Ability of sandfish (*Holothuria scabra*) to utilise organic matter in black tiger shrimp ponds

Satoshi Watanabe[1*], Masashi Kodama[2], Jacques M. Zarate[1,3], Maria J.H. Lebata-Ramos[3] and Marie F.J. Nievales[4]

Abstract

Due to frequent viral disease outbreaks, a large proportion of shrimp aquaculture in South-East Asian countries has switched from black tiger shrimp (*Penaeus monodon*) to *P. vannamei,* an exotic species originally imported from Latin America. One of the causes of disease outbreaks is thought to be poor water and sediment conditions in the shrimp ponds, which may aggravate disease symptoms. To obtain basic information for co-culture methods of black tiger shrimp and sandfish (*Holothuria scabra*) for possible mitigation of shrimp-pond eutrophication and prevention of disease outbreaks, basic laboratory experiments were conducted at the Southeast Asian Fisheries Development Center—Aquaculture Department in Iloilo, the Philippines. A feeding trial of juvenile sandfish showed that they do not grow well with fresh shrimp feed on hard substrate. Another trial indicated that sand substrate enhances the growth of juvenile sandfish fed with shrimp feed. A feeding trial using shrimp tank detritus, shrimp faeces and *Navicula ramosissima* (a benthic diatom) as food sources showed that sandfish grew fastest with the faeces, followed by detritus and *N. ramosissima*. Dissolved oxygen consumption and acid-volatile sulfur levels in the shrimp tank detritus were reduced by sandfish feeding. This suggests that sandfish are capable of growing with organic matter in shrimp ponds, and can bioremediate shrimp-pond sediment.

Introduction

The majority of shrimp aquaculture in South-East Asian countries has changed from black tiger shrimp (*Penaeus monodon*) to *P. vannamei*, which is an exotic species originally imported from Latin America. The change in target species has occurred due to frequent viral disease outbreaks, such as white spot syndrome disease, yellow head disease, hepatopancreatic parvovirus disease and monodon baculovirus disease (Flegel 2006). Effective measures for the prevention of the diseases have not been established. Vaccination against these diseases is still under development, and it is extremely difficult to completely prevent viral intrusion via crustaceans and birds that enter outdoor shrimp ponds. In order to avoid risk of economic loss associated with mass mortality, more farmers culture *P. vannamei*, which can be harvested at a smaller size and earlier than *P. monodon*. However, there is a possibility that *P. vannamei* 'escapees' may reproduce in the wild and cause significant problems to the natural environment. With the introduction of *P. vannamei,* Taura syndrome virus and infectious hypodermal and hematopoietic necrosis virus have now become problematic (Flegel 2006). Thus, it is desirable to establish less risky culture methods of *P. monodon* to revive their production.

---

[1] Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan

[*] Corresponding author: <swat@affrc.go.jp>

[2] National Research Institute of Fisheries Science, Kanazawa-ku, Yokohama, Japan

[3] Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines

[4] Division of Biological Sciences College of Arts and Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines

While extermination of viruses is difficult, it may be possible to suppress disease outbreaks by maintenance of optimal water and sediment conditions. Anecdotally, it is thought that the disease symptoms do not readily manifest in shrimps reared in good environmental conditions, despite the presence of viruses (Lightner and Redman 1998). As an inexpensive technique of environmental control of shrimp ponds, co-culture with commercially important organisms that have bioremediation capability may be a promising approach, and can also provide additional income to farmers. Mitigation of shrimp-pond eutrophication by co-culture may also help reduce environmental deterioration in areas affected by intensive shrimp aquaculture, which has always been an issue in South-East Asian countries. To this end, the feasibility of co-culture of *P. monodon* and sandfish (*Holothuria scabra*), a high-value tropical sea cucumber, was examined in this study. Since sandfish stocks have been heavily depleted in many parts of South-East Asia (Carpenter and Niem 1998; Hamel et al. 2001; Conand 2004), the co-culture may also be beneficial for sea cucumber conservation.

