The effect of food availability on early growth, development and survival of the sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea)

Andrew David Morgan[1]

Abstract

In the aquaculture of marine invertebrates the interaction between the physical environment and the availability of food may affect the quality of larvae in mass cultures. Larvae from fertilised eggs obtained from the sea cucumber Holothuria scabra*, induced to spawn in captivity, were placed in 3 l, aerated, concave aquarium bowls and the quality of larvae and their survival, growth and development in response to the rearing environment was observed. At concentrations of 1 and 2 x 10^4 cells/ml of the alga* Isochrysis galbana*, growth and development of larvae increased substantially. At concentrations of 4 and 8 x 10^4 cells/ml, and in the absence of algae, larval survival was less and growth and development was inhibited. Larval quality was compromised by high concentrations of algae. An increase in the concentration of algae may affect levels of pH and un-ionised ammonia, which can affect the development and survival of larvae. It is important to structure feeding regimes so that concentrations of algae do not compromise the rearing environment.*

Introduction

Since Conand (1990; 1993) and Conand and Byrne (1993) assessed sea cucumber fishery resources and the effects of their exploitation, a number of projects have been developed to assess the potential of sea cucumbers for aquaculture. Aspects of the development of holothurian larvae have been described for the sea cucumbers *Cucumaria elongata*, *Stichopus californicus*, *Psolus chitonoides*, *Psolidium bullatum*, *Actinopyga echinties*, *Holothuria leucospilota*, and *H. pardalis* (Chia and Buchanan 1969; Maruyama 1980; Smiley 1986; McEuen and Chia 1991; Mashanov and Dolmatov 2000). Most aspidochirote holothurians follow the larval cycle of pre-auricularia, early, mid- and late auricularia and subsequent metamorphosis to the doliolaria (non-feeding) stage before settlement. The length of the larval cycle differs between species, but the development of *Holothuria scabra* larvae is typical of holothurian ontogenesis, reaching initial settlement after approximately 10 to twelve days in optimal culture conditions (James et al. 1994; Battaglene et al. 1999).

Species of algae that have been fed to larvae of the sea cucumbers *A. echinites*, *H. gresia*, *P. californicus*, and *S. californicus* include *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Duniella salina*, *D. tertiolecta* and *Pavlova lutheri* (Burke et al. 1986; Smiley 1986; Balser et al. 1993; Dautov 1997). Battaglene et al. (1999) fed *H. scabra* larvae with the algae *Chaetoceros muelleri*, *C. calcitrans*, *P. salina*, *Rhodomonas salina* and *Tetraselmis chuii*. Ito (1995), James et al. (1994) and Ramofafia et al. (1995) indicated that algal concentrations of 2 to 3 x 10^4 cells / ml were optimal for larval ontogenesis of *S. japonicus*, *H. scabra* and *H. atra* respectively, during development in large culture vessels (>100 l). Archer (1996) found that the ingestion rate of larvae of *S. mollis* was reduced when *P. tricornutum* and *D. tertiolecta* was given at concentrations exceeding 6 x 10^3 cells / ml. In the continued presence of high concentrations of algae, larvae stopped feeding.

In the present study I used *H. scabra* larvae produced in captivity from induction of spawning (Morgan 2000) to investigate the effect of different concentrations of the alga *I. galbana* on larval quality. The quality of larvae was determined by observation of symmetry of shape, complexion of the dermis and development of the larval arms.

Materials and methods

Culture vessels

Experiments were conducted in 3 l concave aquarium bowls at 27°C with a 16:8 LD cycle using UV-sterilised seawater filtered to 0.2 mm, to eliminate most bacteria, for use in larval rearing experiments. Concentrations of the alga *Isochrysis galbana* at 0, 1, 2, 4 and 8 x 10^4 cells / ml were assigned to bowls randomly with three replicates per treatment. Larvae were obtained by inducing a number of *H. scabra* to spawn in captivity (Morgan 2000). The fertilised eggs were washed of excess sperm and allowed to develop in a 250 l flow-through hatch tank before

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use in the experiment. The experiment was terminated after 11 days as under optimal nutritional regimes, larvae metamorphose into non-feeding doliolaria around this time. Consequently, it was not necessary to assess the quality of larvae in response to the culture environment after this time.

