Settlement preferences and early migration of the tropical sea cucumber *Holothuria scabra*

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Abstract

Settlement and post-settlement processes of the sea cucumber *Holothuria scabra* Jaeger were studied in the laboratory. Independent and paired choice experiments revealed that several substrates could induce metamorphosis into pentactulae, but that specific substrates favoured settlement. Leaves of seagrass *Thalassia hemprichii*, with or without their natural bio-film, yielded the highest settlement rates (4.8–10.5%). *T. hemprichii* was preferred as a settlement substrate over sand, crushed coral, several other plant species and artificial seagrass leaves with or without a bio-film. Only settlement on the seagrass, *Enhalus acoroides*, was similar to that recorded for *T. hemprichii*. In the absence of a substrate, the larvae delayed settlement for nearly 96 h and survival was less than 0.5%. Sand and crushed coral, either alone or together, induced settlement from < 1.5% of the available larvae. The pentactulae found on sand, coral and in bare containers were 10–35% smaller than those on *T. hemprichii* leaves. Soluble extracts from *T. hemprichii* and *E. acoroides* successfully induced metamorphosis and settlement on clean plastic surfaces. Newly settled juveniles remained on the seagrass leaves for 4–5 weeks before migrating to sand at around 6 mm in length. Prior to this, the juveniles spent 4–5 days moving on and off the leaves. Once on the sand, the juveniles became deposit-feeders, but did not show the typical burrowing behaviour of older specimens until they reached around 11 mm in length. The larvae of *H. scabra* appear to actively select seagrass leaves, possibly through chemical detection. We hypothesise that larvae settling on seagrass have an increased chance of growth and survival because they are provided with a suitable substrate on which to grow, and a bridge to sand substrates as they become deposit-feeders. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

For marine invertebrates with planktonic development, the selection of a suitable habitat by the settling larvae often determines the long-term survival of juveniles and adults (Barker, 1977; Pearce and Scheibling, 1990). This is especially true for sessile species, but also applies to sedentary species with limited ability to relocate from one patch of nursery habitat to another (Gaines and Roughgarden, 1985; Underwood and Fairweather, 1989).

Consequently, the larvae of a wide range of marine invertebrates do not metamorphose and settle unless they encounter specific conditions, ensuring that the correct environment is selected for growth and survival (see review in Giese et al., 1991). Typically, such larvae exhibit a characteristic, and broadly similar, searching behaviour and may react to the physical, chemical or biological features of the substrate, as well as to conspecifics (Barker, 1977; Young and Chia, 1982; Burke, 1983; Butman et al., 1988; Morse, 1990; Pawlik, 1990; Rodriguez et al., 1993). Many invertebrate larvae also exhibit the ability to delay metamorphosis until a suitable substrate is located (Strathmann, 1978; Mladenov, 1985; see review in Giese et al., 1991).

Among echinoderms, the settlement behaviours of ophiuroids, echinoids and asteroids have been the most widely studied. Caspers (1980) demonstrated that the larvae of the brittlestars Ophiura albida and O. ophiura, which occur together in the plankton, settle in different types of sediment. Hinegardner (1969) and Cameron and Hinegardner (1974) observed that laboratory-reared, regular echinoid larvae underwent metamorphosis in response to a microbial film. Similarly, field experiments on larval settlement in Strongylocentrotus franciscanus demonstrated that surfaces with their resident film removed did not induce metamorphosis, whereas settlement did occur on intact films (Cameron and Schroeter, 1980). Henderson and Lucas (1971) and Yamaguchi (1973) found that the larvae of starfish, Acanthaster planci, settled preferentially on coralline algae. Johnson et al. (1991) found the same preference and proposed that the larvae of the species responded to a chemical cue provided by bacteria. Settlement on coralline algae has also been reported for Stichaster australis (Barker, 1977). Birkeland et al. (1971) showed that the larvae of the starfish, Mediaster aequalis, needed to find polychaete tubes to settle, whereas the larvae of Coscinasterias calamaria (Barker, 1977) and Asterias rubens (Barker and Nichols, 1983) settled on any hard substrate covered with a primary film.

Investigations of settlement behaviour in holothuroids are comparatively scarce. Hamel and Mercier (1996) demonstrated that the settling larvae of the sea cucumber, Cucumaria frondosa, favoured gravel and rock surfaces, whereas Young and Chia (1982) indicated that pentactula larvae of another temperate species, Psolus chitonoides, settled gregariously near adults. Also, cultured sea cucumbers Stichopus japonicus and Holothuria scabra are induced to settle with variable success on plates coated with
benthic diatoms, bacterial films and algal extracts (Ito and Kitamura, 1997; Yanagisawa, 1998). Although it seems clear that an active choice occurs during the settlement of holothuroids, there is little information on the processes determining their settlement (Chia and Walker, 1991; Smiley et al., 1991).

