Volume Regulation and Respiration Under Osmotic Stress in *Themiste dyscritum*

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Introduction

Members of the phylum Sipunculida are exclusively marine organisms, inhabiting all but brackish seas in all latitudes. They occur mostly from the intertidal region to the edge of the continental shelves, but are found to 5000 m. (Hyman, 1959). They are simple veriform coelomates with a posterior trunk and anterior slender introvert ending in a small tuft of tentacles. Being positively thigmotaxic, Sipunculids are often found buried in sand or mud, inhabiting old shells or in holes in a variety of substrates.

Volume regulating ability in Sipunculids is most easily studied by noting changes in weight, these changes are assumed to be water that has moved into or out of the body. When placed in dilute sea water the animal gains weight (volume) initially, but, in time, returns to about the original weight. In concentrated sea water the animal looses weight, then returns to original values. This is done most commonly by excretion of water, osmotically active compounds, or both.

The ability to regulate volume in Sipunculids is a subject of debate, with workers reporting various results and conclusions. Kamemoto and Larson (1964), and Kamemoto and Nitta (1964) found *Themiste signifer* and *Sipunculus* sp. to be volume regulators. Gross (1954), working with *T. zostericolum* and Oglesby (1968) with *T. dyscritum* found these animals able to regulate volume in high salinities, but were conformers in diluted sea water. Adolf (1936) and Virkar (1966) reported that *Phascolosoma gouldii* * is a volume conformer. Adolf (1936), however, did find some tendency for *Phascolosoma* to adjust its weight long periods of time after disturbed by injections or extractions of body fluid. This would suggest some regulating ability. On the whole, though, the phylum is considered to be volume conforming.

No information was found on respiration in Sipunculids other than a note in Towle and Giese (1966) that the rate in *Phascolosoma agassizii* is of the order found in other marine invertebrates. Oglesby (1969) mentions trends in respiration rates of various polycheates under osmotic stress.

The present report provides some data on the ability of *Themiste dyscritum* to regulate volume under selected salinities. Some data is given on rates of respiration on worms under salinities higher and lower than those found in its normal habitat.

Methods and Materials

Individuals of the species *Themiste dyscritum* were collected at Boiler Bay, Oregon during the morning low tide Aug. 7, 1975 (6:50 a.m., -1.9 to +1.5 ft. MLLW). Specimens were gathered from the holdfasts of eel grass and from sand filled holes in the shale [illegible]h. The holes were [illegible]

* Oglesby (1968) sites Fisher (1952) and Stephen (1964) for discussion and revision of Sipunculid taxonomy. He states that *Themiste* is the correct genus name for *Dendrostomum* and that *Phascolopsis* is commonly called *Phascolosoma* or *Golfingia*.

beneath. The holes were probably of bivalve origin as Adula californiensis and Saxidomis were found inhabiting clean holes in the area. Some Phascolosoma agassizii was also found in the holdfasts, none in the holes.

The Sipunculids were transported in a bucket to the Marine Science Center lab at Newport, Oregon and placed in a plastic pan of running sea water by 10:00 a.m. One hour later the pan was placed in a cold room, 7°C, where the worms were maintained in non-circulating sea water, with out feeding. The water was changed daily for several days, allowing the gut to empty and to keep the salinity from rising due to evaporation. Prior to running the experiments individual worms were placed in numbered, 3 oz. paper cups.

To collect and standardize the weight data, worms were rolled on a paper towel, weighed, blotted with a Kim-wipe until the skin no longer glistened, and weighed again. All weights were to an accuracy of 0.01 gr. and there was little difference between weights before and after blotting (from zero to 0.05 gr., generally 0.02 gr.). The lower weight was used for calculations. Handling of the worms caused little response, only a slight contraction of the muscles with a resulting increase in turgor of the body, and inversion of the introvert. Adolf (1936) found that this increase was negligible in comparison to the osmotic pressures of the medium. No water was seen issuing from the nephridiopores, as was noted by Oglesby (1968).

Weight changes were noted at increasing intervals over a 50 hour period. Water was added to the containers every 12 hours, and changed at the 24 hour reading. Animals weighing over 2 gr. were used for the volume regulation study, as J. P. Green (private correspondence) has found a significant difference in regulating ability between worms over 2 gr. and under 2 gr.

