OSMOTIC RESPONSES OF TWO SPECIES OF SIPUNCULIDS TO DIFFERENT SALINITIES AND TEMPERATURES

by

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ABSTRACT

Two species of sipunculid worms, Phascolosoma dentigerum and Themiste lissum, inhabit the rocky intertidal zone at Puerto Penasco, Sonora, Mexico. The osmotic concentrations of their coelomic fluid and the changes in body weight were determined while the worms were exposed to 80, 100, and 116 percent sea water and to temperatures of 10°, 25°, and 35°C for varying periods of time up to 48 hours. No differences in the rate or degree of osmotic change were noted between species. There were also no significant differences between the two species in their weight changes. The time for the osmotic pressure of the coelomic fluid of both species to reach equilibrium with the external environment is greatest at the low temperature. There is a difference in mortality of the two species after extended exposure to experimental conditions. Themiste is more sensitive to 35°C and Phascolosoma, under stress, is more sensitive to 10°C. These responses to temperature probably explain differences in the distribution of the two species in the field.

INTRODUCTION

The intertidal zone along a sea coast is a far from stable habitat and may be subject to extremes of temperature, wind velocity, and humidity even during a single excursion of the tide. For example, the maximum vertical excursion of the tide for the intertidal zone in front of the desalting plant at Puerto Penasco, Sonora, Mexico, is 22 feet, yet several hundred feet of reef and boulders can be exposed for about three to six hours when the tide is very low. During this short period of time the desert climate prevails and there can be drying of the surface of the reef, concentration of sea water in the tide pools, exposure of the intertidal animals to extremes in temperatures and, during an occasional rainstorm, dilution of the microhabitat. Cyclical sea breezes tend to modify these conditions, but they are still severe.

What are the ranges of these environmental variables, particularly in the microhabitat? We do not know, but the assumption is made that the concentration of sea water in the burrows of the intertidal animals changes by no more than 20 percent during a low tide and that dry winds are never severe enough to completely desiccate the burrows. One environmental variable that can be determined is

temperature. The mean sea water surface temperature range per year at Puerto Penasco is 13°C in January to 32°C in August (Roden, 1964). My own observations show that the surface temperatures of the exposed reef are usually a degree or two lower than the air temperature. This difference is probably caused by the evaporation of the surface moisture. In addition to the surface temperatures, measurements of temperature were made about 7 cm into the reef. After a hole was drilled and the thermometer inserted, some modeling clay was packed around the thermometer to prevent any air from entering and causing a change in temperature. At this depth, the temperature ranges from 12°C (December) to 31°C (August).

Two species of sipunculids, Phascolosoma dentigerum Selenka and DeMan, and Themiste lissum (Fisher), are distributed in a mosaic pattern in the reefs and rocks of the mid- and low-intertidal zones. Concentrations of Themiste will be found in one small area and concentrations of Phascolosoma will be found in another; seldom are both species found in equal numbers in the same ledge. However, both species are found within 3 to 5 cm of the surface of a ledge or reef. Usually these worms occupy burrows which open out onto the surface or into a tide pool; but sometimes a slab can be lifted off the surface of the reef and

the sipunculids are found in the crevice between the slab and the ledge.

There appear to be further differences in the distribution of the two species in the intertidal zone, according to my observations and those of Taylor (1964). Themiste is rarely found in the mid-intertidal barnacle-covered rocks, while Phascolosoma is more abundant there. Both species are found in about equal numbers from the lower parts of the mid-intertidal levels to the lowest excursion of the tide. The barnacle-covered rocks, because of their high position in the intertidal zone, are exposed for the longest period of time of any of the reef areas during low tide. Taylor (1964) found a difference between the two species in their tolerances to high temperatures and suggested a relationship between tolerance and distribution. Phascolosoma could survive for 30 minutes at temperatures up to 45°C, while Themiste could not survive past 42°C, even after being acclimated at 35°C for 48 hours.

Sipunculids have perhaps the best examples of the hydrostatic skeleton, which is described by Chapman (1958) as any fluid mechanism providing a "means by which contractile elements can be antagonized." In sipunculids this is an open circulatory system contained within the coelom and surrounded by the body wall. The correct volume of body fluid must be maintained in order to allow efficient

movements of the body and extension of the introvert. Zuckerlandl (1950) states that eversion of the introvert in Sipunculus nudus was never observed when there was no coelomic pressure, although the muscles were still able to display activity. When no resistance is met the proboscis can be everted by a pressure as low as 0.9 cm of body fluid. However, during burrowing movements pressures up to 90 cm of body fluid are required. Phascolosoma and Themiste undoubtedly must maintain their hydrostatic pressure in order to remain in their burrows, to extend the introvert for feeding and to facilitate defensive movements such as contracting the body wall and completely withdrawing the introvert. Hydrostatic pressure is dependent on fluid volume and this in turn is dependent upon the fluid's osmotic pressure in relation to the osmotic pressure of the environment.

Sipunculids cannot maintain the osmotic pressure of their body fluids if the external fluids are different; they conform to the osmotic pressure of their surrounding medium (Gross, 1954; Schmidt-Nielson and Laws, 1963). However, they do show some volume regulation in salinities which are not more than 25 percent above and below normal. After an initial gain or loss of weight, in low or high salinities respectively, the animals tend to approach their original weight after several hours. If the salinity varies

more than ±25 percent, the animals are too severely stressed and die quickly. Oglesby (1968) found little volume regulation in Themiste dyscritum after transfers from 98 percent to 49 percent sea water. He postulates that somehow the nephridia are rendered non-functional by the large change in salinity. The nephridia function as volume regulatory organs by eliminating water and salts from the coelomic fluid, thus enabling the animal to maintain its isosmoticity with the environment and also maintain its volume (Kamenoto and Larson, 1964). Gross (1954) found that Dendrostomum zostericolum can release osmotically active particles from its body wall into the coelomic fluid to help compensate for the dilution of the fluid in lower salinities. Virkar (1966) saw no volume control in Golfingia gouldii at lower salinities. However, he did find a release of free amino acids from the body wall in response to lowered salinity. Adolph (1937) first recognized the differential permeability of the body wall of sipunculids; the inward flow of water and salts is greater than the outward flow when the animals are subjected to salinities that are other than normal.

The purpose of this study was to determine whether or not different conditions of salinity and temperature have any effect on the ability of these two species of sipunculids to adapt to the external medium and whether or not there is any difference between the two in this adaptability

that could account for the difference in their distribution in the intertidal zone. Both species are subjected to concentrations of salt water 20 percent above and below normal and to temperatures of 10^{o} and 35^{o}C as well as normal salinity and temperature. The changes in osmotic concentration of the coelomic fluid and the changes in weight of both species have been determined under these various conditions.

MATERIALS AND METHODS

Phascolosoma dentigerum and Themiste lissum were collected from the intertidal area near the desalting plant at Puerto Penasco, Sonora, Mexico. Small samples were taken at various times throughout the year in order to work out the techniques and to perform the preliminary studies. Then, 600 of each species were collected for the osmotic concentration and weight studies on 15 and 16 September 1967. In all cases, the animals were collected at low tide by breaking off pieces of rock and exposing the sipunculids in their burrows by chipping the rock with a pick and hammer. The animals were then kept in sea water and brought back to the laboratory.

In the laboratory, both species were kept together in a 15-gallon aquarium of sea water brought from Puerto Penasco. The bottom of the aquarium contained a half-inch layer of coarse sand into which the animals would burrow. When first put into this stock aquarium, all of the Phascolosoma burrowed rapidly and most had disappeared in a few minutes, while it took several hours for most of the Themiste to burrow. A large sand-filled aerator-filter was kept at one end of the aquarium. Bubbling of the sea water was eliminated to avoid rapid evaporation of water and the

aquarium was covered with clear polyethylene film to reduce surface evaporation. The osmotic concentration of the sea water was checked periodically and distilled water was added as needed to keep the osmotic concentration stable. Under these laboratory conditions, the sipunculids live and remain healthy, with normal activity and reflexes, for at least four months without any food material being added to the aquarium.