Maintenance

Every day 50% of the seawater was siphoned out of each 3 l concave aquarium bowl using a plastic tube with a 100 mm mesh screen glued to one end. The remaining seawater and larvae were drained into a clean bowl containing 1.5 l of 0.2 mm filtered UV-sterilised seawater. The amount of algae was adjusted by taking three 10 ml samples from each bowl and counting the algal density with a high speed particle counter (model FN Coulter Counter; ± 250 cells / ml).

Growth and survival

Larvae were stocked in the 3 l aquarium bowls at a density of 1 larva / 2 ml of filtered sea water. Growth and survival measurements were made preceding the transfer of larvae and remaining seawater to a clean bowl. Every second day, larval survival was measured by taking five 20 ml sub-samples from each bowl and counting the number of surviving larvae using a stereo-dissecting microscope. On alternate days 30 to 50 larvae were sampled from each bowl and total growth in length measured with a compound microscope and micrometer eyepiece. Percentage survival was adjusted for the removal of larvae.

Development and larval quality

Larval stage was recorded by observation of the hydrocoel, left and right somatocoel and lateral folds or 'arms' (Smiley 1986; Pedrotti 1995; Dautov and Kaisheko 1995) using a compound microscope with a micrometer eyepiece (n = 10 larvae / bowl). The hydrocoel is situated to the left of the oesophagus (Fig. 1) and enlarges throughout development. The elaboration of the hydrocoel refers to the thickening and subsequent development of five nodules late in the larval cycle.

Early auricularia larvae had a simple convoluting ciliated band with undeveloped lateral arms, a 'globular' hydrocoel, and very little development of the left somatocoel and no right somatocoel. The mid-auricularia stage was recorded as occurring when the four lateral folds could be seen developing, the left somatocoel extended more than half way down the gut and the hydrocoel was elongated. Late auricularia occurred when lateral folds exhibited overt folding and the hydrocoel had elaborated, the left somatocoel extended to the rear of the stomach and the right somatocoel was clearly visible. The appearance of the dermis and symmetry of shape were also recorded.

Data analysis

A one-way analysis of variance (ANOVA) was used to obtain differences in the mean growth rate of larvae for each concentration of algae from days two to 10. A one-way analysis of variance (ANOVA) was used to obtain differences in the mean survival of larvae for each concentration of algae at the end of the experiment. Differences between treatments were tested for using least square means (LS means) and the Bonferroni correction for multiple

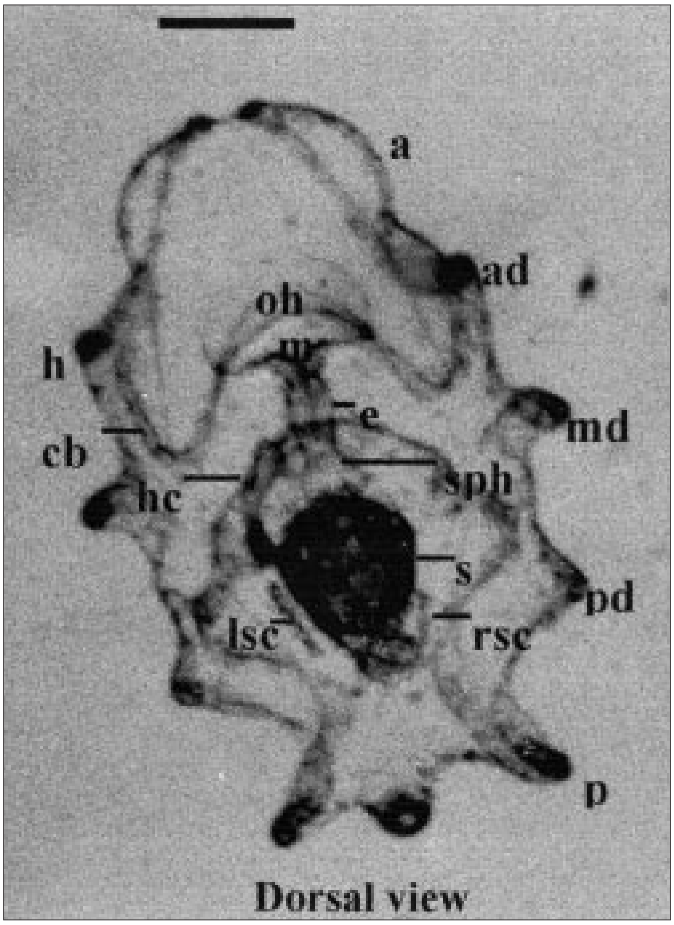

Figure 1

Photograph of *Holothuria scabra* larvae late in development, indicating features used to identify larval stage (scale bar indicates 200 mm). a, anterior fold. ad, anteriodorsal fold. md, mid-dorsal fold. pd, posteriodorsal fold. p, posterior fold. rsc, right somatocoel. lsc, left somatocoel. hc, hydrocoel. m, mouth; s, stomach. cb, ciliary band. h, hyaline sphere. sph, sphincter muscle. oh, oral hood.

comparisons (5% significance level divided by the number of multiple comparisons; n = 10). Differences in the rate of development between early, mid- and late auricularia were graphed.