There are a number of studies on co-culture of sea cucumbers with other organisms, such as teleost fish (Ahlgren 1998), bivalves (Zhou et al. 2006; Slater and Carton 2007, 2009; Paltzat et al. 2008), gastropods (Kang et al. 2003; Maxwell et al. 2009) and shrimps (Pitt et al. 2004; Purcell et al. 2006; Bell et al. 2007). These studies tried to make use of the sea cucumbers' ability to consume particulate organic matter in the sediments. Pitt et al. (2004) studied the effects of size, stocking density and feeding on the feasibility of co-culture of *P. monodon* with *H. scabra*, and reported that co-culture is possible in many situations. However, they encouraged further, more rigorous studies due to statistical uncertainty in their study. In the present study, feeding trials of *H. scabra* using various types of organic matter available in shrimp tanks were conducted in order to ascertain an effective feeding method for *H. scabra* in shrimp ponds. The biomitigating ability of *H. scabra* in tanks was also studied.

Materials and methods

Feeding trial 1: benthic diatoms and shrimp feed

Juveniles for use in the trials were produced at the sea cucumber hatchery of Southeast Asian Fisheries Development Center—Aquaculture Department (SEAFDEC–AQD). In order to compare the relative importance of diatoms and shrimp feed as food sources for juvenile *H. scabra*, feeding trials were conducted using *Navicula ramosissima* (benthic diatom monocultured at SEAFDEC–AQD) and *P. monodon* powdered feed (SEAFDEC–AQD formula). For each treatment described below, five juvenile *H. scabra* were placed in 60-L fibreglass tanks filled with filtered and UV-treated sea water with aeration. Sea water was pumped from offshore, sand filtered, filtered with 10-μm and 1-μm filters, and UV treated before use in the experiments. No substrate was added to the tanks. The juveniles were fed every 2 days with one of the following diets: (1) 1,000 mL *N. ramosissima* (~6.2×10^5 cells/mL); (2) a mixture of 500 mL *N. ramosissima* and 0.5 g powdered shrimp feed; (3) 1 g powdered shrimp feed; or (4) no feed (negative control). Three replicate tanks were used for each treatment, except for the control, where only one tank was used. In order to prevent growth of natural food and accumulation of contaminants, the tanks were cleaned thoroughly and water changed completely prior to feeding every 2 days. To compare the growth rates between treatments after 3 weeks, the juveniles' body length (BL) and weight (BW) were measured to the nearest 0.01 mm and 0.01 g, respectively. To increase size measurement accuracy, the juveniles were anaesthetised with 2% menthol–ethanol solution (Yamana et al. 2005) and blotted dry with paper towels prior to sampling.

Feeding trial 2: effect of sand on sandfish growth

The effects of the presence of sand substrate on the growth of *H. scabra* were studied. Sand collected from the beach in front of SEAFDEC–AQD was sieved through 1-mm mesh, washed with fresh water, bleached with sodium hypochlorite, washed again and sun-dried. The prepared sand was placed in a 60-L fibreglass tank (to approx. 5 cm depth), and filtered (10-μm and 1-μm) and UV-treated sea water was added to the tank. Five *H. scabra* juveniles were placed in the tank and provided with aeration (the with-sand treatment). The same number of juveniles was also placed in another 60-L tank under the same conditions except without sand (the without-sand treatment). The juveniles were fed with powdered shrimp feed (0.5 g/tank) every day. Water in the tanks was changed 100% every 2 days but the sand was not washed during the 2-week trial. Growth in BL and BW over the course of the experiment was compared between treatments.

Feeding trial 3: organic matter from shrimp tanks

Further effects of food types on growth of *H. scabra* juveniles were studied using organic matter that should be available in shrimp ponds: shrimp faeces, shrimp-tank detritus and *N. ramosissima* (*Navicula* species are ubiquitous). Faeces and detritus were collected from *P. monodon* tanks at SEAFDEC–AQD. The tanks were drained to collect the sedimentary detritus, and fresh faeces were manually separated from the total detritus using spoons. Faeces and detritus were stored at –80 °C until used.

