

# Evaluation of nutritional condition of juvenile sandfish (*Holothuria scabra*)

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## Abstract

It is important to accurately evaluate the wellbeing or nutritional condition of organisms when monitoring the wild stock conditions and improvement in aquaculture techniques; however, reliable nutritional condition indexes have not been established for sea cucumbers. In this study, the effects of starvation on condition factor (body weight / body volume), coelomic fluid constituent (protein, carbohydrate and cholesterol) concentrations and coelomic fluid density were analysed in an attempt to establish a method to determine nutritional condition in juvenile sandfish (*Holothuria scabra*). Body length, breadth and weight of juveniles produced at the sea cucumber hatchery of the Aquaculture Department, Southeast Asian Fisheries Development Center, were measured after anaesthetisation with 2% menthol-ethanol. Coelomic fluid protein level was analysed by the bicinchoninic acid method. Carbohydrate level was analysed by the phenol – sulfuric acid method. Cholesterol level was analysed by the Zak method. Coelomic fluid volume and coelomic fluid weight were measured. Starvation caused a concomitant decrease in body length, breadth and weight, resulting in no net change in the condition factor. This result indicated that condition factor cannot be used as a nutritional condition index. Coelomic fluid constituent level could be measured with a small volume of sample (i.e. 10–20 µL). Although no clear pattern was observed in coelomic fluid protein and cholesterol levels during the starvation trial, carbohydrate level increased, as did coelomic fluid density. These results suggest that coelomic fluid density and carbohydrate level may be used as indexes for nutritional condition of sandfish without sacrificing the animal.

## Introduction

Due to overexploitation and increasing demand, fishery stocks of many tropical sea cucumber species have declined drastically in the Pacific and Indian oceans (Carpenter and Niem 1998; Hamel et al. 2001; Conand 2004). In order to increase the fishery production, many studies have been done on hatchery, aquaculture and stock enhancement methods of sea

cucumbers, especially sandfish (*Holothuria scabra*), which is the most valued of tropical sea cucumbers (e.g. Battaglene et al. 1999; Mercier et al. 1999; Purcell et al. 2006; Bell et al. 2007). Nevertheless, there is a basic methodological problem: there has been no standard evaluation method developed for nutritional condition in sea cucumbers, including *H. scabra*. Hatcheries for *H. scabra* have been operating in countries such as New Caledonia, Vietnam, India and the Philippines (James 1999; Pitt and Duy 2004; Agudo 2006; Duy 2010). Slow growth and high mortality of the cultured juveniles are problematic in these hatcheries—the growth of juveniles can sometimes be faster in an earthen pond where supplemental feeding is not conducted than in concrete tanks under controlled conditions (SEAFDEC–AQD, pers. comm.; Pitt et al. 2001; Agudo 2012). Therefore, hatchery

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techniques, particularly feeding methods, should be improved. It is also crucial to establish a method to monitor the condition of released juveniles in stock enhancement programs.

In this study, attempts were made to establish a method to evaluate nutritional condition of sandfish based upon body size to weight relationship and concentration of coelomic fluid constituents.

## Materials and methods

### Measurements of body size, coelomic fluid volume and coelomic fluid density

In order to acquire basic information about the relationship between body size and coelomic fluid, data on *H. scabra* juveniles obtained from the sea cucumber hatchery of the Southeast Asian Fisheries Development Center – Aquaculture Department (SEAFDEC–AQD) in Iloilo, the Philippines, were collected. To increase body measurement accuracy, the juveniles were anaesthetised using a standard anaesthetic solution: ethanol saturated with menthol (i.e. 0.56 g menthol crystal dissolved in 100 mL of 99% ethanol) and diluted with filtered sea water to 2% (Yamana et al. 2005). *H. scabra* ( $n = 15$ ) were placed in the solution at room temperature for 20 minutes. After blotting dry with paper towels, body length (*BL*) and body breadth at the widest point (*BB*) were measured to the nearest 0.01 mm, and body weight (*BW*) was measured to the nearest 0.01 mg. Body volume (*BV*) was calculated as a spheroid according to equation (1):