The three concentrations of salt water used in the experiments were made up from Instant Ocean salts and ordinary tap water. A 40-gallon stock solution of Instant Ocean with a 1.000 osmolal concentration was prepared. From this stock solution dilute salt water was obtained by adding tap water, and concentrated salt water was obtained by adding more salt. Osmotic concentrations of the test solutions used were between 80 and 116 percent of the value of normal sea water at Puerto Penasco, 1.050 osmolal. These salinities are probably a little lower and higher, respectively, than the salinities that actually occur in the habitat. Responses of the two species of worms to higher or lower concentrations than these might yield interesting physiological data, but would be of no ecological significance, since the sipunculids probably are never exposed to greater fluctuations in salinity at Puerto Penasco. Experimental aquaria with the test solutions each contained

five gallons of water and were aerated with an air stone. Filtration was not necessary since only a few animals were kept in the aquarium for periods of not longer than 48 hours. The concentrations of salt water used were 0.830 osmolal, 1.050 osmolal, and 1.210 osmolal for the low, normal, and high salinities respectively, and these correspond to 80, 100, and 116 percent sea water. These were the three standard concentrations for all of the experiments in which changes in internal osmotic concentrations and in weight were determined.

The effect of temperature on osmoregulation was studied by testing animals at 10°, 25°, and 35°C. The two higher temperatures were achieved by using two aquarium heaters in each aquarium. The lowest temperature was obtained by placing the aquaria in a cold room at 4°C and using two heaters to keep the water temperature at 10°C. Ten degrees centigrade is slightly below and 35°C is slightly above the minimum and maximum mean monthly temperatures over a year in the rocky substrate of these sipunculids (personal observation). The 25°C temperature was chosen because it is probably close to the mean temperature of the habitat. Twenty to 25 animals of each species were put into each of three aquaria. The osmotic concentrations of the body fluids of three animals of each species were determined at time zero and at 2, 4, 6, 10, and 24 to 35

hours later. Each animal was used only once and not put back into the test aquarium.

Coelomic fluid was obtained from the sipunculids after each animal was rinsed briefly in distilled water and gently blotted dry with Kimwipes to remove any salts from the skin surface. The coelomic fluid was collected by puncturing the body wall with a hollow glass needle made from 3 mm tubing drawn to a fine point. During this process, the animals were on small squares of Parafilm to prevent the clotting of any coelomic fluid which might be spilled. A new needle was used for each animal. The collected coelomic fluid, usually about 0.06 ml, was then blown out of the needle into a 3 x 25 mm glass tube with one closed end. From the time of collection until dilutions were made, the coelomic fluid and the plasma were kept cool in ice to prevent evaporation. The tubes of coelomic fluid were centrifuged for several minutes and the clear, usually colorless plasma, was drawn off with a long-nosed Pasteur pipet, without disturbing the pellet of cells in the bottom of the tube. The layers of cells in the centrifuged coelomic fluid of both species are similar to those described by Towle and Giese (1967) for Phascolosoma agassizii. The drop of plasma was placed on a small clean square of Parafilm. Ten lambda of the plasma were carefully taken from the drop with a micro-pipet. The

plasma was blown into 0.19 ml of glass-distilled water and the pipet was rinsed several times in this water. Freezing of the plasma was found to increase the osmotic concentration in the same sample by three percent. It is known that freezing can cause changes in the physical properties of plasma (Archer, 1965). To eliminate the need for freezing, it was decided to prepare the samples to be read as they were collected. The reference solutions were made by mixing 20 lambda of sea water in 0.18 ml of glass-distilled water in the same manner.

Dilutions of plasma were made because even from the largest of these sipunculids it was impossible to get 0.2 ml of plasma--the minimum volume that can be used in the osmometer. A 1:20 dilution was chosen since usually 10 lambda could be obtained from the smaller animals. The sea water was also diluted so that the range of the instrument would not have to be changed each time the sea water concentration was read. For the sea water, a 1:10 dilution was used since it was more convenient to have a different size of pipet in this case. A direct relationship was found between the 1:10 and 1:20 dilutions, i.e., sea water diluted 1:20 read half the value of the sea water diluted 1:10. Furthermore, the osmolality of full strength sea water and 1:10 and 1:20 dilutions of it were determined and showed a direct relationship so that it is possible to

convert the value for each sample to its actual value. The effect of these dilutions on the actual values of the osmotic concentration of the plasma and sea water is therefore negligible.

Osmotic concentrations were measured with an Osmette Precision Osmometer. This instrument measures the freezing point depression of a solution. The solution is supercooled to 2°C below the freezing point of the solvent by removal of two calories of heat from each one milliliter of sample and then insulated. The sample is seeded to cause rapid freezing of the solvent. With seeding, the latent heat of fusion is released into the insulated solution until an equilibrium between the formation and melting of ice (the freezing point) is reached. The readings of individual samples on the instrument are reproducible to within ±0.5 milliosmoles. The variation in osmotic concentrations among animals was no more than ±3.6 percent of the mean for animals measured under a given set of conditions.

For the weight studies, the animals were weighed on a Mettler Type H3 Analytical Balance after a brief rinse in distilled water and blotting on a Kimwipe. The same temperatures and salinities used in the osmotic pressure studies were employed. The weights of the animals are used because any changes in the water content of the

coelomic fluid due to differences in salinity would be more accurately measured as a change in weight than as a change in volume. Nine animals of each species were placed in salt water of each of the three salinities. The effects of temperature were determined by changing the temperature of the three aquaria simultaneously on three different days. All 18 animals in each aquarium were weighed carefully just before they were put into the water and at 2, 4, 6, 10, 24 and 48 hours thereafter. Handling animals during weighing did not appreciably affect them. In one critical test, for example, the same animal was weighed seven times in a period of 20 minutes and the recorded weights varied by no more than one percent.

RESULTS

General Procedure - Osmotic Studies

The osmotic concentration of the coelomic fluid of the two species of sipunculids was determined at 35°, 25°, and 10°C and at 1220, 1051, and 840 milliosmoles (116, 100, and 80 percent sea water). The animals were taken from a stock aquarium filled with 100 percent sea water at room temperature and placed into the test aquaria. The osmotic concentrations of the plasma of three animals of each species were read at time zero, before addition to the test aquarium, and at approximately 2, 4, and 6 hours later, because preliminary studies showed that the greatest osmotic changes occurred within the first five hours. The osmotic concentrations of the plasma of two animals of each species were determined also at 10 and 24-35 hours later to see how the animals reacted after more prolonged exposure to the medium.

The means of the osmotic concentrations for each group of three animals are used for plotting the graphs. The ranges of each mean are also shown to indicate the variation among the three animals. Since it took at least one-half hour to obtain coelomic fluid from three animals, the mean of the osmotic concentrations was plotted at the

midpoint of the time interval. For example, the mean for the two hour reading was plotted at 2.5 hours for Themiste and close to three hours for Phascolosoma.

The osmotic concentration of animals kept for over a month in the stock aquarium changed very little. The osmoconcentration of body fluids and sea water, nevertheless, did vary slightly during the experimental period as can be seen in the figures. The mean value for Themiste was 1089 with a range of ±60 milliosmoles and for Phascolosoma was 1073 with a range of ±40 milliosmoles. The mean osmotic concentration of the sea water in the stock aquarium in these experiments also changed a little and was 1050 ±20 milliosmoles.

Not much can be said about changes in behavior with experimental conditions. In all salinities Themiste tend to be more flabby and slower to respond to stimuli at 35°C than Phascolosoma. Animals of both species stay contracted most of the time at 10°C.

Effects of Temperature on Worms in Normal Sea Water

The osmotic concentrations of the plasma of both species decreased slightly after the worms were added to the test aquaria with 100 percent sea water because the water in the stock aquarium from which the animals were taken was about two percent more saline than the test solution of Instant Ocean (Figure 1). The general responses

Figure 1. Changes in osmotic concentration of the coelomic fluid of Themiste lissum and Phascolosoma dentigerum as a function of time, when worms are placed in 100 percent sea water.

