- Kenya on page 12;
- traditional uses of sea cucumbers in the South Pacific, a subject for which not much research had been carried out previously, on page 18; and
- many other interesting topics.

Summaries of the papers and posters presented at the 10th International Echinoderm Conference, 31 January – 4 February 2000, University of Otago, Dunedin, New Zealand are presented on page 35.

Due to the length of this issue (52 pages), we could not include the New Members column. It will be included in the next issue. If you would like to receive this list, please contact the SPC Information Section (see contacts on cover page).

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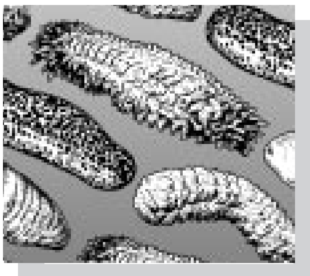
Chantal Conand



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Aspects of sea cucumber broodstock management (Echinodermata: Holothuroidea)

Andrew David Morgan[1]

Abstract

Broodstock *Holothuria scabra* were obtained from Stradbroke Island, Moreton Bay (27°30′ N, 153°24′ E) in Queensland, Australia during the spawning season of November 1996 to February 1997 and October to November 1997. They were placed in a sand bottom, 12-tonne, indoor holding tank, equipped with a sand bio-filter and flow-through seawater. During the first post-spawning period, from March to May 1997, individuals did not recover from the loss in weight that occurred during the period in which they were used in spawn induction, despite the addition of a food supplement. From October to November 1997 the addition of various feed supplements reduced the amount of weight loss of *H. scabra,* which were kept in captivity at a constant temperature and light cycle. After five weeks, animals showed signs of infection, shedding copious amounts of mucoid material. The use of an appropriate diet and the affect of temperature and light conditioning regimes on the coupling of feeding behaviour and the reproductive cycle need to be ascertained.

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Introduction

Information explaining how the captive environment affects the coupling of reproductive synchronicity and the feeding behaviour of sea cucumbers is limited (James *et al.*, 1994; Ito, 1995; Yanagisawa, 1995; Ramofafia *et al.*, 1997; Battaglene & Bell, 1997). The feeding biology of sea cucumbers has been quantified *in situ* (Cameron & Fankboner, 1984; Weidemeyer, 1992; Ahlgren, 1998; Uthicke & Karez, 1999), and may indicate further appropriate conditions and diets to use in captivity. Species of kelp, *Eysenia bicyclis* and *Undaria pinnatifida*, have been ground into a paste and frozen or dried and placed in broodstock tanks, containing *Stichopus japonicus* (Masaki, pers. comm.; Ito, 1995; Yanagisawa, 1995), and consumed the following day. Battaglene and Bell (1997) stated that *Holothuria scabra* broodstock in India and Indonesia were fed soybean powder, rice bran, chicken manure, ground algae and prawn-head waste.

Yingst (1976) noted that in captivity, the sea cucumber *Parastichopus parvimensis* assimilated decomposed sterile crabmeat with a high efficiency whereas sterile plant material was of little or no nutritional value. The meat was removed from the crab and placed in a beaker inoculated with bacteria, which was then placed on a shaker table for 14 days to stimulate decomposition. McClintock *et al.* (1982) found that the urchins *Echinometra lucunter*, *Lytechinus variegatus* and *Eucidaris tribuloides* had an equal or greater preference for pulped or sodden *Donax variablis*, a bivalve, over blades of the seagrass *Thalassia testudinum*, in the captive environment. Food preference is difficult to ascertain in aspidochirote holothurians as they are detritus feeders and more likely to depend on the fungal and bacterial communities promoted by the decomposition of flora and fauna (Yingst, 1976; Moriarty, 1982; Moriarty *et al.*, 1985).

Sea cucumbers have been tagged *in situ* with limited success (Harriott, 1980; Shelley, 1981; Conand, 1989). A small t-bar tag is usually inserted through the body wall using a tagging gun. In these studies localised necrosis of the tissue occurred and in most cases the tag fell out within a few months. However, this method may be adequate for tagging broodstock in captivity over short periods of time.

