

Mr J.X. Rodrigo, Technical Officer, CMFRI, Tuticorin for supplying the micro-algae food.

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# Reactions of the larvae of the sea cucumber *Apostichopus japonicus* to sharp desalination of surface water: a laboratory study

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## Introduction

Larval development of the sea cucumber *Apostichopus (=Stichopus) japonicus* Selenka to settlement occurs in the water column and lasts for 13–20 days depending on seawater temperature and salinity. Early larvae — blastula and gastrula — occur at the surface water, while later stages — dipleurula, auricularia and doliolaria — move into deeper water. Summer monsoon rains and floods of rivers flowing into Vostok Bay (Peter the Great Bay, the Sea of Japan) considerably reduce the salinity of the surface seawater, thereby affecting larval survival of *A. japonicus* (Kashenko 1992, 1997, 1998) and other invertebrates. Distribution, vertical migrations of marine bottom invertebrate larvae, and their behavioural responses to changing salinity in a stratified water column have been extensively studied. However, the conclusions made by the investigators are not unambiguous (Harder 1968; Mileikovskiy 1974, 1981; Seliger et al. 1982; Mann 1986, 1988; Scheltema 1986; Stancyk and Feller 1986; Sulkin and Van Heukelem 1986; Tremblay and Sinclair 1990; Jonsson et al. 1991; Pedrotti and Fenaux 1992; Young 1995; Vazquez and Young 1996; Metaxas and Young 1998; Garrison 1999; Welch et al. 1999).

Reactions of the larvae of the sea cucumber *A. japonicus*, their behaviour, and vertical distribution caused by reduced salinity of surface seawater have not been studied. The aim of this research is a study of this problem under laboratory conditions.

## Materials and methods

Experiments were carried out at the Vostok Marine Biological Station of the Institute of Marine Biology FEB RAS (Vostok Bay, the Sea of Japan) during July and August 1992.

Sea cucumbers in the pre-spawning state were collected on 15 July at 6 m of depth, at a temperature of 19.6°C and salinity of 32.7‰. Spawning proceeded on the same day, in separate vessels for females and males. Fertilisation, maintenance to settlement, and all experiments were carried out at a temperature of 22–23°C and salinity of 32‰ (Kashenko 1992). Larvae were reared in three larval cultures. Filtered and UV-sterilised seawater in aquaria was changed every 1–2 days. It was saturated with oxygen and stirred using a microcompressor that supplied air via glass capillaries to the water surface, inflicting no injury to the larvae.

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Early auricularias were fed with the small microalgae *Nannochloris maculata* and *Pavlova lutheri*, middle and late auricularias with the larger *Phaeodactylum tricornutum* and *Dunaliella salina*.

Responses, behaviour, and vertical distribution of *A. japonicus* in different larval stages (blastula, late gastrula, larvae in transition between gastrula and dipleurula, early auricularia, middle 10-day-old auricularia, late auricularia with hyaline spheres, doliolaria) were studied in relation to dilution of the surface layer of still sea water in the laboratory. One-litre graduated cylinders with a 6-cm diameter and 50-cm water column were used in the experiments. Surface water was diluted by slowly supplying water of reduced salinity to water of normal salinity (32‰) along the vessel's wall. The density gradient in the cylinders was not disturbed for more than one day, i.e., the water layer of markedly decreased salinity was hardly mixed with denser seawater.

Specific volumes of larval culture were emptied into control and experimental seawater cylinders at a depth of 19–20 cm before a diluted water layer was made. In each experiment, nearly the same larval densities were maintained in the cylinders. In some stages, larvae (30 individuals) were measured, and age from the time of fertilisation was estimated.

To model the situation occurring in the natural environment, the experiment was carried out in two variants. Variant I consisted of a sharp dilution. To the cylinder was added 25 ml of fresh water that was about 1 cm high. Variant II was a gradual dilution, with first 25 ml of 20‰ seawater and then 25 ml of 12‰ seawater added to the cylinder. The vessels were placed in front of a source of light. We used natural light but not direct sunlight coming from the window. This makes it possible to record the first response to dilution, follow behaviour, and count the number of larvae in the vessel, as well as to take samples of water from diluted layers that are clearly distinguishable visually.

