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SALINITY TOLERANCE SURVIVAL, BEHAVIOUR AND WEIGHT CHANGES OF THE WEDGE CLAM, *DONAX CUNEATUS*

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The wedge clam, *Donax cuneatus* tolerates salinity dilution upto 22.05‰. In the medium having less than 18.9‰ salinity value, the wedge clams show erratic behaviour and 100% mortality occurred at 9.45‰ salinity at the end of 10 days of experiment. The time of opening of the valves of the wedge clam progressively increased with increase in salinity irrespective of the nature of transfer (sudden or gradual).

On shelling the body of the wedge clam, more weight was lost in the injured than in the non-injured clam, but the mechanism of fluid loss in both the cases was the same. The wedge clams prevented from opening and closing their valves lost weight both in air and water due to the secretion of body fluids. The weight loss in wedged open clams was found to be directly proportional to the dilution of the seawater (31.5‰), whereas the weight changes in intact clams showed no definite correlation with the dilution of the seawater.

INTRODUCTION

SEVERAL investigators have studied the survival and behaviour of marine bivalves with respect to salinity fluctuations (Dodgson, 1928 ; Fox *et al.*, 1936 ; Hopkins, 1936 ; Loosanoff, 1948, 1950, 1952 ; Motwani, 1955 ; Fingerman and Fairbanks, 1956 ; Pierce, 1970). Compared to these studies there appears to be very little work on bivalves from Indian waters on the subject, although Nagabhushanam (1955), Deshmukh (1972) and Mane (1974) have studied the survival and behaviour of *Martesia striata*, *Meretrix meretrix* and *Katelysia opima* respectively, in various salinities.

Donax cuneatus, a wedge clam, occurs in the intertidal zone of open coasts of Mirya and Ratnagiri Bays at Ratnagiri on the west coast of India. The present investigation was undertaken to study the tolerance of the species to low salinities and to obtain data on weight changes and fluid losses generally occurring after shelling the clam body.

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MATERIAL AND METHODS

Most of the experiments were performed from February to April, 1973 except a few ones in September, 1973. The wedge clams were collected from two localities—those used in the experiments for determining the salinity tolerance were

from the Mirya Bay, while those used in other experiments were obtained from the Ratnagiri Bay. In the laboratory, the clams were placed in fresh seawater for few hours before the start of each experiment. No extra food was given. Healthy clams measuring 15-16 mm in length were chosen for the experiments. All experiments were repeated twice. Low and High salinities were obtained by adding distilled water and whole sea salt respectively, to the normal seawater. Salinity of the medium was determined by titration with silver nitrate using potassium chromate as an indicator.

Experiments and Results

Effect of low salinity on survival

The clams were placed in a series of enamel trays each containing one litre of seawater of varying salinity. Twelve different dilutions (from fresh water to 31.5‰) from normal seawater (32.6‰) were prepared. The water was oxygenated twice a day by an aerator and was changed with appropriate salinities once in a day. Water temperature was recorded daily and it was found to vary from 27° to 31°C. during the period of study. The condition of individual clam was noted and it was considered dead when it failed to respond by closing its valves or withdrawing its siphons to a gentle touch with a glass rod. The dead one was replaced immediately by a fresh marked clam, and the survival period from the time of its immersion was then recorded. Each clam was kept under observation for a period of ten days when the percentage of survivors was calculated.

In the media having a salinity value of 22.05‰ all the clams opened their valves within 20 minutes after their sudden immersion in the respective salinity. All the clams extended their siphons and a large quantity of faeces and pseudofaeces was observed at the bottom of each tray. Between 18.9 and 12.6‰ salinity, the clams showed erratic behaviour. There was no regular sequence in the time of valves opening and extension of siphons. At 18.9‰ salinity and a few clams opened their valves within 20 minutes but the majority took 12 hours to do so. At 15.75‰ a few clams opened after one and half hours, some opened on the second day, while the remaining clams did not open their valves at all throughout the experiment salinity medium. All the clams, tested in 12.6‰ remained with their valves closed on the first day except one whereas two opened on the second day and most of them on the fifth day. Of those two clams that opened their valves on the second day, one reclosed again on the third day. The clams placed in 9.45‰ saline water remained with closed valves for first three days but on the fourth day one clam and on the fifth day four clams opened valves, while the remaining clams did not open at all in this salinity. At 6.3‰ and below, the clams did not open their valves until death.