$$BV = 4/3 \times \pi \times BL \times (BB/2)^2 \quad (1)$$

Fulton's condition factor (*K*) was calculated according to equation (2):

$$K = BW/BV \times 10^4 \quad (2)$$

*Holothuria scabra* were then cut longitudinally at the abdomen, and total coelomic fluid was collected with a micropipette into a micro centrifuge tube. Coelomic fluid volume (*CFV*) was measured to the nearest 10  $\mu$ L with micropipettes. Coelomic fluid weight (*CFW*) was measured to the nearest 0.001 mg with a microbalance.

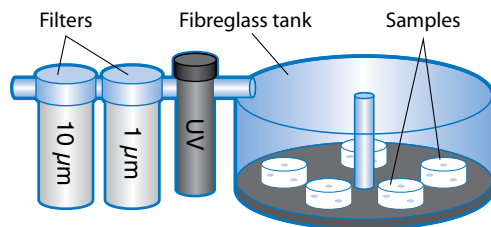
### Effects of starvation on condition of *H. scabra*

*Holothuria scabra* juveniles of similar sizes obtained at the SEAFDEC–AQD sandfish hatchery ( $n = 30$ ) were anaesthetised as described above and body sizes were measured (i.e. *BL*, *BB* and *BW*). For the initial data, five individuals were stored at  $-80^\circ\text{C}$ .

The rest of the *H. scabra* were individually placed in containers made of PVC pipe (10 cm diameter  $\times$  5 cm length) with both ends covered with 5-mm mesh (Figure 1). The containers were placed in a 60-L fibreglass tank (5 containers in each of 5 tanks) and kept under flow-through conditions with aeration but no sediment or supplementary feeding. Tank sea water was sand-filtered, further filtered with 10- $\mu$ m and 1- $\mu$ m filters, and UV-treated. Size measurements were made every 2 days on the same individuals after anaesthetisation, and five individuals (i.e. one tank) were stored at  $-80^\circ\text{C}$  every 2 days until day 10.

At the conclusion of the trial, the frozen samples were thawed in a refrigerator and longitudinally cut at the abdomen. Coelomic fluid was collected to measure protein, carbohydrate and cholesterol concentrations:

- Protein concentration of coelomic fluid was measured by the bicinchoninic acid (BCA) method (QuantiPro BCA Assay Kit, SIGMA-ALDRICH). A 10- $\mu$ L aliquot of coelomic fluid was diluted 100 times with distilled water in 1.5-mL microtubes, and absorbance was read at 562 nm using a microplate reader.
- Carbohydrate concentration of the coelomic fluid was measured by the modified phenol – sulfuric acid method (Kushwaha and Kates 1981). A 10- $\mu$ L aliquot of the coelomic fluid was mixed with 40  $\mu$ L distilled water, 20  $\mu$ L 5% phenol solution and 100  $\mu$ L concentrated  $\text{H}_2\text{SO}_4$  in 2-mL microtubes, vortexed, and incubated in an 80  $^\circ\text{C}$  block heater for 10 minutes. Absorbance was read at 490 nm against a blank, using a microplate reader.
- Cholesterol concentration of the coelomic fluid was measured by the modified Zak method (Zak 1957; Altescu 1965). A 20- $\mu$ L aliquot of the coelomic fluid was mixed with 300  $\mu$ L 0.2%  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  in 99% ethanol and 200  $\mu$ L concentrated  $\text{H}_2\text{SO}_4$  in 1.5-mL microtubes, and incubated in an 80  $^\circ\text{C}$  block heater for 10 minutes. Absorbance was read at 540 nm against a blank, using a microplate reader.