For salinity determination and larval count, after 1 h of experiment, 0.3 mm of water was sampled using a Pasteur pipette with a thin long end to prevent the mixing of layers of different salinity. The water was sampled from different depths: at the surface film (0–0.2 cm and 0.2–1 cm), where there were no larval concentrations; 1–2 cm, where larvae most often concentrated; 2–3 cm, where larvae were few or absent; and then 3–4 cm, 6–7 cm, 18–19 cm, and deeper. In some cases, observations on the lar-

vae and water samples were taken once every 3–4 h or once in a day. Salinity in micro-volumes of water was measured with an accuracy of 0.5‰ using a specially designed device (Tyurin 1994). Larvae were counted either in Bogorov's chamber or in graduated cylinders. Mean density of larvae (6 replicates: 3 larval cultures in each of 2 variants) were calculated. Salinity value at which larvae of a particular development stage concentrated were the same in experiment variants I and II, and larval densities in diluted horizons were also similar; therefore, the data in tables I and II were pooled. In control cylinders, larval densities at different depths were estimated to determine their normal vertical distribution. Analytic procedures followed Urbakh (1963). The hypothesis that treatments within an experiment were significantly different ( $p \leq 0.05$ ) was satisfied in all experiments.

## Results

In experiment variants I and II the width of diluted water layer where there were larval concentrations did not exceed 0.3–0.5 cm. In experimental vessels this horizon was usually at a depth of 1.0–1.5 cm.

In control cylinders, blastulas (at the age of 16 h, size  $195 \pm 24 \mu\text{m}$ ) were constantly moving upward and downward. In the surface layer, their number was increased (Table 1). When water of the top layer was diluted to 9.5–13‰, blastulas accumulated in the horizon at 20–20.5‰. After being in contact with water of reduced salinity, larvae lost motility because of the osmotic shock and increased in size as a result of hydration; however, they rapidly recovered locomotor activity and survived for a long time in this layer but were not able to leave it (Table 1).

**Table 1.** Distribution of blastula larvae of *Apostichopus japonicus* in the water column one hour after dilution of the surface layer. Density data for variants I and II are pooled because the values are very similar. Mean larval densities ( $n = 6$ ), 95% confidence intervals and mean values of salinities for variants I and II.

Depth (cm)	Larval density (nb./ml)		Salinity, ‰
	Control, salinity 32‰	Variants I and II	Variants I and II
0–0.2	7.50±1.12	0	–
0.2–1	3.33±0.64	0	5.5–14
1–1.4	0	34.6±6.91	20–20.5
2–3	0.10±0.01	0.08	30–31
3–4	0.12±0.01	0.08	32
6–7	0.12±0.01	0.08	32
18–19	0.34±0.04	0.08	32

– : not determined

A special experiment was carried out with late gastrulas (age 31 h) (Table 2). In the control, larvae at transition from gastrula to dipleurula were uniformly distributed in the water column, excluding the top 0–1 cm layer. Larvae in large groups were moving up and down. After the upper diluted water layer (6.5–17‰) had been formed, larvae began to stay in the horizon at 20–20.5‰. Disturbance of ciliary movements and hydration were observed in these larvae. After 1 h of experiment, their density in the layer was up to  $17.4 \pm 1.8$  individuals per ml (Table 2). However, after 3 h, larvae already restored the normal work of cilia and began leaving the diluted horizon (Table 2). At the same time, the main mass of larvae in control cylinders descended to a depth below 10 cm and evenly spread in the water column. After 4 h, all larvae in experimental vessels left the diluted horizon. They were dipleurulas.

Auricularia is the sea cucumber's longest development stage; therefore, it was studied in detail. In the control, early auricularias (at the age of 61 h, size  $538 \pm 29 \mu\text{m}$ ) were uniformly distributed in the water column, moving in large groups upward and downward but not entering the top 0–1 cm layer (Table 3). When surface water salinity was lowered to 5–15.5‰, larvae migrated deeper into the water column where salinity was 31–32‰ and did not come into reduced-salinity layers.

In control vessels, middle auricularias (at the age of 10 days, size  $925 \pm 43 \mu\text{m}$ ) were uniformly distributed in the water column, slowly moving up and down and to and fro, not coming into the top layer (Table 3). Their number increased toward the bottom. At 4–15‰ in the top layer, auricularias concentrated in 20‰ water and after 1 h their den-

**Table 2.** Distribution of larvae of *Apostichopus japonicus* in the transition stage between gastrula and dipleurula in the water column 1, 3, and 4 h after dilution of the surface layer. Density data for variants I and II are pooled because the values are very similar. Mean larval densities ( $n = 6$ ), 95% confidence intervals and mean values of salinities for variants I and II.