The factors affecting survival of echinoderms after settlement have received even less attention than settlement processes, even though it is widely accepted that habitat type, food availability and predation may influence the recruitment and final distribution of newly settled juveniles (Gosselin and Qian, 1997; Hunt and Scheibling, 1997). Keesing and Halford (1992) have outlined the relative importance of such factors on the recruitment of the starfish, *A. planci*, and Zann et al. (1987) monitored post-settlement of a cohort of *A. planci* for 2 years in the field. The response of post-settled holothuroids to environmental pressures and predators, and the need for post-settlement migration, have seldom been investigated. Migration of newly settled juveniles, moving from sheltered nursery areas to the exposed adult habitat, was demonstrated in the northern sea cucumber, *C. frondosa* (Hamel and Mercier, 1996), whereas the larvae of *P. chitonoides* were found to settle initially on adults and relocate in nearby shaded habitats (Young and Chia, 1982).

Sea cucumbers, *H. scabra*, are deposit-feeders, widely distributed in the Indo-Pacific and mainly found in sandy to muddy coastal zones. This important commercial species is in world-wide decline and has now been cultured successfully in four countries for the purpose of farming or stock enhancement programs (Battaglene and Bell, 1999). Our field observations in the Solomon Islands indicate a size-specific distribution for *H. scabra*: only individuals > 10 mm were found on soft substrates, whereas newly settled juveniles appear to occur on the leaves of seagrass adjacent to populations of individuals > 10 mm (Mercier et al., in press).

The aim of this research was to identify factors that induced metamorphosis of *H. scabra* doliolaria larvae into pentactulae and their subsequent settlement. We also studied settlement behaviour and determined that larvae have the ability to discriminate among several natural and artificial substrates. Finally, we studied post-settlement migration of the juveniles to obtain a better understanding of the observed field distribution.

2. Materials and methods

2.1. Collection of broodstock and larval rearing

Adult *H. scabra* were collected by snorkelling or SCUBA diving at a depth of 2 m along Kohinggo Island in the Vonavona lagoon, Western Province, Solomon Islands (8°10’S, 157°11’E). The animals were then transported in individual plastic bags within insulated containers to a 1000-l tank within 8 h. The broodstock spawned spontaneously, or were stimulated to spawn using thermal shocks. Spawning females were isolated and their oocytes collected from the tank and fertilised without delay using freshly collected sperm (ca. 4 × 10⁵ spermatozoa ml⁻¹). Developing embryos were kept in 500-l tanks and fed micro-algae (*Chaetoceros muelleri*, *C. calcitrans*, *C. salina* and *Rhodomonas salina*) at 20 000–30 000 cells ml⁻¹ until they reached the auricularia and early
doliolaria stage ca. 10 days after hatch. At that stage, the ciliated larvae were still highly mobile for a minimum of 48 h before settlement. The larvae were considered to have settled when they reached the late pentactula stage with the total disappearance of the ciliae and the development of two tube feet as described by Hamel and Mercier (1996) for *C. frondosa*.

2.2. Experimental design

The study was conducted between September 1997 and June 1998 at the Center for Living Aquatic Resources Management (ICLARM) in the Solomon Islands. The experimental unit consisted of 40 round, polyethylene containers of 1.2 l (15 cm in diameter), distributed in a large water bath to stabilise temperature. Each container was supplied with filtered (1 μm) sea water in flow-through at a rate of 3.5 l h⁻¹. The water was dropping into the container and the outflow was through two small holes, sheathed with 1-μm mesh, on each side of the container. The experimental unit was illuminated by four fluorescent lights adjusted to a 12-h light:12-h dark cycle with an intensity of 65 μmol m⁻² s⁻¹ at the surface of the containers. During the experiments, the salinity fluctuated between 33 and 34 and the temperature between 26 and 29°C. The containers were rinsed for a week in sea water prior to each experiment.

The settlement preferences of *H. scabra* larvae were tested using different substrates, plants and plant extracts. There were three replicate containers for each treatment. Different substrates were tested against each other in pair-wise comparisons to allow determination of preferred settlement under reciprocal influence. Three independent replicates of each substrate were also tested to determine the settlement of larvae in the absence of other substrates. All substrates were used within 24 h of collection. A total of ca. 550 larvae in the auricularia (65.7±3.8%) and early doliolaria (34.3±3.8%) stage were released into each container. Data shown in the methods are given as mean±S.D. The larvae were fed three times a day throughout the experiment, using 25 ml of a mixed diet of diatoms, *C. simplex* and *C. muelleri*, to obtain a final concentration of ca. 25 000 cells ml⁻¹ in each container.

2.3. Settlement preferences between different substrates and seagrass leaves

Our first goal was to determine if *H. scabra* larvae were able to demonstrate a preference toward any of the substrates observed in habitats where adults occurred in the Solomon Islands: *Thalassia hemprichii* seagrass, sand and crushed coral.