At present, no study has determined how the regulation of exogenous cues and its effect on an endogenous rhythm influence feeding and reproduction of sea cucumbers in captivity. With little understanding of the interaction between feeding behaviour and reproductive state, broodstock have been collected late in gametogenesis or the vitellogenic period to avoid the problems associated with feeding and gametogenesis (James *et al.*, 1994; Ito, 1995; Battaglene, pers. comm.; Masaki, pers. comm.). Studies on captive sea cucumbers have addressed some of the preceding issues in part. Information on how the management of broodstock affects the sea cucumber *Holothuria scabra* is presented.

Materials and methods

Captive management

In October and November 1997, approximately 100 *H. scabra* were collected from Stradbroke Island, Moreton Bay (27°30′ N, 153°24′ E), and re-located to Bribie Island Aquaculture Research Centre (BIARC) using a 750-litre fish transporter aerated with oxygen. Individuals were placed into closed mesh cylinders (Figure 1). The bottom layer of mesh cylinders contained no animals and was used to keep the cylinders containing *H. scabra* off the bottom of the transporter. Animals were placed in a sand bottom, 12-tonne indoor holding tank, equipped with a sand bio-filter and flow-through seawater.

At the end of the 1996/97 summer spawning season (November to January), forty broodstock were separated into four areas by placing mesh screens in the holding tank. Animals in three areas were

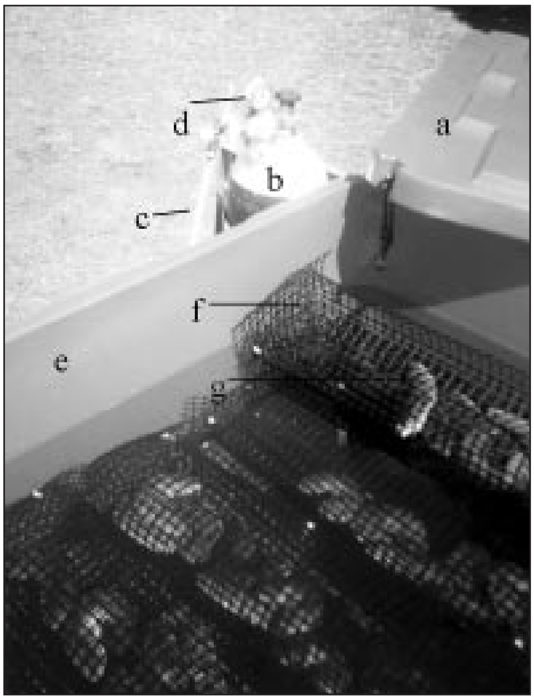

Figure 1.
Photograph of fish transporter (750-litre polycarbonate container) used to collect broodstock. a. Transporter lid; b. Oxygen cylinder; c. Air hose; d. Regulator; e. Fish transporter; f. Mesh screens; g. Broodstock of *Holothuria scabra.*

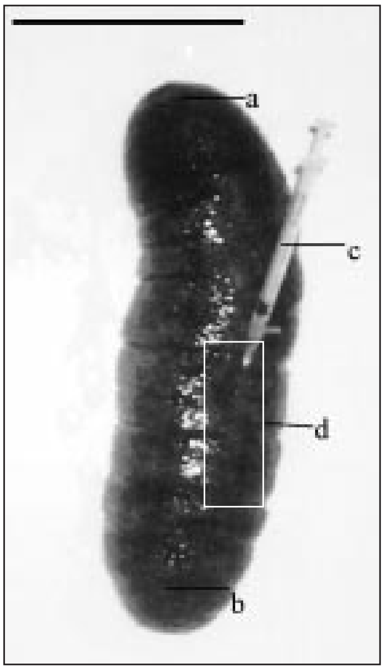

Figure 2.

Photograph of biopsy needle orientation during gonad sampling of *Holothuria scabra* (scale bar = 10 cm).
a. Posterior; b. Anterior; c. Syringe; d. Area of epidermis from which the biopsy was taken.