There was a progressive decrease in the amount of faeces with decreasing salinity from 31.5 to 22.05‰, wherein the clams could perform their normal activities. At 18.9 and 15.7‰ saline condition, very little quantity of faeces was observed and at 12.6‰ and below, no faeces was present. The length to which the siphons were extended was also gradually reduced with decrease in salinity.

From Fig. 1 it could be seen that from fresh water to 9.45‰, 100% mortality was occurred at the end of eight days. At 12.6‰, 80% mortality was observed after nine days. Further, in 15.75‰, 60% animals died within eight days while at 18.9‰ a similar mortality was occurred after ten days. At 22.05‰, 25.25‰ and

28.35‰ salinity media, respectively, 40%, 20% and 10% clams, were found dead within ten days. There was no mortality in the medium with 31.5‰ salinity. It is thus clear that the lethal salinity for *D. cuneatus* is within the range of 22.05 and 18.9‰ and that the lowest salinity that would be tolerated by the species is 20.5‰ at 50% survival.

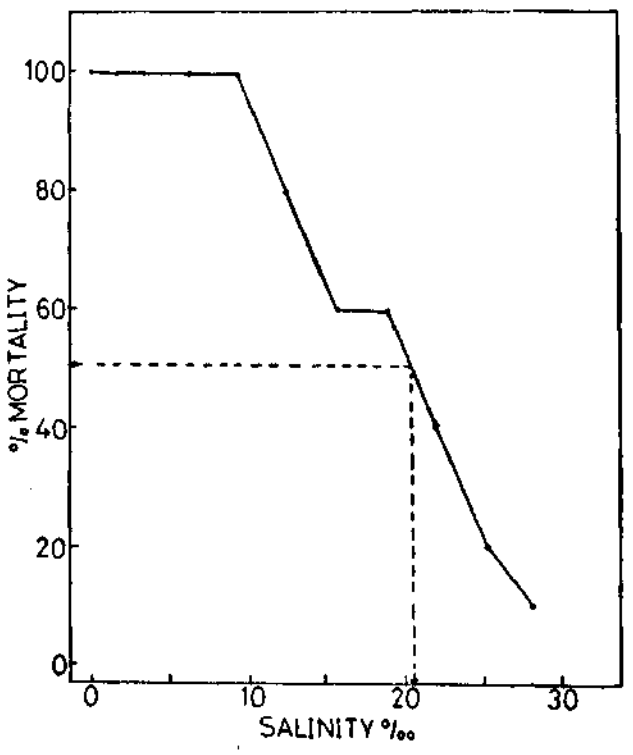

Fig. 1. Low salinity tolerance in *D. cuneatus*.

Time of opening the valves of clams in relation to external conditions

While performing the above experiments it was observed that the clams took more time to open valves when the salinity of the medium was lowered. Four sets of experiments involving progressive (step by step) dilution of seawater ; progressive increase of salinity ; sudden dilution and sudden increase were conducted to study the relation of valve opening of clams to external conditions.

In progressive dilution experiments, the clams were taken out of aquaria and dried externally by blotting towles and placed in the media having 32.6‰ salinity. The time of immersion, initial opening of the valves, and of fully opened condition was recorded. The clams were allowed to remain in this salinity for 24 hours. After this, they were transferred to the next dilution series of 29.34‰ and the time required for the initial and full opening of valves was noted. In this way,

experiments were conducted in different salinity gradients namely 26.08‰, 22.82‰, 19.56‰ and 16.3‰. Similar method was employed in the experiment with increasing salinity in which the clams were transferred to seawater having salinity valves 32.6‰, 35.86‰, 39.12‰, 42.38‰, 45.64‰ and 48.9‰.

In the sudden dilution and concentration experiments, the clams were conditioned in normal seawater for 24 hours before transferring them to the experimental saline medium.

Much diversity in the behaviour and response of clams to the environmental conditions was observed. The results therefore, are presented on the basis of the average data on ten clams (Fig. 2).