Results

Survival

Survival of larvae at 2 x 10^4 cells / ml differed from all other concentrations of algae except 1 x 10^4 cells / ml ($p < 0.005$; Table 1). Few larvae died at 2 x 10^4 cells / ml while 55 ± 6.7 percent of larvae remained at 0 x 10^4 cells / ml at the end of the experiment (Figure 2). Other concentrations of 1, 4 and 8 x 10^4 cells / ml had 81 ± 7, 63 ± 21 and 68 ± 2.2 % of larvae left respectively, on day eleven (Figure 2).

Table 1. Analysis of variance (ANOVA) results for numbers of *Holothuria scabra* larvae surviving after 11 days and p-values for Least squares means at 0, 1, 2, 4, and 8 x 104 cells / ml of the algae *Iscochrysis galbana* ($p < 0.005$).

Source	DF	SS	MS	F value	Pr>F
Model	5	136	27	5	0.0164
Error	9	47	5		
Corrected total	14	183			
LS Means	1	2	4	8	
0	0.0379	0.0016	0.4932	0.4065	
1		0.062	0.118	0.1652	
2			0.0042	0.005	
4				0.8551	
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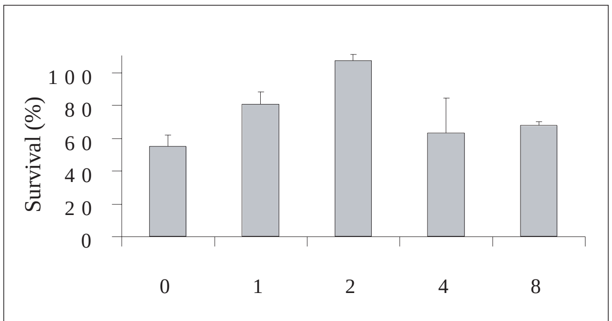

Figure 2.

Mean number (n = 3) of *Holothuria scabra* larvae surviving after eleven days at concentrations of 0, 1, 2, 4, and 8 x 10^4 cells / ml of the algae *Isochrysis galbana* (mean ±S.E.).

Growth

Growth of larvae at 1 and 2 x 10^4 cells / ml differed from 0, 4 and 8 x 10^4 cells / ml ($p < 0.005$; Table 2). Growth of starved larvae and growth of larvae between 4 and 8 x 10^4 cells / ml did not differ significantly, as was the case between 1 and 2 x 10^4 cells / ml ($p > 0.005$). Larvae at 1 and 2 x 10^4 cells / ml grew an average of 32 ± 7.3 and 32 ± 3.1 µm / day, respectively (Fig. 3). Larvae at these two concentrations reached a total length of 899 and 866 µm respectively on day 10 (Table 3).

Table 2. Analysis of variance (ANOVA) results for growth rate of *Holothuria scabra* larvae over 10 days and p-values for Least squares means at 0, 1, 2, 4, and 8 x 10^4 cells / ml of the algae *Iscochrysis galbana* ($p < 0.005$).

Source	DF	SS	MS	F value	Pr>F
Model	4	12387	3097	22.99	0.0001
Error	10	1347	135		
Corrected total	14	13734			
LS Means	1	2	4	8	
0	0.0001	0.0001	0.8151	0.887	
1		0.96	0.0001	0.0001	
2			0.0001	0.0001	
4				0.7077	
8					

Table 3. Summary of measurements of growth and survival of *Holothuria scabra* larvae at different concentrations of the algae *Isochrysis galbana.*

Conc. x 10^4 cell/ml	Length µm/day Day 10	Growth rate µm/day	Initial no. per litre	Final no. per litre	% Survival
0	654	2.64	478	263	55
1	899	32	462	373	81
2	866	32	437	467	107
4	642	3.78	468	294	63
8	568	1.95	433	293	68