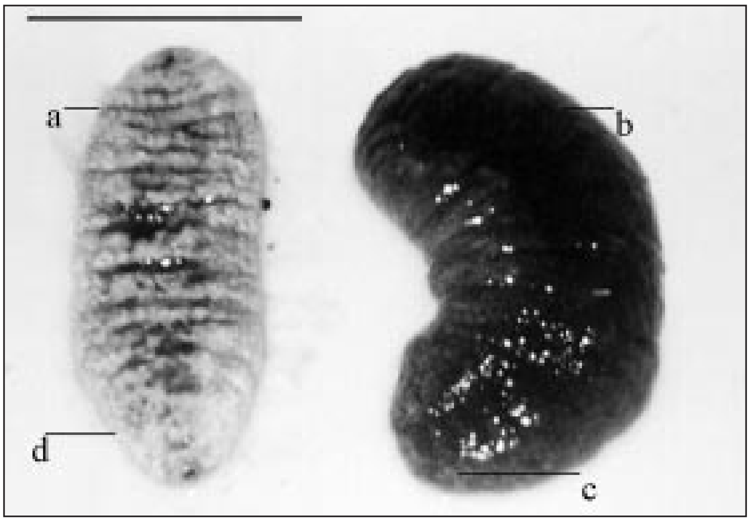

Figure 3.

Photograph of heavily infected sea cucumber contrasted with an individual at the early stages of infection (scale bar = 10 cm).
a. Heavily infected sea cucumber;
b. Sea cucumber with normal epidermal complexion;
c. Lesions appearing on the epidermis;
d. Loss of pigmentation.

fed either prawn pellets, lucerne pellets (grass) or mixed lucerne and prawn pellets. Food was fed *ad libitum* to these three quadrats and the fourth quadrat was a control and contained no added food. The weights of individuals were recorded periodically to determine change in size over time.

During the 1997/98 summer, animals were placed in one of four divided areas in the holding tank (n = 18 animals per area), and conditioned using a 16:8 L:D cycle at a seawater temperature of 27°C. Animals in three areas were fed either a formulated powdered blue crab or abalone diet, available from Gulf Feeds South Australia, or a mixture of species of dried, powdered brown kelp (*Durvillaea potatorum*, *Ecklonia radiata*, *Macrocystis angustifolia*, *Cystophora platylobium* and *Cystoseira trinodis*) obtained from Beachport Sea Products, South Australia. Animals in the remaining area acted as controls and their food was limited to naturally-occurring detrital matter contained in the sand. Before feeding, the incoming seawater was turned off and any current allowed to dissipate. Some transfer of material occurred in the water column through the mesh screens that separated each quadrat. Nutrient loading of the sediment was assessed visually. Too much food resulted in the growth of fungus on the substrate and the inability of the bio-filter to handle the excess loading.

Once the animals were obtained from the wild, individuals were removed from the tank and placed in a tub containing 20 to 30 cm of seawater. They remained undisturbed for approximately 10 minutes and were tagged using a Dennison model 3030 tagging gun (Harriott, 1980), with a small t-bar tag (available from Hallprint South Australia). The tagging needle was inserted mid-dorsally, penetrating the body wall before the tag was fired from the gun. Tagged animals were replaced in the holding tank and animals losing tags were immediately retagged and their new tag number recorded.

One day later, a biopsy on animals was done to determine the sex of individuals. A portion of gonad was extracted using a hypodermic needle and syringe, inserted at an angle to the left of the mid-dorsal line approximately one third to half way down the body (Figure 2), and examined under a dissecting microscope. Penetration of the dermis with the needle at too steep an angle (> 45° from horizontal) resulted in the puncturing of visceral organs and caused evisceration.

Detection and treatment of infectious pathogens

During week five of captivity, broodstock obtained in October 1997 became infected, resulting in 95 per cent mortality within three to seven days. Four animals (two in late stage, two in early stage of infection) were sent to the Yeerongpilly Veterinary Laboratory for pathological examination (Figure 3).