The osmotic concentration is expressed in milliosmoles. The temperatures are 35°, 25°, and 10°C for (a), (b), and (c), respectively. The dotted line indicates the osmotic concentration of the test medium throughout the experiment. The average of three values is plotted at each point. The vertical bars indicate the range. Open circles indicate Phascolosoma; closed circles indicate Themiste.

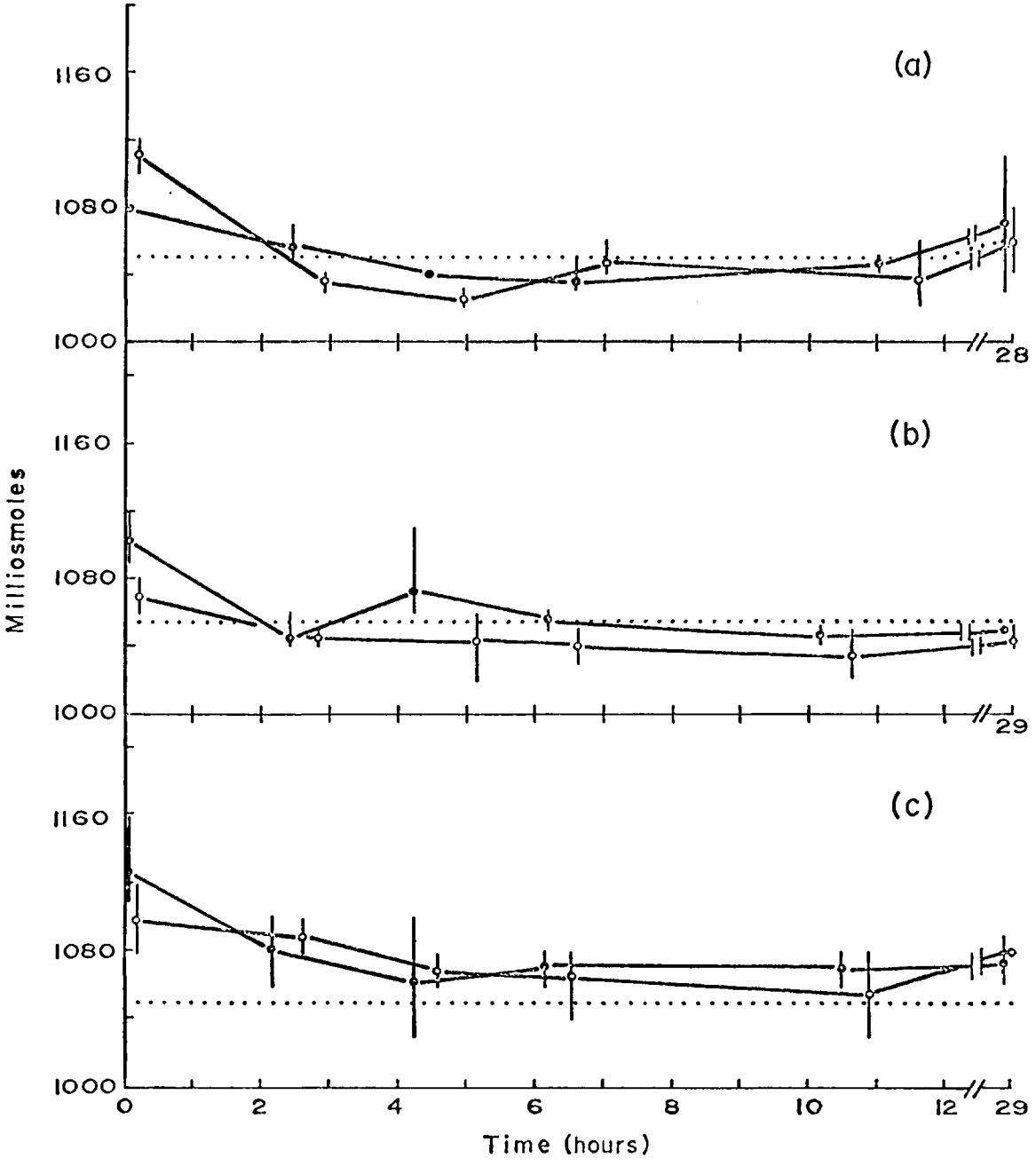

Figure 1. Osmotic changes in 100 percent sea water.

of both species at all three temperatures are very similar. The plasma osmoconcentration reaches that of the test water within two to three hours and then generally remains unchanged for at least 29 hours. Slight differences can be noted however. In Figures 1a and 1b at 35° and 25°C the plasma osmoconcentration of both species tends to be a little lower than the water osmoconcentration. (In Figure 1b at four hours, the mean for Themiste is above that of sea water because of a high value for one animal.) On the other hand, at 10°C (Figure 1c) both curves are above the osmoconcentration of the salt water. On reanalysis of the results it is concluded that these high values are artifacts resulting from instrument malfunction in this single experiment and it is concluded that the osmoconcentration of the body fluid is close to that of the test solution. A change in salinity of the test solution at high temperatures cannot be avoided as some evaporation takes place. As expected, after 28 hours there is a slight increase in the osmoconcentration of sea water (Figure 1a), and the plasma osmoconcentration of both species rises slightly as well.

Effects of Temperature on Worms in Hypersaline Water

In Figure 2 both species at all three temperatures show an increase in the osmotic concentration of the plasma

Figure 2. Changes in osmotic concentration of the coelomic fluid of Themiste lissum and Phascolosoma dentigerum as a function of time, when worms are placed in 116 percent sea water.

For other information refer to legend of Figure 1.