The term ‘clean’ applies to any substrate from which the bio-film, and encrusting plants and animals, were removed. This was done by gentle scrubbing, followed by bathing for 30 min in freshwater, and 5 min in 10% methanol, and then rinsing in freshwater for 10 min. The cleaning procedure was used for some *T. hemprichii* leaves and artificial seagrass leaves, which were used later to test the physical role of the substrate on settlement preference. Otherwise, the plants used were supporting a bio-film with flora and fauna intact. Sand (0.36±0.15 mm in grain diameter) and crushed coral (4.58±1.43 mm) substrates were washed thoroughly for 3 h in running sea water and heated at 500°C for 5 h to reduce their organic matter content. When tested against each
other in the same treatment, the two substrates were spread within the containers in two semi-circles, covering the entire bottom, which had a total surface area of ca. 175 cm². In similar tests between seagrass and sand, the containers were covered uniformly with a thin layer of sand and seagrass leaves were planted in half of the container (total leaf surface area of ca. 40–50 cm²). Similar procedures were used when testing combinations of seagrass and crushed coral. Each substrate was also tested independently. Independent testing of seagrass leaves consisted of placing three floating leaves, with a total average surface area of 70–80 cm², into the container. The treatment with 3 ml of sand was used to test the possible influence of chemical cues in the otherwise bare containers as opposed to containers filled with sand. Sand collected under adult H. scabra was also tested, without rinsing or burning, and is referred to as adult-conditioned sand. The sand used during all experiments was the substrate of choice for juvenile H. scabra > 10–140 mm during a previous study (Mercier et al., 1999). Clean containers filled only with filtered sea water were used as controls.

To minimise the possibility of an orientation effect, the three replicate containers were each oriented differently. All containers were distributed randomly within the water bath. In all cases, the surface of the plastic container was an inherent condition, but its impact on larval settlement was minimised by cleaning the sides every day to remove the developing bio-film. In addition, each experiment lasted < 96 h to minimise the confounding effect of bio-films developing on the clean substrates. The few larvae found on the sides of the containers at the end of the experiment were omitted from the analyses.

2.4. Settlement preferences between seagrass leaves and other plants

The results obtained during the first experiment indicated clearly that larvae settled preferentially on the leaves of T. hemprichii. The second experiment therefore focused on assessing whether: (1) all plant substrates found in soft bottom or hard bottom habitats would be accorded a similar preference; and (2) there were physical and chemical cues involved in the substrate selection.

2.4.1. Plant species of soft bottom habitats

Like T. hemprichii, the seagrass Enhalus acoroides, the calcareous alga Halimeda gracilis and the alga Caulerpa racemosa occur in the natural habitat of H. scabra. Floating leaves or fronds of these other plant species were presented to settling larvae, alone or in combinations, as described for seagrass leaves, but the surface area could only be determined accurately for E. acoroides.

2.4.2. Plant species of hard bottom habitats

Plants of hard bottom habitats, absent from the typical habitat of H. scabra, were also tested. Sargassum cristaefolium, Boodlea coacta and Ectocarpus breviarticulatus were collected from rocky habitats. Again, the surface area offered by a given substrate could only be estimated accurately for seagrass leaves.
2.4.3. Artificial seagrass

Artificial seagrass leaves were cut out of soft semi-transparent polyethylene plastic. Conditioned artificial seagrass leaves were left for 2 months in an outdoor tank supplied by running sea water and, at the time of use, were covered by a rich fouling layer composed of a variety of encrusting coralline algae and microscopic animals, mainly bryozoa. Artificial seagrass leaves were cut to provide a similar surface area and shape to the natural seagrass leaves.

2.5. Experimental procedures

During the first experiment, development of larvae, mortality and settlement were monitored in two of the three replicates of each treatment following initial stocking and every 24 h until definitive settlement, whereas only final settlement was determined in subsequent experiments. To assess the abundance of the larval stages present, a sub-sample of 120 ml of water was collected. In addition, whenever applicable, either half the leaves, half the surface area of sand, 10 pieces of crushed coral or 16 cm of the bottom surface area of the container were examined for settling larvae under a binocular microscope. These data were then extrapolated to the total respective surface areas or volumes. When no more pelagic and early benthic stages were observed in the samples, we counted the remaining larvae on the substrates in all three replicates. The sand was sieved and inspected while each leaf, coral fragment and plant surface was examined thoroughly under a binocular microscope. During the first experiment, the size of the settled pentactula was recorded as the distance between the anus and the mouth with an ocular micrometer at a magnification of 40×.

