Dietary effects on shell microstructures of cultured, maculate top shell (Trochidae: *Trochus maculatus*, Linnaeus, 1758)

Suraphol Chunhabundit[1], Panjit Chunhabundit[2], Porcham Aranyakananda[1] and Nudol Moree[1]*

Abstract

Maculated top shells grew rapidly when co-cultured in tanks with fish and fed on leftover fish feed and fish wastes. During rapid growth on this high protein diet, top shells deposited a red band on their outer shell surface. Top shells reared in tanks without fish and fed only algal diets decreased in size. With the nutritionally deficient diet, top shells deposited a purple band on their outer shell surface. Shell microstructures were observed and described for these two conditions using a scanning electron microscope, which revealed substantial differences between shell structures under well-fed and nutritionally deficient conditions.

Introduction

An individual's life history, or ontogenetic growth is often preserved in its mineralised or otherwise refractory structures. With shell-forming molluscs, changes in the animal's environment are reflected in shell structures. These preserved shell records include growth increments and discontinuities; or changes in shell allometry structure, mineralogy, and/or chemistry. These features are observed on whole shell surfaces, or using special shell preparations. Environmental factors known to effect shell structures include: temperature (Phillips et al. 1973), salinity, dissolved oxygen, substratum conditions, water turbidity (Rhoads and Lutz 1980), and food concentrations (Ino 1949, 1953).

There are about 40,000 described species of living gastropods (Brusca and Brusca 1990), or about three-quarters of the phylum Mollusca. Molluscs have a mantle, which is a fold of the dorsal body wall that creates a mantle cavity. The mantle cavity usually houses the ctenidia, anus and pores of the nephridial and reproductive systems. Just under the mantle epithelium are shell glands responsible for biomineralisation and shell formation.

Maculated top shell (*Trochus maculatus*) is one of three top shell species found in Thailand. Before it became depleted due to overfishing and perhaps pollution, this species was economically important for island residents in the upper Gulf of Thailand (Chunhabundit and Thapanand 1994). The outer shell is covered with light green, radiating lines that create a maculated surface (Thapanand and Chunhabundit 1993).

The aim of this study was to document changes in shell microstructures associated with dietary changes and food availability in top shells. This study was part of a larger programme to develop appropriate propagation methods for top shell stock enhancement.

Materials and methods

Food types

Top shells of 14.5–45.0 mm shell base diameter were collected from the ocean in their natural habitat and held in an acclimation tank for one week at Sichang Marine Science Research and Training Station (SMaRT), Sichang Island. After acclimation, 35 and 36 top shells were transferred to two red snapper (*Lutjanus argenticulatus*) fish-rearing tanks (designated as Tanks 2 and 3, respectively). Stocking densities were 35 top shells/m^2. Top shells in both tanks were fed organic fish wastes (mostly faeces) and on uneaten fish pellets that remained on the tank bottom. Another 80 top shells were reared in a separate high-density tank (designated as Tank 1), and fed only algae (*Enteromorpha* sp. and diatoms). Seawater at 32 ppt salinity and 29° C was flow-through in all rearing tanks. After one week, shell diameters and heights were measured, and whole body weights calcu-

1. Aquatic Resources Research Institute, Chulalongkorn University, Phyathai Road, Phthumwan, Bangkok 10330, Thailand.
2. Department of Anatomy, Faculty of Dentistry, Mahidol University, Yothee Road, Rajathevee, Bangkok 10400, Thailand.
* corresponding author e-mail: csuraph1@chula.ac.th

lated using the equation (Thapanand and Chunhabundit 1993): $W = 9.5 \times 10^{-5} L^3$; where W = whole body weight (g), and L = shell diameter (mm). At that time, colour bands that appeared on the shell surfaces were also measured.

Scanning electron microscopic analyses

Five top shells were collected from the high density rearing tank (Tank 1) and from the two red snapper rearing tanks (Tanks 2 and 3) at SMaRT. The living animals were removed, and the shells thoroughly rinsed with distilled water three times. Each shell was cut cross-sectionally through its midline with a cutting disc. All samples were washed again with distilled water and air-dried overnight in a dust free cabinet. They were then mounted on metal stubs and gold coated, before being examined using a scanning electron microscope (JEOL JSM-5410LV) with 20 kV acceleration voltage.

