ eggs.ml}^{-1}}$

for ET# 3; $V_1 = \dfrac{(30\text{-}40 \text{ eggs.ml}^{-1})(350,000 \text{ ml})}{_____ \text{ eggs.ml}^{-1}}$

for ET# 4; $V_1 = \dfrac{(30\text{-}40 \text{ eggs.ml}^{-1})(350,000 \text{ ml})}{_____ \text{ eggs.ml}^{-1}}$

from ET# 1, stock: V_1 = _____ ml per LT # ___
from ET# 2, stock: V_1 = _____ ml per LT # __
from ET# 3, stock: V_1 = _____ ml per LT # ___
from ET# 4, stock: V_1 = _____ ml per LT # ___

IV. Estimating Percentage of Fertilization
2 hrs post-fertilization

Sample	Unfertilized		Fertilized	
#	#	%	#	%
1				
2				
3				

n=100 zygotes per sample
% Fertilization: _____ ± _____ %

DATA SHEET C
DAY 2

Species: _____   Time: _____
Date: _____   By: _____

I. Estimating Number of Larvae

LT #	1	2	3	4
Volume (ml):				
Parents:				
sample #				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
mean (D_1; larvae.ml^{-1}):				
SD ±:				
D_1 x LT volume (ml)				

TOTAL LARVAE: _____
Larvae. tank^{-1}: _____ ; _____
_____ ; _____
_____ ; _____
_____ ; _____

III. Size Measurement
Eyepiece micrometer readings:

Calibr. factor: _____(check CF data on microscope)
Average larvae size: _____ ± _____ μm

Larval tank (LT) assignments:

LT #	Larvae. tank^{-1}	Parents (birthyear / tag #)	
		Male gametes	Female gametes
1			
2			
3			
4			
5			
6			
7			
8			

II. Stocking 3-4 larvae.ml^{-1} in larval tanks:

$$D_1V_1=D_2V_2$$

Where: D_1= mean larval density in bin (larvae.ml^{-1})
V_1= unknown volume of larvae to be stocked (ml)
D_2= stocking density (larvae.ml^{-1})
V_2= volume of larval tank (ml)

Given: D_1= _____ larvae.ml^{-1}
V_1= unknown volume of larvae to be stocked (ml)
D_2= 3 - 4 larvae. ml^{-1}
V_2= e.g. 350,000 ml

for LT # 1; $V_1 = \dfrac{(3 - 4\ \text{larvae.ml}^{-1})(350,000\ \text{ml})}{\_\_\_\_\_\ \text{larvae.ml}^{-1}}$

for LT # 2; $V_1 = \dfrac{(3 - 4\ \text{larvae.ml}^{-1})(350,000\ \text{ml})}{\_\_\_\_\_\ \text{larvae.ml}^{-1}}$

for LT # 3; $V_1 = \dfrac{(3 - 4\ \text{larvae.ml}^{-1})(350,000\ \text{ml})}{\_\_\_\_\_\ \text{larvae.ml}^{-1}}$

for LT # 4; $V_1 = \dfrac{(3 - 4\ \text{larvae.ml}^{-1})(350,000\ \text{ml})}{\_\_\_\_\_\ \text{larvae.ml}^{-1}}$

from LT # 1, stock: $V_1 =$ _____ ml per LT # ___
from LT # 2, stock: $V_1 =$ _____ ml per LT # ___
from LT # 3, stock: $V_1 =$ _____ ml per LT # ___
from LT # 4, stock: $V_1 =$ _____ ml per LT # ___

IV. Feeding
Microalgal sp: _____   Days: _____
Hemocytometer counts: _____ ; _____ ; _____
Mean count x 10 000 = _____ cells.ml^{-1}

$$D_1V_1=D_2V_2$$

Where: D_1= mean algal density (cells.ml^{-1})
V_1= unknown volume of algae to be fed (ml)
D_2= feeding density (15,000 cells ml^{-1})
V_2= volume of larval tank (e.g.350,000 ml)

Feed: _____ ml vol. of cultured microalgae per tank

DATA SHEET D
DAY 4

Species: _____   Time: _____
Date: _____   By: _____

I. Estimating Number of Larvae

LT #	1	2	3	4
Volume (ml):				
Parents:				
sample #				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
Mean $(D_1$; Larvae.ml^{-1}):				
SD ±:				
D_1 x LT volume (ml)				

TOTAL LARVAE: _____
Larvae. tank^{-1}: _____ ; _____
_____ ; _____
_____ ; _____
_____ ; _____

III. Size Measurement
Eyepiece micrometer readings:

Calibr. factor: _____ (check CF data on microscrope
Average larval size: _____ ± _____ μm

Larval tank (LT) assignments:

LT#	Larvae. tank^{-1}	Parents (birthyear / tag #)	
		Male gametes	Female gametes
1			
2			
3			
4			
5			
6			
7			
8			

II. Stocking 3-4 larvae.ml^{-1} in larval tanks:

$$D_1V_1=D_2V_2$$

Where: D_1= mean larval density in bin (larvae.ml^{-1})
V_1= unknown volume of larvae to be stocked (ml)
D_2= stocking density (larvae.ml^{-1})
V_2= volume of larval tank (ml)

Given: D_1= _____ larvae.ml^{-1}
V_1= unknown volume of larvae to be stocked (ml)
D_2= 3 - 4 larvac. ml^{-1}
V_2= e.g. 350,000 ml

for LT# 1; $V_1 = \dfrac{(3 - 4\ \text{larvae.ml}^{-1})(350,000\ \text{ml})}{\text{_____ larvae.ml}^{-1}}$

for LT# 2; $V_1 = \dfrac{(3 - 4\ \text{larvae.ml}^{-1})(350,000\ \text{ml})}{\text{_____ larvae.ml}^{-1}}$

for LT# 3; $V_1 = \dfrac{(3 - 4\ \text{larvae.ml}^{-1})(350,000\ \text{ml})}{\text{_____ larvae.ml}^{-1}}$

for LT# 4; $V_1 = \dfrac{(3 - 4\ \text{larvae.ml}^{-1})(350,000\ \text{ml})}{\text{_____ larvae.ml}^{-1}}$

from LT# 1, stock: V_1 = _____ **ml per LT#** ___
from LT# 2, stock: V_1 = _____ **ml per LT#** ___
from LT# 3, stock: V_1 = _____ **ml per LT#** ___
from LT# 4, stock: V_1 = _____ **ml per LT#** ___

IV. Feeding
Microalgal sp: _____   Days: _____
Hemocytometer counts: _____ ; _____ ; _____
Mean count x 10 000 = _____ cells.ml^{-1}

$$D_1V_1=D_2V_2$$

Where: D_1= mean algal density (cells.ml^{-1})
V_1= unknown volume of algae to be fed (ml)
D_2= feeding density (15,000 cells ml^{-1})
V_2= volume of larval tank (e.g.350,000 ml)

Feed: _____ **ml vol. cultured microalgae per tank**

DATA SHEET E
DAY 6

Species: _____   Time: _____
Date: _____   By: _____

I. Estimating Number of Larvae

LT#	1	2	3	4
Volume (ml):				
Parents:				
sample #				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
mean (D_1; larvae.ml^{-1}):				
SD ±:				
D_1 x LT volume (ml)				

TOTAL LARVAE: _____

Larvae. tank^{-1}: _____ ; _____
_____ ; _____
_____ ; _____
_____ ; _____

III. Size Measurement

Eyepiece micrometer readings:

Calib. factor: _____ (check CF data on microscope)
Average larval size: _____ ± _____ μm

Larval tank (LT) assignments:

LT#	Larvae. tank^{-1}	Parents (batch / tag #)	
		Male gametes	Female gametes
1			
2			
3			
4			
5			
6			
7			
8			

II. Stocking 3-4 larvae.ml^{-1} in larval tanks:

$$D_1V_1=D_2V_2$$

Where: D_1= mean larval density in bin (larvae.ml^{-1})
V_1= unknown volume of larvae to be stocked (ml)
D_2= stocking density (larvae.ml^{-1})
V_2= volume of larval tank (ml)

Given: D_1= _____ larvae.ml^{-1}
V_1= unknown volume of larvae to be stocked (ml)
D_2= 3 - 4 larvae. ml^{-1}
V_2= e.g.350,000 ml

for LT# 1; $V_1 = \dfrac{(3 - 4 \text{ larvae.ml}^{-1})(350,000 \text{ ml})}{\_\_\_\_\_ \text{ larvae.ml}^{-1}}$

forLT # 2; $V_1 = \dfrac{(3 - 4 \text{ larvae.ml}^{-1})(350,000 \text{ ml})}{\_\_\_\_\_ \text{ larvae.ml}^{-1}}$

for LT# 3; $V_1 = \dfrac{(3 - 4 \text{ larvae.ml}^{-1})(350,000 \text{ ml})}{\_\_\_\_\_ \text{ larvae.ml}^{-1}}$

for LT# 4; $V_1 = \dfrac{(3 - 4 \text{ larvae.ml}^{-1})(350,000 \text{ ml})}{\_\_\_\_\_ \text{ larvae.ml}^{-1}}$

from LT# 1, stock: $V_1 =$ _____ **ml per LT#** ___
from LT# 2, stock: $V_1 =$ _____ **ml per LT#** ___
from LT# 3, stock: $V_1 =$ _____ **ml per LT#** ___
from LT# 4, stock: $V_1 =$ _____ **ml per LT#** ___

IV. Feeding

Microalgal sp: _____   Days: _____
Hemocytometer counts: _____ ; _____ ;
Mean count x 10 000 = _____ cells.ml^{-1}

$$D_1V_1=D_2V_2$$

Where: D_1= mean algal density (cells.ml^{-1})
V_1= unknown volume of algae to be fed (ml)
D_2= feeding density (15,000 cells ml^{-1})
V_2= volume of larval tank (e.g.350,000 ml)