2.6. Metamorphosis and settlement cue

An experiment was conducted to determine whether: (1) seagrass extracts would be enough to induce metamorphosis and settlement, as opposed to previously tested physical contact; and (2) there was a variation in the number of larvae settling in response to extracts of two seagrass species and an alga that had previously been proved to be a poor settlement substrate. About 550 larvae in the auricularia and doliolaria stages were stocked into each of 12 clean 1.6-l containers. After 40 h, the proportions of larval stages in the different stages of development was established in each container as described previously. Extracts of clean T. hemprichii, H. gracilis and E. acoroides were then added to nine of the containers chosen at random, using three replicates for each extract, and three containers left without addition of extracts as controls. The extracts were prepared by macerating the clean grounded leaves (ca. 195–205 cm²) in 25 ml of sea water and filtering the solution on Nitex mesh (1.0 μm). Fifty ml of sea water were added for rinsing. A 10-ml aliquot of the crude extract was then added to each container. The proportion of larval stages, and the initial survival rate, were recorded for each container, including the controls, at the beginning of the experiment, then again after 3, 6, 12, 21 and 24 h, using the technique described above.
2.7. Settling behaviour and post-settling migration

Three 1.2-l containers and two larger ones (75 l, ca. 52 × 33 cm), each filled with shoots of *T. hemprichii* anchored into sand, were used for this series of observations. Each container initially received some 550 larvae as described previously.

Samples of larvae were observed daily under a binocular microscope to assess the behaviour of the late doliolariae and early pentactulae before settlement. We noted whether the larvae were swimming or in contact with the substrate, and the different stages of exploration with the feeding and ambulacral podia. Observations continued until all larvae had settled. We monitored the general behaviour and migration of newly settled *H. scabra* juveniles in the laboratory because they were not found on the sand with the adults in the wild until they reached 10–20 mm in length (Mercier et al., in press).

The behaviour and movement of settled juveniles were monitored for 2 months by inspecting the seagrass and sieving about 10% of the sand present in the container every 12–24 h. Small, newly settled sea cucumbers were detectable with the naked eye. The plastic sides of each container were cleaned of bio-film every day to minimise their attractiveness as a feeding substrate and hence avoid a biased response from the juveniles searching for food. As they moved onto the sand, we recorded the behaviour of juveniles, including their feeding status, as indicated by the presence of faecal pellets, and their ability to burrow. The length of the sea cucumber was recorded as the distance between the mouth and the anus in fully extended animals using a caliper.

2.8. Data analysis

The survival rate of each larval stage was expressed as the proportion of the estimated total number released at the beginning of the experiment. The survival rate corresponded to the settlement rate when settled pentactulae were counted at the end of an experiment. The abundance of each larval stage was estimated by multiplying the number of larvae, found either in the 120-ml sample of water or substrate aliquots, by the appropriate factor. In the initial trial, the development of larvae was monitored in two of the three replicates for each treatment. Although it was not possible to determine statistically if disturbed and undisturbed replicates were significantly different, scatter plots of the data revealed no such differences. Results were expressed as mean±S.D. for the three replicates of each treatment.

Settlement data were analysed using analysis of variance. Normality and homogeneity of variance were evaluated using Kolmogorov–Smirnov and Cochran’s tests, respectively, and the data were transformed using square root of $x$ where necessary. Student–Newman–Keuls (SNK) multiple range tests were used for a posteriori comparisons among treatments where appropriate. Paired and Student $t$-tests were used to compare settlement between paired substrates and the mean sizes reached by the larvae on different substrates. For these tests, normality was determined by Kolmogorov–Smirnov test and homogeneity of variance was evaluated using Levene’s test. In the cases where heterogeneity of variances occurred, a Mann–Whitney $U$-test was performed.
3. Results

3.1. Metamorphosis and settlement

During the late doliolaria and early pentactula stages, the larvae repetitively touched the substrate for hours each day, crawling and exploring the bottom with their five buccal podia. Given the relatively short monitoring period of 1 h, no behavioural difference was noted between night and day. Overall, late doliolaria larvae spent 84±3% of the time swimming and attached to the substrate only for short periods of time, around 60±35 s. Doliolaria larvae were estimated to move ˃3 m h⁻¹ under static laboratory conditions. Early pentactula larvae lost their ciliated band and their capacity to swim. They moved by small successive jumps, spending 93±2% of their time attached to the substrate, restricted roughly within 1 cm² and exhibiting only minimal movements. Once settled using their ambulacral podia, the larvae moved on the substrate and fed using their feeding podia. Most of the time, four buccal podia were attached to the substrate and one was transferred to the mouth. At this stage, the larvae were negatively phototaxic and migrated to the shaded side of the substrate. Feeding of older pentactula larvae was continuous for periods of 2.1±0.5 h, separated by intervals of immobility lasting 30 min to 1 h.