Results

Feeding trials

After one-week of culture, top shell size increased substantially in the fish-rearing tanks. Average shell lengths increased from 28.4 to 29.2 mm in Tank 2, and from 26.8 to 28.4 mm in Tank 3 (Table 1). At the same time, average whole body weights increased from 2.32 to 2.55 g in Tank 2, and from 2.14 to 2.45 g in Tank 3. The "red band" phenomenon appeared on top shell surfaces in the fish tanks, starting from the outer lip of the shell aperture. Average red band area in Tank 2 was 18.8 mm^2, which on average covered 5.4% of the shell's outer surface. In Tank 3, the red band area averaged 19.0 mm^2 and 8.5%, respectively. At the same time, top shell size in the algal fed tank (Tank 1) increased (Table 1) while the whole body weight decreased. Whole body weight decreased from 3.5 to 2.7 g during the 17 days. The "purple band" phenomenon appeared on shell surfaces of these top shells starting from the shell apexes, extending to the middle of the shell. Average purple band area was 477 mm^2, which covered 56% of the outer shell surface.

Microstructures of a normal shell

Normal *T. maculatus* shells consisted of layers of crystalline calcium carbonate separated by thin sheets of protein (Fig. 1). The outer surface was mostly covered by periostracum, which is a thin organic layer composed mainly of sclerotized protein. Periostracum protects shells from corrosion and erosion, provides an initial substratum for mineral deposition at shell's edge, seals the extrapallial space, protects shells from infestation by organisms, and possibly helps camouflage top shells (Fig. 2). There are four microstructures in a normal *T. maculatus* shell composition (Table 2). The first microstructure consists of spherulitic, prismatic structures (Fig. 3 and Fig. 4). These include sub-units radiating in three dimensions from a single nucleation site, or spherulite, towards the depositional surface. This first microstructure consists of aragonite and calcite. The second microstructure group consists of laminar, columnar nacreous structures (Fig. 5). These structures are deposited near the shell margin.

The tablets are stacked in columns with coinciding centers, an arrangement only found in some gastropods and cephalopods. The mineralogy of this group is aragonite. The third microstructure group is a fibrous, prismatic structure (Fig. 5). The prisms are simple, but have large length / width ratios. The mineralogy of this group is aragonite with fibrous calcite prisms. Prism boundaries are well defined and generally non-interdigitating. The fourth microstructure group is an irregular complex, cross-foliated. This group commonly appeared in the inner shell layer, and was a distinct type of shell structure at the point of pallial muscle attachment (Fig. 5). This group was adjacent to aggregations of numerous parallel, elongate sub-units that showed three or more predominant dip directions. The sub-units were calcite blades or laths.

Microstructure of defective shells

Defective shell conditions occurred in top shells when they were reared under high density with nutritional deficiencies. Shell deformities included purple bands on the outer shell surface, shell dissolution (Fig. 6), and variation in periostracum thickness (Fig. 7). In addition, a thick spherulitic structure was deposited with an organic matrix, and the thickness of the columnar nacreous structure increased. At the same time, the thickness of the fibrous, prismatic structure and the irregular, complex cross-lamella structures were reduced (Fig. 8 and Fig. 9).

The prism boundaries of the spherulitic, prismatic structures were poorly defined and non-interdigitating. Prism length and height ratios were more complicated. The columnar, nacre structure interdigitated and increased in thickness. The irregular complex, cross-foliated structure also interdigitated, but the layer thickness decreased (Fig. 9). The interface zones between normal and defective areas (the purple bands) of defective shells (Fig. 10 and Fig. 11) were clearly different compared with normal shells (Fig. 2). Figure 12 shows the nature of this transition zone, with an exposed cut through the purple band.

Table 1. **Average shell dimensions and whole body weights of maculated top shells (*Trochus maculatus*) reared in an algal tank (Tank 1), and in two fish culture tanks (Tanks 2 and 3). Shell "red bands" and "purple bands" were measured and respective areas calculated for each shell, where: PL = length of purple band, PW = width of purple band, RL = length of red band, and RW = width of red band. Numbers in parentheses are standard deviations.**