**Figure 1.** Schematic drawing of the sandfish rearing system used in this study

## Effects of starvation on coelomic fluid volume and coelomic fluid density

To determine changes in coelomic fluid density (*CFD*) and *CFV* relative to the body size (*CFV'*), another starvation trial was conducted for 20 days. Body size measurements were made after anaesthetisation ( $n = 25$ ) and five individuals were stored at  $-80\text{ }^{\circ}\text{C}$  for the initial data. In each of four 60-L fibreglass tanks, five individuals were placed (not individually separated). Every 5 days, five individuals (i.e. all five individuals from one tank) were measured and stored at  $-80\text{ }^{\circ}\text{C}$ . The frozen samples were thawed in a refrigerator; total coelomic fluid was drawn from the body cavity to measure *CFV* and *CFW*. *CFD* was obtained as  $CFW/CFV$ . *CFV'* was calculated as  $CFV/BV$ .

### Statistical analysis

Relationships between the studied parameters were examined by linear, hyperbolic or exponential regression analyses. For comparisons of more than three datasets, ANOVA was performed followed by a Tukey test for posteriori comparisons. For comparisons of paired data sets, t-tests were used. Differences were considered significant if  $P < 0.05$ .

## Results and discussion

### Body size, coelomic fluid volume, coelomic fluid density and condition factor

*CFV* of *H. scabra* had a significant positive correlation with *BL* (Figure 2, equation (3)):

$$CFV = 1.73 \times e^{0.11BL} \quad (3)$$

*CFV* increased linearly as *BW* (Figure 2, equation (4)):

$$CFV = 123.0 \times BW - 93.7 \quad (4)$$

and *BV* (equation (5)) increased:

$$CFV = 0.01 \times BV - 60.40 \quad (5)$$

This is common for many animals since *BV* and *BW* increase exponentially with *BL*.

There were no significant correlations between *CFD* and *BL* ( $r^2 = 0.13$ ,  $P = 0.19$ ), *BW* ( $r^2 = 0.058$ ,  $P = 0.39$ ) or *BV* ( $r^2 = 0.068$ ,  $P = 0.35$ ).

Condition factor (*K*) had a significant negative linear correlation with *BL* (Figure 2), *BV* and *BW*, according to equations (6), (7) and (8), respectively:

$$K = -0.01 \times BL + 1.22 \quad (6)$$

$$K = -2.8 \times 10^{-6} \times BV + 1.05 \quad (7)$$

$$K = -0.0032 \times BW + 1.05 \quad (8)$$

Since *K* is correlated with *BL*, *BV* and *BW*, it must be standardised for size and weight if it is to be used for condition comparisons of *H. scabra* of different sizes.

### Starvation, condition factor and coelomic fluid constituent concentrations

*BV* and *BW* of individual *H. scabra* decreased concomitantly during the 10-day starvation period (Figure 3). While *BW* gradually decreased over the experimental period, the trend in *BV* was less clear, perhaps due to limited accuracy of body size measurements despite anaesthetisation. The plasticity of the body shape of sea cucumbers is problematic for size measurements (Sewell 1990; Battaglene et al. 1999). Nevertheless, both mean *BV* and *BW* significantly decreased in 10 days ( $P < 0.05$  and  $P < 0.01$ , respectively, t-test). Because of this, *K* stayed constant during the starvation period (Figure 3), with no significant difference between day 1 and day 10 ( $P = 0.16$ , t-test). *K* is one of the most widely used indexes for determination of condition, well-being or 'plumpness' of organisms in fisheries and general fish biology studies (e.g. Nash et al. 2006). However, unlike vertebrates and invertebrates with exoskeletons, *K* is not a useful index for the evaluation of nutritional condition in *H. scabra* because of the concomitant changes in body size and weight.