The doliolaria and pentactula larvae responded differently to a variety of settling conditions (Fig. 1). Metamorphosis and settlement were delayed for nearly 96 h in the absence of an adequate substrate. Ultimately, the vast majority of larvae did not reach the late auricularia and pentactula stages in control containers and died before settlement (Fig. 1). Metamorphosis into pentactulae occurred after ca. 48 h in the presence of crushed coral, 3 ml of sand or complete sand cover, whether conditioned or not (Figs. 1 and 2), but settlement rates remained comparable to controls (one-way ANOVA, F = 19.0, df = 10.22, P < 0.0001; SNK test, P > 0.05). Rates of settlement were greater on all other substrates and substrate combinations than for the controls (SNK test, P < 0.05). Overall, significantly higher proportions of settled pentactulae (ca. 4.8–10.5%) were observed whenever *T. hemprichii* leaves were present (SNK test, P < 0.05). Cleaned and uncleaned seagrass leaves yielded equally high settlement rates when present in a combination (SNK test, P > 0.05).

3.2. Settlement preferences between different substrates and seagrass leaves

Settlement was consistently greater on *T. hemprichii* leaves than on all other substrates (Fig. 2). When clean and uncleaned leaves were offered in separate trials, a greater number of pentactulae were recovered from the former (one-way ANOVA, F = 21.4, df = 6,14, P < 0.0001; SNK test, P < 0.05). However, when clean and unclean leaves were offered to larvae simultaneously, there was no significant difference in settlement (paired t-test, P = 0.640). The number of settled pentactulae on sand and crushed coral was consistently low (<20 larvae), regardless of whether they were offered as a single substrate or in combination with another (Fig. 2). There was no significant difference in abundance of pentactula larvae on adult-conditioned sand and ordinary sand (SNK test, P > 0.05). However, the presence of 3 ml of sand resulted in
Fig. 1. *Holothuria scabra*. Abundance of larval stages (mean±S.D.) on different substrates during settlement. The final settlement rate (%) is indicated as a mean for the three replicates of each treatment.
significantly greater settlement than complete coverage with ordinary or adult-conditioned sand (SNK test, $P < 0.05$). Crushed coral supported significantly greater settlement than sand, both in paired comparisons (paired $t$-test, $P = 0.033$) and when essayed alone (SNK test, $P < 0.05$).

3.3. Settlement preferences between seagrass and other natural or artificial plants

In the trial comparing settlement on different species of plants from soft-bottom habitats, pentactula larvae settled preferentially on seagrass leaves (Fig. 3). Among single substrate treatments, there was significantly greater settlement on $E$. acoroides compared to $C$. racemosa and $H$. gracilis (one-way ANOVA, $F = 27.8$, df = 2.6, $P = 0.0009$; SNK test, $P < 0.05$). Pairwise comparisons of combination assays showed...
that *T. hemprichii* leaves were preferred to *C. racemosa* and *H. gracilis* (paired t-tests, $P < 0.05$), whereas there was similar settlement on *T. hemprichii* and *E. acoroides* (paired t-test, $P = 0.585$; Fig. 3).

Very few late pentactulae settled on plants from hard substrates compared to the numbers settling on *T. hemprichii* leaves (Fig. 4). In single treatments, *B. coacta* received more larvae than *S. cristaefolium* (one-way ANOVA, $F = 8.42$, df = 2.6, $P = 0.0181$; SNK test, $P < 0.05$), but similar settlement to *E. breviarticulatus* (SNK test, $P > 0.05$).

Conditioned artificial seagrass was an effective substrate for settlement of *H. scabra* larvae but clean artificial leaves were not (Fig. 5). Significantly more larvae settled on *T. hemprichii* than conditioned artificial seagrass in paired comparisons (paired t-tests, $P < 0.05$). However, there was no significant difference in settlement of *H. scabra* larvae on conditioned artificial seagrass and the alga *C. racemosa* (paired t-test, $P < 0.5$).
3.4. Effect of plant extracts

Settlement varied significantly among containers given different plant extracts (one-way ANOVA, $F = 248.3$, df = 3.8, $P < 0.0001$; Fig. 6). *H. gracilis* extracts did not induce high rates of settlement; the larvae remained pelagic until they died, responding similarly to the larvae that were kept under control conditions (SNK test, $P > 0.05$). However, extracts of *E. acoroides* and *T. hemprichii* induced a similar response (SNK test, $P > 0.05$), both yielding significantly higher settlement rates than controls or *H. gracilis* after 24 h (SNK test, $P < 0.05$). Nonetheless, the fastest response was obtained when the larvae were exposed to extracts from *T. hemprichii* (Fig. 6): the larvae began to settle ca. 6 h after the first addition of extract and 100% of individuals were found on the bottom after 21 h. In comparison, few larvae exposed to extracts from *E. acoroides* were still floating after 24 h (Fig. 6).
3.5. Effect of substrate on growth

Pentactulae that settled on cleaned and uncleaned *T. hemprichii* leaves were of the same average size at the end of the experiment (Student *t*-test, *P* > 0.05; Table 1). Pentactulae that settled on sand and crushed coral were also the same average size (Mann–Whitney *U*-test, *P* > 0.05). However, pentactulae from *T. hemprichii* leaves were about 10–35% larger than those from all other substrates, including sand and coral. This difference in size was significant (Mann–Whitney *U*-test, *P* > 0.05) (Table 1).
3.6. Post-settling migration