Feed: _____ **ml vol. cultured microalgae / tank**

DATA SHEET F
DAY 8

Species: _____   Time: _____
Date: _____   By: _____

I. Estimating Number of Larvae

LT#	1	2	3	4
Volume (ml):				
Parents:				
sample #				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
mean (D_1; larvae.ml^{-1}):				
SD ±:				
D_1 x LT volume (ml)				

TOTAL LARVAE: _____

Larvae. tank^{-1}: _____ ; _____
                            _____ ; _____
                            _____ ; _____
                            _____ ; _____

III. Size Measurement

Eyepiece micrometer readings:

Calib.factor: _____ (check CF data on microscope)
Average larval size: _____ ± _____ μm

Settlement tank (ST) assignments:

ST#	Larvae. tank^{-1}	Parents (birthyear / tag #)	
		Male gametes	Female gametes
1			
2			
3			
4			
5			
6			
7			
8			

II. Stocking 1-5 larvae.cm^{-2} in settlement tanks:

$$D_1V_1=D_2A_2$$

Where: D_1= mean larval density in bin (larvae.ml^{-1})
        V_1= unknown volume of larvae to be stocked (ml)
        D_2= stocking density (larvae.cm^{-2})
        A_2= floor area of settlement tank (cm^{-2})

Given: D_1= _____ mean larvae.ml^{-1}
        V_1= unknown volume of larvae to be stocked (ml)
        D_2= 1-5 larvae.cm^{-2}
        A_2= 100,000 cm^{-2}

for bin # 1; $V_1 = \dfrac{(1\text{-}5 \text{ larvae.cm}^{-2})(100{,}000 \text{ cm}^{-2})}{\underline{\hspace{1cm}} \text{ larvae.ml}^{-1}}$

for bin # 2; $V_1 = \dfrac{(1\text{-}5 \text{ larvae.cm}^{-2})(100{,}000 \text{ cm}^{-2})}{\underline{\hspace{1cm}} \text{ larvae.ml}^{-1}}$

for bin # 3; $V_1 = \dfrac{(1\text{-}5 \text{ larvae.cm}^{-2})(100{,}000 \text{ cm}^{-2})}{\underline{\hspace{1cm}} \text{ larvae.ml}^{-1}}$

for bin # 4; $V_1 = \dfrac{(1\text{-}5 \text{ larvae.cm}^{-2})(100{,}000 \text{ cm}^{-2})}{\underline{\hspace{1cm}} \text{ larvae.ml}^{-1}}$

from LT# 1, stock: V_1 = _____ ml per ST # ___
from LT# 2, stock: V_1 = _____ ml per ST # ___
from LT# 3, stock: V_1 = _____ ml per ST # ___
from LT# 4, stock: V_1 = _____ ml per ST # ___

IV. Feeding

Microalgal species: _____   Days: _____
Heamocytometer counts: _____ ; _____ ; _____
mean count x 10 000 = _____ cells.ml^{-1}

$$D_1V_1=D_2V_2$$

Where: D_1= mean algal density (cells.ml^{-1})
        V_1= unknown volume of algae to be fed (ml)
        D_2= feeding density (15 000 cells.ml^{-1})
        V_2= volume of settlement tank (4 000 000 ml)

Feed: _____ ml, vol. cultured microalgae per tank

DATA SHEET G
ZOOXANTHELLAE

I. Calculating the feeding volume:

$$\boxed{D_1V_1=D_2V_2}$$

Where: D_1= mean zooxanthellae density (cells.ml^{-1})
        V_1= unknown volume of zooxanthellae culture or Freshly Isolated Zoox suspension to be fed (ml)
        D_2= feeding density (100 cells.ml^{-1})
        V_2= volume of larval tank (e.g.350 000 ml); volume of settlement tank (4 000 000 ml)

II. Feeding:

Day 5
Hemocytometer counts: _____ ; _____ ; _____
mean zooxanthellae count: _____ x 10 000
= _____ cells.ml^{-1}
feed: _____ ml per tank

ST#	Zoox source
1	
2	
3	
4	
5	
6	
7	
8	

Day 7
Hemocytometer counts: _____ ; _____ ; _____
mean zooxanthellae count: _____ x 10 000
= _____ cells.ml^{-1}
feed: _____ ml per tank

ST #	Zoox source
1	
2	
3	
4	
5	
6	
7	
8	

Day 9
Hemocytometer counts: _____ ; _____ ; _____
mean zooxanthellae count: _____ x 10 000
= _____ cells.ml^{-1}
feed: _____ ml per tank

ST #	Zoox source
1	
2	
3	
4	
5	
6	
7	
8	

Day 13
Hemocytometer counts: _____ ; _____ ; _____
mean zooxanthellae count: _____ x 10 000
= _____ cells.ml^{-1}
feed: _____ ml per tank

ST #	Zoox source
1	
2	
3	
4	
5	
6	
7	
8	

DATA SHEET H
DAY 30, 60 and 90 (Settlement tanks)

Species: _____
Date (day **30**): _____
Time: _____
By: _____

I. Estimating No. of Juveniles

ST #				
Parents:				
Sample Area (100 cm^2)				
1				
2				
3				
Mean (**x**) (juvs.100 cm^{-2})				
SD $\pm$:				
x · tank floor area (cm^2)				

TOTAL: _____
Juveniles. tank^{-1}: _____ ; _____
                            _____ ; _____
                            _____ ; _____
                            _____ ; _____

II. Size Measurement
Eyepiece micrometer readings:

Calibr. factor:____(check CF data on microscope)
Mean juvenile shell length: _____ $\pm$ _____ μm

Species: _____
Date (day **60**): _____
Time: _____
By: _____

I. Estimating No. of Juveniles

ST#				
Parents:				
Sample Area (100 cm^2)				
1				
2				
3				
Mean (**x**) (juvs.100 cm^{-2})				
SD $\pm$:				
x · tank floor area (cm^2)				

TOTAL: _____
Juveniles. tank^{-1}: _____ ; _____
                            _____ ; _____
                            _____ ; _____
                            _____ ; _____

II. Size Measurement
Eyepiece micrometer readings:

Calibr. factor:____(check CF data on microscope)
Mean Juvenile shell length: _____ $\pm$ _____ μm

Species: _____
Date (day **90**): _____
Time: _____
By: _____

I. Estimating No. of Juveniles

ST#				
Parents:				
Sample Area (100 cm^2)				
1				
2				
3				
Mean (**x**) (juvs.100 cm^{-2})				
SD $\pm$:				
x · tank floor area (cm^2)				

TOTAL: _____
Juveniles. tank^{-1}: _____ ; _____
                            _____ ; _____
                            _____ ; _____
                            _____ ; _____

II. Size Measurement
Eyepiece micrometer readings:

Calibr. factor:____(check CF data on microscope)
Mean juvenile shell length: _____ $\pm$ _____ μm

DATA SHEET I

SUMMARY of DATASHEETS A-H

SPECIES: _____ RECORDED: _____

BIRTH YEAR: _____ RECORDED: _____

DATE	DAY	ACTIVITY	STAGE	SIZE	% SURVIVAL	AERATION			TEMPERATURE		
						0800H	1200H	1700H	1000H	1300H	1600H
	0	Spawning									
	1	Trocophoe check									
	2	Change water, algal feed									
	3	Algal feed									
	4	Change water, algal feed									
	5	Zoox infection, algal feed									
	6	Change water, algal feed									
	7	Zoox infection, algal feed									
	8	Transfer to settlement tanks, algal feed									
	9	Zoox infection, algal feed									
	10	Algal feed									
	11	Algal feed									
	12	Zoox infection, algal feed									
	13	Zoox infection, algal feed									
	14	Increase FSW inflow, daily algal/ greenwater feeding									
	30	Sample #live juvs, ave size									
	60	Sample #live juvs, ave size; remove shade; grazers									
	90	Harvest									
		Deploy to ocean nursery									

APPENDIX 2

Microalgal culture

Summary of procedures
- Sterilize glassware and other materials for use in microalgal culture.
- Prepare the nutrient solutions for the microalgal cultures. To make Walne's Medium, first prepare the Trace Metal Primary Stock Solution, then the Vitamin Primary Stock solution (Walne 1974, see Tech 1981).
- Establish and maintain monospecific cultures (*Isochrysis galbana*) as food for clam larvae.
- As needed, establish outdoor scaled-up microalgal cultures as supplementary food for juveniles, and large clams.

Materials to be used for algal cultures need to be sterilized to destroy all microorganisms, including spores. Sterilization is achieved with heat, using a commercial autoclave, pressure cooker type laboratory autoclave, or a hot air oven. In contrast, disinfection is the destruction of most microorganisms, and some bacterial spores. Disinfection is achieved using chemicals, as in acid wash. Materials that are sensitive to heat are sterilized by chemical disinfection (WHO-SEARO 2001).

How to sterilize glassware and other materials
- Clean glass bottles, flasks, stirring rods, and micropipette tips with liquid detergent, and rinse thoroughly in tap water.
- Glass tubings, plastic hoses, and bottles with carbonate deposits or which have stubborn dirt should be acid washed. First wash in tap water, then acid wash by soaking in 2N HCl acid bath (= 1.67ml commercially pure HCl + 1000ml distilled water) from 20 min (if not too dirty) to several hours (if very dirty). Sensitive plastic materials should not be left in the acid bath for too long. After soaking, rinse thoroughly with tap water, and finally with distilled water several times. Drip dry, or dry in a low temperature oven.

To sterilize by autoclave, you will need:
  2 x 250ml glass Erlenmeyer flasks with 100ml UVFSW (UV-treated 0.2µm-filtered seawater). Plug the mouth of the flasks with gauze.
  10 x 1L dextrose bottles with 1L UVFSW. Plug the mouth of the bottles with aluminum foil.
  1L glass reagent bottle, clear, with ground glass stopper, with 900ml distilled water.
  250ml glass reagent bottle, amber, with ground glass stopper, with 200ml distilled water.
  40ml glass vial, clear, autoclavable screw cap, with 20ml distilled water.