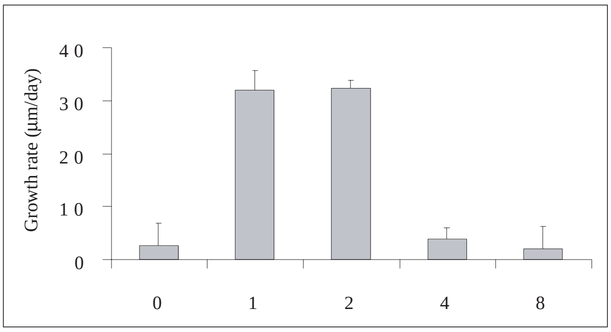

Figure 3. Mean growth rate (n = 3) of *Holothuria scabra* larvae measured over eight days at concentrations of 0, 1, 2, 4, and 8 x 10^4 cells / ml of the algae *Isochrysis galbana* (mean ±S.E.).

Development

Highest proportions of late auricularia larvae were observed at 1 and 2 x 10^4 cells / ml on day ten while little or no development to late auricularia occurred in other algal concentrations (Fig. 4). On day eight there was a slight increase in the numbers of early auricularia larvae recorded at 4 and 8 x 10^4 cells / ml due to inaccuracies in distinguishing between early and abnormally developing larvae.

After two days, only early auricularia were present in all concentrations. Mid-auricularia were seen at varying levels in all concentrations from days four to ten, and late auricularia was observed only at days eight and ten (Fig. 4). From four to ten days the proportion of mid-auricularia larvae did not change markedly. However, early auricularia were observed less frequently at 1 and 2 x 10^4 cells / ml.

Larval quality

The physical appearance of larvae differed between algal concentrations. The appearance of larvae was assessed to provide an indicator of the effects of the culture environment on larval quality (Fig. 5). Early auricularia appeared uncompromised during the first few days in each algal concentration.

However, by the time larvae reached mid-auricularia there were differences in larval form (Fig. 5; a1, b1, and c1). Two features were obvious in distinguishing the quality of larvae; the appearance of the dermis of larvae and the curvature and degree of folding in the lateral arms. A minimal amount of folding and a pointed apex, together with an anterior-posterior contraction of the body length indicated an extreme of larval abnormality (Fig. 5; c, c1 and c2). This occurred predominantly at 8 x 10^4

