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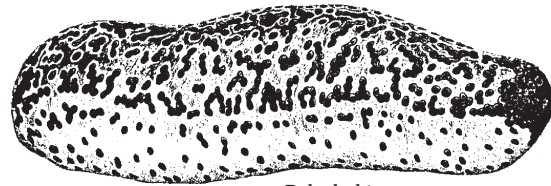# INFO

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Spawning and early larval rearing of *Holothuria atra*

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Introduction

The Coastal Aquaculture Centre (CAC) of the International Center for Living Aquatic Resources Management (ICLARM) in Solomon Islands has started to assess the potential of enhancing populations of sea cucumbers associated with coral reefs (Anon, 1993). The first step in this process is to determine whether it is possible to produce juvenile sea cucumbers *en masse* at low cost. To this end, the CAC is holding broodstock of a variety of species for experiments on induction of spawning and larval rearing. Although the emphasis is on species of high commercial value, such as white teatfish (*Holothuria fuscogilva*), the locally abundant, low-value species, *Holothuria atra*, is also being maintained in captivity as an experimental animal.

This short note presents the results of an initial attempt to spawn *H. atra* in captivity using heat stress, and to rear the larvae using methods similar to those developed by the CAC for larvae of giant clams. These larval rearing methods centre on the use of large rearing tanks and 'off-the-shelf' diets, such as dried *Tetraselmis* algae and Frippak microcapsules. The rationale for using these methods in the first instance was that, if successful, they could be applied easily in developing countries. During this initial research on *H. atra* we experimented with a wide variety of rearing methods to generate observations on factors affecting the survival of cultured larvae. Several trends emerged which can be used to form useful hypotheses for testing in future research on this species.

Materials and methods

Induction of spawning

Adult *H. atra* were induced to spawn by using heat stress. Two tanks with holding capacities of 2500 l were supplied with flow-through seawater filtered to 25 μm. Water flow to one of the tanks was continual at an ambient temperature of 30° C. Flow in the second tank was held static until the temperature increased by 2 – 3° C above ambient. Adults were introduced into the tanks at 11h00 on 3 December 1993. Seven animals were placed in the static tank and six in the ambient tank. Blended gonads of *H. atra* were added to the static tank to stimulate spawning. Animals were transferred between the two tanks every 30 minutes until spawning occurred. Spawning females were placed in individual 50 l bins filled with UV-sterilised seawater filtered to 1 μm, and left to complete ovulation. A small quantity of sperm (~ 20 ml) was then added to each 50 l bin.

Larval rearing

The CAC has six 700 l larval rearing tanks and all were stocked with fertilised eggs at a density of 2.7 eggs/ml. The tanks were supplied with UV-sterilised seawater filtered to 1 μm, and aeration. The antibiotic, *Streptomycin*, was added to each tank at a concentration of 10 ppm. The water in each tank was changed completely every second day. This was done by draining the tanks and retaining the larvae in sieves of 80 μm. The antibiotic was added to the water as soon as tanks were refilled.

Until Day 20, water flow was kept static in two of the six tanks between the regular drainings. For two other tanks, 100 l was replaced daily by fresh seawater that had been filtered to 1 μm and sterilised by UV. In the remaining two tanks, 300 l of the rearing water was replaced each day. The number of larvae surviving in each tank was estimated every second day by counting the number of larvae in six 1 ml subsamples. These subsamples were taken from a well-stirred nally bin holding the larvae that had been sieved from the 700 l rearing tank during complete water changes.

Feeding of larvae

We also made a limited comparison of the suitability of different 'off-the-shelf' diets for larval *H. atra*.

Larvae in three of the 700 l tanks were fed 50 per cent *Tetraselmis* (T) and 50 per cent Frippak (F). Larvae in the other three tanks were fed 33 per cent T, 33 per cent F and 33 per cent Selco yeast (S). The three tanks used for each feeding treatment comprised one static tank, one with a daily exchange of 100 l and one with an exchange of 300 l each day. Both diets were given at a concentration of 40 000 cells/ml. After 10 days, it was evident that survival of larvae fed on the diet including the yeast was lower (see *Results* below), so this feeding regime was abandoned. The remaining larvae were then redistributed equally among the six tanks and fed on one of two diets. These diets were 50 % T:50% F fed at 40 000, and at 80 000 cells/ml. There were three replicate 700 l tanks for each treatment. The density of the 10-day-old larvae in this trial was 1.4 larvae/ml.