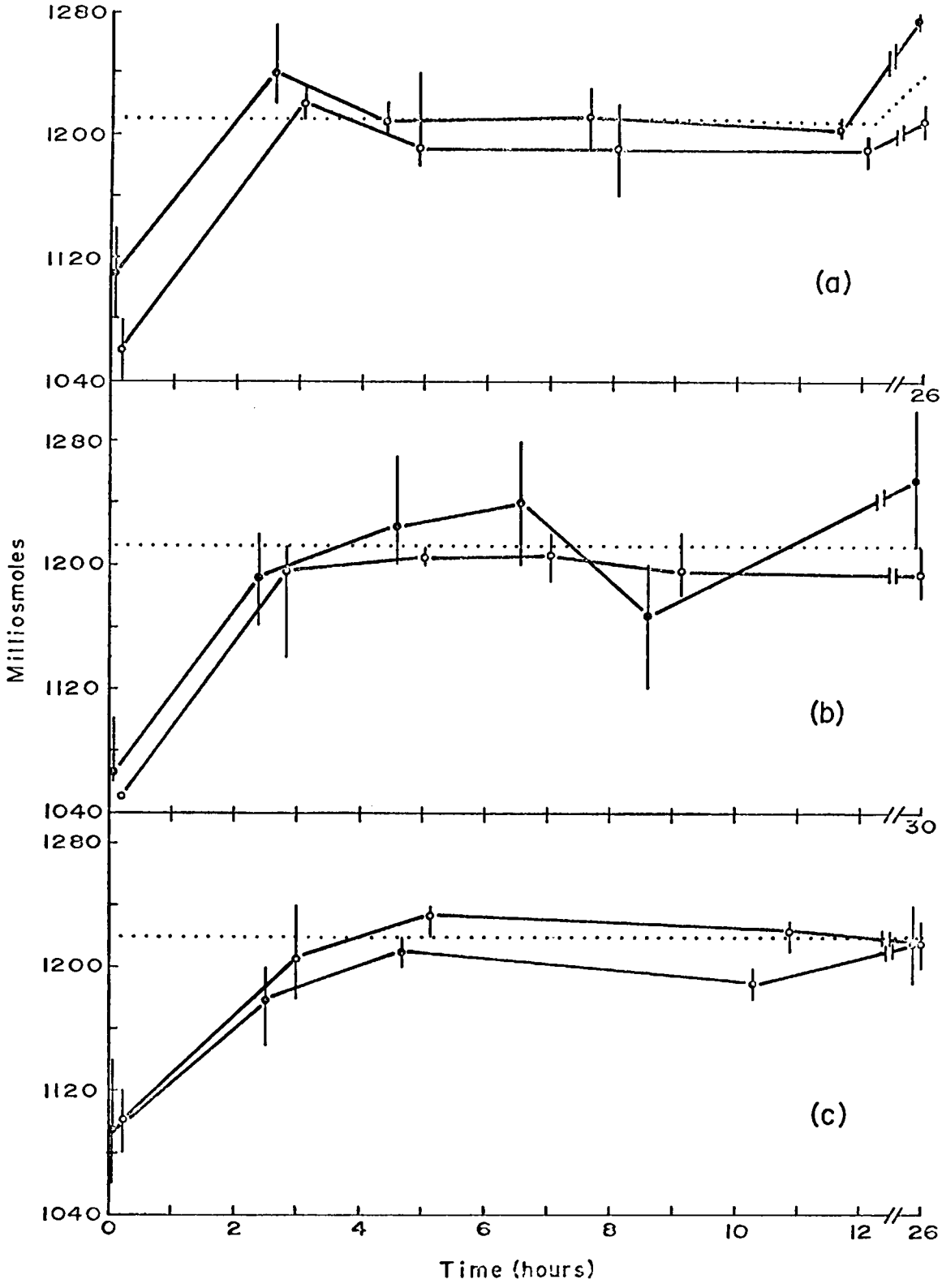

Figure 2. Osmotic changes in 116 percent sea water.

until it conforms to the external medium by the end of five or six hours. Then, for the next 20 to 24 hours the plasma osmoconcentration generally parallels the osmotic concentration of the salt water. At 10°C (Figure 2c) the initial response of both species appears to be slower than at higher temperatures, i.e., the slope is less than in Figures 2a and 2b. Conversely, at 35°C (Figure 2a) the peak concentration is reached more quickly than at the lower temperatures.

In Figure 2a between 12 and 26 hours the osmotic concentration of the salt water had increased due to a high rate of evaporation at 35°C. The plasma osmoconcentration of both species increases concurrently, but the two means are widely separated at 26 hours. Themiste was much more erratic than Phascolosoma at 25°C (Figure 2b) with widely varying ranges and at 30 hours the two means are also quite widely separated. Whether these differences in means at the higher temperatures are transient differences or whether they are biologically significant is still to be determined. At 10°C (Figure 2c) at 26 hours the means and ranges of both species are quite close to one another and to the osmotic concentration of the salt water.

Effects of Temperature on Worms in Hyposaline Water

When placed in dilute sea water Themiste and Phascolosoma show decreases in the osmotic concentration of the plasma until it approaches the osmotic concentration of the surrounding medium within 2 to 5 hours (Figure 3). The plasma osmoconcentrations roughly parallel the water osmoconcentration at all three temperatures but both species remain hyperosmotic to the medium until after 12 hours. Again, the slope is less at 10°C (Figure 3c) than at the two higher temperatures, indicating a slower rate of adjustment at the lower temperature.

The plasma osmoconcentration of Themiste at 35°C (Figure 3a) at 35 hours and the plasma osmoconcentrations of both species at 10°C (Figure 3c) at 30 hours are slightly below the osmotic concentration of the salt water, while at 25°C (Figure 3b) the plasma osmoconcentrations of both species at 29 hours are above the concentration of the medium.

Effects of Salinity at Moderate and Extreme Temperatures

At all salinities and temperatures the osmotic concentrations of the plasma of both species of sipunculids tend to conform to the external salinity. The rate at which the adjustments occur is virtually the same in all

Figure 3. Changes in osmotic concentration of the coelomic fluid of Themiste lissum and Phascolosoma dentigerum as a function of time, when worms are placed in 80 percent sea water.

For other information refer to legend of Figure 1.

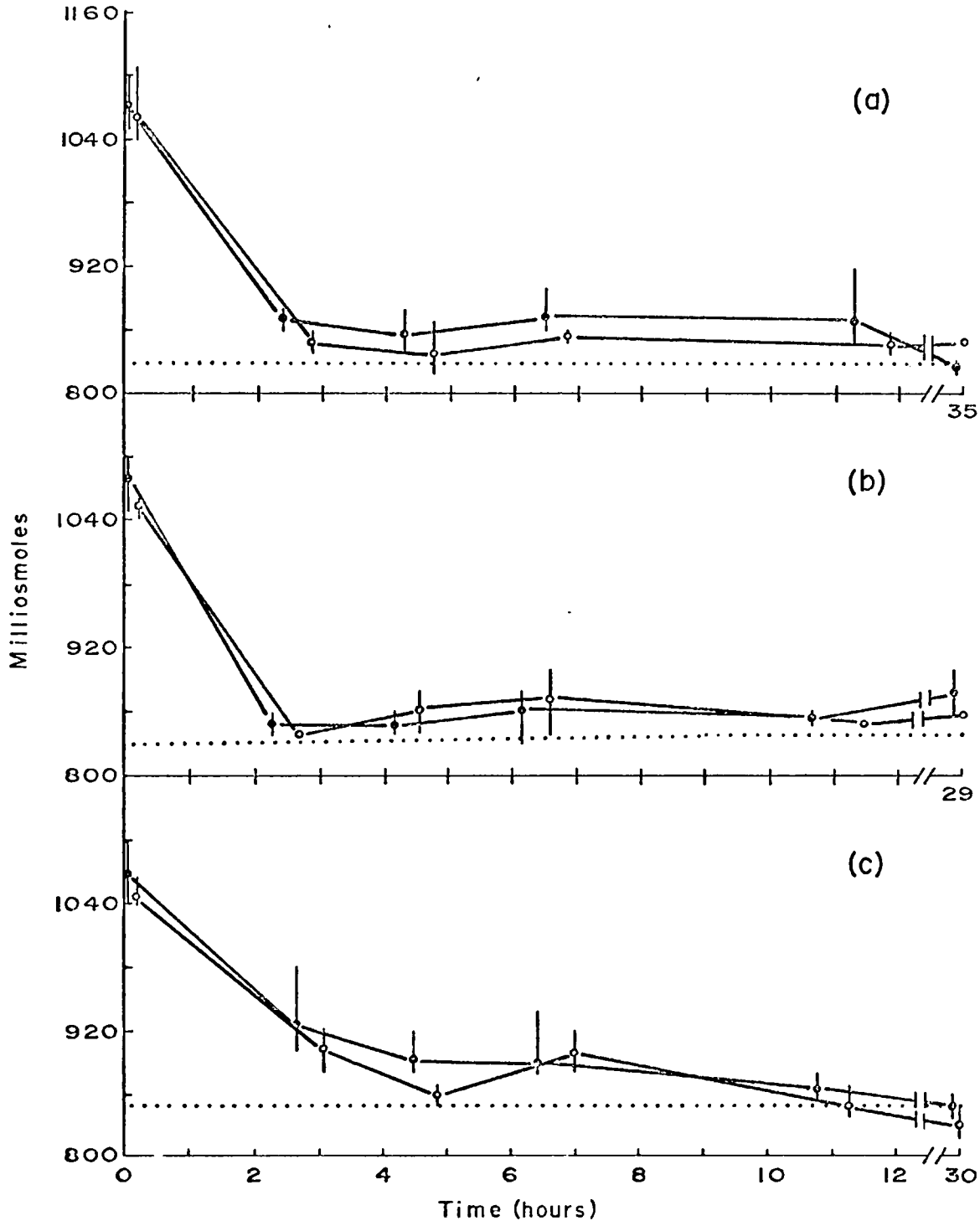

Figure 3. Osmotic changes in 80 percent sea water.

cases except at the lowest temperature in which there is a time lag for complete adjustment.