Tanks	Shell diam. (mm)	Shell height (mm)	Whole weight (g)	Shell area (mm^2)	PL (mm)	PW (mm)	RL (mm)	RW (mm)	Shell area (mm^2)	Band area (%)	N
Tank 1 Day 0	29.5 (5.5)	26.2 (6.1)	3.48 (1.69)	875 (332)	-	-	-	-	-	-	79
Tank 1 Day 17	32.2 (5.7)	27.5 (5.1)	2.68 (1.39)	836 (290)	20.9 (10.2)	18.5 (9.2)	-	-	477 (326)	56.1 (27.5)	80
Tank 2 Day 0	28.4 (5.5)	20.1 (4.3)	2.32 (1.11)	595 (211)	-	-	-	-	-	-	35
Tank 2 Day 7	29.2 (5.0)	22.4 (4.3)	2.55 (1.21)	664 (230)	-	-	18.8 (3.6)	1.9 (2.2)	40 (54)	5.4 (5.2)	31
Tank 3 Day 0	26.8 (6.4)	19.2 (5.4)	2.14 (1.42)	546 (274)	-	-	-	-	-	-	36
Tank 3 Day 7	28.4 (5.9)	21.8 (5.4)	2.45 (1.43)	648 (287)	-	-	19.0 (4.7)	2.4 (2.1)	49 (53)	8.5 (9.7)	36

Table 2. **Shell microstructure guide for maculated top shell *Trochus maculatus* Linnaeus, 1758.**

Microstructure group and Figures	Microstructure varieties	Mineralogy
1. Prismatic		
Figures 3 and 4	**Sp** - Spherulitic prismatic structure. Prisms show a substructure of elongate subunits radiating in three dimensions from a single nucleation site of spherulite toward the depositional surface.	Aragonite+Calcite
Figure 5	**Lc** - Fibrous prismatic structure. Prisms lack a substructure of elongate subunits diverging toward the depositional surface. Prismatic boundaries are well defined and generally noninterdigitating. Prisms show a large length / width ratio.	Aragonite+Calcite
2. Laminar		
Figure 5	**Lr** - Nacreous structure. Laminae consist of polygonal to rounded tablets lying essentially parallel to the general depositional surface. Spiral growth of the tablets may locally disrupt the laminar arrangement. Columnar nacreous structure. Deposited near the margin of the shell, and the tablets show vertical stacking in all vertical sections.	Aragonite
3. Crossed		
Figure 5	**Ic** Irregular complex cross-foliated structure. This structure is a particular variety of crossed lamellar structure (adjacent aggregations of elongate subunits show three or more predominant, or they are arranged on the surfaced cones) in which the elongate subunits are calcitic blades or laths.	Calcite

Discussion

In our trials, top shells readily fed and grew well on fish wastes and uneaten, prepared red snapper feed. This protein and nutrient-rich diet resulted in the formation of a red band starting from the outer lip of the shell aperture and extending to the outer shell surface. A purple band formed on top shells that were fed an inadequate algal diet. This band started at the shell's apex and continued to the middle zone of the shell. These red and purple bands contrasted with the natural maculated green coloration of wild top shells. In their natural habitat, top shells forage on a mixture of plants, animals, and detritus.

Most gastropod growth studies measure shell size and shape. Thapanand and Chunhabundit (1993) found that *Trochus maculatus* growth was isometric, keeping the same proportions throughout life. Body weight was directly proportional to internal shell volume. Shell growth can occur even during starvation (Rhoads and Lutz 1980). Since a more or less complete record of post-larval ontogeny is preserved in gastropod shells, and since their shells reflect environmental conditions, gastropod shells are valuable indicators of environmental changes. Environmental conditions such as temperature and food have profound effects on gastropod shell form, growth rate and sculpture (Phillips et al. 1973).

Figure 1.
SEM microphotograph of normal maculated top shell (*Trochus maculatus*) rearing under low density condition, shell length was 35 mm. Photography shows the apex area of the shell, the outer most surface is protected by a periostracum layer. Bar = 0.5 mm.

Figure 2.
SEM microphotograph of the mid-shell area of normal maculated top shell. The outer layer of organic periostracum protects the shell from corrosions and provides an initial substratum for mineral decomposition at the shell's edge. Bar = 20 µm

Figure 3.
SEM microphotograph of cross-section of normal maculated top shell showing shell layers: Sp = Simple prismatic layer; Lc = Laminar columnar, nacreous layer; Lr = Laminar, regularly foliated layer; and Ic = Irregular complex, cross-lamellar layer. The outermost layer is the periostracum (not labeled). Bar = 20 µm

Figure 4.
High magnification view of Figure 3 showing: simple prismatic layer (Sp) and laminae columnar, nacreous layer (Lc). Bar = 10 µm

Figure 5.
High magnification view of Figure 3 showing: the middle layer; laminae columnar nacreous layer (Lc); laminae regularly foliated layer (Lr); and the inner layer of irregular complex, cross-lamellar layer (Ic). Bar = 10 µm

Figure 6.
Microphotograph of defective shell (the apex area) of maculated top shell caused by rearing under high density with inadequate nutrition. The periostracum was reduced and shell structure was dissolved and replaced by fouling organisms.