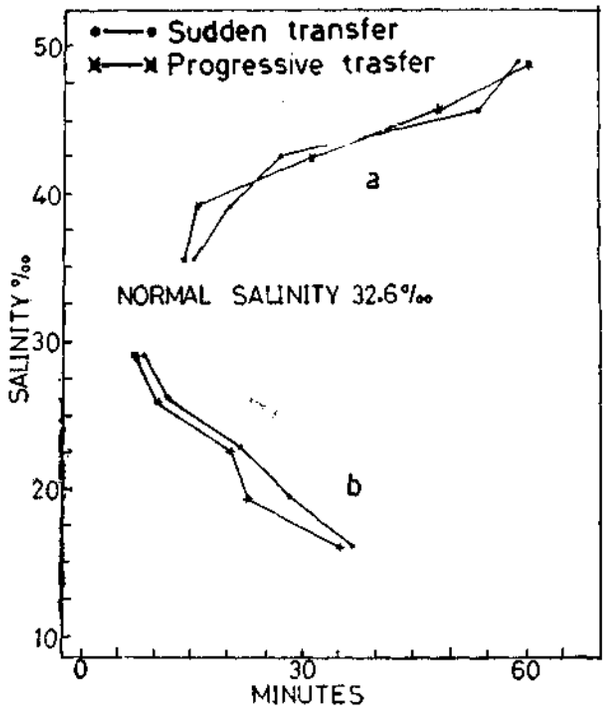

Fig. 2. Time to open shell valves in *D. cuneatus* transferred to different seawater salinities: a. concentration and b. dilutions.

A progressive increasing delay in the time of opening of valves was observed with increase in salinity, irrespective of the nature of change. It was noted that the time taken by clams 'to begin to open' as well as 'to fully open' valve condition was progressively increased when the salinity of medium was departed from that of normal seawater. The interval between these two stages was also correspondingly increased. The time taken to open valves in a given salinity was approximately the same regardless of the nature of change. From Fig. 2, it is seen that there was no marked difference between the time taken to open valves by the clams subjected to progressive changes and those subjected to sudden transfer.

Effect of shelling on body weight

Two batches of thirty clams were removed from the aquaria, blotted and individually weighed. Each clam was then shelled separately in the manner described by Fingerman and Fairbanks (1956) so as to give no injury to the clam body in first batch and injury to the other. The clam body from two batches was then individually weighed at intervals of 15 minutes for the first 60 minutes and then at 30 minutes interval, for 120 minutes. Prior to weighing, the body was blotted to remove excess moisture. The results are expressed as percentage change in body weight in both the batches of clams (Fig. 3 a, b). In the first 15 minutes, more loss in body weight took place in both the batches and later on it decreased. 69.59% of the original body weight was lost in the first batch whereas 74.35% in the second

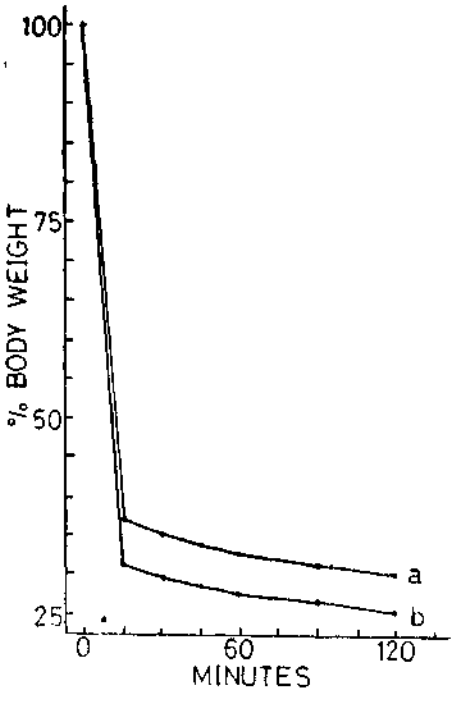

Fig. 3. Effect of removing the clam body on body weight changes in *D. cuneatus*: a. without injury and b. with injury.

batch of clams at the end of experiment. The mechanism of fluid loss from 15 to 120 minutes was the same in all the clams irrespective of the method of shelling of the clam body. In clams with injury 4.75% additional weight loss was occurred than those without injury to the body parts. It is possible that the weight loss of clams might be due to fluid exuded from the sinuses in the mantle and from the cut surfaces of the adductor muscles.