General Procedure - Weight Studies

The experiments in which changes in weight of Themiste and Phascolosoma were determined as a function of changes in salinity and temperature were carried out at 35°, 25°, and 10°C and at 116, 100, and 80 percent sea water. Each of nine animals of both species was put into a small labelled beaker in a test aquarium and was weighed before addition to the test solution and at exactly 2, 4, 6, 10, 24 and 48 hours later for the 35° and 25°C experiments and at 2, 5, 7, 10, 24 and 48 hours later for the 10°C experiments. In the figures, the initial weight at time zero is taken as 100 percent weight; and the changes in weight with time are expressed as a percent of the original weight, that is, the ratio of the new weight to the original weight.

The worms were taken from a stock aquarium filled with 100 percent sea water at room temperature and, after weighing, were placed into the test aquarium at staggered intervals to allow each animal to be weighed at the exact time specified. The same animals were weighed throughout the experiment at any one temperature and salinity. The means of the percent weight change are plotted on the graphs with the ranges shown to indicate the variations among the animals. Weighings were taken also on nine animals of both

species in the stock aquarium at the same time as weighings of animals in the 35° and 25°C experiments. The animals were again isolated in small beakers and handled in the same manner to observe the amount of variation that might be caused solely by handling over a long period of time.

There were a number of fatalities in these experiments, with Themiste affected more than Phascolosoma. There were also some fatalities in the osmotic studies, but among Themiste only. These latter occurred at 35°C and in all salinities to which animals were exposed for more than 24 hours. However, both species suffered fatalities in the weight studies and fatalities among Themiste were much more common in the weight studies than in the studies of osmotic concentrations. A possible explanation is that weight change experiments were conducted about a month and a half after the osmotic studies were begun and the animals may have begun to be affected by starvation and were less able to adapt. In the weight change experiments, at 24 hours seven of the nine Themiste were dead in the 35°C and high salinity test aquarium. After 48 hours at 35°C and in all the salinities, half or more of the Themiste were dead. After ten hours some of the Themiste would seem to lose muscle tonus and become flabby. They would react slowly to touch and contract after 10 to 15 minutes instead of immediately as in normal worms. However,

these animals were weighed because they were assumed to be alive, even though they were dead at 24 to 48 hours. Before dying they seemed to fill with water, extend the introvert and tentacles, and become covered with a mucoid substance. The only fatalities among the Phascolosoma were at 48 hours at 10°C in all salinities. When the worms were placed on a paper towel prior to weighing at 24 hours, they would burst open towards the anterior end of the introvert, blood would spurt out, and the animal would be limp but still responsive. At 48 hours they would be dead. The 24 and 48 hour weights of these animals were not used.

Effects of Temperature on Worms in Normal Sea Water

Figure 4 is the graph of the weight changes of Themiste and Phascolosoma at 22°C in the stock aquarium with 100 percent sea water. The means are close to the original weights of both species with Phascolosoma generally retaining its original weight and Themiste slowly decreasing in weight even under control conditions. There are slight fluctuations of the means within these general trends. At 48 hours the means of the two species are widely separated and the ranges about each mean are larger than before but the weights of the majority of the 18 animals lie within ±5 percent of the original weights. Just before the ten hour weighing, one Phascolosoma escaped from its

Figure 4. Percent change in weight of Themiste lissum and Phascolosoma dentigerum as a function of time, when worms remain in stock aquarium with 100 percent sea water, at 22°C.

The mean of nine values is plotted at each point. The vertical bars indicate the range. Open circles indicate Phascolosoma; closed circles indicate Themiste.

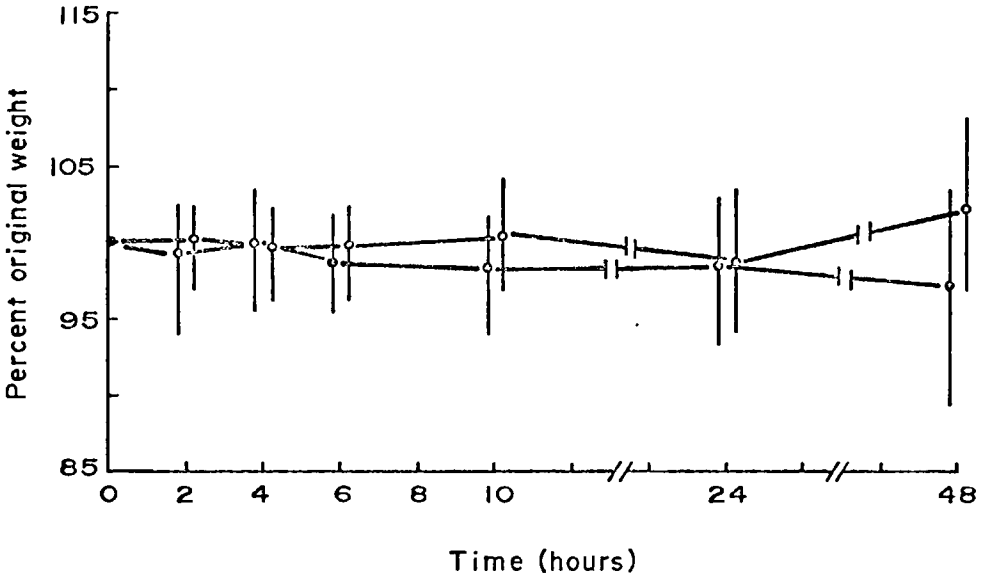

Figure 4. Weight changes in 100 percent sea water at 22°C.

beaker and mingled with the other worms kept in the stock aquarium. Therefore the means for this species from ten hours on represent eight animals each. The 48 hour mean for Themiste also represents eight animals since there was one fatality.

When worms were transferred from the stock aquarium to 100 percent sea water (Figures 5 and 6) the mean percentages of weight changes over a 48 hour period were within five percent of the original weight. The only exception is Themiste at 10°C (Figure 6b) where the weights drop initially and continue to drop to about 10 percent below the original weight by 24 hours. At this same temperature Phascolosoma also decreases in weight initially but stays between 95 and 100 percent until 48 hours. Due to fatalities this last point represents the mean weight change of seven animals. Comparing the worms kept in the stock aquarium at 22°C (Figure 4) with those transferred to 100 percent sea water at 25°C (Figure 5), it can be seen that the variation in weight change is much greater in the latter. What is responsible for this variation is not known. In fact the range of variation is greater in most of the experiments in this section. Due to mortality the weight change for Themiste at 24 hours is an average of five animals and at 48 hours of four animals. Although there is much fluctuation in the means throughout the

Figure 5. Percent change in weight of Themiste lissum and Phascolosoma dentigerum as a function of time, when worms are placed in 100 percent sea water, at 25°C.

The mean of nine values is plotted at each point (except as indicated in the text). The vertical bars indicate the range. Open circles indicate Phascolosoma; closed circles indicate Themiste.