The second group of *H. scabra* broodstock (obtained in November 1997) was transported to BIARC and weighed, tagged and biopsied after the holding tank at BIARC was chlorinated, rinsed with fresh tap water and the biofilter restarted. During weeks three and four of captivity the same infection was observed in this group. Holding and conditioning of broodstock was halted and the remaining healthy animals were separated and placed in another tank through which clean seawater flowed at > 20 L/min. The remaining animals continued to show signs of infection but the infection progressed at a slower rate.

Results

Prawn and lucerne pellets placed in the broodstock tank to enrich the substrate and provide a food source after the 1996/97 spawning season did not halt the weight loss of *Holothuria scabra* that occurred during the spawning season. During May 1997 the epidermis in some animals in all treatments started to split open but no pathogenic infection was observed. During the 1997/98 summer spawning season the decline in the weight of *H. scabra* for all four diets containing either kelp, no food, blue crab and abalone was reduced (n = 18; Figure 4). Approximately 10 per cent weight loss occurred in the first week of captivity while a net weight gain was observed in the second week of captivity. The mean weight of *H. scabra* continued to decline in the kelp, control and abalone diets for weeks three and four. Weight loss after four weeks, was least in the blue crab diet, where it fluctuated between 5 to 10 per cent. During week five, a marked weight loss was observed in all diets at a time corresponding with a visible sign of infection with a pathogen that killed most animals within three to seven days (Figure 4).

After two weeks of captivity, tag-loss was five per cent, increasing to eight per cent after a further two weeks. At five weeks of captivity the cumulative percentage tag-loss of *H. scabra* was 10 per cent.

Diagnosis of infectious pathogens

The infection that occurred in broodstock during the 1997/98 summer was first observed around the mouth and/or anus of an animal and then spread laterally before encompassing the whole animal in the worst cases. At the collection site, 10 per cent of animals had similar lesions. Surface damage to the epidermis of *H. scabra* was not a prerequisite for infection, but infection progressed more rapidly from an existing wound. Infection of animals in the