Once the larvae had reached the doliolaria stage at Day 20, they were again redistributed equally among the tanks so that a comparison could be made between the survival of older larvae fed on diets with and without diatoms. The diets used in this trial were 50% T:50% F at 40 000 cells/ml, and the same diet supplemented with 'wild' diatoms. The diatoms were obtained by soaking clear fibreglass plates measuring 24 x 200 cm in outdoor flow-through seawater tanks. After seven days the plates were removed and placed in larval tanks. Plates were replaced with others that had soaked for at least nine days each time the water was changed. When removing the plates from the larval rearing tanks, care was taken to rinse any larvae from the surface. The tanks containing the diatoms were moved outdoors on Day 21 to enhance the growth of the live algae. However, survival rates were very poor compared to the tanks kept indoors (see *Results* below) so the tanks were returned to the hatchery on Day 26.

Results

Induction of spawning

After two hours of exchanging animals between the static and ambient tanks, some of the *H.atra* in the ambient tank began to adopt typical spawning behaviour, i.e. they raised the anterior half of the body and swayed from side to side. Between 13h00 and 15h00 a total of five males (three from the ambient tank and two from the static tank) and four females (three from the ambient tank and one from the static tank) spawned.

Gametes were released from the genital papilla in strands of varying lengths. Gametes were negatively buoyant, sinking to the bottom of the tank or onto the animal itself. Eggs were pink and sperm were white. The release of gametes was moderately slow. Upon disturbance, gametes spilt from the strand into the water. The spawning period varied between individuals. Two of the male animals spawned continuously for more than an hour. Approximately 9.7×10^6 eggs were fertilised.

Development of larvae

The unfertilised eggs had a mean size of 138.65 ± 1.6 SD µm (n = 10). After fertilisation, the embryonic and larval developmental pattern observed was similar to that described for other species of tropical sea cucumbers by Preston (1993). The development times to the 2-, 4-, 32- and 64-cell stages, and the blastula, auricularia and doliolaria stages, are given in Table 1.

Table 1: Developmental stages of *Holothuria atra* reared in captivity

Developmental stages	Time
2 – cell	1 – 2 hours
4 – cell	3 – 4 hours
32 – cell	5 hours
64 – cell	9 hours
Blastula	1 day
Auricularia (early)	2 days
Auricularia (late)	10 days
Doliolaria	20 days

The early auricularia stage had developed by Day 2 post-fertilisation. The mean size of these larvae was 431 ± 41.70 SD µm (n = 30). The late auricularia stage was observed at Day 10. The mean size of this stage was 402.13 ± 40.66 SD µm (n = 30), and not significantly different from the earlier stage (t = 4.02, df =28, P > 0.05). The late auricularia stage persisted until Day 19. Metamorphosis to the doliolaria stage began on Day 20 and there was complete metamorphosis of all surviving larvae to doliolaria on Day 23. The mean size of a doliolaria larva was 355.48 ± 56.86 SD µm. On Day 28, all larvae were still at doliolaria stage. By Day 30, all larvae had died.

Effects of partial water exchanges on survival

The patterns of survival for the different levels of water exchange were similar between Day 2 and Day 8 for both diets, and so data for each level of water exchange were pooled across the two diets for comparison. Until Day 8, survival in the tanks with a daily water exchange of 300 l was markedly lower than for the other treatments (Fig. 1a). This

type of comparison was not possible for Day 10 because there was massive mortality of larvae in all three tanks fed with the T:F:S diet on that day (*see below*). Survival in the three tanks fed the T:F diet on Day 10 ranged from 44 per cent for the tank with a 300 l exchange to 72 per cent for the static tank.