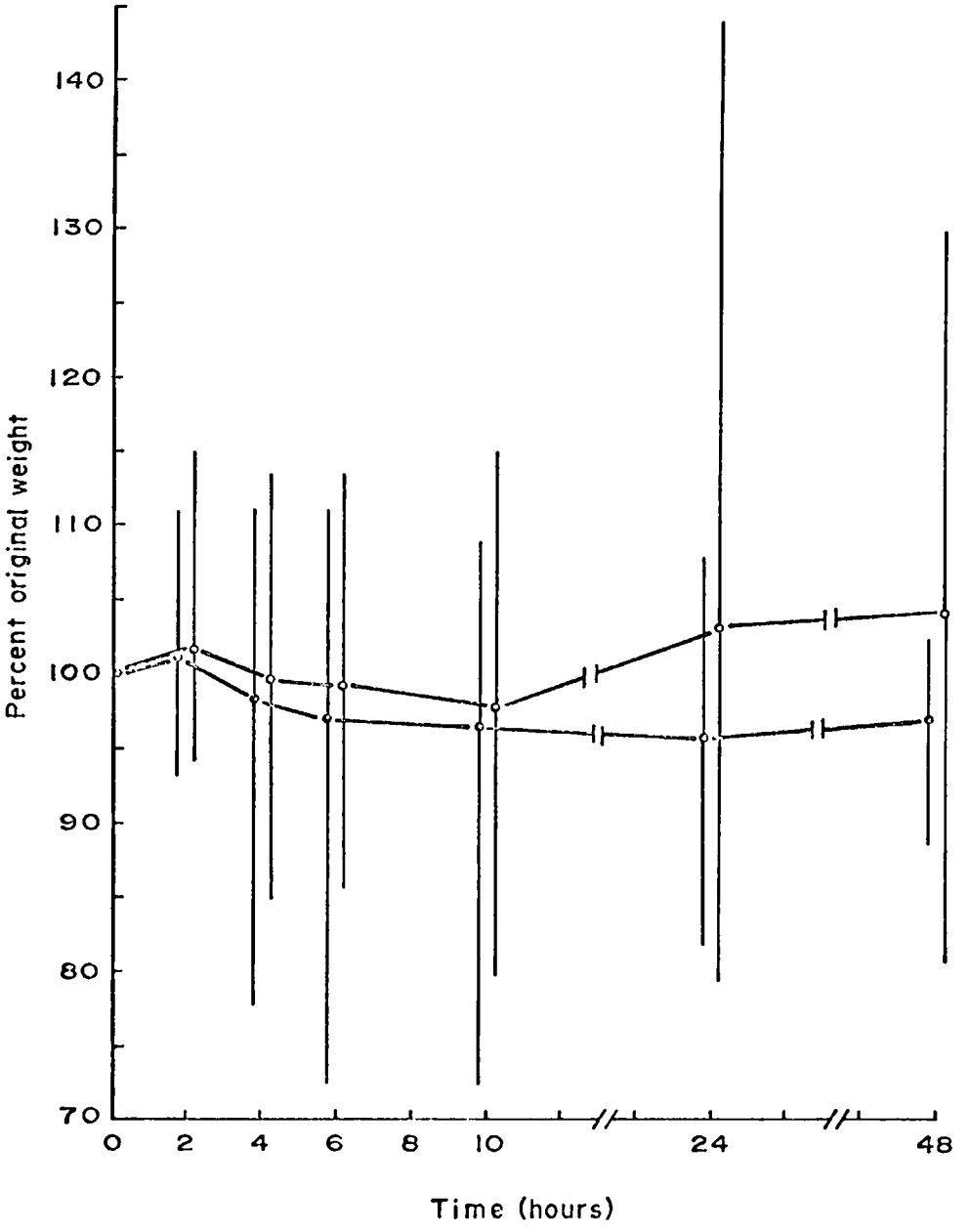

Figure 5. Weight changes in 100 percent sea water at 25°C.

Figure 6. Percent change in weight of Themiste lissum and Phascolosoma dentigerum as a function of time, when worms are placed in 100 percent sea water, at 35° and 10°C.

The temperatures are 35° and 10°C for (a) and (b), respectively. For further information refer to legend for Figure 5.

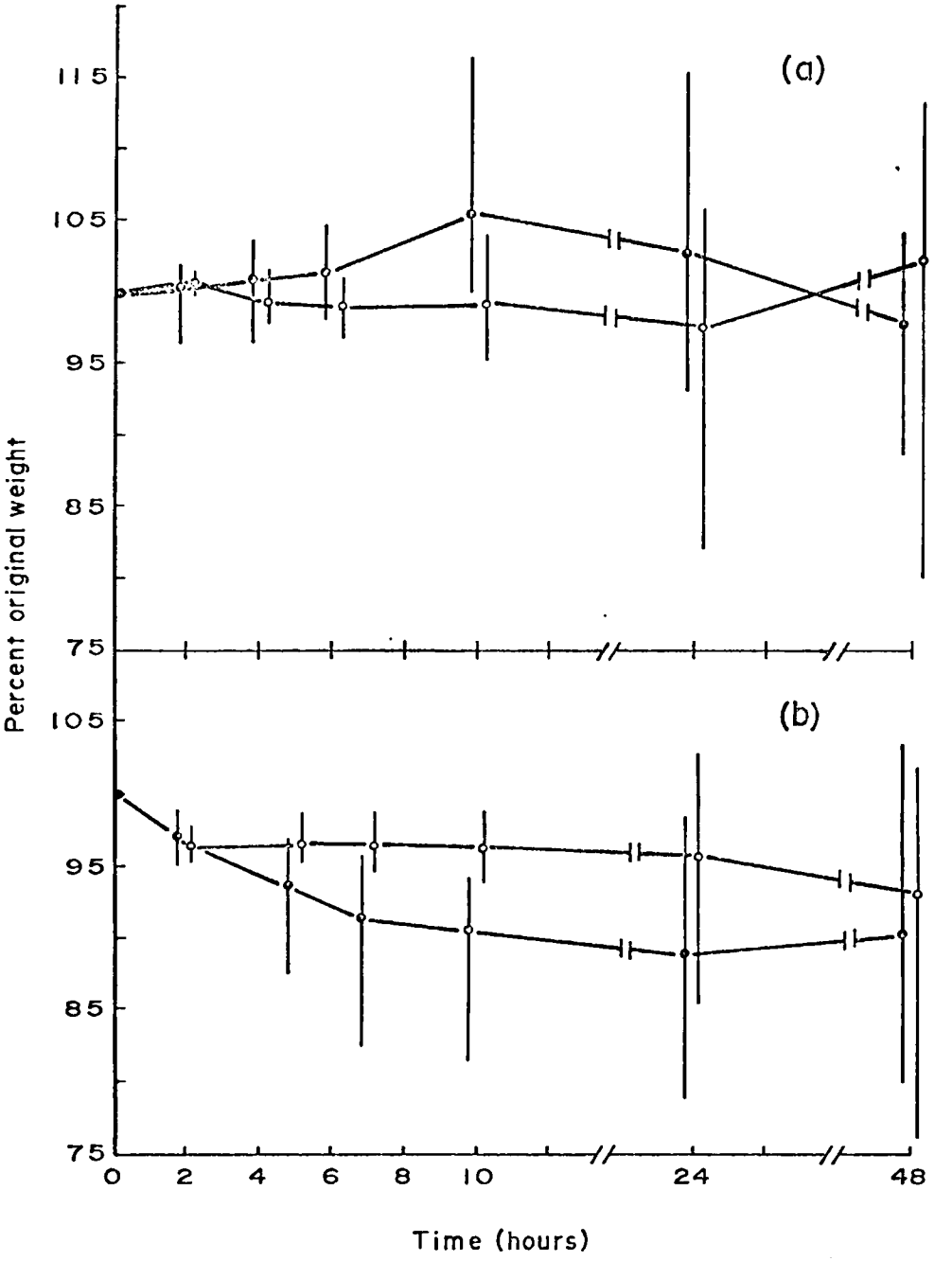

Figure 6. Weight changes in 100 percent sea water at 35° and 10°C.

48 hour period, the worms of both species generally remain fairly close to the original weight.

Effects of Temperature on Worms in Hypersaline Water

At all temperatures the initial response to the transfer to hypersaline water is a drop in weight. The greatest decrease in both species occurs at 10°C (Figure 8b) and the least decrease in both species occurs at 35°C (Figure 8a). After 48 hours most of the animals are still less than 95 percent of their original weight, but Themiste at 25°C (Figure 7) approaches its original weight by that time. The only other exception to this occurs at 35°C. Here, due to high mortality, there were only two Themiste left at 24 hours and they were well over 115 percent of their original weight. This was an unexpected finding, and indicative that they were no longer functioning correctly. These two were dead at 48 hours. A few worms died in another experiment as well. At 10°C (Figure 8b) there were eight Phascolosoma left at 24 hours and seven left at 48 hours.

In general the means for Themiste at all temperatures were slightly above those for Phascolosoma throughout the whole time interval.

Figure 7. Percent change in weight of Themiste lissum and Phascolosoma dentigerum as a function of time, when worms are placed in 116 percent sea water, at 25°C.

For further information refer to legend for Figure 5.