- Keep the caps loose on the bottles.
- Wrap stirring rods separately in aluminum foil. Place micropipette tips in a beaker. Wrap glass tubings, and plastic hoses (fill both ends with fiberfill) separately in paper.
- Load materials in a commercial autoclave, and sterilize in the autoclave at 121°C for 15min. Follow the manufacturer's operating instructions for the autoclave.
- After autoclaving, retrieve sterilized materials and cool before use.

To prepare nutrient solutions, you will need:

Disposable gloves

Weighing boats

Spatula

Paper wipes

Glass rods

Square-shaped wax papers (to form a funnel)

Analytical balance

10ml sterilized distilled water in a clear glass vial, for Trace Metal Primary stock solution

200ml sterilized distilled water in an amber reagent bottle, for Vitamin Primary stock solution

900ml sterilized distilled water in 1L clear glass reagent bottle, for Walne's Medium

Some notes regarding Walne's Medium:

- The preferred form of H_3BO_3 is fine granules rather than crystalline. The granules dissolve faster when immersed in warm water bath.
- Although MSI has not observed precipitation when dissolving $NaH_2PO_4.2H_2O$, other workers have reported so. In this case, it may be necessary to autoclave or mix this chemical last.

To prepare Walne's Medium, refer to Table 6. Note that the chemicals for the Trace Metal Primary Stock solution readily absorb moisture from the air, hence this solution must be prepared first. Use gloves when handling the trace metal chemicals. Use wax paper funnels when transferring any weighed chemical to the nutrient bottle. Weigh the chemicals for the Trace metal Primary stock solution. Dissolve the chemicals one at a time, in sequence, in 10ml sterilized distilled water. Mix well until a clear solution is achieved. If the solution is cloudy, add 1N HCl drop by drop, until the solution becomes clear.

Weigh the chemicals for the Vitamin Primary Stock solution. Dissolve the vitamins one at a time in sequence in 200ml sterilized distilled water. Mix well.

To prepare the Walne's Medium, weigh the 6 chemicals. Dissolve them one at a time, in sequence, in 900ml sterilized distilled water. Note that NaH_2PO_4 is added last, to avoid precipitation. Mix well until a clear solution is achieved. Then, add 100ml of Vitamins Primary Stock solution, and mix well. Then, add 1ml of Trace Metal Primary Stock solution, and mix well.

Store the Walne's Medium, and the remaining Primary Stock solutions (Vitamins, Trace Metals) in the refrigerator. Note that although their shelf life is about 6 months, any solution must be discarded as soon as bacterial growth is observed.

To establish cultures of* Isochrysis galbana*, you will need:

2 x 250ml clear glass Erlenmeyer flasks, with 100ml UVFSW, for maintenance culture

10 x 1L glass dextrose bottles, with 1L UVFSW, to upscale from maintenance culture

20 x 10L clear barrel jars, with 9L UVFSW for further upscaling of cultures

Walne's Medium

Algal starter culture

Maintenance cultures

Glass tubings mounted on rubber caps, for dextrose bottles

Plastic hose with fiberfill at both ends

Micropipettes 200 µl and 5000 µl, with corresponding tips

UV transfer hood

Alcohol lamp (denatured), box of matches

Employ aseptic methods when working with culture media, and culture micro-algae. Activate the UV transfer hood, and allow to irradiate for 3 hours. Remember to switch off the UV lamp while working under the UV transfer hood. During the transfer of algae between culture vessels, sterilize the vessel's mouth (gauze removed) immediately before and after transfer, by passing the vessel's mouth over the blue flame of alcohol lamp. This procedure kills any surface and airborne contaminants, such as bacteria, that might enter the culture.

Table 5. Recipe for Walne's Medium (see Tech 1981)

Trace metal Primary stock solution	Amount required	Walne's medium	
Sterilized distilled water	10.0 ml	Sterilized distilled water	900.0 ml
$ZnCl_2$	0.21 g	$NaNO_3$	100.00 g
$CoCl_2.6H_2O$	0.20 g	Na_2EDTA	45.00 g
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.09 g	H_3BO_3	33.60 g
$CuSO_4.5H_2O$	0.20 g	$FeCl_3.6H_2O$	1.30 g
Vitamin Primary stock solution		$MnCl_2.4H_2O$	0.36 g
Sterilized distilled water	200.0 ml	$NaH_2PO_4.2H_2O$	20.00 g
B_1 (or Thiamine)	0.20 g	Vitamin Primary Stock Solution	100.0 ml
B_{12}	0.01 g	Trace Metal Primary Stock solution	1.0 ml

Dispense the 150 µl of Walne's Medium into 250ml Erlenmeyer flasks with 100ml UVFSW; 1.5 ml into 1L dextrose bottles with 1L UVFSW, and 15ml into 10L clear plastic barrel jars.

With an algal starter stock (in test tubes), transfer the contents (about 10 ml) into the 250ml Erlenmeyer flasks, constituting the maintenance cultures. Plug the flasks with sterile gauze. Arrange the flasks in a row, exposing the cultures to the light bank (Fig. 19). Swirl the flasks everyday.

To subculture using maintenance stock
Transfer 10% of flask contents into the 1L dextrose bottles (or to 9L carboy). If there is an immediate need for microalgal feed (i.e. to be ready in 5-6 days), up to 50% of the stock may be transferred. Subculture the maintenance algal stock weekly, before half the total life span of the culture has passed (Guillard 1995). Any remaining stock culture may be used to upscale the algal culture to larger volumes. Or they may be stored in the refrigerator for up to 3 weeks before starting a new batch.

Plug the inoculated dextrose bottles with the rubber caps. For aeration, attach the plastic hose (with fiberfill to filter the incoming air) to the aeration source. Then, attach the glass tubing from the dextrose bottle to the plastic hose (Fig. 18).

To upgrade using dextrose cultures
Transfer 10% of dextrose contents into the 10L barrel jars. All dextrose and barrel containers must be exposed to light at 4000-6000 lux (equivalent to 2 fluorescent lamps, per row of culture vessels), and vigorously aerated (Fig. 19).

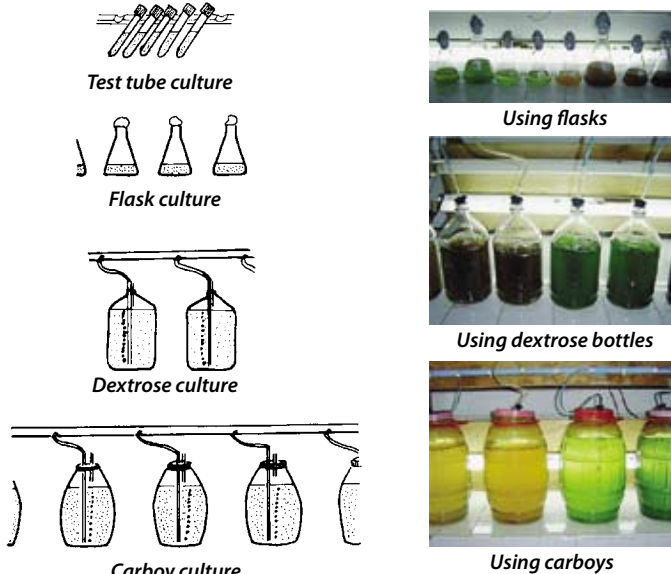*Test tube culture*

Flask culture

Dextrose culture

Carboy culture

Using flasks

Using dextrose bottles

Using carboys

Fig. 19. Culture set-ups of microalgae, from starter cultures in test tubes to flask, dextrose and carboy cultures

APPENDIX 3

Zooxanthellae culture

Summary of procedure

Sterilize glassware and other materials for use in zooxanthellae culture. Follow procedures for sterilization in Appendix 2.

Prepare the nutrient solutions for the zooxanthellae cultures. To make the f/2 medium (Guillard & Ryther 1962, Guillard 1975, Belda & Yellowlees 1995), first prepare the Trace Metal Primary Stock solution, then the Vitamin Primary Stock solution.

Acquire zooxanthellae starter culture, and establish culture of zooxanthellae. This culture is crude (being a mixed population), unless identified as a particular strain or clone.

Maintain the crude cultures by subculturing weekly.

If desired, establish clones of zooxanthellae using single cell isolates, from crude cultures or zooxanthellae freshly isolated from the clam mantle.

To sterilize by autoclave, you will need:
   2 x 250ml glass Erlenmeyer flasks with 100ml UVFSW (UV-treated 0.2µm-filtered seawater), for crude culture. Plug the mouth of the flasks with gauze.
   20 x 150mm screw cap test tubes, for zooxanthellae culture
   1L glass reagent bottle, amber, with ground glass stopper, for 0.45µm FSW
   250ml glass reagent bottle, amber, with ground glass stopper, for vitamin stock solution
   4 x 250ml glass reagent bottle, clear, with ground glass stopper, for stock solutions of
   $NaNO_3$, NaH_2PO_4, trace metals, ferric citrate
   250 ml, 1L glass beakers
   20ml glass vials, for vitamin aliquots
   Vacuum flask
   Glass Petri dishes

Keep the caps loose on the bottles.

Wrap several Pasteur pipettes in aluminum foil. Wrap glass rods separately in foil wrapper. Place micropipette tips in a beaker, and cover with foil. Load materials in a commercial autoclave, and sterilize in at 121°C for 15 min. Note that if other kinds of autoclave are used, always follow the manufacturer's operating instructions for the autoclave. After autoclaving, retrieve sterilized materials and cool before use.