Between Day 12 and Day 20 there was little variation in survival of larvae among the three levels of water exchange when data from the two diets were pooled (Fig. 1b). Rather, survival decreased sharply between Day 12 and Day 16 for all three treatments of water exchange (Fig. 1b).

Comparisons of survival between tanks with different water exchange were not possible for Day 18 and 20 because there was total loss of all larvae in most of the tanks fed on 80,000 cells/ml (*see below*).

Effects of diet on survival

The mean survival of larvae fed on 50%T:50%F and the diet containing yeast, was compared by pooling the data from the three tanks with different water exchange for each diet. Mean survival for both diets was more than 60 per cent until Day 8 (Fig. 2a). All larvae examined during this period had ingested *Tetraselmis*. On Day 10, however, there was a significant difference in survival between the two diets, with survival of larvae fed the diet containing yeast dropping to 10.1 per cent whereas survival of those fed 50%T:50%F remained at 59.7 per cent (Fig. 2a). The sharp decrease in survival of the larvae fed with the more complex diet may have been due to the difficulty of maintaining good water quality in the presence of yeast.
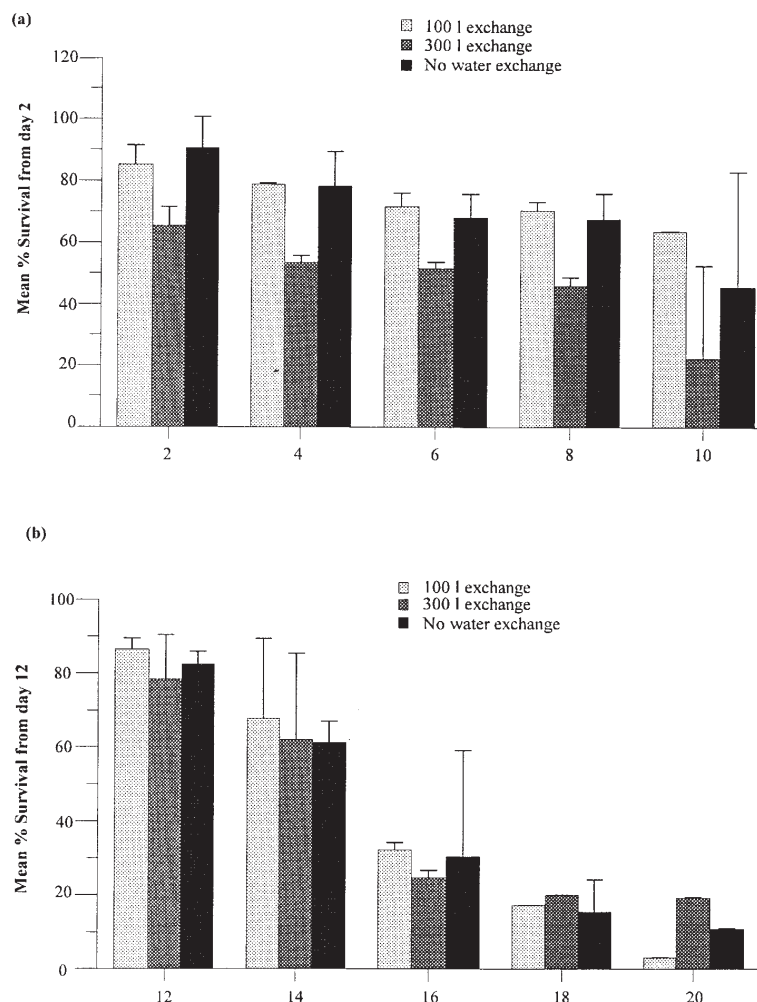

Figure 1. Mean (n = 2) survival of the larvae of *Holothuria atra* reared under three different levels of water exchange in 700 l tanks

a) Day 2 – Day 10; larvae stocked at 2.7 larvae/ml
b) Day 12 – Day 20; larvae stocked at 1.4 larvae/ml.
Note that data for each level of water exchange are pooled across two diets (*see text*). Error bars are standard deviations. Where no error bar is drawn, all larvae in one of the two tanks had died.