Juvenile *H. scabra* were individually placed in containers made of PVC pipe (10 cm diameter × 5 cm length) with both ends covered with 5-mm mesh. For each treatment described below, 10 containers were placed in each 60-L fibreglass tank with sand and aeration. Each container was partially embedded in the sand. Juveniles were fed every 2 days with the following: (1) 2 g shrimp-tank detritus; (2) 2 g shrimp faeces; (3) 430 mL *N. ramosissima* (~ 6.2×10^5 cells/mL); and no feed (negative control). Two replicate tanks were used for each treatment except for the control, where only one tank was used. Water was changed 100% every 2 days and sand in the tanks was not cleaned. The trial ran for 10 days.

The BL and BW of all juveniles were measured after 10 days. The juveniles were anaesthetised before recording the measurements. Carbohydrate concentration in the coelomic fluid of the juveniles was measured after the size measurements. Coelomic fluid was collected with a micropipette though an incision along the abdomen, and the carbohydrate concentration was measured by a modified phenol – sulfuric acid method (Kushwaha and Kates 1981). A 10-µL aliquot of coelomic fluid was mixed with 40 µL distilled water, 20 µL 5% phenol solution and 100 µL concentrated H_2SO_4 in 2-mL microtubes; vortexed; and incubated in a block heater at 80 °C for 10 minutes. Absorbance was read at 490 nm against a blank, using a microplate reader.

Biomitigation of sediment eutrophication by sandfish

In order to examine the ability of *H. scabra* to mitigate sediment eutrophication, two aspects of sedimentary organic matter were studied: (1) reduction of acid-volatile sulfur (AVS) level in detritus after ingestion and excretion by *H. scabra;* and (2) reduction of dissolved oxygen (DO) consumption by detritus after ingestion and excretion by *H. scabra*.

Holothuria scabra (about 20 cm in BL) were allowed to defecate in a bare tank for 2 days, then were separately placed in bare 60-L fibreglass tanks ($n = 3$) with aeration, and allowed to feed on shrimp tank detritus for 2 days. Their faeces were then collected with a spatula. AVS levels in both the faeces and the detritus were measured by Hedorotech-S kit (GASTEC Co.)

About 3 g of both the faeces and detritus samples ($n = 3$ each) were mixed with 330 g filtered sea water, placed in sealed Erlenmeyer flasks, shaded with aluminum foil, and placed in an incubator at 27 °C. A control flask containing filtered sea water was also incubated. DO levels were measured every hour with a DO meter until DO in one of the treatments was depleted. DO consumption rate (mg O_2/g/hour) was determined as the largest difference between two successive readings.

Results and discussion

Feeding trial 1: benthic diatoms and shrimp feed

Diatoms and epiphytic algae are suggested to be important food sources for juvenile *H. scabra* by Battaglene et al. (1999), who hypothesised that reduced light decreased growth in *H. scabra* juveniles through lower algal production. Shrimp starter feed is also reported to be good food for *H. scabra* (Pitt et al. 2004). Results from these trials confirmed that there was no mortality of *H. scabra* in any feed treatment during the 3-week study. However, unlike the previous study (Pitt et al. 2004), negative growth was observed in *H. scabra* fed with powdered shrimp feed (Figure 1; –0.026 to –0.022 g/day, –0.27 to –0.17 mm/day). Positive growth was observed in *H. scabra* fed with a mixture of powdered shrimp feed and *N. ramosissima* (Figure 1), in which the growth rates (0.065 to 0.12 g/day, 0.36 to 0.45 mm/day) were comparable to those of those fed only with *N. ramosissima* (0.036 to 0.12 g/day, 0.30 to 0.52 mm/day). Thus, powdered shrimp feed by itself was found to be ineffective for the growth for *H. scabra* under the rearing conditions used in this trial.