Figure 7.
Enlargement of a "purple band" on the defective shell of maculated top shell. The shell surface was particle dissolved.

Figure 8.
Microphotograph cross-section of defective maculated top shell, shell showing all shell layers. The outermost layer has fouling deposits, and the inner most layer was reduced in thickness.

Figure 9.
Enlargement of defective shell layers of maculated top shell.

Figure 10.
Microphotograph of maculated top shell showing the interface zone between normal shell area (N), and the "purple band" area (P) of a detective shell.

Figure 11.
Enlarged section of Figure 10. Benthic diatoms (*Cocconeis* sp.) were attached to the shell's surface.

Figure 12.
High magnification of a cross-sectional area of the simple prismatic layer of defective maculated top shell in the "purple band" area. The simple prismatic layer of the defective shell changed form. Boundaries were not well defined, and generally interdigitating.

Figure 1

Figure 2

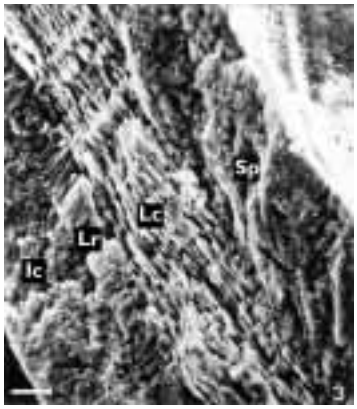

Figure 3

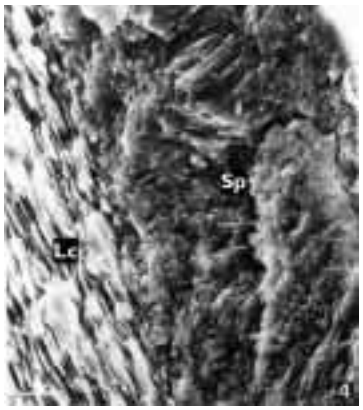

Figure 4

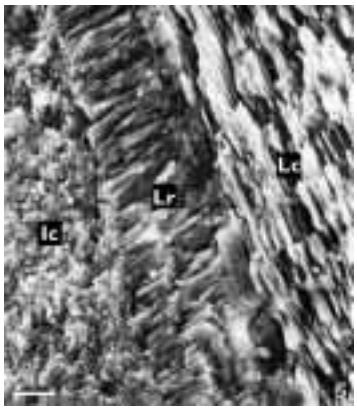

Figure 5

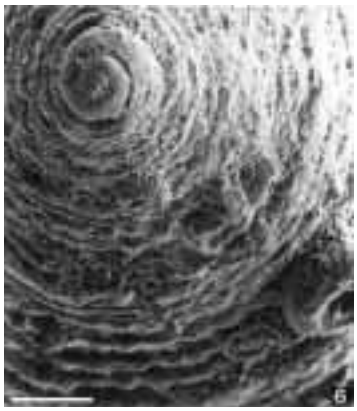

Figure 6

Figure 7

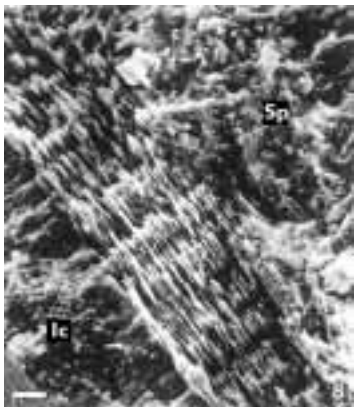

Figure 8

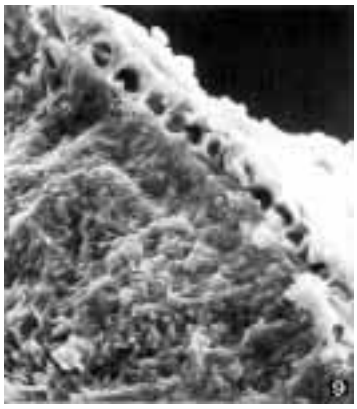

Figure 9

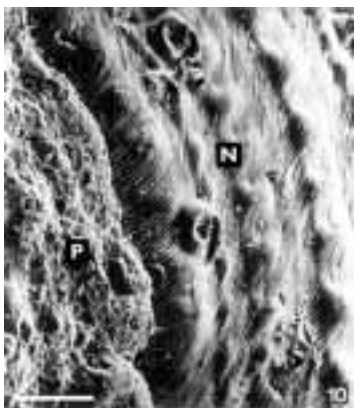

Figure 10

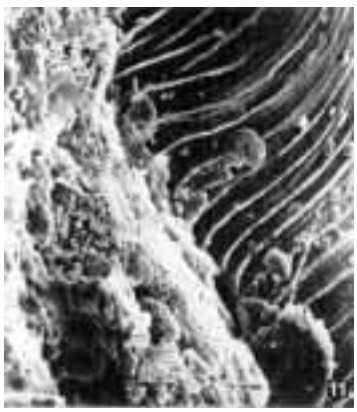

Figure 11

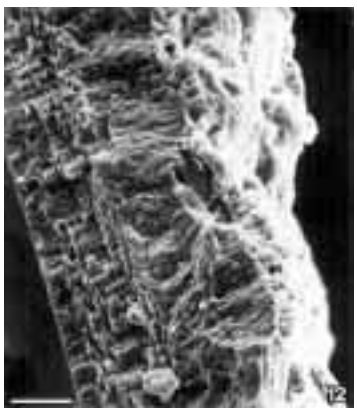

Figure 12

With archaeogastropods, and perhaps other snails, shell pigmentation is affected by diet. When Japanese turban snails (*Turbo cornatus*) were fed with brown algae (*Eisenia bicyclis*), the shell became white, whereas those fed red algae (*Cheilosporum maximum* and *Corallina pilulifera*) retained the greenish-brown colour typical of specimens collected in the field (Ino 1949, 1953). Red abalone (*Haliotis rufescens*) shells in California were white when fed the holdfasts of brown kelp (*Macrocystis pyrifera*), whereas their shells were dark, brick-red when fed red algae (Leighton 1961). With maculated top shell (*Trochus maculatus*) reared using commercial abalone diet, we found in our earlier studies that shell colour became red starting on the outer lip, whereas shell colour was greenish when the snail fed green algae (*Enteromorpha* sp.) (Chunhabundit and Thapanand 1995). In our present study with maculated top shells, the red band appeared on the outermost portion of the shell when reared with red snapper and fed fish wastes and excess fish feed.