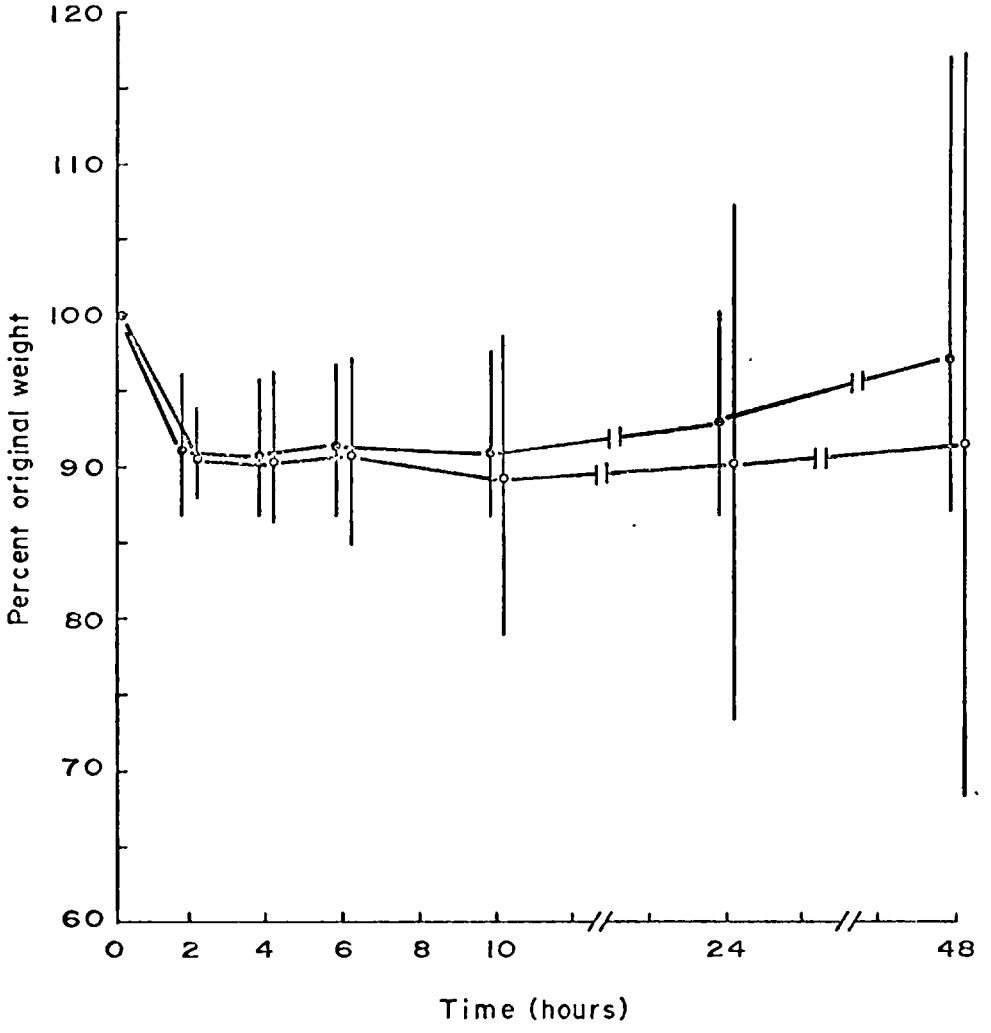

Figure 7. Weight changes in 116 percent sea water at 25°C.

Figure 8. Percent change in weight of Themiste lissum and Phascolosoma dentigerum as a function of time, when worms are placed in 116 percent sea water, at 35° and 10°C.

The temperatures are 35° and 10°C for (a) and (b), respectively. For further information refer to legend for Figure 5.

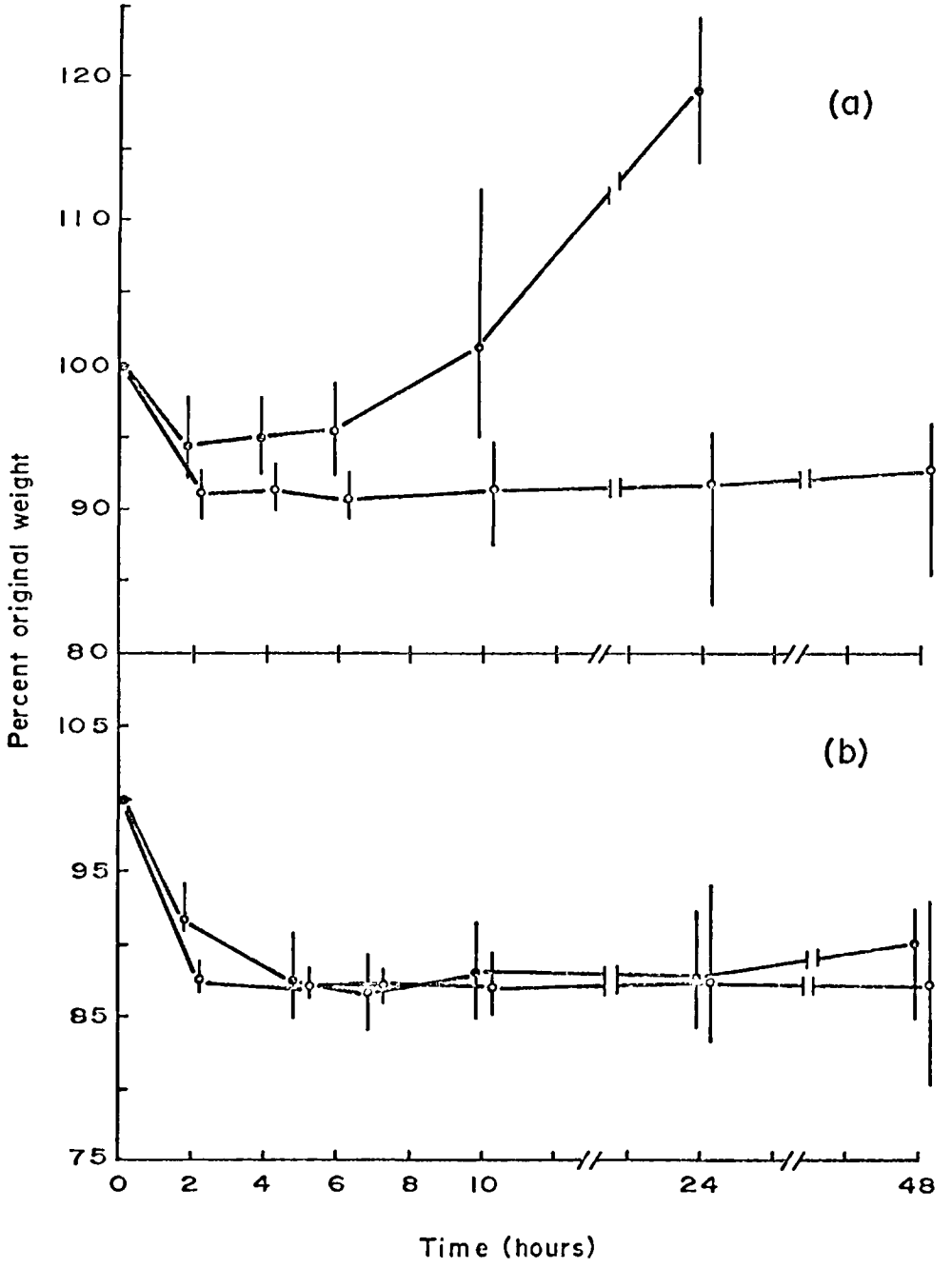

Figure 8. Weight changes in 116 percent sea water at 35° and 10°C.

Effects of Temperature on Worms in Hyposaline Water

The initial responses of the two species at all temperatures (Figures 9, 10, and 11) are rapid increases in weight as a result of exposure to lower salinity. The greatest increase is shown by Themiste at 25° and 35°C (Figures 9 and 10) and the smallest change is shown by both species at 10°C (Figure 11). After two hours the mean weights gradually decrease until they are within ±5 percent of the original weight at 48 hours. The only exception to this is the response of Phascolosoma at 35°C (Figure 10) where at 48 hours it weighs nearly 10 percent less than it did originally. In some cases, e.g. Themiste at 10°C (Figure 11) and Phascolosoma at 25°C and 35°C (Figures 9 and 10), the mean weights are below the original weight within 24 hours.