Feeding trial 2: effect of sand on sandfish growth

Battaglene et al. (1999) reported that the growth rate and survival of *H. scabra* juveniles reared on sand was higher than those on a hard substrate when fed with dried powdered algae and natural biofilm. Kihara et al. (2009) observed faster growth and better survival of Japanese sea cucumber juveniles (*Apostichopus japonicus*) with the presence of sand, compared with those provided with no sand, when fed with dried powdered algae. Although the effect of sand on the survival of juveniles is not consistent, sand substrate seems to have a positive effect on the growth of sea cucumbers.

In this study, a similar result was observed. *H. scabra* juveniles provided with powdered shrimp feed showed positive growth (0.068 g/day, 0.25 mm/day) in the presence of sand (Figure 2), whereas juveniles reared without sand showed negative growth (–0.13 g/day, –0.42 mm/day). The growth rate observed in the 'with-sand' treatment was smaller than that reported for similar sized individuals by Pitt et al. (2004), in which shrimp feed and sand substrate were used. Nevertheless, the presence of sand substrate seems important for feeding and/or assimilation of food for *H. scabra*. Kihara et al. (2009) reported that, although *A. japonicus* ingested dried powdered algae, it did not bring about positive growth in the absence of sand. Since faeces were constantly observed in the 'without-sand treatment' during this trial, *H. scabra* seem to ingest powdered shrimp feed without sand. Therefore, sand may assist digestion of food particles in the gut of sea cucumbers. It is also possible that partially decomposed shrimp feed that could not be removed from the sand was more readily digestible or assimilable than fresh feed for sea cucumbers, which have been shown to have low digestive enzyme activity (Yingst 1976).

Feeding trial 3: organic matter from shrimp tanks

H. scabra juveniles showed positive growth when fed with shrimp tank detritus, shrimp faeces and *N. ramosissima*, except in one of the replicates of the detritus treatment (Figure 3). The growth rate was fastest with the faeces (0.018 to 0.052 g/day, 0.18 to 0.38 mm/day), followed by

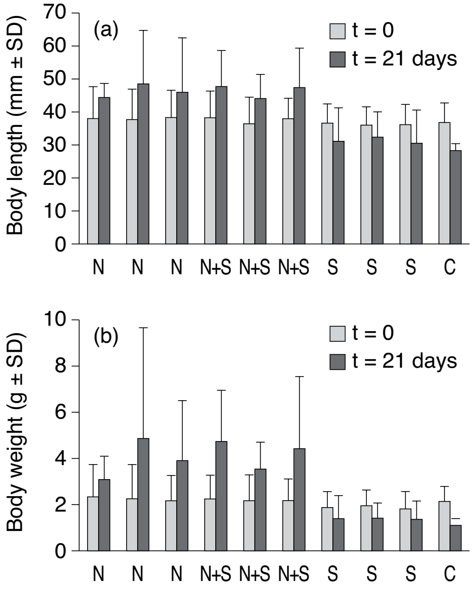

Figure 1. Mean (a) length (mm) and (b) weight (g) of *Holothuria scabra* juveniles ($n = 5$) reared with: *Navicula ramosissima* (N); a mixture of *N. ramosissima* and powdered shrimp feed (N+S); powdered shrimp feed (S); and no feed (C) for 21 days; error bars represent standard deviation.

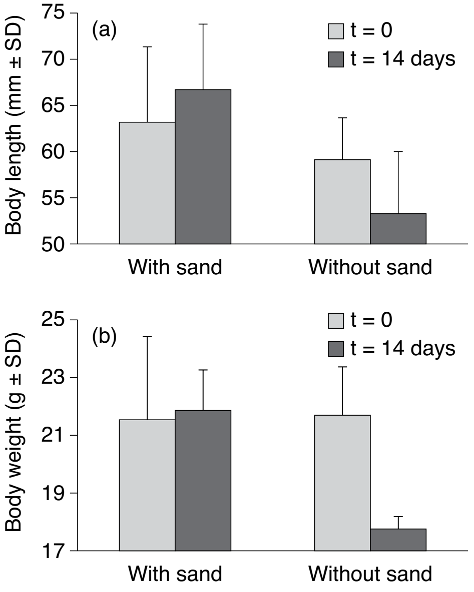

Figure 2. Mean (a) length (mm) and (b) weight (g) of *Holothuria scabra* juveniles ($n = 5$) reared with and without sand substrate in fibreglass tanks and fed powdered shrimp feed for 14 days; error bars represent standard deviation.