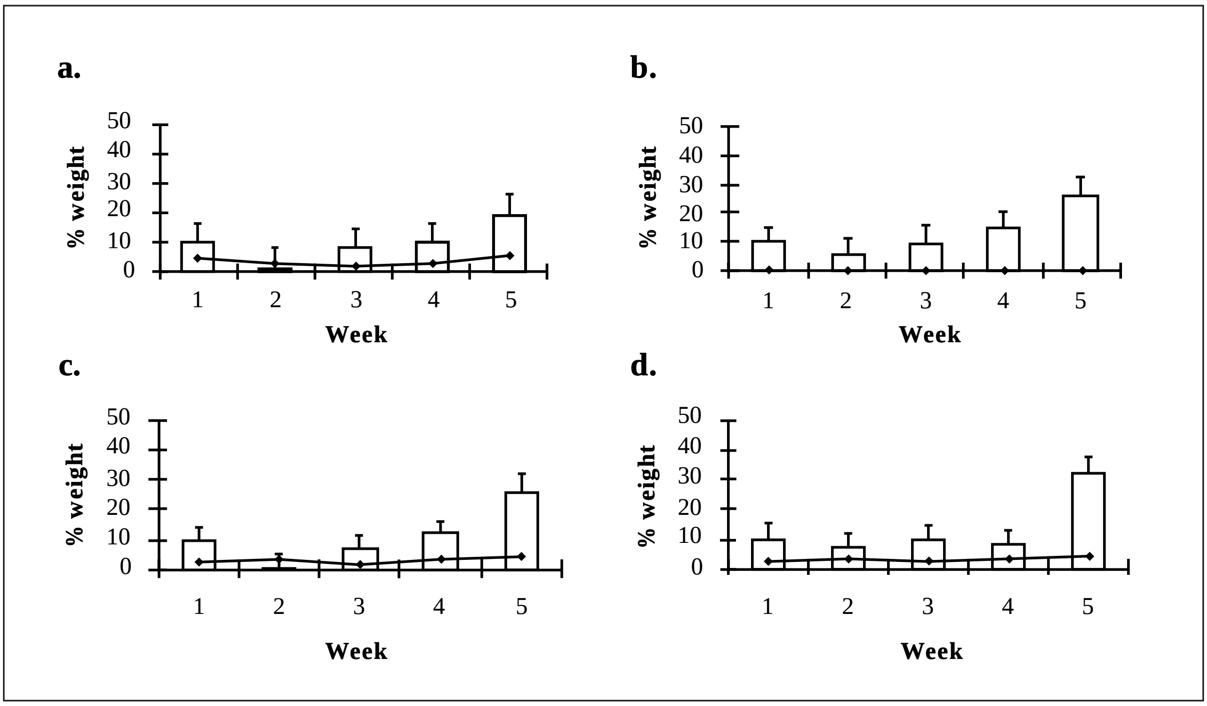

Figure 4.

Percentage weight gain or loss, based on a proportion of total weight (g), of *Holothuria scabra* broodstock in each area containing a diet of either kelp, no food, abalone or blue crab formulated feed (n = 18 tagged individuals per treatment). a. Kelp diet; b. Control; c. Abalone diet; d. Blue crab diet (line represents amount of feed as a proportion of biomass fed to animals).

holding tank occurred in all treatments and was slow to start (five to seven days), but spread to the remaining sea cucumbers rapidly (three days).

The pathology report from the Queensland Department of Primary Industries (QDPI) Yeerongpilly Veterinary Laboratory indicated that the two animals with the most overt signs of infection exhibited a loss of epidermal pigmentation associated with the presence of copious amounts of viscous mucus. Minimal mucus production and discolouration of the epidermis was seen in apparently healthy animals. There was no obvious pathological condition present in the viscera of the four animals. Wet mounts of skin scrapes taken from the four animals differed only in that copious filamentous mucoid material was present in the scrapes from the two 'sick' sea cucumbers.

Giemsa-stained sections of skin scrapes were examined in all four sea cucumbers. Slides from the two apparently healthy animals contained moderate numbers of mixed populations of bacteria. Slides from the two sick animals contained fine, stringy, mucoid material plus moderate numbers of bacteria in which one population appeared to predominate. *Vibrio harveyi* was the predominant bacterium on skin mucus samples from sick animals. There were also low numbers of a motile gram-negative rod bacterium that were probably not significant. No diagnosis was possible from existing results.

Discussion

Management during the spawning season

Weight loss during the 1997/98 summer may have been due to the captive environment, and/or inadequate nutrition. Masaki (pers. comm.) found that broodstock of the Japanese sea cucumber *Stichopus japonicus* did not retain weight and initiate gametogenesis outside of the spawning season. Active feeding by captive animals during the reproductive season may not compensate entirely for the diversion of resources to reproduction. Animals from the control containing no diet supplement, for the 1997/98 period of captivity, exhibited a similar degree of weight loss over one month (approximately 20 per cent). Animals in the areas containing the kelp, abalone and blue crab diets, during the 1997/98 summer, did not lose as much weight.

The regulation of temperature and photoperiod during the 1997/98 summer may have affected the feeding behaviour of *H. scabra*. Ito (1995) and Masaki (pers. comm.) collected *Stichopus japonicus* several months prior to spawning, during gametogenesis. The animals were maintained on a long light cycle at a lower than normal ambient summer seawater temperature and fed quantities of dried algae, which was consumed by the following morning. Temperature regimes may influence the feeding behaviour and metabolic activity of *H. scabra* in captivity. The use of temperature and light regimes to promote gametogenesis and vitellogenesis may come at a cost to feeding and other metabolic processes.

Post-spawning recovery

Despite the addition of feed supplement appropriate to promote bacterial and fungal growth in the substrate after the 1996/97 summer spawning season, re-absorption of the epidermis still occurred, indicating that the experimental animals were unable to utilise the nutrient resources available. Once a critical low weight was exceeded, *Holothuria scabra* may not have been able to recover successfully. Perhaps only large animals should be collected from the natural environment to avoid problems associated with starvation and re-absorption of the epidermis over extended periods in captivity.