Survival of late-stage auricularia larvae fed on a diet of 50%T:50%F at 40 000 and 80 000 cells/ml declined markedly between Day 12 and Day 20, irrespective of the number of cells/ml (Fig. 2b). A significant difference in survival of larvae fed on the two diets occurred on Day 14, when survival was better for the 40 000 cell/ml treatment (Fig. 2b). From then on, survival in tanks fed with 80000 cells/ml was highly variable: it was greater than for the 40 000 cells/ml diet on Day 16 (Fig. 2b), but by Day 18 all larvae in two of the three tanks had

died and by Day 20 all larvae in the remaining tank were also dead.

Survival of dololaria larvae fed on the diets with and without diatoms was very poor (Fig. 2c). On Day 22 survival was significantly better in the tanks kept indoors and fed the diet without diatoms. By Day 24, entire tanks of larvae fed on both diets had been lost and by Day 28 only 1.8 per cent of the 21-day old larvae stocked into this feeding trial were alive (Fig. 2c). All larvae were dead by Day 30.
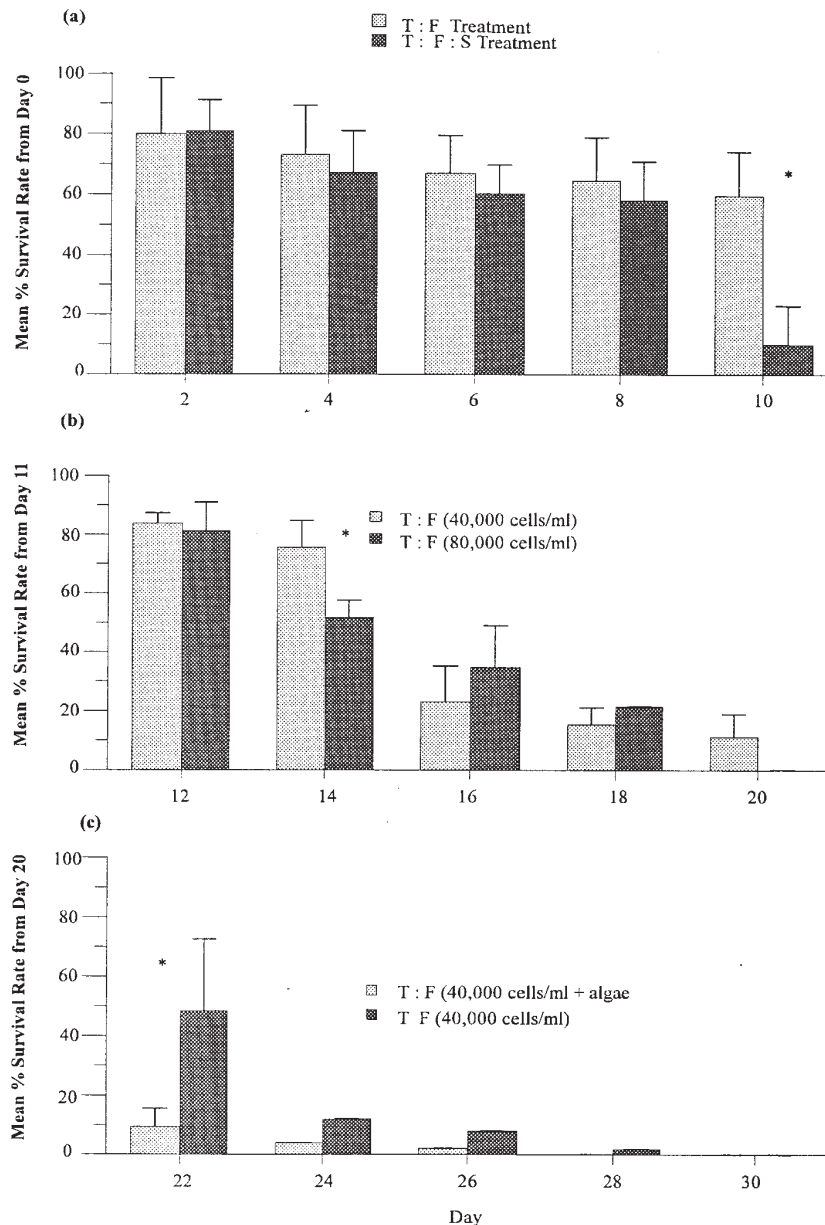