Depth (cm)	Larval density (nb./ml)					Salinity, ‰
	Control, salinity 32‰		Variants I and II			Variants I and II
	(in 1 h)	(in 4 h)	(in 1 h)	(in 3 h)	(in 4 h)	
0–0.2	0	0	0	0	0	–
0.2–1	0	0	0	0	0	6.5–17.0
1–1.3	$0.82 \pm 0.14$	0	$17.4 \pm 0.18$	$16.5 \pm 0.24$	0	20–20.5
2–3	$0.60 \pm 0.12$	0	0	0	0	27–26
3–4	$0.40 \pm 0.08$	0	0	0	0	32
6–7	$0.50 \pm 0.10$	0	$0.10 \pm 0.02$	$0.16 \pm 0.04$	0	32
12–13	$0.50 \pm 0.07$	$1.00 \pm 0.13$	$0.24 \pm 0.06$	$0.90 \pm 0.12$	$1.58 \pm 0.31$	32
18–19	$0.50 \pm 0.00$	$1.20 \pm 0.18$	$0.97 \pm 0.11$	$1.00 \pm 0.14$	$1.72 \pm 0.38$	32

– : not determined

**Table 3.** Distribution of early (age 61 h) and middle (age 10 d) auricularia larvae of *Apostichopus japonicus* in the water column one hour and one day after dilution of the surface layer. Density data for variants I and II are pooled because the values are very similar. Mean larval densities ( $n = 6$ ), 95% confidence intervals and mean values of salinities for variants I and II.

Depth (cm)	Larval density (nb./ml)					Salinity, ‰	
	Control, salinity 32‰		Variants I and II			Variants I and II	
	(in 1 h) Early	(in 1 h) Middle	(in 1 h) Early	(in 1 h) Middle	(in 1 d) Middle	(in 1 h) Early	(in 1 h) Middle
0–0.2	0	0	0	0	0	–	–
0.2–1	0	$0.22 \pm 0.09$	0	0	0	5–15.5	4–15
1–1.5	$0.82 \pm 0.17$	$0.12 \pm 0.01$	0	$13.02 \pm 0.24$	$12.93 \pm 1.08$	20–21	20
2–3	$1.04 \pm 0.24$	$0.24 \pm 0.07$	0	$1.14 \pm 0.36$	$0.36 \pm 0.05$	28–29	26–27
3–4	$0.72 \pm 0.09$	$0.24 \pm 0.05$	$2.71 \pm 0.72$	$0.20 \pm 0.04$	$0.38 \pm 0.04$	31–32	31–30
6–7	$0.62 \pm 0.03$	$0.32 \pm 0.06$	$1.02 \pm 0.28$	$0.15 \pm 0.03$	$0.18 \pm 0.03$	32	32
18–19	$0.69 \pm 0.18$	$0.48 \pm 0.10$	$1.51 \pm 0.22$	$0.12 \pm 0.03$	–	32	32

– : not determined

sity was  $13.02 \pm 1.76$  individuals per ml. Larvae did not move because of the osmotic shock and were markedly hydrated. After one day, the vertical distribution pattern was somewhat different. Larvae that came into 20‰ water moved little, and at 25–26‰ some larvae left the layer.

In the control, late auricularias with hyaline spheres did not come into the surface layer (Table 4). Near the bottom their number increased. At 5–18.5‰ in the surface layer, larvae migrated to a depth of 18 cm where salinity was 32‰. Late auricularias were more motile and significantly decreased in size ( $763 \pm 35 \mu\text{m}$ ).

In the doliolaria, the main mass of control larvae remained near the bottom and only a small quantity of larvae approached the surface. They also actively migrated away from diluted surface layer (5–15‰) to a depth of 17–18 cm with normal salinity (Table 4).

## Discussion

Most studies of the mechanisms of larval distribution in estuaries or in coastal waters have been performed on crustaceans, molluscs, and fishes. Larvae of different invertebrates respond to environmental changes in a different manner. In estuaries, larval transport is largely dependent on rapidly changing environmental factors, currents, and turbulence. The mechanisms and significance of larval transport cannot be separated from the overall biology of the organisms (Stancyk and Feller 1986). Dispersal of marine invertebrate larvae is related to the circu-

lation of oceanic and estuarine water masses, as well as the length of development in plankton, age, and adaptability in each development stage. It has been suggested that either larvae are capable of active dispersal — that is, they control their spatial distribution in the water column (Mileikovskiy 1974, 1981; Mann 1986, 1988; Scheltema 1986; Stancyk and Feller 1986; Jonsson et al. 1991; Young 1995; Vazquez and Young 1996; Metaxas and Young 1998; Welch et al. 1999) — or they are merely transported passively by currents or drifting in the estuarine systems (Seliger et al. 1982; Sulkin and Van Heukelem 1986; Garrison 1999).