detritus (–0.021 to 0.026 g/day, –0.26 to 0.29 mm/day) and *N. ramosissima* (0.011 to 0.013 g/day, 0.156 to 0.158 mm/day). This, together with the result of feeding trial 2, indicates that *H. scabra* can grow in a *P. monodon* pond without additional feeding. Growth rates obtained in this trial are comparable with those reported by Battaglene et al. (1999), in which diatoms and epiphytic algae were fed to *H. scabra*, but almost an order of magnitude slower than those reported by Pitt et al. (2004). Therefore, shrimp feed in the presence of sand substrate, rather than decomposed leftovers, shrimp faeces or naturally occurring micro-algae, seems to be suitable for the growth of *H. scabra*. Yuan et al. (2006) reported that the mixed diets of bivalve faeces and powered algae showed promising results for cultivation of sub-adult *Apostichopus japonicus*, while the sea cucumber fed with powdered algae or faeces alone could not obtain the best growth. *Holothuria scabra* may be able to consume fresh leftovers by *P. monodon* more efficiently, rather than deteriorated sludge in the shrimp pond.

The carbohydrate concentration in the coelomic fluid had a significant negative correlation with growth in BL (Figure 4; $r = –0.30$, $p < 0.05$) and BW ($r = –0.29$, $p < 0.05$, $n = 70$). Watanabe et al. (2012) found that carbohydrate concentration in the coelomic fluid is positively correlated with starvation period. Therefore, carbohydrate concentration may be correlated with growth rate through nutritional condition, which presumably affects the growth rate of *H. scabra*. However, although the correlations were significant, the correlation coefficient had small values, and carbohydrate concentration values were highly variable, especially at low growth rates. Since feeding condition or nutritional condition is not the only factor affecting growth rate, one should be careful in the interpretation of carbohydrate concentration when analysing it in relation to growth rate.

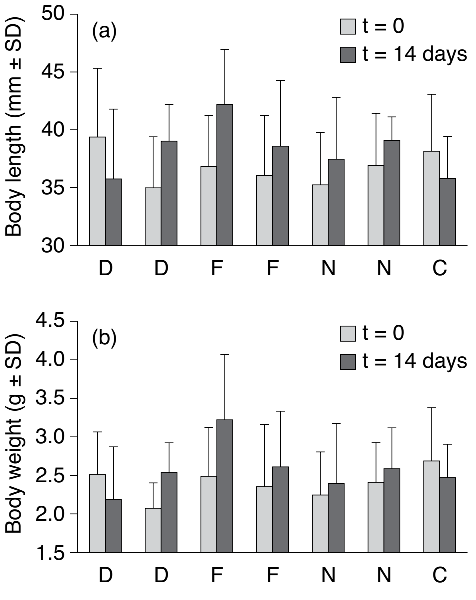

Figure 3. Mean (a) length (mm) and (b) weight (g) of *Holothuria scabra* juveniles (n = 10) reared with detritus collected from *Penaeus monodon* rearing tanks (D); *P. monodon* faeces (F); *Navicula ramosissima* (N); and no feed (C) for 14 days; error bars represent standard deviation.