After 16–17 days of pelagic stages, the larvae of *H. scabra* settled on the leaves of the seagrass *T. hemprichii* and remained there for about 12 days until they reached a size of 3.9±0.4 mm long (Fig. 7). The next ca. 6 days were spent moving between the leaves and the sand until day 35 after fertilisation. A negative phototaxic response was not observed during this period. The juveniles of 6.1±0.3 mm long were associated only with the sandy habitat, although the characteristic burrowing behaviour of the adults and
Table 1

*Holothuria scabra*: size and survival rate of late pentactulae settled under different conditions

<table>
<thead>
<tr>
<th>Conditions tested</th>
<th>Length (μm)</th>
<th>n</th>
<th>Survival rate in bowl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean <em>T. hemprichii</em></td>
<td>592.8±91.1</td>
<td>83</td>
<td>10.5±1.9</td>
</tr>
<tr>
<td>vs <em>T. hemprichii</em></td>
<td>574.1±86.7</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Clean <em>T. hemprichii</em></td>
<td>594.3±89.1</td>
<td>88</td>
<td>5.2±1.7</td>
</tr>
<tr>
<td>vs sand</td>
<td>500.0±50.0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><em>T. hemprichii</em> vs sand</td>
<td>578.5±88.6</td>
<td>100</td>
<td>6.0±2.3</td>
</tr>
<tr>
<td>Clean <em>T. hemprichii</em></td>
<td>577.9±103.5</td>
<td>122</td>
<td>8.3±0.7</td>
</tr>
<tr>
<td>vs crushed coral</td>
<td>521.9±103.2</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td><em>T. hemprichii</em> vs crushed coral</td>
<td>575.0±84.0</td>
<td>78</td>
<td>4.8±1.1</td>
</tr>
<tr>
<td>vs crushed coral</td>
<td>470.0±57.0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Sand</td>
<td></td>
<td>0</td>
<td>1.1±0.5</td>
</tr>
<tr>
<td>vs crushed coral</td>
<td>415.8±55.4</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td><em>T. hemprichii</em></td>
<td>625.9±93.2</td>
<td>111</td>
<td>6.7±1.5</td>
</tr>
<tr>
<td>Crushed coral</td>
<td>419.6±49.4</td>
<td>23</td>
<td>1.4±0.6</td>
</tr>
<tr>
<td>Adult-conditioned sand</td>
<td>421.8±60.1</td>
<td>4</td>
<td>1.2±0.7</td>
</tr>
<tr>
<td>Sand</td>
<td>450.0±100.0</td>
<td>3</td>
<td>0.2±0.8</td>
</tr>
<tr>
<td>Three-ml sand</td>
<td>375.0±143.6</td>
<td>7</td>
<td>0.8±0.4</td>
</tr>
<tr>
<td>Water only</td>
<td>416.7±60.6</td>
<td>6</td>
<td>0.5±0.5</td>
</tr>
</tbody>
</table>

The number (n) of pentactulae measured on each substrate is shown and survival rate was calculated for three replicate containers. All data as mean±S.D.

![Fig. 7. *Holothuria scabra*. Growth (mean±S.D., n = 38–183) and behaviour of settling larvae and newly settled juveniles over time.](image-url)
larger juveniles (Mercier et al., 1999) was recorded only when individuals reached ca. 11 mm, approximately 45 days after fertilisation (Fig. 7).

4. Discussion

Our data are consistent with the conclusion that larvae of *H. scabra* demonstrated relatively high selectivity of substrates upon settlement. In particular, they settled preferentially on the leaves of *T. hemprichii* or *E. acoroides*, two seagrass species that occur on the margins of habitats occupied by adult *H. scabra* in the Solomon Islands. This finding matches the results of field studies in which we were able to find newly settled sea cucumbers in the wild by collecting and examining seagrass leaves (Mercier et al., in press).

Does the greater abundance of pentactula larvae on seagrass leaves actually represent selection, or is it due to differential survival? There are three reasons to conclude that it truly represents selection. First, there were no predators in the containers and the larvae were fed throughout the experiment so it is unlikely that there was predation or starvation among treatments. Second, the rate of mortality during experiments was similar to that recorded in routine culture of *H. scabra* larvae at that time (S. Battaglene, unpubl. data). Third, similar numbers of larvae were found on seagrass occupying 100 or 50% of the container, indicating that abundance was not a function of surface area of the substrate.

The settlement results were also consistent between experiments. For example, treatments tested on several occasions, such as the bare control containers, clean and fouled *T. hemprichii*, and *C. racemosa* yielded similar numbers of settled larvae on each occasion. There was strong evidence for a preference pattern: true seagrass leaves were favoured over artificial conditioned ones, although the latter yielded a good settlement rate. Other plant species were comparatively poor settlement inducers, but better than clean plastic surfaces and sand. Finer discriminative abilities between non-optimal substrates were difficult to establish, possibly due to the influence of gregarious settlement. As demonstrated by Gotelli (1990), the propensity of larvae to settle gregariously can affect statistical evidence in substrate selection trials when the preference is weak or moderate. However, strong larval selection remains virtually unaffected by this behaviour (Gotelli, 1990).