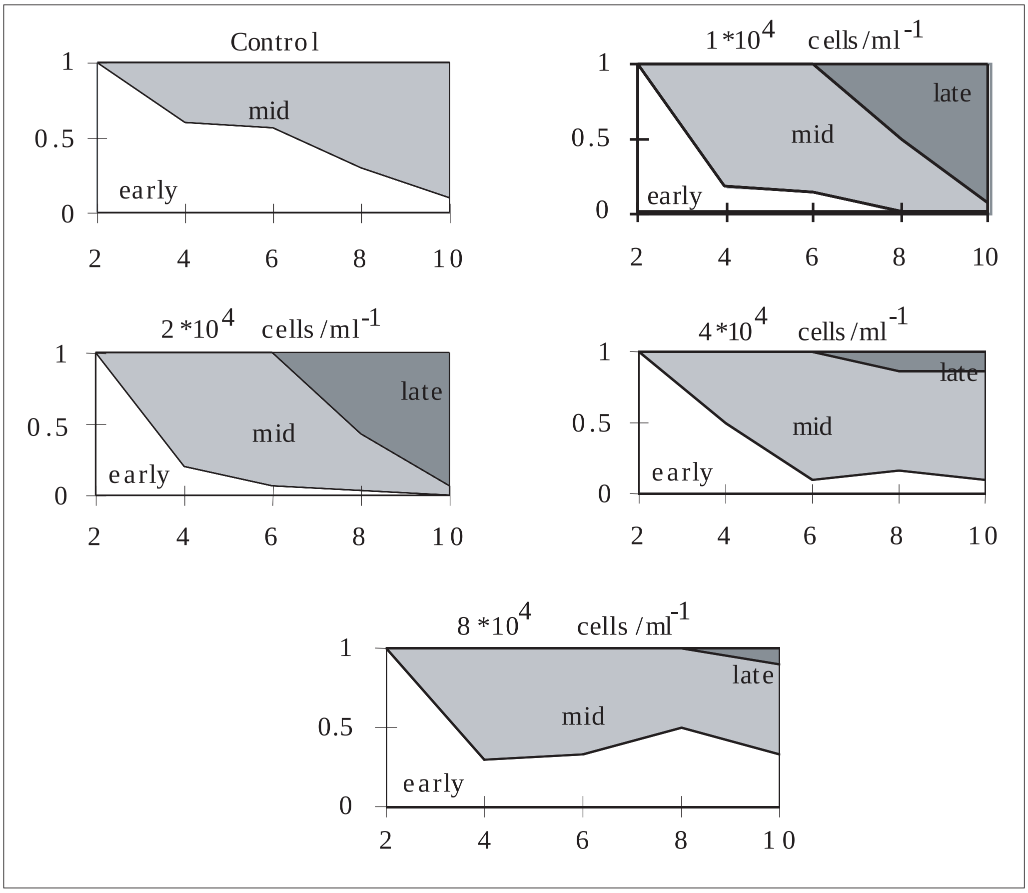

Figure 4.

Development of *Holothuria scabra* larvae observed over ten days at concentrations of 0, 1, 2, 4, and 8 x 10^4 cells / ml of the algae *Isochrysis galbana*, (three replicates pooled for each concentration; mean ±S.E.; n = 30 to 50 larvae per day / bowl).

cells / ml but was less frequent at other concentrations. Discolouration of the dermis could be seen (Fig. 5; b1 and b2) but did not always occur with other abnormalities described above. Some variation in pH occurred at 4 and 8 x 10^4 cells / ml (7.5 to 8.5), which may have reflected variation in the level of un-ionised ammonia, affecting the quality of larvae.

Early stages of *H. scabra* larvae were affected by the placement of the aeration pipette as they were not strong enough to swim out of eddies created at the base of the bowl. The pipette was situated about 2 cm off the bottom of each bowl and larvae were placed in bowls at 60 to 72 hour's post-fertilisation as they were better able to maintain their position in the water column.

Discussion

In the present study optimal levels of algae for maintaining the quality of larvae in culture were 1 and 2 x 10^4 cells / ml or 2 to 4 x 10^4 cells / larva / day. Increasing the algal concentration to 4 and 8 x 10^4 cells / ml did not result in increased growth and development but compromised the quality of larvae and the culture environment.

It was likely that the maintenance of excess concentrations of algae disrupted the process of filtration, ingestion and digestion in *H. scabra* larvae. When observing the state of the gut of *H. scabra* larvae, James et al. (1994) considered a concentration of 2 to 3 x 10^4 cells / ml to be optimal for growth and development. Ito (1995) states that in the early devel-