Respirometry was done using a Gilson Respirometer. Animals were removed from their individual cups and placed into the respirometer vials with either 40% or 120% sea water. The vials were then placed into the machine and remained there for the 24 hour period of measurement. The system was allowed to equilibrate for two hours before readings were taken. Worms under two grams were used as larger ones would not fit the vials, adjustments of the machine could not be done, and there was not enough time to do repetitive runs.

A 10 gallon jug was filled with sea water from Yaquina Bay and used as a standard for all water changes, dilutions, and concentrations. Dilutions were made with distilled water, concentration by evaporation on a hot plate. Four salinities were used; 40%, 80%, 100%, and 120% sea water. Osmotic concentration was determined using a Wescor inc. 5100 Vapor Pressure Osmometer, and results are given in Table I. The standard, 100%, did not change concentration over the two week period used.

Five worms were used for each salinity in the volume regulation experiment and all were kept at room temperature during the run, 18°C to 21°C. The respirometer was kept at 9.1°C during the measurement period, and three worms were used at each salinity.

Per cent Sea Water	Osmotic Concentration mOsm/kg
40 %	415
80 %	787
100 %	985
120 %	1213

Table I - Osmotic concentrations of the selected dilution and concentration of sea water used.

Results

Respiration

No significant difference ($\alpha = 0.20$) was found in the rate of respiration between specimens of Themiste dyscritum in 40% and 120% sea water. Data collected is given in Table II with standard deviations. The small sample size and limited number of data pionts restricts the use of this data for detailed observations and conclusions,however, there does appear to be a slight difference in rate between the two salinities.

Time from transfer (hours)	Per cent sea water	
	40 %	120 %
2	13.57 ± 0.35	8.20 ± 4.00
3	12.90 ± 0.46	8.07 ± 0.25
4	9.73 ± 0.95	7.07 ± 1.33
15	11.30 ± 1.30	9.07 ± 1.47
23	9.80 ± 1.00	6.00 ± 1.25
24	11.53 ± 1.40	8.47 ± 0.46

Table II - Rate of respiration (μl O_2/hr/gr initial weight) after transfer to indicated salinity

Volume Regulation

After transfer there was an immediate gain or loss of water proportional to the magnitude of change in salinity. Changes by the control (100 % sea water) were negligible, though the broad dip in all curves from 8 to 36 hours is interesting. This trend could be due to a natural rythm, which would suggest a slight regulatory ability, or due to some factor in handling . In either case a longer period of observation would be needed, and greater care in methods.

On the whole *Themiste dyscritum* appears to be unable to regulate volume over the salinities used. Data is given in Table III and plotted in Figure I. At 16 hours after transfer there were significant differences (α =0.10) in weight change between those worms kept in 100 % sea water and those in other salinities. These results are in agreement with Oglesby (1968). He also noted a regulation in worms transfered to slightly concentrated sea water, and none in hyposaline conditions. This is indicated here, in fact the only case to be made for regulation is in the concentrated medium where a slight trend is seen in its return to initial weight. After 24 hours the difference between 100 % and 120 % sea water is seen to be decreasing, and may continue if observed over a longer period of time.

Time from transfer (hours)	Per cent sea water			
	40 %	80 %	100 %	120 %
1	17.53	7.40	1.39	-2.04
2	26.58	11.28	1.30	-3.44
4	47.08	14.66	1.50	-7.10
8	64.40	13.46	0.80	-9.76
16	70.60	10.02	-1.32	-13.44
24	79.18	17.70	-1.82	-11.26
36	101.64	15.92	2.58	-9.24
49	98.96	16.86	2.70	-8.46

Table III - Per cent change in weight from initial weight after transfer to indicated salinity, in Themiste dyscritum. All values are averages of five animals.