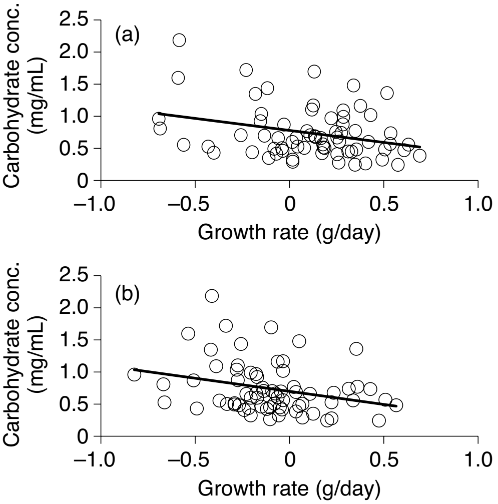

Figure 4. Relationship between growth rate of (a) length (mm/day, and (b) weight (g/day) and carbohydrate concentration (mg/mL) in the coelomic fluid of *Holothuria scabra* juveniles (n = 70) reared with detritus collected from *Penaeus monodon* rearing tanks; *P. monodon* faeces and *Navicula ramosissima*. Linear correlations were significant in both graphs.

Biomitigation of sediment eutrophication by sandfish

Dissolved oxygen consumption by faeces of *H. scabra* fed with shrimp tank detritus was 22% of that consumed by the shrimp tank detritus alone (from 1.0 ± 0.1 SD to 4.5 ± 1.0 SD g O_2/g dry wt/hour, $p < 0.001$, $n = 3$, t-test) (Figure 5). This may be attributable to reduction of organic matter in the detritus through assimilation by *H. scabra* (i.e. less organic matter equals less oxygen consumed by micro-organisms during decomposition of organic matter). Grazing of the brown sea cucumber (*Australostichopus mollis*) reduces total organic carbon, chlorophyll *a* and phaeopigment, as well as the chlorophyll *a* : phaeopigment ratio of sediments impacted by green-lipped mussel depositions (Slater and Carton 2009). Conversely, it is reported that, while *H. scabra* bioturbate sediments and eat organic deposits in tanks with blue shrimp (*Litopenaeus stylirostris*), they did not significantly reduce the organic content of the sand in the tanks (Purcell et al. 2006).

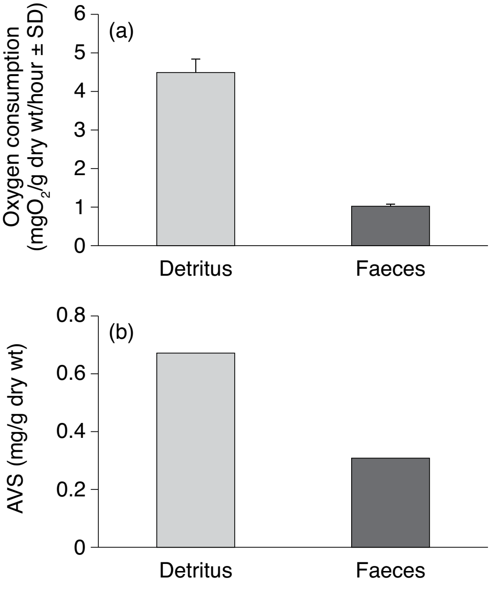

Figure 5. (a) Dissolved oxygen consumption rate and (b) acid-volatile sulfur (AVS) level of detritus collected from *Penaeus monodon* rearing tanks and faeces of *H. scabra* fed with the detritus ($n = 3$ for oxygen consumption rate and $n = 1$ for AVS level); error bars represent standard deviation.

If this also applies to the detritus derived from *P. monodon*, *H. scabra* may somehow change the nature of organic matter in the detritus so that oxygen consumption is reduced. Regardless of the actual mechanism, reduction of oxygen consumption is an important consequence of *H. scabra* feeding since hypoxia in the sediment and bottom water is a major problem in shrimp ponds (Suplee and Cotner 1996; Avnimelech and Ritvo 2003).