The vertical distribution of the sea cucumber *Apostichopus japonicus* larvae in different development stages is known. Like other echinoderms (Rumrill 1989; Pedrotti and Fenaux, 1992; Metaxas and Young 1998), early larvae of *A. japonicus* occur in surface water, while late larvae migrate toward deeper horizons. There is no information on diel vertical migrations of larvae of sea cucumber in the natural environment. In this study, upward and downward migrations of larvae in control and experimental vessels are likely to reflect their behaviour in still water in the natural environment. At the same time, blastulas and gastrulas tended to occur near the surface layer, while later stages remained in the lower area of vessels.

In Vostok Bay, the surface water can be markedly freshened (1–10‰) by heavy rains, and in windless weather the stratified water column can persist for a long time (Kashenko 1997). In the morning hours, salinity of the surface water could lower to

**Table 4.** Distribution of larvae of the late auricularia with hyaline spheres and doliolaria of *Apostichopus japonicus* in the water column one hour after dilution of the surface layer. Density data for variants I and II are pooled because the values are very similar. Mean larval densities ( $n = 6$ ), 95% confidence intervals and mean values of salinities for variants I and II.

Depth (cm)	Larval density (nb./ml)				Salinity, ‰	
	Control, salinity 32‰		Variants I and II		Variants I and II	
	Late auricularia	Doliolaria	Late auricularia	Doliolaria	Late auricularia	Doliolaria
0–0.2	0	0	0	0	–	–
0.2–1	0	0	0	0	5–18.5	5–15
1–2	0.18±0.05	0	0	0	23–25	25–26
2–3	0.24±0.04	0	0	0	31	31–31.5
3–4	–	0.01	0	0	32	32
6–7	0.28±0.04	0.04±0.01	0	0	32	32
18–19	0.36±0.06	150	0.27±0.07	0.04±0.01	32	32
25–26	0.32±0.05	0.08±0.02	0.53±0.09	0.08±0.01	32	32
36–37	0.78±0.06	0.76±0.12	0.89±0.16	0.98±0.04	32	32

24–27‰ in calm weather. It is at this time that newly hatched blastulas of sea cucumber ascend by moving their cilia to the water surface; adult sea cucumbers, as suggested by our observations, spawn either late in the evening or around midnight. Meeting with the diluted horizon, blastulas stopped moving upward at salinity causing the osmotic shock. The larvae soon adapted to 20‰ and began swimming but were still unable to leave the diluted horizon because they became less dense in the iso-osmotic environment, as indicated by their enlarged size (hydration), and could not overcome the density gradient to penetrate into the underlying horizon where density was higher. Blastulas and gastrulas can descend only when they stop the work of cilia on their bodies.

Like blastulas, gastrulas have no locomotor organ and hence were also unable to leave the diluted horizon. Previous studies showed that if the blastula and gastrula stages were exposed to reduced salinity (20‰), larvae in the subsequent stages became more resistant to decreased salinity (Kashenko 2000). An experiment with larvae transiting from gastrula to dipleurula showed that some larval stages can actively respond to reduced salinity. In the dipleurula of sea cucumber, the nervous system is laid down (Dautov et al. 1991) and the larval locomotor organ, a ciliary band, appears. Therefore, dipleurulas were probably able to leave the reduced salinity horizon. Thus, after the nervous system and larval locomotor organ have been formed, larvae began to actively respond to unfavourable conditions, in particular to the change in salinity.

In the 20‰ horizon, there were also middle auricularias. It has been shown that at 22–24‰ of the locomotor activity in the auricularia can be recovered in 2.5 days. Larvae in this stage are more vulnerable and perish at 20‰ (Kashenko 1992). Moreover, middle auricularias have by far greater size and more complex body shape, compared to other larvae. This probably does not allow them to rapidly react to sharp changes in the environment. Scheltema (1986) noted that at a change of the environmental conditions 'the response of a bipinnaria or polychaete larva will therefore necessarily differ from that a bivalve or decapod larva.'

Our studies showed that early auricularias, late auricularias with hyaline spheres, and doliolarias are able to respond to environmental signals and avoid decreased salinity in non-turbulent water. Thus, early larval stages of sea cucumber cannot actively avoid adverse environmental conditions, while later larvae exhibit preferential response to salinity changes.