Continuous feeding by *H. scabra* may be needed to optimise ingestion and digestive processes (Hammond, 1982; Penny & Jumars, 1987) and mobility may depend on food availability and the energy required to search for it (Cameron & Fankboner, 1985). Penny and Jumars (1987) stated that the gut of sea cucumbers was like a continuous flow, stirred tank reactor while Hammond (1982) indicated that continuous feeding by the sea cucumber *Stichopus chloronotus* may be needed to assist peristalsis because gut muscles were weak. Mobility in the urchin *Lytechinus variegatus* and the sea cucumber *Parastichopus californicus* was shown to be dependant on food availability and the energy required to search for it (Cameron & Fankboner, 1985). The lack of food during the 1996/97 spawning season may have reduced the capacity, over time, of *H. scabra* to move. Cameron and Fankboner (1985) reported that after *P. californicus* had been transferred to aquaria lacking detritus, they ceased all locomotor and feeding activity. The same occurred for *H. scabra* in the present study after approximately three months in captivity, post-spawning, during the 1996/97 summer.

It may be necessary to manipulate temperature and photoperiod to ensure that broodstock are not affected adversely in the period of post-spawning by the disturbance of interactive feeding and reproductive rhythms. The time in which animals can be kept in captivity without seriously affecting metabolic processes may be limited if food is not provided. When extending the spawning season artificially, it may be important to minimise body wall weight loss and the effect of temperature and photoperiod regimes on conditioning for spawning and subsequent post-spawning recovery.

The distribution of food in the holding tank, boundary effects and photoperiod and temperature regimes may have influenced the activity patterns and feeding behaviour of *Holothuria scabra*. The effect of fine scale regulation of environmental parameters in captivity on feeding behaviour and egg production, in combination with species-specific feeding processes, is likely to be important in obtaining successful spawning. Further, the type of diet may not be as critical as the bacterial and fungal communities the diet sustains.

Handling

Collection and tagging

The ability to recognise individuals was essential for interpretation of spawning behaviour (Morgan, 1999). A red-heat wire was used successfully in Japan to brand the sea cucumber *Stichopus japonicus* (Yanagisawa, 1995) eliminating dermal puncture of the animals as occurs when t-bar tags are used. In the present study, tag-loss (10 % of animals after five weeks of captivity) was preceded by necrosis of the tissue surrounding the tag, resulting in the tag being expelled or taken into the coelomic cavity. Loss of tags can be controlled to some extent by ensuring that the t-bar is pushed right through the dermis and that the puncture wound is as small and clean as possible.

Previous researchers have used fluorescent dye to stain the calcareous plates surrounding the buccal cavity of sea cucumbers (Harriott, 1980) with limited success. Successful staining depended on the relative timing of injection of the dye and deposition of calcium for growth of the mouthparts. The hypodermic extraction of gonadal material also involved handling, which may have had deleterious effects. Extraction of gonadal material from *H. scabra* in the present study was useful in determining the sex of sea cucumber broodstock. Once this technique had been refined, no animals eviscerated the gonad, stomach and respiratory trees because of puncture of the viscera with the biopsy needle.

Infectious pathogens

The unidentified infection that spread through broodstock during collection from Stradbroke Island in the 1997/98 summer did not occur during the 1996/97 summer. Lesions were not observed on animals collected during the 1996/97 summer nor were they observed on individuals obtained from the north end of Moreton Bay during the 1995/96 summer.

Removing animals from the holding tank as soon as symptoms were noticed prevented the spread of infection. Animals were then placed in a tub with a high rate of flow-through seawater where lesions stopped secreting mucus and eventually closed, leaving scar tissue. The predominance of one type of bacterial community on heavily infected animals warrants further investigation as to the effects of pathogens on bacteria inhabiting the epidermis of *H. scabra*. Investigation of this relationship between host and bacteria may assist in identification of the pathogen affecting broodstock in the present study.

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