Protein and cholesterol concentrations in the coelomic fluid of *H. scabra* initially increased and then decreased after day 6 during the 10-day starvation period (Figure 4), according to equations (9) and (10):

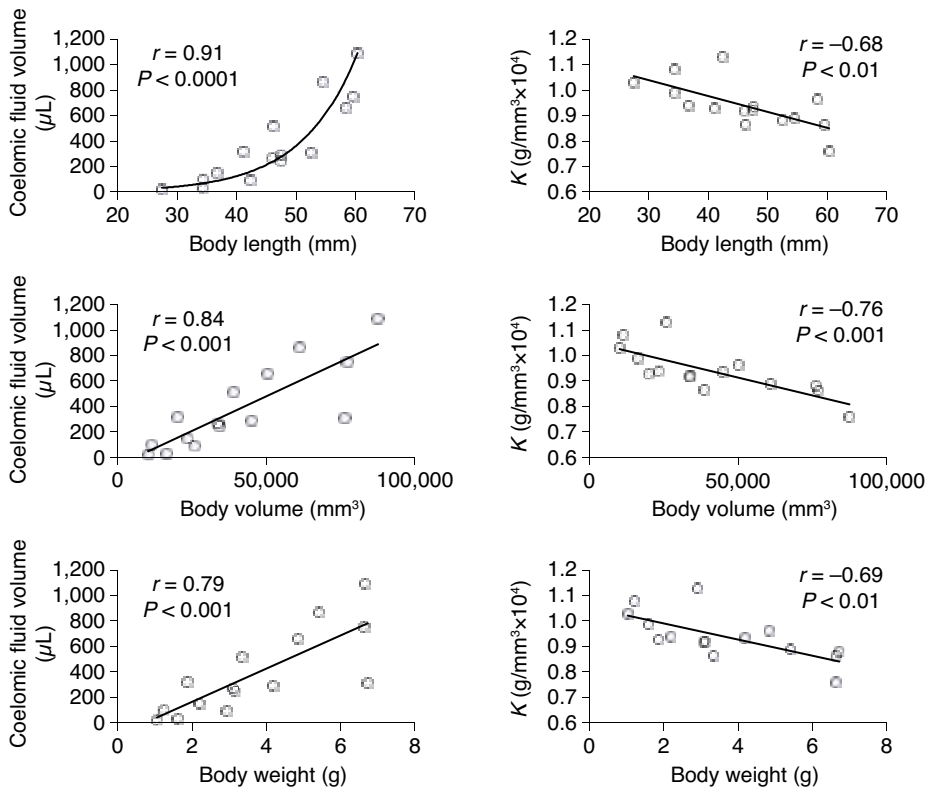
$$C_p = -0.050 \times d^2 + 0.53 \times d + 2.70 \quad (9)$$

$$C_{ch} = -0.0016 \times d^2 + 0.021 \times d + 0.17 \quad (10)$$

where  $C_p$  is protein concentration and  $C_{ch}$  is cholesterol concentration, and  $d$  is day of starvation.

Therefore, it is difficult to use them as indexes of nutritional condition in *H. scabra*. On the other hand, carbohydrate concentration ( $C_c$ ) increased linearly (Figure 4) as starvation continued, according to equation (11):

$$C_c = 0.076 \times d + 0.86 \quad (11)$$



**Figure 2.** Relationships between body size and coelomic fluid volume and condition factor ( $K$ ) in *Holothuria scabra* ( $n = 15$ )

Therefore,  $C_c$  may be suitable for determination of nutritional condition. Reasons for increased  $C_c$  despite starvation are not known. In fact,  $C_c$  in the coelomic fluid in Japanese sea cucumber *Stichopus japonicus* is reported to decrease and non-protein nitrogen level stay constant during starvation (Tanaka 1958). Further studies on the physiological processes of *H. scabra* during starvation are needed.

### Starvation, coelomic fluid volume and coelomic fluid density

$CFD$  had a significant positive linear correlation with the starvation period (Figure 5), according to equation (12):