Harder (1968), who studied planktonic organisms, found that most plankters stopped moving

at the freshwater-seawater interface. This response by plankters was associated with markedly different characteristics of density in these layers. A similar response was reported for larvae of three mactrid bivalves and it was attributed to differential behaviour along the salinity gradient (Mann et al. 1991). In the stratified water column sea scallop larvae *Placopecten magellanicus* showed subsurface peaks in concentration above the pycnocline (Tremblay and Sinclair 1990). Pedrotti and Fenaux (1992) also remarked that the stratification of the water column keeps larval distribution of echinoderms near the surface waters. Chinese investigators (Zhenzu and Wenxiong 1993) suggested that salinity stratification in natural environments greatly influences vertical migrations of larval oysters *Crassostrea gigas*. Metaxas and Young (1998) also showed that larvae of sea urchins crossing a halocline encountered water of lower salinity that might induce osmotic stress. From the above, it can be concluded that the stratification of the water column in estuaries and bays leads to the accumulation of marine invertebrate larvae in waters of reduced salinities, causing osmotic shock and loss of locomotor activity in the larvae. Depending on age and adaptability, larvae either will be able to leave waters of reduced salinity or will stay there. It can be assumed that this is one of the reasons why larvae are retained in the near surface water or above or below the pycnocline.

## Conclusions

At desalination of surface water, larvae of *A. japonicus* in the blastula, gastrula, dipleurula, and auricularia stages lost motility and accumulated in those horizons where seawater salinity caused an osmotic shock. Blastulas and gastrulas concentrated at 20–20.5‰. Although blastulas were not able to leave this horizon, they survived in it. Larvae that developed from gastrula to dipleurula in the diluted water horizon left it. Middle auricularias were not able to leave the 20‰ water horizon and perished. At dilution of surface water, early and late auricularias and doliolarias migrated into deeper water of normal salinity. Thus, adaptive plasticity and behavioural mechanisms allow larvae in some stages of the sea cucumber *A. japonicus* to survive and avoid the adverse effects of reduced salinity in surface layers of seawater.

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## market news

beche-de-mer

### Beche-de-mer prices on the Asian markets (August 2001 to April 2002)

Species	Size		Indicative price in US\$/kg (C&F) Aug. 2001 – Apr. 2002										Market area	Origin
			Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.			
White teatfish (skin-on)	3–5 pc/kg	A	25.0	23.0	24.0	23.0	21.0	21.0	23.0	23.0	23.0	SE Asian ports	S. Pacific	
		B	18.0	16.0	16.0	16.0	13.0	13.0	13.0	13.0	13.0	SE Asian ports	S. Pacific	
Prickly Redfish ( <i>Thelenota ananas</i> )	6–15 pc/kg		12.0	10.0	10.0	10.0	10.0	10.0	12.0	12.0	12.0	SE Asian ports	S. Pacific	
Black teatfish	3–5 pc/kg	A	18.0	17.0	17.0	15.0	15.0	15.0	18.0	18.0	18.0	SE Asian ports	Australia	
		B	15.0	14.0	14.0	13.0	12.0	12.0	12.0	12.0	12.0	SE Asian ports	Australia	
Sandfish		A	33.0	30.0	32.0	32.0	32.0	32.0	34.0	34.0	34.0	Singapore	Indonesia	
	10–30 pc/kg		58.0	50.0	50.0	45.0	43.0	45.0	45.0	45.0	45.0	Singapore	Australia	
	15–40 pc/kg		40.0	35.0	35.0	35.0	35.0	35.0	40.0	40.0	40.0	Singapore	S. Pacific	
Greenfish ( <i>Stichopus chloronotus</i> )	50–120 pc/kg		10.0	10.0	10.0	10.0	10.0	10.0	13.0	13.0	13.0	Singapore	S. Pacific	
Lollyfish			2.0	2.0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	Singapore	S. Pacific	
Stonefish			15.0	14.0	15.0	15.0	15.0	12.0	14.0	14.0	14.0	Singapore	Indonesia	
Surf redfish ( <i>Actinopyga mauritiana</i> )	15–35 pc/kg		12.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	Singapore	S. Pacific	
Tigerfish	25–55 pc/kg		3.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	Singapore	S. Pacific	
Brown sandfish ( <i>Boadschia marmorata</i> )	25–110 pc/kg		3.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	Singapore	S. Pacific	
Curryfish ( <i>Stichopus variegatus</i> )	30–50 pc/kg		17.0	15.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	Singapore	S. Pacific	
	70–120 pc/kg		8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	Singapore	S. Pacific	
Elephant trunkfish	3–8 pc/kg		2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	Singapore	S. Pacific	

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