To prepare nutrient solutions, you will need:
    Distilled water
    Vacuum filter holder, vacuum flask, vacuum pump,
       0.45 Millipore filter for seawater
    Forceps
    1L x 0.45 millipore FSW
    Disposable gloves
    Weighing boats
    Spatula
    Paper wipes
    Glass rods for stirring
    Square-shaped wax papers (to form a funnel)
    Analytical balance
    10ml syringe, syringe filter
    Micropipette 1000 µl, with tips
    Alcohol lamp (denatured alcohol), box of matches
    Labels, fine marker pen
    Transfer chamber

First filter 1L seawater through 0.45 millipore filter into 1L reagent bottle. Prepare the seawater medium for zooxanthellae culture using the FSW. All transfers of solutions and cultures must be done within a fumehood or transfer chamber. Employ aseptic methods when working with culture media, and zooxanthellae cultures. Refer to Appendix 2 for proper laboratory techniques using aseptic methods.

Add 0.5ml of the following stock solutions ($NaNO_3$, trace metals, and ferric citrate), one at a time, and mix well until the seawater solution is clear. Then autoclave. After the autoclaved seawater solution has cooled, add 0.5ml NaH_2PO_4. Then, pass a 5-ml vitamin solution aliquot through a syringe-filter, and into a sterile glass vial. Dispense 0.5 ml to complete the medium f/2. Mix well. Label the complete 1L-medium f/2 to indicate date of the preparation.

Store the remaining medium f/2, and the remaining stock solutions in the refrigerator. Note that although their shelf life is about 6 months, any solution must be discarded as soon as bacterial growth is observed.

To establish culture of zooxanthellae (from mixed starter), you will need:
    Medium f/2 stock solutions
    Mixed zooxanthellae starter culture
    Micropipette, 1000 µl, with tips
    250ml clear glass sterile Erlenmeyer flasks
    20 x 150mm screw cap sterile test tubes
    Alcohol lamp (denatured alcohol), box of matches
    Labels, fine marker pen

Table 6. Recipe for medium f/2

Trace Metal Primary Stock solution	Amount required
Distilled water	500 ml
$ZnSO_4.7H_2O$	22 mg
$CoCl_2.6H_2O$	11 mg
$Na_2MoO_4.2H_2O$	6.3 mg
$CuSO_4.5H_2O$	9.8 mg
$MnCl_2.4H_2O$	180 mg
Vitamin Primary Stock solution	
Distilled water	100.0 ml
B_1 (or Thiamine)	20 mg
Biotin	0.1 mg
B12	0.1 mg
Ferric Citrate Stock solution	
Distilled water	500 ml
Ferric citrate	4.5 g
Citric acid	4.5 g
MEDIUM f/2 STOCK SOLUTIONS (Guillard & Ryther 1962, Guillard 1975)	
0.45 Millipore FSW	1 L
$NaNO_3$ Stock solution=15.0 g in 100ml distilled water	0.5 ml
Trace Metal Primary Stock solution	0.5 ml
Ferric Citrate Stock solution	0.5 ml
Autoclaved seawater medium (as above)	1 L
$NaH_2PO_4.2H_2O$ Stock solution (autoclaved)= 1g in 100ml distilled water	0.5 ml
Vitamin Primary Stock solution (thawed 5ml aliquot), syringe filter-sterilized	0.5 ml

Employ aseptic methods. Activate the fumehood, or transfer chamber for 2 hrs (or follow operating procedures).

Prepare the medium f/2 as above. Dispense the 100ml of this medium into 250ml Erlenmeyer flasks. Transfer about 10ml of the zooxanthellae starter culture into the flasks. Plug the flasks with sterile gauze. Label properly and indicate the date of transfer. Arrange the flasks in a row, exposing the cultures to the light bank. Swirl the flasks gently everyday.

Subculture the crude zooxanthellae cultures weekly, or before half the total life span of the culture has passed (Guillard 1995). To subculture, remember to swirl the source culture gently before transferring 10% of flask contents into new autoclaved flasks.

To establish pure culture of zooxanthellae (from single-cell isolate)
Medium f/2 stock solutions, micropipette 1000 µl, with tips
Mixed zooxanthellae starter culture, or freshly isolated zooxanthellae
Sterile Pasteur pipettes, capillary pipettes, bulbs
Alcohol lamp (denatured alcohol), box of matches
Petri dishes
UVFSW (UV-treated 0.2µm-filtered seawater) in wash bottle
Compound microscope
Labels, fine marker pen
Test tube slant racks

Prepare the medium f/2 as above. Dispense the 10 ml of this medium into 20ml test tubes (screw cap). Make a capillary pipette (see Pringsheim 1964, Kurosawa 1994) from a sterile Pasteur pipette. Heat the thin tube of a Pasteur pipette over a blue flame, while holding the tip with a pair of forceps. When the thin tube softens, quickly remove it from the flame while drawing it out with forceps to make a capillary tube. Break the capillary tube to a suitable length, and attach a bulb at the other end.

Sample the zooxanthellae with a new Pasteur pipette. Slightly press the bulb of the Pasteur pipette to form a droplet at the pipette's tip. Consecutively, put 6 small droplets on a Petri dish in a circular manner. In so doing, the last droplet would have the fewest cells compared to the first droplet.

Put the Petri dish under a compound microscope to inspect the last droplet for zooxanthellae. While viewing under the microscope, pick-up a single cell with a capillary pipette. Inoculate the cell into a test tube with culture medium. Cover the test tube with the screw cap but keep it loose. Label properly, indicate the date of transfer, and expose the tubes to a bank of lights on slant racks. Observe the tubes weekly for any presence of brownish colored growth, usually at the bottom of the tube. Gently shake the tubes daily to dislodge any growth adhering to the tube walls. Record all observations per tube. The coloration of the culture should intensify after about 2 weeks. Top up the seawater medium in the tube to compensate for loss of medium from evaporation. Ensure that the additional seawater medium is of same temperature as the medium in the tubes prior to addition. Subculture after 2 weeks, and regularly thereafter.

Subculture the zooxanthellae cultures weekly, or before half the total life span of the culture has passed (Guillard 1995). To subculture, remember to swirl the source culture gently before transferring 10% of flask contents into new autoclaved flasks.

APPENDIX 4

Data sheets for the ocean nursery

STOCK INVENTORY SUMMARY

Broodstock or Juveniles
(encircle one)

Date _____
Recorder _____

SPECIES	BIRTHYEAR				NO. CLAMS	AVERAGE SL
		(Indicate Site ID)				(mm or cm)

Broodstock or Juveniles
(encircle one)

Date _____
Recorder _____
Site ID _____
Species _____

				(Indicate Birthyear)					
Average SL									
s.d.									

(N=30 to 50)

GLOSSARY

acclimatize – v, to adapt to a new environment, or condition, e.g. temperature, salinity

acid wash – n, sterilization method that uses a chemical, like sulfuric or hydrochloric acid

adduct – v, to pull toward the body's median axis

algal fouling – n, undesirable presence of algal overgrowth in culture

aliquot – adj, fractional

ammonium sulfate – n, $(NH_4)_2SO_4$; a water-soluble chemical compound of fine white granules, used in the fertilizer industry

analytical balance – n, a laboratory instrument that accurately and precisely measures the mass of an object. Proper functioning requires that it be stationed on a plain solid surface, free of draft and vibration.

anterior – adj, located toward the front

appressed – adj, lying low, or close to a surface

aseptic – adj, free from disease-causing microorganisms

asymmetrical – adj, cannot be divided into two similar halves along a longitudinal plane

axenic – adj, free from other living organisms

bacteria – n, group of unicellular organisms classified under Prokaryotes. They lack nuclei but nonetheless possess genetic material. Bacteria have specific shapes: rod-shaped, spherical, and spiral. May exist as single cells, in pairs, or clusters. Size range is 0.2 – 5μm.

barrier reef – n, a kind of coral reef generally adjacent to land but separated from it by a lagoon

bleaching – n, as in mantle *bleaching*; whitening of the clam mantle due to loss of zooxanthellae

breed – v, to produce a better or superior kind of organisms of species or closely related species, by sexual propagation or by genetic manipulation, often under controlled conditions

broodstock – n, as in clam *broodstock*; giant clams that are male or male-female mature and can be used as parent stock for mariculture

broodstock maturity – n, condition of broodstock when they are ready for reproduction, having ripe male and female gonads

byssal aperture – n, also byssal gape; the ventral opening between the valves, through which the bivalve foot and the byssus protrude

byssal gland – n, the bivalve organ which secretes the byssus for attachment to the substratum

byssus – n, a tuft of proteinaceous filaments secreted by the byssus gland, adheres strongly to substratum

calibrate – v, to improve the accuracy and precision of a measuring instrument by determining its deviations in function against an accepted standard, and to apply the proper correction factor; to mark the graduations of a measuring instrument against an accepted standard.

caliper – n, a measuring instrument that has two adjustable jaws, to measure length, diameter or thickness

chilling – n, a physiological condition of cultured clams, responding to large temperature fluctuations, or to low temperatures.

clade – group of organisms that belong to the same taxon, sharing common characteristics arising from a common descent

cohort – n, group of individuals having the same parentage and age

conditioning – n, culture method whereby the culture organism is given supplemental nutrition to enhance its growth and reproductive development.

conical siphon – n, inverted funnel-shaped part of the clam mantle, surrounding the excurrent aperture at the anterior region

coral head – n, a conspicuous coral formation, referring to colonial massive forms

coral rubble – n, substratum consisting of fragmented coral

culture – v, as in aqua*culture* or mari*culture*; the farming of aquatic (marine) organisms, involving their capture, breeding, or rearing at densities usually higher than in wild populations

culture medium – n, an artificial solution or gel preparation consisting of particular elements, compounds, vitamins and/or nutrients, to serve as food for specific organisms or specimens

decant – v, to pour off the solution component of a heterogeneous mixture, and retaining the denser component

density – n, as in stocking *density*; the number of organisms in a specified area or volume

dichotomous key – n, a taxonomic tool that distinguishes between two groups or two taxa of organisms, based on a particular, observable, and consistent characteristic

dinoflagellate – n, unicellular algae that bear two dissimilar flagella. Endosymbiotic dinoflagellates exist in a non-motile state within the host.