$$CFD = 0.0087 \times d + 0.92 \quad (12)$$

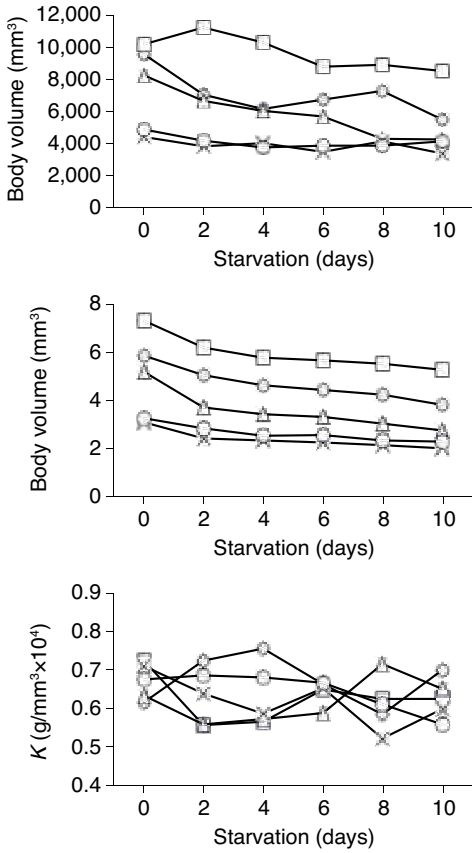
$CFV'$ , on the other hand, had a significant negative linear correlation with the starvation period (Figure 4) according to equation (13):

$$CFV' = -0.0015 \times d + 0.084 \quad (13)$$

These relationships may indicate that increased  $C_c$  due to starvation may be related to thickening of the coelomic fluid. In addition, since protein is reported to be the major energy source for small *Apostichopus japonicus* (synonym for *S. japonicus*) during aestivation (Yang et al. 2006), the protein concentration may increase during the initial phase of starvation due to thickening of the coelomic fluid, and subsequently decrease due to energy consumption.

### Recommended index for nutritional condition

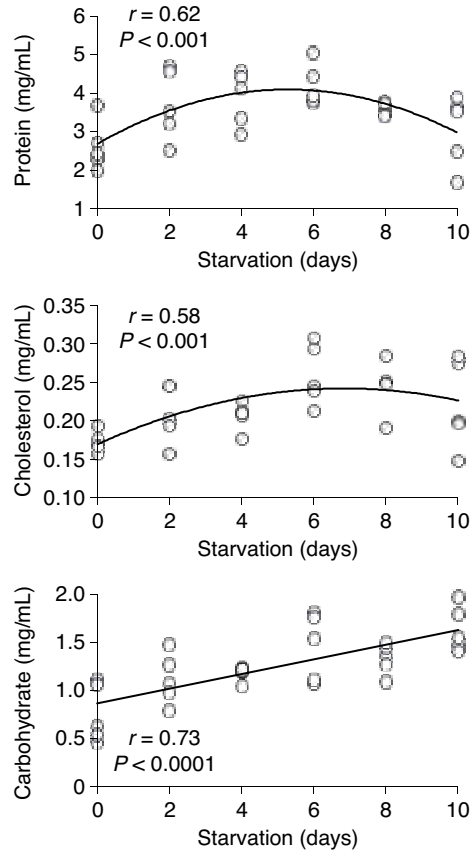
Although mechanisms of changes in coelomic fluid constituent concentrations during starvation are not understood, the  $C_c$  seems to be a good indicator of nutritional condition in *H. scabra*. Since it requires a very small amount of coelomic fluid sample (10 µL) for the colorimetric carbohydrate measurement, it



**Figure 3.** Changes in body volume, body weight and condition factor ( $K$ ) of *Holothuria scabra* ( $n = 5$ ) during a 10-day starvation trial. Different symbols refer to specific individuals.

may be possible to monitor the time-course change of nutritional condition of an individual without sacrificing it. A method to sample the coelomic fluid using cannulation from live specimens should be further investigated. Although  $CFD$  may also be a good indicator of nutritional condition, it requires a larger amount of sample for the measurement. In this study, the entire coelomic fluid of each individual was used for the density measurement to increase accuracy of the measurements. While the use of more sensitive devices may increase the measurement accuracy of  $CFD$ , colorimetric methods are recommended.