The innermost layer of top shell contains the nacreous, or mother-of-pearl (MOP) layer. After grinding or otherwise removing the prismatic layers of red band shell, the nacreous layer remains. Potential exists for enhancing the lustre or orientation of the aragonite, columnar nacreous structure by feeding appropriate diets. This enhancement can greatly increase the retail value of shell products, and correspondingly also greatly increase the value of raw, unprocessed shells. Trochus shells are used to produce high quality pearl buttons, while shell wastes after button production are used to produce MOP chips, or crushed into powder for use in making paint and nail polish (Hahn 1989).

There are four categories of microstructure in the shell layers of *Trochus maculatus.* The first category is an aragonite and spherulitic, prismatic structure, which probably occurs in all bivalves (Taylor et al. 1973). The second category is a columnar, nacreous layer. This category is found in some gastropods and cephalopods, such as *Haliotis cracherodi, Tectus pyramis* and *Perotrochus quoyanus* (Hedegaard and Wenk 1998). This microstructure category consists of flattened aragonite blades or laths, common to Bivalvia, including Pterioda, Pectinacea, Anomiacea and Ostreacea (Waller 1972, 1978). With gastropods, Hedgaard and Wenk (1998) reported that the nacreous structures are "argonitic laminar" consisting of polygonal to rounded tablets, broadly arranged. The third category of top shell microstructure is an aragonitic, fibrous and prismatic structure. The structure's prisms lack a substructure of elongate sub-units diverging toward the depositional surface. The prisms have a large length / width ratio. The fourth category is an irregular complex, cross-foliated. This complex, cross-lamellar structure consists of elongate sub-units of calcite blades or laths. A summary of maculated top shell, shell microstructure is shown in Table 2. There are two nacreous layers with top shells; one which is a columnar, nacreous layer, while the other layer is a fibrous, prismatic structure. This top shell feature differentiates it from other mollusc shells.