dissolved inorganic nitrogen – n, nitrogen available as dissolved nutrients in the form of nitrate, nitrite, ammonium, and ammonia

dorsal – adj, referring to the backside, away from the underside

embryo – n, an animal at its early development stage undergoing cleavage, tissue differentiation; a very young plant having rudimentary leaves, stem, and roots

emersion – n, act of rising from a fluid medium

endangered – adj, as in endangered species; in danger of total disappearance, in the absence of measures to preserve remaining numbers

epibiont – n, a sedentary organism that adheres to a surface, either for part or the whole of its life cycle

epibiota – n, the flora and fauna of a specified area or region

equator – n, the circular band whose plain is perpendicular to the earth's axis, and dividing the earth in two exact hemispheres, north and south

equilateral – adj, having equal sides

ex situ **spawning** – n, release of sperm and eggs by broodstock in a culture tank

excurrent aperture – n, opening in the mantle, and serving as an exit for water, gametes, feces, etc.

export – n, to move (something) out of the country of origin

extant – n, not extinct

extinct – adj, no longer existing

eyepiece – n, as in microscope *eyepiece*; that part of the microscope closest to the viewer, for viewing a specimen

fathom – n, unit of measurement for water depth, or length of rope or material for similar purpose, equivalent to 6 feet

fertilize – v, as in to *fertilize* the eggs with sperm; to introduce the male germ cell (sperm) into the female germ cell (egg)

fiberfill – n, lightweight synthetic fibers used as filling material for pillows and the like, or for insulation

fiberglass – n, a material made of extremely fine glass fibers, used to make different materials including structural materials

filamentous alga – n, any aquatic plant that exhibits a threadlike growth form, often in clumps

flagellate – adj, bearing a flagellum or flagella

flowrate – n, volume of fluid passing through a given unit of time

fungus – n, a eukaryotic (nucleus-bearing) filamentous organism, neither plant nor animal, may be uni- or multicellular. They are parasitic and may thrive on any organic substrate.

gamete – n, mature reproductive cell that is haploid (bears only one set of unpaired chromosomes), and capable of fusing with another such cell from the opposite sex to form a new individual that is diploid (bears one set of paired chromosomes)

gas bubble disease – n, a physiological condition in the giant clam, having air blisters at the mantle surface

grazer – n, an animal that feeds on plants

greenhouse heating – n, the simulation of a greenhouse effect using an enclosure of glass or clear plastic, with controlled temperature and humidity conditions

guard tentacle – n, short to long extensions of the clam mantle surrounding the incurrent aperture, serve as an initial filter against large particles entering the incurrent chamber

harvest – v, to collect or gather together a resource

hatchery – n, a place where eggs are hatched

hatching tank – n, a culture tank where fertilized eggs of the culture organism are placed and hatched

heat stress – n, a heat-related stimulus that challenges the physiological ability of an organism to continue its metabolic functions; physiological condition responding to an extreme heat stimulus.

hemocytometer – n, a microscopy tool, especially a slide bearing two mirror calibrated grids, to count unicellular organisms

heterotrophy – n, utilization of different sources of food, often complex compounds of nitrogen and carbon for metabolic synthesis

hinge – n, as in shell hinge; the flexible joint of tough connective tissue, where the shell valves meet

holding tank – n, a culture tank used for various purposes

husbandry – n, the economical management of farmed plants and animals

hyaline organ – n, a small organ (numerous) in the clam mantle, surrounded by zooxanthellae. Has the ability to concentrate light that is then harnessed by surrounding zooxanthellae for photosynthesis.

immerse – v, to put in, as to cover, with a fluid

import – v, to allow entry (of something) from another country

in situ **spawning** – n, release of gametes (sperm and eggs) in a natural habitat

inbreeding – n, sexual breeding of organisms of the same species or closely related species, under controlled conditions, to preserve or eliminate a particular trait

incurrent aperture – n, elongated opening of the clam mantle at the posterior end, serves as entrance of water and food into the incurrent chamber. Sometimes, also serving as exit of clam pseudofeces

inoculation – n, the implantation of microorganisms into a culture medium

insulated – adj, being protected from external stimulus

interdigitating – n, as in *interdigitating* processes of a clam valve; pronounced dorsal extensions of a valve, interlocking with the other valve

interlocking teeth – n, matching depressions and protuberances of shell material on the ventral area of the inner shells, used as a diagnostic characteristic in bivalve systematics

invasive – n, pathogenic microorganism, or disease-causing agent; a species that establishes residency in a habitat not its own

iridescent – adj, exhibiting changing colors, or a shifting display of an array of rainbow colors

juvenile – n, as in clam *juvenile*; the young immature bivalve, almost resembling the adult form, but differing in body proportions

landbased nursery – n, the place where juveniles are reared until they reach a size suitable for the ocean nursery; broodstock may also be kept in the landbased nursery

larva – n, as in clam *larva*; the planktonic stage in clam development; different in form and function from the juvenile and adult. Typically bears a velum for swimming.

lateral – adj, at or toward a side

light attenuation – n, decreased manifestation of the properties of light

macroalga – n, large aquatic plant

mantle – n, the clam's large fleshy organ bordering the shells, the outer and median lobes fused to form the retractable excurrent siphon and incurrent aperture, the inner lobe responsible for shell calcification and reaching down to the byssal gape. Exhibits various colorations and patterns, and harbors populations of zooxanthellae.

metabolic – adj, converting carbon sources to energy that will fuel cellular functions

metamorphosis – n, transformation in form and function

microalga – n, microscopic unicellular aquatic plant

micropipette – n, a calibrated measuring tool able to deliver micro volumes of fluid

microscope stage – n, part of the microscope where the slide is placed for viewing

mother clam – n, broodstock clam that releases eggs for larval culture

mottled pattern – n, appearance that is mosaic, showing large irregular patches of color

nitrogen deficiency – n, lack of nitrogen in an organism's nutrition; the physiological state of an organism that lacks nitrogen in its diet

objective – n, as in microscope *objective*; the lens or system of lenses used to magnify an image of an object under the microscope

ocean nursery – n, a place in the sea where broodstock, sub-adults, and juveniles are reared

ocular micrometer – n, calibrated scale inserted through a microscope eyepiece

parasite – n, organism that lives off another organism (host), and uses energy resources that would otherwise be used by its host. Often renders the host metabolically deficient, to the point of death

pathogen – n, organism that causes a disease

pediveliger – n, transitional stage in the development of the giant clam larva exhibiting crawling and swimming behavior. The larva's velum is almost gone or has been resorbed, and an extensible foot has developed to aid crawling.

pH – n, the condition of a fluid ranging from acidity to alkalinity, resulting from the effective hydrogen ion concentration in that fluid

photosynthesis – n, the production of carbohydrate compounds in the tissues of chlorophyll-bearing plants, with the aid of light

phototrophy – n, utilization of food directly derived from photosynthesis for metabolic synthesis

planktotrophy – n, life history strategy among invertebrates with a planktonic larval stage that must feed on external energy sources for metamorphosis and settlement; in contrast to lecithotrophy, which is the strategy of larvae relying on maternal (yolk) energy resources

plunge-type mixer – n, (in culture) a hand tool consisting of a perforated disc attached to a short pole; used to mix eggs or larvae in a small tank.

poach – v, to take by illegal means, often encroaching or trespassing

polyspermy – n, penetration of the egg's outer (vitelline) membrane by two or more sperm

population genetics – n, branch of science dealing with statistical analyses of inheritance and the transmission of genes in populations

posterior – adj, at or toward the rear or back

protandric hermaphrodite – n, an organism having both male and female reproductive organs (gonads), with the initial development of the male gonads, followed by the development of the female gonads.

protocol – n, plan or procedure (to conduct a scientific activity)

protozoa – n, large group of nucleus-bearing, single-celled microorganisms possessing either flagella or pseudopodia for feeding or motility. Includes flagellates, amoebas, ciliates, and sporozoans.

pyramidellid – n, a group of small gastropods that are parasitic on bivalves, including giant clams. Possesses an extensible proboscis for sucking the body fluids of its host.

quarantine – n, period of isolation during which (something) is subjected to observations or studies to detect disease or contamination, and contain or eliminate pathological agents.

raceway – n, any large container, usually longer than wider, often used for culture.

reef moat – n, a submarine trough in a reef

reef slope – n, the sloping area from the reef crest (the margin of active coral growth), to deeper water

reproduce – v, to give rise to new individuals by sexual or asexual means

reproductive health – n, the condition of the body, particularly its potential to reproduce

restock – v, reseed; to add individuals, often young, of a particular species, to allow repopulation of that area or neighboring areas.

rib – n, ribbing; ridge; wave-like shell sculpture that prominently radiates from the umbonal area to the shell margin

salinity stress – n, a stimulus that challenges the ability of an organism to regulate osmotic pressure in its body, as to affect its metabolic functions; physiological condition responding to an extreme osmotic stimulus.

sandbox filter – n, a device containing coarse to fine particles, to strain unwanted organisms and particles

scute – n, plate-like shell sculpture, occurring externally and often in series on the shell rib

seagrass – n, any of the flowering marine plants, possessing rhizomes for attachment to substratum

seed stock – n, the young of a cultured stock

serotonin – n, (as spawning inducer), 5-hydroxytryptamine creatinine sulfate complex, $C_{14}H_{19}N_5O_2.H_2SO_4$; a white powder, at the proper dose, may be used to induce giant clams to spawn; (in bivalves) a natural compound occurring in the nerves, and controls muscle contraction.