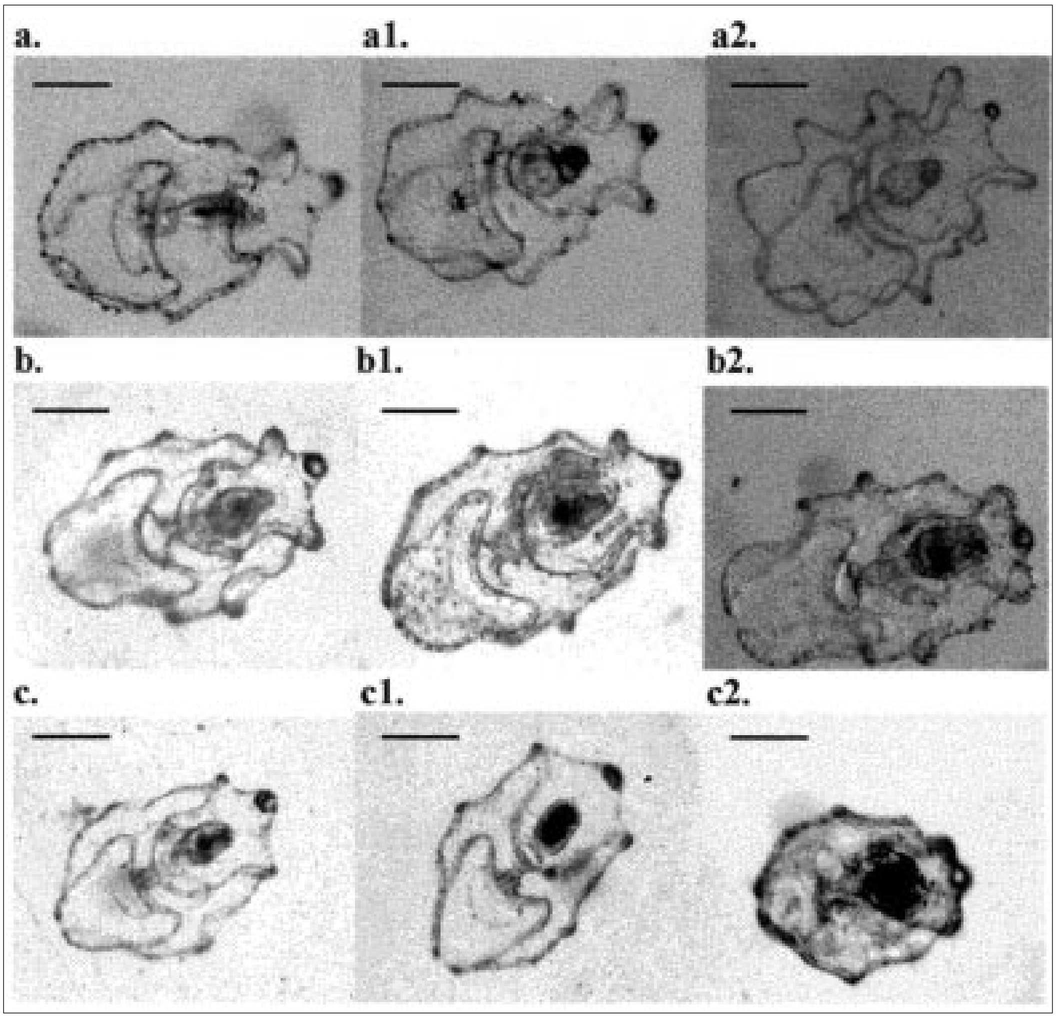

Figure 5.

Quality of *Holothuria scabra* larvae reared in different concentrations of the alga *Isochrysis galbana*. a: normally developing larvae at early, mid-and late auricularia in moderate amounts of algae (1 and 2 x 10^4 cells / ml). b; abnormal development of larvae at increased algal concentrations (4 x 10^4 cells / ml). c: abnormal development of larvae in excessive amounts of algae (4 x 10^4 cells / ml; scale bar approximately 200 µm).

opment of larvae of the sea cucumber *Stichopus japonicus*, a concentration of 0.5 x 10^4 cells / ml was adequate but that this was increased to around 3 x 10^4 cells / ml late in the larval cycle. It was likely that the survival of *H. scabra* larvae in the absence of algae resulted from the use of stored nutrients. Archer (1996) found that larvae of the sea cucumber *S. mollis* eventually stopped feeding when concentrations of algae continuously exceeded 0.6 x 10^4 cell / ml as ingestion rates peaked at 18.2 cells / min at this level.

Ito (1995) indicated that the growth in length and width of the stomach may be an important indicator of larval quality especially late in development. In the present study the shape of the gut of most larvae in moderate amounts of algae was spherical, but in increased amounts of algae this was more variable and the gut was often contracted along the lateral axis (Fig. 5 c1 and c2).

Some asymmetry in development of the lateral folds of larvae was evident in excess amounts of algae (Fig. 5 b and c). If larvae were not developing normally, there was a greater chance of observer error in recording developmental stages as early, mid- or late auricularia. The lack of a skeletal structure in holothurian larvae made it difficult to distinguish transition to subsequent larval stages. In the present study large numbers of abnormally developing larvae stayed in the water column for the duration of the experiment and in some instances exhibited normal developmental features but had contracted lateral folds. In cultures containing excess food the quality of the lateral folds, symmetry of shape, growth in total length and the shape of the gut best indicated the response of larvae to the culture environment.

The observation and recording of development of the left and right somatocoel was difficult in cultures containing excess food. In the early auricularia larva the hydrocoel is connected to the left somatocoel, which breaks from the hydrocoel during larval development and divides into the left and right somatocoel (Balser et al. 1993). The timing of breakage of the left somatocoel from the hydrocoel and the right somatocoel from the left somatocoel may indicate the transition of larvae to subsequent stages of auricularia development.

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Sea cucumber retail market in Singapore

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The retail market for sea cucumbers is active in Singapore and many shops are selling diverse dried sea products (Fig. 1). The price for sandfish *Holothuria scabra* was around SGD 180 per kg in October 2000 [Note from editor 1SGD ≈ 0.58 USD].

In the main town market, there are many sellers of sea cucumbers already soaked. It is difficult to determine the species in that case. They sell for between SGD 14 and 18 per kg (Fig. 2) and an assortment of 6-7 pieces at SGD 2 (Fig. 3).

Figure 1.

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