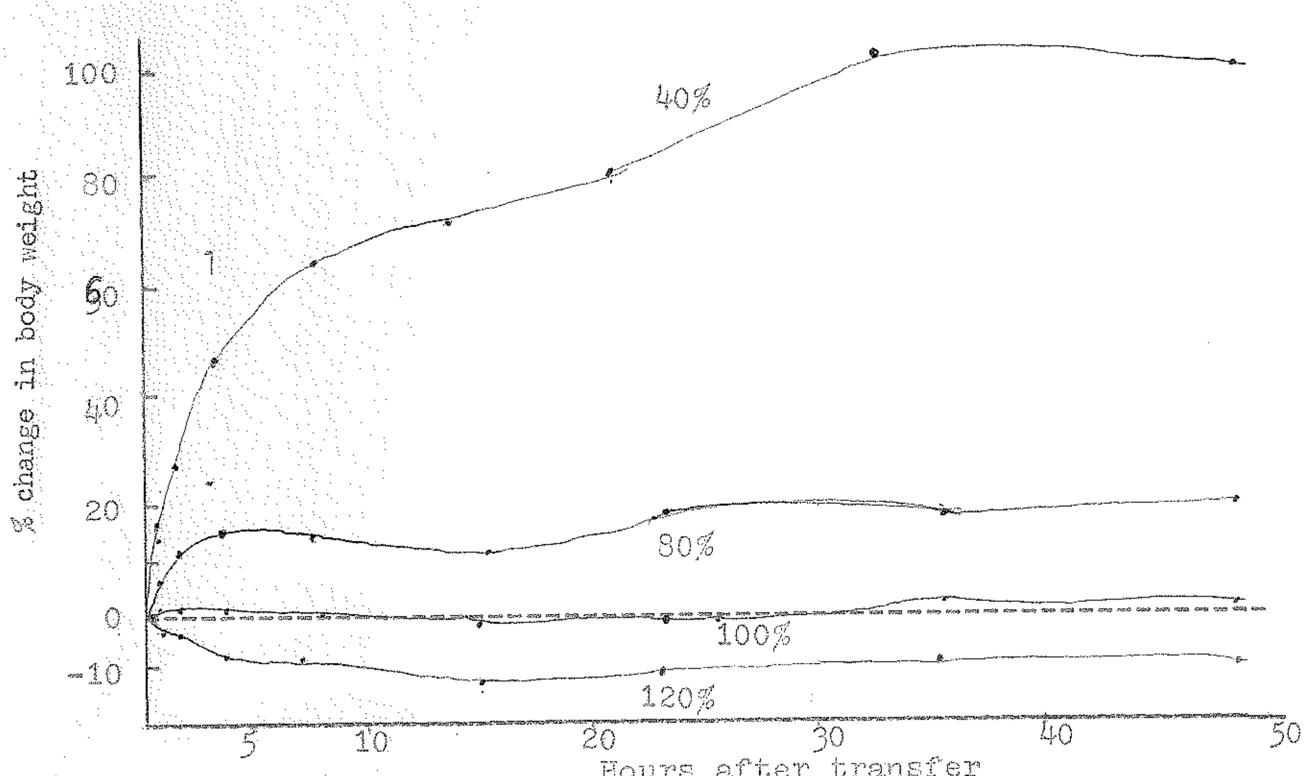

Figure I - Time course of weight change in T. dyscritum from time of transfer.

Discussion

The above results are in general agreement with those reported by other investigators with respect to volume regulation in Sipunculids in general, and *T. dyscritum* in particular. *T. dyscritum* was found not to be a volume regulator. It was also found that there is no significant difference in respiration between individuals under osmotic stress in high and low salinities.

With a limited access to, and time for use of the respirometer it was decided to look for differences in the extreme salinities. It was felt that this would be the most likely method of finding any difference. There was no desire to establish a base rate, or absolute value of respiration, only to note relative changes with respect to both time and salinity. After the values were taken it was called to my attention that it would have been better to look for differences between stressed and unstressed animals.

No other work has been found relating to respiration in Sipunculids, but this data seems to support some idea of rates in this animal. No change in respiration was expected as a volume conformer would not need to expend energy in maintaining a salinity gradient across the body wall. This would in turn cause no change in the need for oxygen.

Along with work in volume regulation there has been studies in determining osmotic concentrations of the coelomic and nephridial fluids. In both cases they vary only slightly with the medium. Determinations of ionic concentration in the coelomic fluid are inconclusive, but reported levels of proteins, amino acids and other nitrogenous compounds apparently contribute little to the total osmotic concentration of the coelomic fluid (Oglesby, 1968; Virkar, 1966; Towle and Giese, 1966). In one paper, (Virkar, 1966) it was noted that these vary with nutritional and reproductive states of the animal. Comparable studies are outside the scope of this paper, though many of them would not be difficult to carry out and would be rewarding to the investigator.

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