settlement – n, as in *larval settlement*; the stage in larval metamorphosis during which the larva develops a foot and is able to crawl on the tank bottom

shell length – n, the size of the shell at its longest horizontal axis

siltation – n, presence of fine particles, such as silt or mud, in the water

sleeve pipe – n, a piece of pipe with a serrated bottom end, vertically enclosing a standpipe in a culture tank

solar radiation – n, energy from the sun

spawning – n, the release of male and female gametes

species – a group of similar individuals that can sexually interbreed and give rise to offspring that possesses similar interbreeding potential

spore – n, a small primitive cell produced by plants and some invertebrates, and has the capability to either directly develop into, or fuse with another such cell to produce a new individual dissimilar to the parent

spring tide – n, the highest and lowest tide in a month, coinciding with the lunar phases, new moon or full moon

stage micrometer – n, slide with a calibrated scale, placed on the microscope stage, and used during calibration of the ocular micrometer

stand pipe – n, a piece of pipe often with a threaded bottom end, vertically inserted or screwed into a drainage hole of a culture tank

starter algae – n, a culture of microalgae often used as inoculum for maintenance culture

stock – n, (something) at hand or available; in culture, the individuals produced from culture

streptomycin sulfate – n, $(C_{21}H_{39}N_7O_{12})_2 . 3H_2SO_4$, with molecular weight 1457.38; water-soluble aminoglycoside used as antibiotic, interferes with protein synthesis of gram-negative bacteria

styropore – n, insulation material made from plastic polystyrene, useful in reducing heat flow, exhibits resistance against low temperatures

rhomboidal – adj, similar to a rhomboid, in having four sides, the opposite sides being parallel and equal

subculture – v, to culture microorganisms by using a fraction from the source

substrate – n, a medium, usually at the bottom of a container, for growing (something), or to which (something) is attached

superphosphate – n, usually available as $Ca(H_2PO_4)_2$, water-soluble, with at least 20% available phosphate content; used as fertilizer for plants, and important in the formation of nucleic acids, proteins, etc.

suspension – n, mixture with undissolved particles in a fluid or solid, often differing in density or colloidal size

symbiosis – n, an intimate relationship between at least two organisms, generally both benefiting from the relationship

temperature shock – n, a stimulus involving an abrupt change in temperature; the physiological condition of an organism responding to an abrupt change in temperature, affecting its metabolic functions

thermal pollution – n, contamination, usually of a water source, by heat effluents

threatened – adj, as in *threatened* species; close to being endangered

tissue culture plate – n, a laboratory apparatus; a polycarbonate block containing a number of wells or receptacles to hold samples (tissue, microorganisms, etc.)

trochophore – n, a planktonic larval stage of giant clam, typically bearing an apical tuft of filaments and a ciliated band

turbidity – n, the state of having particles, either dissolved, undissolved, or suspended, in water or in a solution

turnover – n, as in water *turnover*; replacement of water

umbo – n, prominent part of the bivalve shell closest to the hinge

UVFSW or ultraviolet-treated filtered seawater – n, filtered seawater that has passed through ultraviolet treatment to kill most microorganisms

UV transfer hood – n, a contained chamber with an ultraviolet lamp, used for tasks requiring a sterile environment

veliger – n, planktonic larval stage of giant clam, typically with a ciliated velum for motility

ventral – adj, referring to the stomach area, or underside

vestigial – adj, having a rudimentary structure and/or function, in contrast to formerly exhibiting structure and/or function that was more fully developed

vulnerable – adj, susceptible; open to attack

winter mortality – n, death of clams attributed to temperatures that are low, or below the clams' lower limit of tolerance

zooxanthellae – n, single-celled dinoflagellate algae that are endosymbiotic with several reef organisms, such as the giant clams and scleractinian corals.

REFERENCES CITED

Alcala AC, EP Solis, SN Alcazar. 1986. Spawning, larval rearing and growth *of Hippopus hippopus* (Linn.) (Bivalvia: Tridacnidae). Silliman J. 33: 45-53

Alder J, RD Braley. 1988. Mass mortalities of giant clams on the Great Barrier Reef, p. 230. In: Copland JW, JS Lucas (eds), Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

Allison GW, J Lubchenco, MH Carr. 1998. Marine reserves are necessary but not sufficient for marine conservation. Ecol Appl. 8: 79-92

Belda-Baillie CA, M Sison, V Silvestre, K Villamor, V Monje, ED Gomez, BK Baillie. 1999. Evidence for changing symbiotic algae in juvenile tridacnids. J Exp Mar Biol Ecol. 24(2): 207-221

Belda CA, D Yellowlees. 1995. Phosphate acquisition in the giant clam zooxanthellae symbiosis. Mar Biol. 124: 261-266

Bell JD. 1999. Restocking of giant clams: progress, problems and potential, pp 437-452. In: Howell BR, E Moskness, T Svasand (eds), Stock Enhancement and Sea Ranching. Blackwell Science, Oxford

Bell JD, DM Bartley, K Lorenzen, NR Loneragan. 2006. Restocking and stock enhancement of coastal fisheries: Potential, problems and progress. Fish Res. 80: 1-8

Benzie JAH. 1993. Review of the population genetics of giant clams, pp 1-6. In: Munro, P (ed), Genetics aspects of conservation and cultivation of giant clams. ICLARM Conf Proc No 39, International Center for Living Aquatic Resources Management, Makati

Benzie JAH, ST Williams. 1992. Genetic structure of giant clam (*Tridacna maxima*) populations from reefs in the Western Coral Sea. Coral Reefs. 11: 135-141

BFAR (Bureau of Fisheries and Aquatic Resources). 1990. Fisheries Administrative Order No. 168. Rules and regulations governing the gathering, culture and exportation of shelled mollusks (Phylum Mollusca)

Blake SG. 1995. Late quaternary history of Henderson Island, Pitcairn Group. Biol J Linnean Soc. 56(1-2) 43-62

Blidberg E, M Tedengren, T Elfwing. 2004. Chapter 5. Effects of petroleum on bioenergetics and DNA integrity in the giant clam *Hippopus hippopus*, 20p. Doctoral dissertation, Department of Systems Ecology, Stockholm University

Braley RD. 1989. A giant clam stock survey and preliminary investigation of pearl oyster resources in the Tokelau Islands. South Pacific Aquaculture Development Project, Food and Agriculture Organization of the United Nations, Suva, Fiji

Braley RD (ed). 1992. The giant clam: Hatchery and nursery culture manual. ACIAR Monograph No. 15, Australian Center for International Agricultural Research, Canberra, Australia, 144 p

Bryan PG, DB McConnell. 1976. Status of giant clam stocks (Tridacnidae) on Helen Reef, Palau, Western Caroline Islands, April 1975. Mar Fish Rev. 38(4): 15-18

Caddy JF. 1993. Towards a comparative evaluation of human impacts on fishery ecosystems of enclosed and semi-enclosed seas. Rev Fish Sci. 1: 57-95

Caddy JF, O Defeo. 2003. Enhancing or restoring the productivity of natural populations of shellfish and other marine invertebrate resources. FAO Fisheries Tech Paper No 448. Food and Agriculture Organization of the United Nations, Rome. 159 p

Calumpong HP (ed). 1992. The giant clam: An ocean culture manual. ACIAR Monograph No. 16. Australian Center for International Agricultural Research, Canberra, Australia. 68 p

Carr MH, DC Reed. 1993. Conceptual issues relevant to marine harvest refuges: Examples from temperate reef fishes. Can J Fish Aquat Sci. 50: 2019-2028

CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). 1979. Amended copy of CITES, June 22, 1979, 8 p

Copland JW, JS Lucas (eds). 1988. Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia, 274 p

Cumming RL. 1988. Pyramidellid parasites in giant clam mariculture systems, pp 231-236. In: Copland JW, JS Lucas (eds), 1988. Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

de Perio NS, CA Belda. 1988. Predators and parasites of giant clams (Bivalvia: Tridacnidae) in the land- and oceanbased nurseries in Bolinao, Pangasinan. pp 75-80. In: Zaragosa, EC, DL de Guzman, EP Gonzales (eds), Proc. Symp. Workshop on the Culture of Giant Clams (Bivalvia: Tridacnidae). Silliman University, Dumaguete City

Dillwyn LW. 1817. A descriptive catalogue of recent shells. 1: 215

Fisher CR, WK Fitt, RK Trench. 1985. Photosynthesis and respiration in *Tridacna gigas* as a function of irradiance and size. Biol Bull. 169: 230-245

Fitt WK. 1984. The role of chemosensory behavior of *Symbiodinium microadriaticum*, intermediate hosts, and host behavior in the infection of coelenterate and molluscs with zooxanthellae. Mar. Biol. 81: 9-17

Fitt WK, CR Fisher, RK Trench. 1984. Larval biology of tridacnid clams. Aquaculture. 39: 181-195

Gomez ED, CA Belda. 1988. Growth of giant clams in Bolinao, Philippines, pp 178-182. In: JW Copland, JS Lucas (eds). Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

Gomez ED, SS Mingoa-Licuanan. 1998. Mortalities of giant clams associated with unusually high temperatures and coral bleaching. In: Watson, M (ed), Reef Encounter No. 24 (December 1998)

Gomez ED, PC Cabaitan, KC Vicentuan. 2005. Coral Culture and Transplantation and Restocking of Giant Clams in the Philippines. Proc of the Regional Tech Consultation on stock Enhancement for Threatened Species of International Concern. Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan 5021, Iloilo, Philippines

Gomez ED, SS Mingoa-Licuanan. 2006. Achievements and lessons learned in restocking giant clams in the Philippines. Fish. Res. 80: 46-52