4.1. Delay of metamorphosis and settlement

The persistent presence of swimming larvae in control containers deprived of a well established bio-film strongly infers that *H. scabra* larvae were able to delay metamorphosis and settlement when no suitable substrate was available. This behaviour has previously been observed in echinoids (Cameron and Hinegardner, 1974; Tani and Ito, 1979; Highsmith, 1982) and asteroids (Birkeland et al., 1971). Our study demonstrates that *H. scabra* can postpone settlement for a relatively short period (< 96 h) compared to the echinoderms listed above, and to other holothuroids, such as *S. californicus* and *C. frondosa* whose larvae survive for up to 10 or 8 days, respectively, in the absence of
substrate under laboratory conditions (Smiley et al., 1991; Hamel and Mercier, 1996). The warm temperature at which *H. scabra* were reared, and the rapid embryonic development compared to polar and temperate species, could explain the relatively short window during which larvae remain competent to settle. However, larval competency of *H. scabra* in large 500-l hatchery tanks is up to a week and compares better to other species, possibly because the environment is less restrictive and more efficiently buffered.

Although metamorphosis may be induced by a ‘preferred’ substrate that aids survival later in life, it is likely that a small portion of larvae will settle on any type of surface. A small proportion (ca. 0.5–1.5%) of *H. scabra* larvae always settled during our trials, although this proportion was usually an order of magnitude lower than the rates observed on the preferred substrate. Settlement on suboptimal substrates by species that express a clear substrate preference was also observed in sea urchins (Highsmith, 1982; Burke, 1984; Pearce and Scheibling, 1990) and starfish (Johnson et al., 1991).

### 4.2. Induction of metamorphosis and settlement

Preferential settlement is likely to begin with a specific cue associated with a given substrate, habitat or assemblage that stimulates metamorphosis. In *H. scabra*, metamorphosis into pentactulae was induced by the presence of several of the natural and artificial substrates presented. During direct exposure to sand, coral, plants and even conditioned artificial substrates, a minimum of 60% of the larvae metamorphosed. However, the control containers and clean artificial seagrass leaves induced less than 5% metamorphosis. It would appear that the proximity of any type of natural substrate can entice the larvae to initiate metamorphosis, at least to a certain degree. The cue itself does not seem to be unique or very precise, perhaps consisting of a relatively broad range of environmental compounds. Similarly, Strathmann (1978) observed that the larvae of several species of sea urchins responded to numerous substrates by metamorphosing. Bio-films and adult-associated factors are the commonest proposed cues of echinoderm metamorphosis (Burke, 1984; Pearce and Scheibling, 1990; Johnson et al., 1997). Although the matter was not investigated thoroughly, we found that adult-conditioned sand did not induce metamorphosis more efficiently than ordinary sand.

Metamorphosis was followed by a high rate of mortality before settlement in the presence of most substrates, suggesting that the proper conditions for settlement or post-settlement growth were not always found. Seagrass leaves yielded settlement rates that were around five times higher than the other materials which induced metamorphosis. A similar differentiation between settlement induction and metamorphosis was observed by Cameron and Hinegardner (1974) in their study of echinoid larvae. Our results suggest that two distinct processes are involved in successful settlement: metamorphosis, triggered by a broad range of factors and attachment of the pentactula larva to the substrate and definitive settlement, which are induced by a more refined signal.

Only a few studies, all related to temperate species, have previously dealt with holothuroid settlement preferences. Young and Chia (1982) demonstrated that pentactula larvae of *P. chitonoides* settled gregariously, either on the adult or around the base of the
adult sole. Hamel and Mercier (1996) showed that gravel or rocks were favoured by larvae of the sea cucumber *C. frondosa* during settlement, and that the settling larvae seemed to be responding to light and tactile stimuli.

The possibility that species requiring a particular habitat type should settle in response to cues associated with that habitat (Chia et al., 1984) was mostly studied in echinoids and asteroids (Chia and Walker, 1991; Pearse and Cameron, 1991). Cameron and Schroeter (1980) studied the larvae of the sea urchin *S. purpuratus* and concluded that they could not distinguish between natural substrates and most probably settled in response to a bacterial film. Alternatively, Rowley (1989), while testing the same species, obtained different settlement rates on natural and artificial substrates and proposed that larvae responded to more than a microbial film. Johnson et al. (1991) conducted settlement trials with larvae of the crown-of-thorns starfish *A. planci* and revealed that the highest rates of settlement and metamorphosis occurred on coral rubble and crustose coralline algae. Further studies on *A. planci* emphasised the role of bacterial induction in settlement and metamorphosis (Johnson et al., 1991, 1997).