Weight losses induced by draining the free fluid between the shells

In the first experiment, ten clams were blotted externally to remove excess moisture and exposed to atmospheric air. These clams were weighed at 0, 15, 30, 45, 60, 90 and 120 minutes individually after blotting. The clams were shelled after two hours and their body weights including the fluid were determined individually. The weight losses were then calculated as the percentage of original body weight. The weight loss in the clams was due to evaporation of water from the surface of shells. The control clams lost 5.44% of their original body weight after two hours (Fig. 4 a).

In the second experiment wedges were placed between the shells of each of ten clams to prevent complete closure of the shell valves. Such clams were taken out from the aquaria, the water between the shells was discarded, blotted externally and exposed to atmospheric air. The clams were then weighed at the same time intervals as the control clams. The fluid accumulated between the shells was discarded prior to each weighing. After two hours these clams were also shelled and the body weight was determined. The weight changes are expressed as the percentage of original body weight (Fig. 4 b).

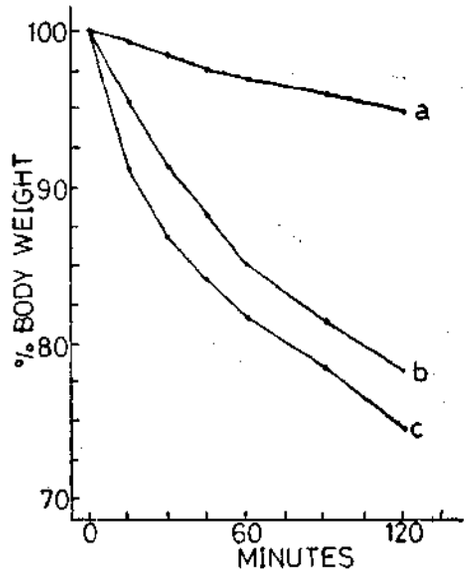

Fig. 4. Weight loss in *D. cuneatus* after draining the free fluid between the shells : a. due to evaporation ; b. due to wedged open and c. due to forced open.

In the third experiment ten clams were taken from the aquaria, blotted and exposed to atmospheric air for 120 minutes similar to earlier batches. These clams were weighed at the same intervals as was done previously. Before taking weight the valves of each clam were forced apart slightly and the free fluid between the shells was discarded. At the end of experiment the clams were shelled, their original body weights were determined and the percentage was calculated (Fig. 4 c).

The results of all the three experiments show that 25.65% of the original body weight was lost by forcing the shells of clams apart while 22.07% of the original body weight was lost due to wedged agape. However, a small portion of 5.44% loss was due to evaporation of water from the outer surface of the shells of control clams. It may be concluded that in order to regulate its body weight the clams must be free to open and close their shells. The differences between the weight losses of the last two groups was insignificant.

Weight loss of clams by draining the free fluid between the shells and subsequently returning to the aquaria

Ten clams were taken out from the aquaria, and the free fluid between the shells was drained by forcing the shells apart and the shells were blotted. The clams were exposed to atmospheric air and weighed at 15 minutes intervals for one hour. Prior

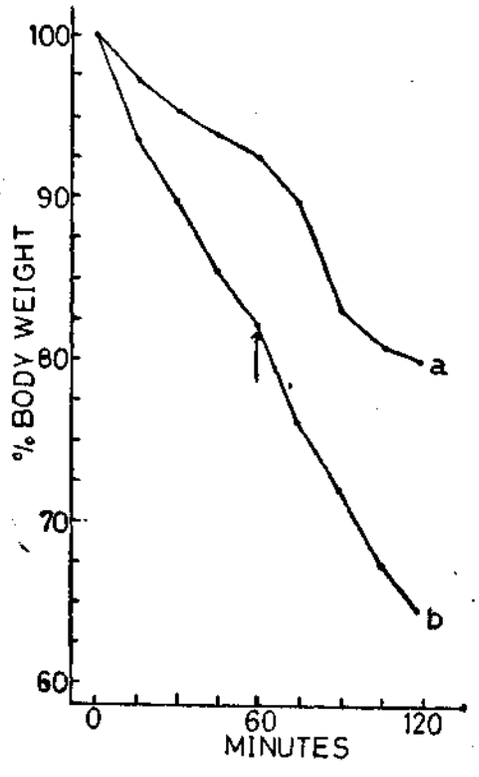

Fig. 5. Weight loss in *D. cuneatus* after draining the free fluid and subsequently returning to sea water : a. due to forced open ; b. due to wedged open ; ↑ -returned to aquaria.

to each weighing the free fluid between the shells was drained. After one hour these clams were returned to normal seawater and weighed at 15 minutes intervals for the next one hour. The shells of the clams were blotted and the free fluid between the shells was drained prior to each weighing.