Govan H.1992. Chapter 5: Predators and predator control, pp. 41-50. In: Calumpong, HP (ed), The Giant Clam: An Ocean Culture Manual. ACIAR Monograph No.16. Australian Center for International Agricultural Research, Canberra, Australia

Govan H. 1994. Predators of maricultured tridacnid clams. Proc 7th Intl Coral Reef Symp. 2: 749-753

Govan H. 1995. *Cymatium muricinum* and their ranellid gastropods: major predators of cultured tridacnid clams. ICLARM Tech Rep. 49. International Center for Living Aquatic Resources Management, Makati.136 p

Govan H, LY Fabro, E Ropeti. 1993. Controlling predators of cultured tridacnid clams, pp 111-118. In: Fitt WK (ed), The Biology and Mariculture of Giant Clams: A workshop held in conjunction with the 7th Intl Coral Reef Symp, 21-26 June 1992, Guam, USA. ACIAR Proc. No. 47. Australian Center for International Agricultural Research, Canberra, Australia

Govan H, PV Nichols, H Tafea. 1988. Giant clam resource investigations in Solomon Islands, pp 54-57. In: Copland JW, JS Lucas (eds), Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

Griffiths CL, DW Klumpp. 1996. Relationships between size, mantle area and zooxanthellae numbers in five species of giant clam (Tridacnidae). Mar. Ecol. Prog. Ser. 137:139-147

Guillard RRL. 1975. Culture of phytoplankton for feeding marine invertebrates, pp 29-60. In: Smith WL, MH Chanley (eds), Culture of Marine Invertebrate Animals. Plenum Publishing Corp, New York

Guillard RRL. 1995. Culture Methods, pp 45-62. In: Hallegraeff GM, DM Anderson, AD Cembella (eds), Manual of Harmful Marine Microalgae. IOC Manuals and Guides No. 33. UNESCO

Guillard RRL, JH Ryther. 1962. Studies on marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula conferva* (Cleve) Gran. Can. J. Microbiol. 8: 229-239

Heslinga GA.1993. Palau country report, pp. 38-40. In: Munro P (ed). Genetic Aspects of Conservation and Cultivation of Giant Clams. ICLARM Conf Proc No. 39, International Center for Living Aquatic Resources Management, Makati

Heslinga GA, WK Fitt. 1987. The Domestication of Reef-dwelling Clams. BioScience 37(5): 332-339

Heslinga GA, FE Perron, O Orak. 1984. Mass culture of giant clams (F. Tridacnidae) in Palau, Aquaculture 39: 197-215

Heslinga GA, T Watson, T Isamu. 1990. Giant clam farming. Pacific Fisheries Development Foundation (NMFS/NOAA), Honolulu, Hawaii. 179 p

Hester FJ, EC Jones. 1974. A survey of giant clams, Tridacnidae, on Helen Reef, a Western Pacific atoll. Mar. Fish. Rev. 36(7): 17-22

Huguenin JE, J Colt. 1989. Design and operating guide for aquaculture seawater systems. Developments in Aquaculture and Fisheries Science. Vol. 20. Elsevier, Amsterdam

Humphrey JD. 1988. Disease risks associated with translocation of shellfish, with special reference to the giant clam *Tridacna gigas*, pp 241-245 . In: Copland, JW, JS Lucas (eds), Giant Clams in Asia and the Pacific. ACIAR Monograph No 9. Australian Center for International Agricultural Research, Canberra, Australia

Hutsell KC, LL Hutsell, DL Pisor. 1997. Registry of World Record Size Shell. Snail's Pace Productions: San Diego. 104 p

Ishikura M, K Hagiwara, K Takishita, M Haga, K Iwai, T Maruyama. 2004. Isolation of new *Symbiodinium* from Tridacnid giant clam (*Tridacna crocea*) and sea slug (*Pteraeolidia ianthina*) using culture medium containing giant clam tissue homogenate. Mar. Biotechnol. 6: 378-385

Kurosawa K. 1994. Hatchery manual for bivalve researchers in the Philippines. Philippine Human Resources Development Center, Philippines/Japan International Cooperation Agency, Tokyo. 31 p

Langi V, H Aloua. 1988. Status of giant clams in Tonga, pp 58-59. In: Copland JW, JS Lucas (eds), Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

Lewis AD, TJH Adams, E Ledua. 1988. Fiji's giant clam stocks – A review of their distribution, abundance, exploitation and management, p 66-72. In: Copland JW, JS Lucas (eds), Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

Lewis AD, E Ledua. 1988. A possible new species of *Tridacna* (Tridacnidae: Mollusca) from Fiji, pp 82-84. In: Copland JW, JS Lucas (eds), Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

Lucas JS. 1988. Giant clams: description, distribution and life history, p 21-32. In: Copland JW, JS Lucas (eds), Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

Lucas JS. 1994. The biology, exploitation, and mariculture of giant clams (Tridacnidae). Rev. Fish. Sci. 2(3): 181-223

Lucas JS, E Ledua, RD Braley. 1991. *Tridacna tevoroa* Lucas, Ledua, and Braley: a recently- described species of giant clam (Bivalvia: Tridacnidae) from Fiji and Tonga. The Nautilus 105(3): 92-103

Miner RW. 1938. On the bottom of a pearl lagoon. The National Geographic Magazine 74(3): 365-382

Mingoa SSM. 1988. Photoadaptation in juvenile *Tridacna gigas,* pp 145-150. In: Copland JW, JS Lucas (eds), Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia.

Mingoa SSM, LAB Menez. 1988. A comparison of two benthic survey methods. Mar Biol 99: 133-135

Mingoa-Licuanan SS. In press. Effects of physical tags and chemical markers on the shell growth of tridacnids. J. Med. & Appl. Malacol. 12: 47-57

Mingoa-Licuanan SS. 1993. Oxygen consumption and ammonia excretion in juvenile *Tridacna gigas* (Linne 1758): Effects of emersion. J. Exp. Mar. Biol. Ecol. 171: 119-137

Mingoa-Licuanan SS, DT Dumale, ED Gomez. 2000. Giant clam culture manual. *Gabay sa pag-aalaga ng taklobo sa dagat*. How to grow giant clams in the ocean. University of the Philippines, and Bureau of Agricultural Research, Diliman, Philippines. 48 p

Mingoa-Licuanan SS, JS Lucas. 1995. Bivalves that "feed" out of water: Phototrophic nutrition during emersion in the giant clam, *Tridacna gigas* Linne. Journal of Shellfish Research 14(2): 283-286

Munro JL. 1986. Status of giant clam stocks and prospects of clam mariculture in the Central Gilbert Islands Group, Republic of Kiribati. Report to Fisheries Division, Tarawa, Kiribati and South Pacific Regional Fisheries Development Programme, Suva, Fiji. 36 p

Munro JL. 1993. Strategies for re-establishment of wild giant clam stocks, pp 17-20. In: Munro P (ed), Genetic Aspects of Conservation and Cultivation of Giant Clams. ICLARM Conf Proc No 39. International Center for Living Aquatic Resources Management, Makati

Newkirk G. 1993. A discussion of genetic aspects of broodstock establishments and management, pp. 6-13. In: Munro, P (ed), Genetic Aspects of Conservation and Cultivation of Giant Clams. ICLARM Conf. Proc No 39. International Center for Living Aquatic Resources Management, Makati

Newman WA, ED Gomez. 2000. On the status of giant clams, relics of Tethys (Mollusca: Bivalvia: Tridacnidae). Proc 9th International Coral Reef Symp. Bali, Indonesia. 2: 927-936

Norton JH, GW Jones. 1992. Giant clams: An anatomical and histological atlas. ACIAR Monograph No. 14. Australian Center for International Agricultural Research, Canberra, Australia. 142 p

Pasaribu BP. 1988. Status of giant clams in Indonesia, pp 44-46. In: Copland JW, JS Lucas (eds). Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

Paulay G. 1987. Biology of Cook Islands' bivalves. Part I. Heterodont families. Atoll Res. Bull. 298: 1-31

Paulay G. 1989. Marine invertebrates of the Pitcairn Islands: species composition and biogeography of corals, molluscs, and echinoderms. Atoll Res. Bull. 326: 1-28

Pearson RG. 1982. Assessment and management of fisheries for sessile invertebrates, pp. 123-159. In: Munro, JL (ed), Ecological aspects of coastal zone management: marine and coastal processes in the Pacific. Proc. Seminar at Motupore Island Research Centre-Papua New Guinea. UNESCO-ROSTEA, Jakarta

Perron FE, GA Heslinga, JO Fagolimul. 1985. The gastropod *Cymatium muricinum*, a predator on juvenile tridacnid clams. Aquaculture 48: 211-221

Price CM, JO Fagolimul. 1988. Reintroduction of giant clams to Yap State, Federated States of Micronesia, pp 41-43. In: Copland JW, JS Lucas (eds). Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia.

Pringsheim, EG. 1964. Pure cultures of algae: their preparation and maintenance. Hafner Publishing Company: New York & London. 119 p.