Top shells are herbivores and detritivores (Hahn 1989), and non-selective grazers (Thapanand and Chunhabundit 1993). They can readily adapt to seasonally changing food availability when their preferred food items are not available. During adverse food or other environmental conditions, shell growth is represented by a purple band of many closely packed rings, as shown in Figures 9 and 12. The outer shell is dissolved, while the nacreous, inner layer increases in thickness. The inner aragonite, fibrous prismatic structure becomes thinner. The prismatic layer dissolves and is replaced by an organic matrix. The columnar, nacreous layer increases in thickness and becomes more interdigitating. In this condition, the columnar, nacre layer accretes faster than the sheet nacre layers, such as those found in shell microstructures of the Bivalvia. Vertically stacked, columnar nacre exposes greater numbers of growing tablet edges per unit area of depositional surface, thus allowing more rapid shell deposition. This agrees with the hypothesis concerning correlations between columnar nacre and low aperture expansion rates in the gastropod and cephalopod, and between sheet nacre and high aperture expansion rates in the Bivalvia. Wise (1970) stated that columnar nacre can accrete faster than sheet nacre because its vertical stacking exposes more growing tablet edges per unit area of depositional surface. Nacre is particularly strong, but because it is so often absent from the nacreous layer, it appears that its energetic expense sometimes outweighs its structural advantages.

Hedegaard and Wenk (1998) stated that shell textures may correlate with microstructures (as with cross-foliated structures), or similar microstructure may have very different textures (such as with nacre). The strength and thickness of the nacreous layer in top shells can be enhanced through diet, thus providing higher quality MOP. From our present work, it appears that it may be feasible to rear top shells with the carnivorous animals in pond rearing systems (polyculture systems) and thereby increase top shell quality and value.

Three species of *Trochus* top shells are fished in Thai waters. Local fishermen readily consumed meats of *T. maculatus*, *T. niloticus* and *T. pyramis* until natural stock were depleted (Thapanand and Chaunhabundit 1993; Chunhabundit and

Thapanand 1994). Propagation of juvenile top shell for reseeding depleted natural stocks offers an opportunity to re-establish the *Trochus* fishery at a higher level in Thailand (Chunhabundit and Thapanand 1993a, b).

While *Trochus* top shells are used to make jewellery, inlays in carvings, and paint additives, their greatest economic value is for production of MOP, for making buttons. Such buttons are still in high demand in the fashion industry. The potential exists for re-establishing wild populations of top shells using mariculture and ocean ranching. This would provide both a supplemental food resource for local fishermen, and an added source of cash income from sale of shells for commercial uses.

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An improved method of packing to minimise mortality in juvenile trochus during transport

Graeme Dobson[1]

Summary

The improved method described for transporting juvenile trochus gave a mortality rate of <10% after being packed 24 hours, and <20% after being packed for up to 36 hours for large juveniles 15–30 mm in diameter. Smaller juveniles between 5 and 12 mm showed slightly higher mortality.

Introduction

Sites designated for trochus reseeding are often remote, and in some cases thousands of kilometres from the hatchery. To get stock from the hatchery to the field may involve complex travel arrangements, with stock being transferred from one form of transport to another a number of times before finally being released.

The ACIAR/NTU Trochus Reseeding Project conducted extensive trochus reseeding trials in two locations in the Kimberley, West Australia, using juveniles produced in the pilot hatchery managed by the Northern Territory University, Darwin. These trials called for the air transport of juvenile trochus from Darwin to Broome, a distance of more than 1000 km, followed by a journey of more than 200 km on dirt track to One Arm Point before reseeding them on selected sites at Dampier Peninsula. For reseeding on Sunday Island, sea travel is also involved. Total travel time often exceeds 24 hours and is extremely stressful for the juveniles involved.

Initially the trochus were packed for transport in plastic bags with pieces of damp paper or cloth to maintain humidity. The bags were inflated with industrial oxygen, sealed with rubber bands and packed into polystyrene boxes for transportation. This oxygen-charged plastic (OCP) method proved unsatisfactory even over comparatively short periods (12 hours), as mortality was unacceptably high. If the trochus were alive, they were often lethargic and slow to recover. There was a clear need to develop a specific method of packing juvenile trochus that would ensure survival over periods of at least 24 hours.

Methods

Observations of juvenile trochus in the hatchery and mature trochus in their natural environment showed they commonly remained out of the water for long periods. This suggested that, if near natural conditions could be recreated in a packaging system, survival during transit could be enhanced.

There were two major factors that appeared to contribute to the survival of the trochus: high humidity and a solid substrate. Packing in oxy-

1. School of Biological and Environmental Science, Northern Territory University, Darwin 0909, NT, Australia