A second group of ten clams was wedged open and taken out from the aquaria. The free fluid between the shells was drained and the shells were blotted. This group was also exposed to atmospheric air and weighed at the same time intervals as the forced open clams. After one hour the wedged open clams were also returned to normal seawater and weighed for next one hour.

After two hours both groups of clams were shelled. The original body weight was determined by substracting the weight of shells from the original weight of intact clams. The per cent weight changes were calculated on the basis of original body weight. The results of these two experiments are shown in Fig. 5 a, b. The differences between the weight loss of two groups of clams was 15.14%. Wedges between the shells or forcing apart the shells at regular intervals prevented the clams from regulating their weight and consequently their volume both in air and in seawater.

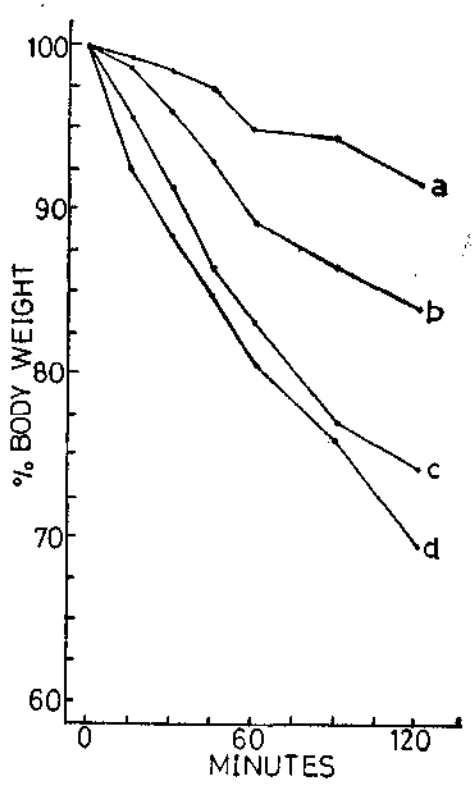

Fig. 6. Weight changes in wedged open *D. cuneatus* exposed to seawater salinities : a. 31.5‰ ; b. 25.2‰ ; c. 18.9‰ and d. 12.6‰.

Weight changes of wedged open clams in different dilutions of seawater

Four batches of clams (each comprising of five clams of equal size) were wedged open. The free fluid between the shells was discarded and the clams were blotted. Each batch was then placed in seawater of 31.5‰, 25.2‰, 18.9‰ and 12.6‰

salinity respectively. These clams were weighed at 15 minutes intervals during the first one hour and later, at 30 minutes interval in the next hour. Before weighing the free fluid between the shells was discarded. The original weight was determined as in the previous experiment and the weights were converted to the percentage of original body weight. The results plotted in Fig. 6 show that the weight losses are directly proportional to the decrease in salinity.

Weight changes of clams in seawater with different salinity

It is generally accepted that the increase in water content in tissues of isomotic animals is directly proportional to the decrease in salinity of the surrounding water and therefore it may be expected that the weight of isosmotic animals would be decreased in most concentrated waters. It was, therefore, felt necessary to study the effect of low and high concentrations of seawater on the behaviour of this clam. Seven batches of four clams, were selected and blotted. Each clam was weighed and immersed in seawater with salinity valves 11.0‰, 19.25‰, 22.0‰, 27.5‰, 35.0‰, 40.0‰ and 45.0‰. The clams were weighed at 6, 12, 18, 24, 36 and 48 hours. The percentage of weight changes are based on the body weight including shell weight and are shown in Table 1. As the clams behaved erratically no definite tendency towards increase or decrease in the body weight was observed though the experiments were repeated several times. The weight changes are found to be insignificant in various salinities.