AVS level (hydrogen sulfide and iron sulfide ≈ total sulfur) in the shrimp tank detritus (0.67 mg/g dry wt) was reduced by 55% by *H. scabra* (0.31 mg/g dry wt) feeding (Figure 5). Dissimilative sulfate reduction by sulfate-reducing bacteria (Fenchel and Blackburn 1979) results in the release of hydrogen sulfide into the environment, which is very toxic to many aquatic organisms (Bagarinao and Vetter 1992). Sulfide diffused out of sediments into bottom water is quickly oxidised biotically and abiotically (Jorgensen 1977); therefore, high sulfide levels in the sediment can aggravate hypoxia in the bottom water. Thus, AVS reduction in shrimp-tank detritus by *H. scabra* should bring about positive effects to the environment of a shrimp pond. Further quantitative analysis should be carried out to determine the proper stocking density of *H. scabra* for effective biomitigation of a shrimp pond.

Conclusions

The series of experiments conducted in this study showed that, although more quantitative data are needed, *H. scabra* has potential to biomitigate the eutrophication and improve sediment quality in a shrimp pond. Studies on the relationship between stocking density of *H. scabra* and the extent of biomitigation, as well as relationships between the shrimp-pond environment and shrimp disease manifestation, should also be carried out.

Agudo (2006) suggested that, although the availability of cultured juvenile *H. scabra* provides potential for farming *H. scabra* in earthen ponds or sea pens, it should not be reared in ponds together with shrimp because shrimp prey on *H. scabra*. Bell et al. (2007) reported that co-culture of *H. scabra* with blue shrimp (*Litopenaeus stylirostris*) is not viable due to death and morbidity of *H. scabra*. Therefore, in co-culture with *P. monodon*, *H. scabra* should be either protected in cages or reared in a separate pond, to which shrimp-pond effluents are introduced before the water is returned to the shrimp pond. Rotational culture is another possible approach. Further studies to establish practically feasible co-culture methods should be conducted.

Acknowledgments

The authors are grateful to Joemel Sumbing, Jesus Rodriguez, Harold Figurado and Esteban Garibay at SEAFDEC–AQD for assistance in sandfish culture and laboratory analysis. This study was funded by the Japan International Research Center for Agricultural Sciences (JIRCAS).

References

Agudo N.S. 2006. Sandfish hatchery techniques. Australian Centre for International Agricultural Research, Secretariat of the Pacific Community and WorldFish Center: Noumea, New Caledonia.

Ahlgren M.O. 1998. Consumption and assimilation of salmon net pen fouling debris by the red sea cucumber *Parastichopus californicus*: implications for polyculture. Journal of the World Aquaculture Society 29, 133–139.

Avnimelech Y. and Ritvo G. 2003. Shrimp and fish pond soils: processes and management. Aquaculture 220, 549–567.

Bagarinao T. and Vetter R.D. 1992. Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. Aquatic Toxicology 24, 21–62.

Battaglene S.C., Seymour J.E. and Ramofafia C. 1999. Survival and growth of cultured juvenile sea cucumbers *Holothuria scabra*. Aquaculture 178, 293–322.

Bell J.D., Agudo N.N., Purcell S.W., Blazer P., Simutoga M., Pham D. et al. 2007. Grow-out of sandfish *Holothuria scabra* in ponds shows that co-culture with shrimp *Litopenaeus stylirostris* is not viable. Aquaculture 273, 509–519.

Carpenter K.E. and Niem V.H. 1998. The living marine resources of the Western Central Pacific, Vol. 2: cephalopods, crustaceans, holothurians and sharks. FAO species identification guide for fishery purposes. Food and Agriculture Organization of the United Nations: Rome.

Conand C. 2004. Present status of world sea cucumber resources and utilization: an international overview. In ‘Advances in sea cucumber aquaculture and management’, ed. by A. Lovatelli, C. Conand, S. Purcell, S. Uthicke, J.-F. Hamel and A. Mercier. FAO Fisheries Technical Paper No. 463, 13–23. Food and Agriculture Organization of the United Nations: Rome.

Fenchel T. and Blackburn T.H. 1979. Bacteria and mineral cycling. Academic Press: London.

Flegel T.W. 2006. Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. Aquaculture 258, 1–33.

Hamel J.-F., Conand C., Pawson D.L. and Mercier A. 2001. The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): its biology and exploitation as beche-de-mer. Advances in Marine Biology 41, 129–223.