Figure 2 : Mean (n = 3) survival of the larvae of *Holothuria atra* during three feeding trials

The three trials involved larvae of different ages:
a) Day 2 – Day 10 auricularia larvae fed on dried algal and Frippak diets with and without Selco yeast (see text);
b) Day 12 – Day 20 auricularia larvae feed on dried algae and Frippak diets at 40 000 and 80 000 cells/ml; and
c) Day 22 – Day 30 larvae fed on dried algae and Frippak diets with and without diatoms. Asterisks indicate that the two means compared on that day were significantly different by t test (df = 4, P < 0.05).
Note that data for trials (a) and (b) were pooled across three tanks with different levels of water exchange. Error bars are standard deviations. Where no error bar is drawn, all larvae in two of the three tanks had died.

Discussion

Our initial attempt to spawn *H. atra* and rear their larvae demonstrated that spawning can be induced by a simple method of heat stress. James (1988) also sucessfully spawned *H. scabra* using this method. The period of the year when *H. atra* can be induced to spawn has not yet been determined, but in the case of Solomon Islands, it also includes November, because *H.atra* kept in captivity have been observed to shed gametes spontaneously in that month (Anon, 1994).

Fertilisation of the eggs was straightforward and the timing of development to the late auricularia stage was well within the range described for several other species of aspidochirate sea cucumbers (Preston, 1993). For example, it was slower than the 10 days taken by *H. scabra* (James et al., 1988), and the 15 days needed by *Actinopyga echinites* (Chen & Chian, 1990) to develop to the doliolaria stage, but considerably faster than the temperate species *Stichopus californicus*, which reaches the doliolaria stage after 65 days (Cameron & Fankboner, 1989).

Survival of the late auricularia larvae of *H. atra* under all our rearing protocols was relatively high for the first eight days, although it was reduced when large partial exchanges of water were made each day. This may have been because the antibiotics were diluted. The sharp increase in mortality on Day 10 in all tanks supplied with the diet containing yeast points to a deterioration in water quality caused by this dietary component.

The most likely explanation for the dramatic decrease in survival from Day 16 onwards is a deficiency in the diet. Larvae of other species of sea cucumbers have been reared successfully when fed on freshly cultured microalgae and diatoms (Preston, 1993; James et al., 1988; D. Sarver, pers. comm.). The reason for incorporating Frippak in the diet was to supplement the monospecific diet of dried *Tetraselmis*. We could not ascertain whether the larvae of *H. atra* ingested the Frippak microcapsules, so we do not know whether this source of nutrients was unavailable to them, or whether it was ingested but provided inadequate nutrition. Doubling the concentration of food between Day 10 and Day 20 did not improve survival. On the contrary, the mortality rate of larvae fed on the diet of 80 000 cells/ml was even faster than for those fed the diet of 40 000 cells/ml, suggesting that much of the food was not being eaten and that it had reduced water quality.

The addition of diatoms to the diet at Day 21 did not improve survival. In fact, total loss of larvae occurred sooner in the tanks supplied with wild diatoms. We cannot say whether this was due to the diatoms being contaminated by bacteria or to the increased risk of bacterial contamination, since the tanks with diatoms were kept outdoors.

Although the lack of more than six tanks limited our ability to design unconfounded experiments, our data suggest that survival of *H. atra* larvae will be improved by the use of static tanks and by diets that do not contain yeast. Above all, they show that the relatively simple methods that can be used for giant clam larvae may not be suitable for *H. atra*. On the basis of other research, larval sea cucumbers appear to need a variety of live algae.

Future experiments on larval rearing of *H. atra* and other species of sea cucumbers at the CAC will concentrate on comparing the effects of a wider variety of feeds on survival. These feeds will include some containing cultured live algae. All diets will be introduced at a range of times, starting at Day 2, to identify which diet and feeding regime maximises survival.

Acknowledgements

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