TABLE 1. *Weight changes of* Donax cuneatus *in several concentrations of seawater (Weight change is expressed as the percentage of the original weight. Weight of the shell is included in calculations)*

Salinity (‰)	Time in hours						
	0	6	12	18	24	36	48
11.00	100.00	99.97	99.88	99.68	99.61	99.31	99.15
19.25	100.00	99.93	99.72	99.61	99.79	99.49	99.36
22.00	100.00	99.72	99.70	99.61	99.74	99.63	99.49
27.50	100.00	99.93	100.00	99.88	100.00	99.79	99.72
35.00	100.00	100.20	100.30	100.30	100.30	100.20	99.88
40.00	100.00	100.20	100.20	100.20	100.10	100.00	99.91
45.00	100.00	100.10	100.20	100.10	100.30	100.20	99.33

DISCUSSION

The present study showed that *D. cuneatus* in the sandy intertidal zone could survive upto 22.05‰ dilution. Below 18.9‰ salinity, the clams behaved erratically. Though a few clams did open valves in 9.45‰, their activities were ceased

practically below this salinity. The lethal salinity based on 50% survival is 20.5‰ which showed the marine habitat of *D. cuneatus* compared to other bivalves. In *Teredo navalis*, Blum (1922) observed that this pholad could be active in 9‰ salinity below which the activity was decreased. Identical results were obtained by Atwood and Johnson (1924) for *Bankia gouldi*. Motwani (1955) found considerable variation in the survival period of individual *Mytilus edulis* in the same salinity and suggested that the salinity tolerance would fall within 20‰ and 50‰. Outside these limits a sharp fall in resistance in this mussel occurred. *Rangia cuneata* could survive in salinities ranging from 0 to 39‰ for a period of at least two months, if gradually acclimated (Bedford and Anderson, 1972). Two distinct lethal salinities of *Katelysia opima* were observed by Mane (1974) wherein he found that this clam in summer can survive at 16‰ salinity whereas in monsoon at 10‰.

The time taken by *D. cuneatus* to open valves was progressively increasing dilutions as well as concentrations of seawater. The clams subjected to higher concentrations took more time to open their valves than those subjected to dilutions. No marked difference was noted between the time taken by clams subjected to progressive changes and the time taken by those subjected to sudden changes. The mussel, *Mytilus edulis* was found to be very sensitive to dilutions of seawater and valve opening of this species was progressively delayed with increasing dilutions (Dodgson, 1928 ; Milne, 1940). Motwani (1955) in *Mytilus edulis* and Mane (1974) in *Katelysia opima* obtained identical results. These workers, however, noted that the time taken to open valves was greater for the clams subjected to progressive changes than for those subjected to sudden transfers. It has been demonstrated that the osmotic pressure of outside medium acts as the principle stimulus controlling the initial opening of valves and a small amount of salts in the external medium is necessary for fully opening of valves (Motwani, 1955 ; Mane, 1974).

The body fluids play a vital role in the physiology of animals. It was found that the clams with the injured mantles, adductor muscles and underlying parts lost more weight than those without injury. The clams lost weight due to exudation of fluids from the sinuses in the mantle and the cut surfaces of adductor muscles and additional loss in injured clam body occurred due to fluid loss from the ruptured body parts. It was found that the clams could not regulate their weights when disturbed. Disruption of the rhythmic opening and closing of the shells interfered with the ability of clams to regulate weight and hence volume. Removal of the free fluid between the shells stimulated further secretion to replace the first one at the expense of the internal body fluids. A small portion of the weight loss in the forced open clams was due probably to injury of the blood spaces within the adductor muscles caused by tearing of muscle fibres.

The weight of wedged open clams was increasingly reduced with decrease in salinity. A simple osmotic phenomenon is the probable explanation of this type of behaviour. But the weight changes of intact clams in all salinities were not significant. In the first case, the freedom of clams to open and close their valves might be reduced by the presence of wedges between their shells and osmosis might have occurred, as was suggested by Mane (1974). In the later experiment, if any weight change occurred one would expect the weight to decrease rather than increase in the most concentrated seawater and therefore there might be some phenomenon playing its role other than osmosis which caused increase in the weight in higher salinities (Fingerman and Fairbanks, 1956; Mane, 1974). The results presented in Table 1 show that there is a lack of significant loss of weight after 48 hours in the gradient of salinities which in turn indicates that *D. cuneatus* might be able to regulate volume,

but before arriving at any definite conclusion additional experiments would be worthwhile.

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