Roa, HT. 1997. Reproductive conditioning of *Tridacna crocea* (Tridacnidae) using algae and dissolved inorganic nitrogen as nutritional supplements. MSc thesis, University of the Philippines, Diliman

Romimohtarto K, P Sianipar, MGL dan Sutomo Panggabean. 1987. *Kima: Biologi, Sumberdaya, dan Kelestarian-nya, Puslitbang Oseanolog*i-LIPI. Jakarta

Rosewater J. 1965. The family Tridacnidae in the Indo-Pacific. Indo-Pacific Mollusca 1: 347-396

Rosewater J. 1982. A new species of *Hippopus* (Bivalvia: Tridacnidae). The Nautilus 96: 3-6

Schneider JA, DO Foighil. 1999. Phylogeny of giant clams (Cardiidae: Tridacnidae) based on partial mitochondrial 16S rDNA gene sequences. Molecu. Phylogen. Evol. 13(1): 59-66

Schonberg CHL. 2002. Substrate effects on the bioeroding demosponge *Cliona orientalis*. I. Bioerosion rates. Mar. Ecol. *Publicazioni Della Stazione Zoologica Di Napoli I.* 34(4): 313-326

Shelley CC, JS Glazebrook, E Turak, L Winsor, GRW Denton. 1988. Trematode (Digenea: Bucephalidae) infection in the burrowing clam *Tridacna crocea* from the Great Barrier Reef. Diseases of Aquatic Organisms 4: 143-147

Shelley CC, RGB Reid. 1988. An improved gonad biopsy technique for *Hippopus hippopus,* pp 95-97. In: Copland JW, JS Lucas (eds). Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

Sims NA. 1993. Pearl oysters. In: Wright A, L Hill (eds), Nearshore Marine Resources of the South Pacific, pp. 414-423. Institute of Pacific Studies, Suva

Sims NA, NT Howard. 1988. Indigenous tridacnid clam populations and the introduction of *Tridacna derasa* in the Cook Islands, pp 34-40. In: Copland JW, JS Lucas (eds), Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

Sirenko BI, OA Scarlato. 1991. *Tridacna rosewateri* (sp.n.). A new species of giant clam from Indian Ocean (Bivalvia: Tridacnidae). *La Conchiglia* (261): 4-9

Stasek CR. 1965. Behavioral adaptations of the giant clam *Tridacna maxima* to the presence of grazing fish. Veliger 8(1): 29-35

Stephenson TA, A Stephenson, G Tandy, M Spender. 1931. The structure and ecology of Low Isles and other reefs. Scientific Reports, Great Barrier Reef Expedition 1928-1929 3(2): 17-112

Tech E. 1981. Culture of phytoplankton. In: Guerrero RD, CT Villegas (eds), Report of the Training Course on Growing Food Organisms for Fish Hatcheries. FAO/UNDP

Thomas FR. 2001. Mollusk habitats and fisheries in Kiribati. An assessment from the Gilbert Islands. Pacific Science 55(1): 77-97

Tisdell C, KM Menz. 1992. Chapter 1. Giant clam farming and sustainable development: An overview, pp 3-16. In: Tisdell, C (ed), Giant clams in the sustainable development of the South Pacific: Socioeconomic issues in mariculture and conservation. ACIAR Monograph No. 18. Australian Center for International Agricultural Research, Canberra, Australia

Todd JC, AH Sanford, I Davidson, JB Henry (eds). 1979. Clinical Diagnosis and Management by Laboratory Methods. 16th ed. WB Saunders Co: Philadelphia, Pa. 1574 p

Valentine JW, D Hedgecock, GS Zumwalt, FJ Ayala. 1973. Mass extinctions and genetic polymorphism in the "killer clam", *Tridacna*. Geol Soc America Bull. 84: 3411-3414

Villegas CT. 1981. Culture and screening of food organisms as potential larval food for finfish and shellfish. In: Guerrero RD, CT Villegas (eds), Report of the Training Course on Growing Food Organisms for Fish Hatcheries. FAO/UNDP

Wada SK. 1942. On some shells of the Family Tridacnidae from Palau. *Kagaku Nan-yo* (Science of the South Seas) 5(1): 62-29.

Walne, PR. 1974. Culture of bivalve molluscs. Fishing News (Books) Ltd., 23 Rosemount Ave, West Byfleet, Surrey, England. 173 p

Watanabe T, A Suzuki, H Kawahata, H Kan, S Ogawa. 2004. A 60-year isotopic record from a mid-Holocene fossil giant clam (*Tridacna gigas*) in the Ryukyu Islands: physiological and palaeoclimatic implications. Palaeogeog. Palaeoclim. Palaeoecol. 212(3-4): 343-354

World Health Organization-Regional Office for Southeast Asia (WHO-SEARO). 2001. Guidelines on standard operating procedures for Microbiology

Zann LP, AM Ayling. 1988. Status of giant clams in Vanuatu, pp 60-64. In: Copland JW, JS Lucas (eds). Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9. Australian Center for International Agricultural Research, Canberra, Australia

RECENT PUBLICATIONS OF SEAFDEC

AEM 16 Diseases of Penaeid Shrimps in the Philippines. CR Pitogo et al. (2000) 83 pp

AEM 21 Feeds and Feeding of Milkfish, Nile Tilapia, Asian Sea Bass and Tiger Shrimp. Feed Development Section 97 pp

AEM 22 Pag-aalaga ng Tilapya. RV Eguia et al. (2007). 55 pp. 3rd ed.

AEM 23 Pagpapaanak ng Tilapya. RV Eguia et al. (2007). 52 pp. 3rd ed.

AEM 24 Grouper Culture in Brackishwater Ponds. DD Baliao et al. (1998). 18 pp

AEM 26 Pen Culture of Mudcrab in Mangroves. DD Baliao et al. (1999). 10 pp

AEM 29 Grouper Culture in Floating Net Cages. DD Baliao et al. (2000). 10 pp

AEM 30 Net Cage Culture of Tilapia in Dams and Small Farm Reservoirs. DD Baliao et al. (2000). 14 pp

AEM 32 The Farming of the Seaweed *Kappaphycus*. AQ Hurtado, RF Agbayani (2000). 26 pp (in English or Filipino)

AEM 33 Induced Breeding and Seed Production of Bighead Carp. AC Gonzal et al. (2001). 40 pp

AEM 34 Biology and Hatchery of Mud Crabs *Scylla* spp. ET Quinitio, FD Parado-Estepa (2003) 39 pp

AEM 35 Best Management Practices for Mangrove-Friendly Shrimp Farming. DD Baliao, S Tookwinas (2002). 50 pp (Filipino version also available)

AEM 36 Tilapia Farming in Cages and Ponds (in print or CD). RV Eguia, MRR Eguia (2004). 40 pp

AEM 37 Giant Clam Hatchery, Ocean Nursery and Stock Enhancement. SS Mingoa-Licuanan, E Gomez (2007). 109 pp

AEM 38 Tilapia broodstock and hatchery management. R Eguia, MRR Eguia (2007). 48 pp

Proceedings of the Regional Technical Consultation on Stock Enhancement. JH Primavera, ET Quinitio, MR Eguia (eds.) (2006). 150 pp

Seaweeds of Panay. AQ Hurtado et al. (2006). 50 pp 2nd ed.

State of the Art Environment-Friendly Schemes in Intensive Shrimp Farming. DD Baliao (2000). 24 pp

State of the Art Closed Recirculating Shrimp Farming System. S Tookwinas (2000). 28 pp

Ecology and Farming of Milkfish. TU Bagarinao (1999). 117 pp

Health Management in Aquaculture (textbook). GL Po et al., eds (2001). 187 pp

Nutrition in Tropical Aquaculture (textbook). CM Millamena et al., eds (2002) 221 pp

Handbook of the Mangroves of the Philippines - Panay. JH Primavera et al., (2004). 106 pp

Mangrove-Friendly Aquaculture (proceedings). JH Primavera et al., eds (2000). 217 pp

Responsible Aquaculture Development in Southeast Asia (proceedings). LMB Garcia, ed (2001). 274 pp

An Assessment of the Coastal Resources of Ibajay and Tangalan, Aklan. LMB Garcia, ed (2001). 60 pp

Diseases in Farmed Mud Crabs *Scylla* spp.: Diagnosis, Prevention and Control. CR Lavilla-Pitogo, LD de la Peña (2004). 89 pp (in print or CD)

Diseases of Cultured Groupers. K Nagasawa, ER Cruz-Lacierda, eds (2004). 81 pp (in print or CD)

Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training (proceedings). CR Lavilla-Pitogo, K Nagasawa, eds (2004). 254 pp (in print or CD)

Laboratory Manual of Standardized Methods for the Analysis of Pesticide and Antibiotic Residues in Aquaculture Products. IG Borlongan, Chuan JNP (2004). 46 pp (in print or CD)

Laboratory Manual of Standardized Methods for Antibicrobial Sensitivity Tests for Bacteria Isolated from Aquatic Animals and Environment. Ruangpan L, Tendencia EA (2004). 55 pp (in print or CD)

For more information, visit the SEAFDEC/AQD website:

www.seafdec.org.ph

The Southeast Asian Fisheries Development Center (SEAFDEC) is a regional treaty organization established in December 1967 to promote fisheries development in the region. The member countries are Brunei Darussalam, Cambodia, Indonesia, Japan, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam. The policy-making body of SEAFDEC is the Council of Directors, made up of representatives of the member countries.

SEAFDEC conducts research on fisheries problems; generates appropriate fisheries technologies; trains researchers, technicians, fishers and aqua-farmers, and managers; disseminates information on fisheries science and technologies; and recommends policies pertaining to the fisheries sector.

SEAFDEC has four departments that focus on different aspects of fisheries development:

- The Training Department (TD) in Samut Prakan, Thailand (1967) for training in marine capture fisheries
- The Marine Fisheries Research Department (MFRD) in Singapore (1967) for post-harvest technologies
- The Aquaculture Department (AQD) in Tigbauan, Iloilo, Philippines (1973) for aquaculture research and development

- The Marine Fishery Resources Development and Management Department (MFRDMD) in Kuala Terengganu, Malaysia (1992) for the development and management of fishery resources in the exclusive economic zones of SEAFDEC member countries.

SEAFDEC/AQD is mandated to:
- Conduct scientific research to generate aquaculture technologies appropriate for Southeast Asia
- Develop managerial, technical and skilled manpower for the aquaculture sector
- Produce, disseminate and exchange aquaculture information

SEAFDEC/AQD maintains four stations: the Tigbauan Main Station and Dumangas Brackishwater Station in Iloilo province; the Igang Marine Station in Guimaras province; and the Binangonan Freshwater Station in Rizal province.