Jorgensen B.B. 1977. The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnology and Oceanography 22, 814–832.

Kang K.H., Kwon J.Y. and Kim Y.M. 2003. A beneficial coculture: charm abalone *Haliotis discus hannai* and sea cucumber *Stichopus japonicus*. Aquaculture 216, 87–93.

Kihara M, Tamoto J. and Hoshi T. 2009. Effects of sand particles on feeding behavior of sea cucumber *Apostichopus japonicas* reared in tank. Journal of Fisheries Technology 2, 39–43.

Kushwaha S.C. and Kates M. 1981. Modification of phenol-sulfuric acid method for the estimation of sugars in lipids. Lipids 16, 372–373.

Lightner D.V. and Redman R.M. 1998. Shrimp diseases and current diagnostic methods. Aquaculture 164, 201–220.

Maxwell K.H., Gardner J.P.A. and Heath P.L. 2009. The effect of diet on the energy budget of the brown sea cucumber *Stichopus mollis* (Hutton). Journal of the World Aquaculture Society 40, 157–170.

Paltzat D.L., Pearce C.M., Barnes P.A. and McKinley R.S. 2008. Growth and production of California sea cucumbers (*Parastichopus californicus* Stimpson) co-cultured with suspended Pacific oysters (*Crassostrea gigas* Thunberg). Aquaculture 275, 124–137.

Pitt R., Duy N.D.Q., Duy T.V. and Long H.T.C. 2004. Sandfish (*Holothuria scabra*) with shrimp (*Penaeus monodon*) co-culture tank trials. SPC Beche-de-mer Information Bulletin 20, 12–22.

Purcell S.W., Patrois J. and Fraisse N. 2006. Experimental evaluation of co-culture of juvenile sea cucumbers *Holothuria scabra* (Jaeger) with juvenile blue shrimp *Litopenaeus stylirostris* (Stimpson). Aquaculture Research 37, 515–522.

Slater M.J. and Carton A.G. 2007. Survivorship and growth of the sea cucumber *Australostichopus* (*Stichopus*) *mollis* (Hutton 1872) in polyculture trials with green-lipped mussel farms. Aquaculture 272, 389–398.

Slater M.J. and Carton A.G. 2009. Effect of sea cucumber (*Australostichopus mollis*) grazing on coastal sediments impacted by mussel farm deposition. Marine Pollution Bulletin 58, 1123–1129.

Suplee M.W. and Cotner J.B. 1996. Temporal changes in oxygen demand and bacterial sulfate reduction in inland shrimp ponds. Aquaculture 145, 141–158.

Watanabe S., Zarate J.M., Lebata-Ramos M.J.H. and Nievales M.F.J. 2012. Evaluation of nutritional condition of juvenile sandfish (*Holothuria scabra*). In ‘Asia–Pacific tropical sea cucumber aquaculture’, ed. by C.A. Hair, T.D. Pickering and D.J. Mills. ACIAR Proceedings No. 136, 50–56. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Yamana Y., Hamano T. and Yamamoto K. 2005. Anesthetizer of the adult sea cucumber *Apostichopus japonicus*. Nippon Suisan Gakkaishi 71, 299–306 (in Japanese with English abstract).

Yingst J.Y. 1976. The utilization of organic matter in shallow marine sediments by an epibenthic deposit-feeding holothurian. Journal of Experimental Marine Biology and Ecology 23, 55–69.

Yuan X., Yang H., Zhou Y., Mao Y., Zhang T. and Liu Y. 2006. The influence of diets containing dried bivalve feces and/or powdered algae on growth and energy distribution in sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea). Aquaculture 256, 457–467.

Zhou Y., Yang H., Liu S., Yuan X., Mao Y., Liu Y. et al. 2006. Feeding and growth on bivalve biodeposits by the deposit feeder *Stichopus japonicus* Selenka (Echinodermata: Holothuroidea) co-cultured in lantern nets. Aquaculture 256, 510–520.