Although the role of bacteria cannot be completely discarded because of their rapid growth, the experimental procedures involved in preparing clean substrates should have minimised their influence. Settlement rates on clean and fouled seagrass leaves were either statistically similar or in favour of clean leaves, and the size reached by the settled pentactulae were comparable on both substrates. Nonetheless, settlement, while lower than on true seagrass leaves, was more important on fouled artificial leaves than on cleaned ones. The stimulus for settlement does not appear to be tactile since the physical presence of the leaves was not required. Extracts from two seagrass species induced metamorphosis and settlement of *H. scabra* larvae in bare containers. While it seems to be of a chemical nature, settlement in *H. scabra* apparently requires more than a non-specific fouling film: settlement on artificial substrates allowed to foul for several weeks was significantly lower than on clean or natural seagrass leaves. However, conditioned artificial seagrass leaves yielded more pentactulae than control bare containers or substrates such as sand and crushed coral, whereas the settlement of starfish *A. planci* on fouled tiles was zero or negligible (Johnson et al., 1991). Combining these results, we might postulate that the chemical and, to a certain degree, the physical nature of seagrass leaves favour the growth of an inductive fouling layer or bacterial film, as proposed by Johnson et al. (1997), and that chemicals exuded by seagrass subsequently act on the larvae to promote their growth and survival. The possibility that growth and ultimate survival of the pentactulae on a substrate might be related to feeding processes is supported by the different size reached by the larvae on the substrates tested. The few pentactulae that survived on the suboptimal substrates were significantly smaller than those which survived in larger quantities on the preferred substrate.

What advantages would there be for the larvae to be so selective in the choice of a substrate upon settlement? Barker (1977) showed that *S. australis* larvae only settled on the encrusting coralline alga *Mesophyllum insigne*, which appears to provide a stable and abundant source of food to juveniles. This indicates that this alga has a considerable ecological significance to the starfish. Among asteroids, the only similar case of associated behaviour is that described by Birkeland et al. (1971), who found that
Phylochaetopterus tubes were chosen as settlement sites by the larvae of *M. aequalis*. Such associative behaviours further supports the possibility that *H. scabra* larvae may benefit from seagrass leaves, or one of its constituents or epiphytes, as a nutritional source. Juvenile *H. scabra* settled on seagrass leaves may also benefit from the lower predation intensity usually associated with vegetation (Hunt and Scheibling, 1997).

4.3. Post-settlement migration

It appears that seagrass is an important indicator in the sense that, while sand is the natural habitat of adult *H. scabra*, it does not seem appropriate for newly settled larvae, whereas seagrass shoots that grow directly in the sandy environment would provide the food and shelter needed by the early life stages. The leaves enable recently settled juveniles to grow until they reach the size at which they can move and feed at ease on the underlying sandy habitat. A similar migration implicating a dietary transition has been observed in the starfish *S. australis* (Barker, 1977).

Post-settlement processes in sea cucumbers have rarely been studied. A size-dependent migration similar to the one observed in *H. scabra* was previously observed in two dendrochirotids. Hamel and Mercier (1996) reported that, after reaching ca. 2.8 mm in length, juvenile *C. frondosa* moved to sheltered, illuminated areas of the rocky substrate, and later migrated from protected to exposed areas after they reached 35 mm in length. Young and Chia (1982) found that newly settled juveniles of *P. chitonoides* migrated from near the adults toward shaded substrates. While migration of *H. scabra* seems to be induced by food availability, or a related factor, Young and Chia (1982) and Hamel and Mercier (1996) found that the main factors regulating the post-settlement migration of juvenile holothuroids was the distribution of shaded substrates and vulnerability to predatory pressure, respectively.

5. Conclusion

The settling larvae of *H. scabra* express a relatively high substrate selection. While metamorphosis from pelagic to benthic form and onset of exploration phase seem to be triggered by several substrates, the preferred conditions for settlement are more defined. High rates of settlement of *H. scabra* on seagrass leaves seem to be favoured by the very nature of the leaves, possibly because it promotes the growth of a distinctive fouling layer. Chemicals emitted by the leaves, and to a secondary degree their shape and exposure to light, might be involved. Seagrass leaves thus appear to constitute the most suitable substrate for post-settlement growth of *H. scabra* larvae. Realistically, factors other than substrate selection are likely to be influencing recruitment of the species, especially since metamorphosis is induced by a broad range of conditions. The proximity of the spawning adults to seagrass beds and the physical characteristics of the leaves, which might act as collectors, may also account for the occurrence of newly settled *H. scabra* juveniles on seagrass leaves. A post-settlement migration, occurring when the juveniles acquire the ability to move and to feed on sand, is responsible for their
distribution a few weeks after settlement. Overall, larval behaviour in the laboratory appears to be consistent with juvenile distribution in the field.

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References