Studies on improvement of *H. scabra* production at hatcheries and aquaculture facilities, as well as stock

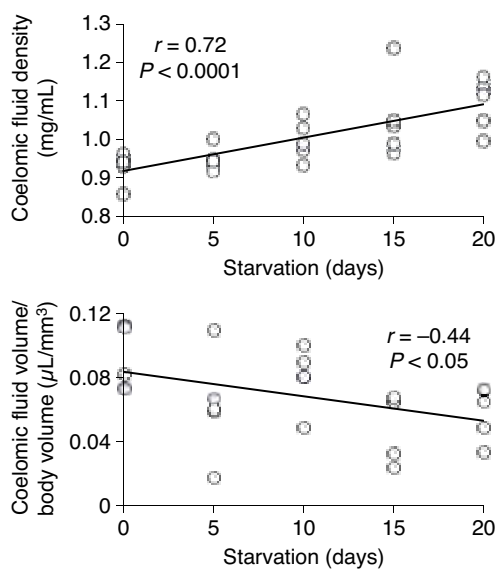


**Figure 4.** Relationships between starvation period and levels of protein, cholesterol and carbohydrate in the coelomic fluid of *Holothuria scabra*

enhancement technologies, should be carried out with the methods for monitoring *H. scabra* conditions described here.

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**Figure 5.** Relationships between starvation period and coelomic fluid density and coelomic fluid volume relative to body volume of *Holothuria scabra*

## 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.
- Agudo N.S. 2012. Pond grow-out trials for sandfish (*Holothuria scabra*) in New Caledonia. In 'Asia-Pacific tropical sea cucumber aquaculture', ed. by C.A. Hair, T.D. Pickering and D.J. Mills. ACIAR Proceedings No. 136, 104–112. Australian Centre for International Agricultural Research: Canberra. [These proceedings]
- Altescu E.J. 1965. New reagent for the direct determination of serum cholesterol. *Journal of Clinical Pathology* 18, 824.
- 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.S., 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, Volume 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.
- Duy N.D.Q. 2010. Seed production of sandfish (*Holothuria scabra*) in Vietnam. Aquaculture Extension Manual 48. Southeast Asian Fisheries Development Center: Iloilo, Philippines.
- 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, 129–202.
- James D.B. 1999. Hatchery and culture for the sea cucumber *Holothuria scabra* Jaeger in India. *Naga, ICLARM Quarterly* 22, 12–16.
- Kushwaha S. C. and Kates M. 1981. Modification of phenol-sulfuric acid method for the estimation of sugars in lipids. *Lipids* 16, 372–373.
- Mercier A., Battaglene S.C. and Hamel J.-F. 1999. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. *Journal of Experimental Marine Biology and Ecology* 239, 125–156.
- Nash R.D.M., Valencia A.H. and Geffen A.J. 2006. The origin of Fulton's condition factor—setting the record straight. *Fisheries* 31, 236–238.
- Pitt R. and Duy N.D.Q. 2004. Breeding and rearing of the sea cucumber *Holothuria scabra* in Viet Nam. 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, 333–346. Food and Agriculture Organization of the United Nations: Rome.
- Pitt R., Thu N.T.X., Minh M.D. and Phuc H.N. 2001. Preliminary sandfish growth trials in tanks, ponds and pens in Vietnam. *SPC Beche-de-mer Information Bulletin* 15, 17–27.
- 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.
- Sewell M.A. 1990. Aspects of the ecology of *Stichopus mollis* (Echinodermata: Holothuroidea) in north-eastern New Zealand. *New Zealand Journal of Marine and Freshwater Research* 24, 97–103.
- Tanaka Y. 1958. Feeding and digestion processes of *Stichopus japonicus*. *Bulletin of Faculty of Fisheries Hokkaido University* 9, 14–28.
- 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)

- Yang H., Zhou Y., Zhang T., Yuan X., Li X., Liu Y. et al. 2006. Metabolic characteristics of sea cucumber *Apostichopus japonicus* (Selenka) during aestivation. *Journal of Experimental Marine Biology and Ecology* 330, 505–510.
- Zak E. 1957. Simple rapid microtechnic for serum total cholesterol. *American Journal of Clinical Pathology* 27, 583–588.