In Figure 11 at 48 hours the point represents the mean for only eight Phascolosoma because two of the animals had died. Also in this figure all the means of Themiste represent eight animals because the initial weight of the ninth worm was not recorded.

In general, in all three parts of this experiment the ranges varied more widely throughout the 48 hour period than in any of the other experiments.

Figure 9. Percent change in weight of Themiste lissum and Phascolosoma dentigerum as a function of time, when worms are placed in 80 percent sea water, at 25°C.

For further information refer to legend for Figure 5.

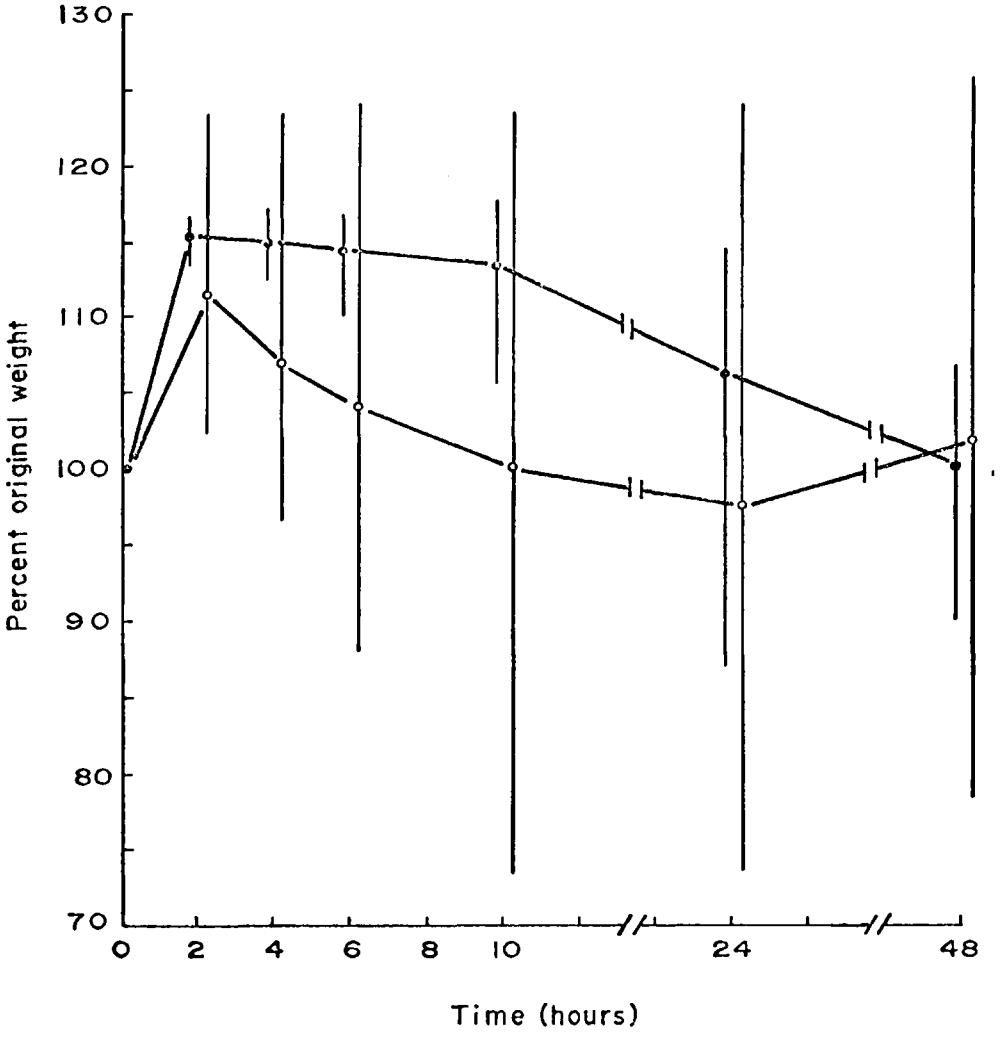

Figure 9. Weight changes in 80 percent sea water at 25°C.

Figure 10. Percent change in weight of Themiste lissum and Phascolosoma dentigerum as a function of time, when worms are placed in 80 percent sea water, at 35°C.

For further information refer to legend for Figure 5.

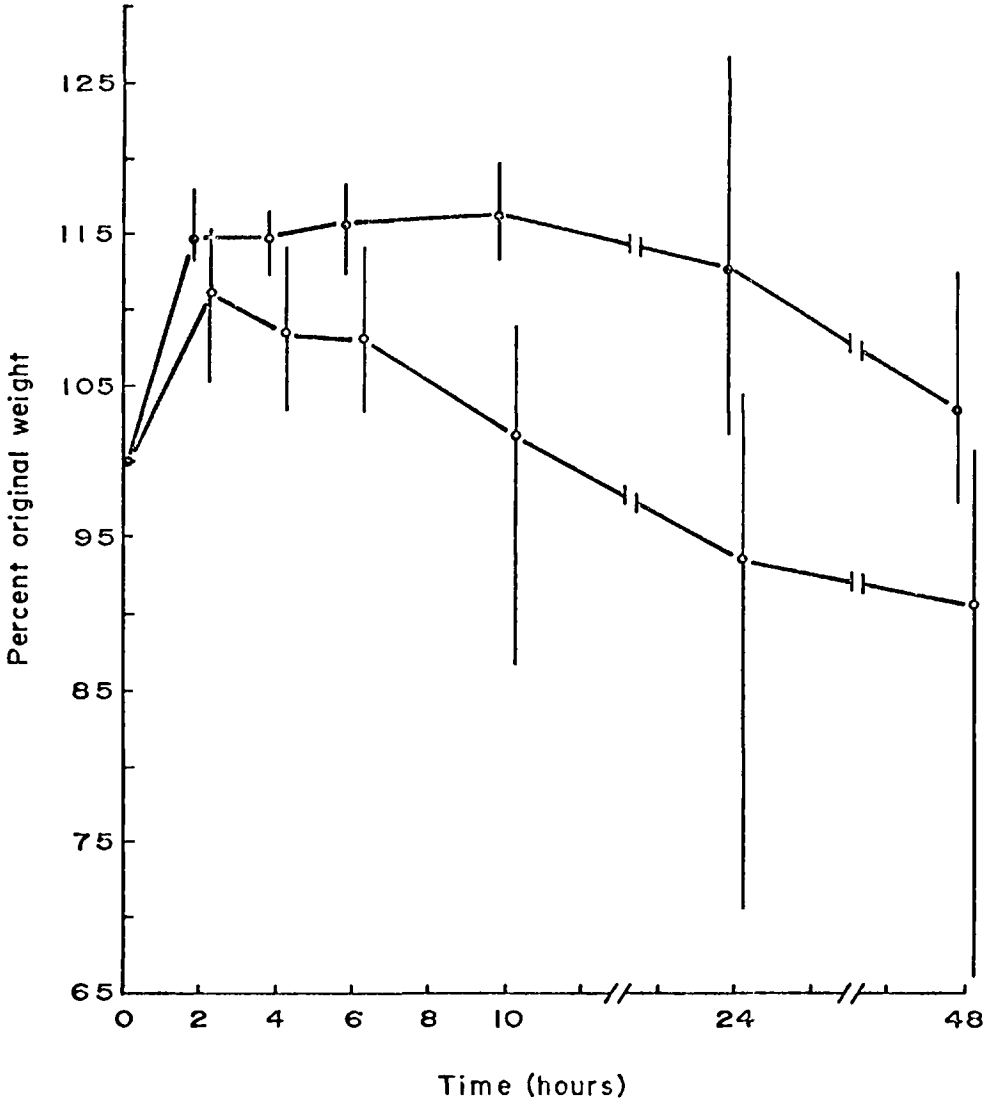

Figure 10. Weight changes in 80 percent sea water at 35°C.

Figure 11. Percent change in weight of Themiste lissum and Phascolosoma dentigerum as a function of time, when worms are placed in 80 percent sea water, at 10°C.

For further information refer to legend for Figure 5.

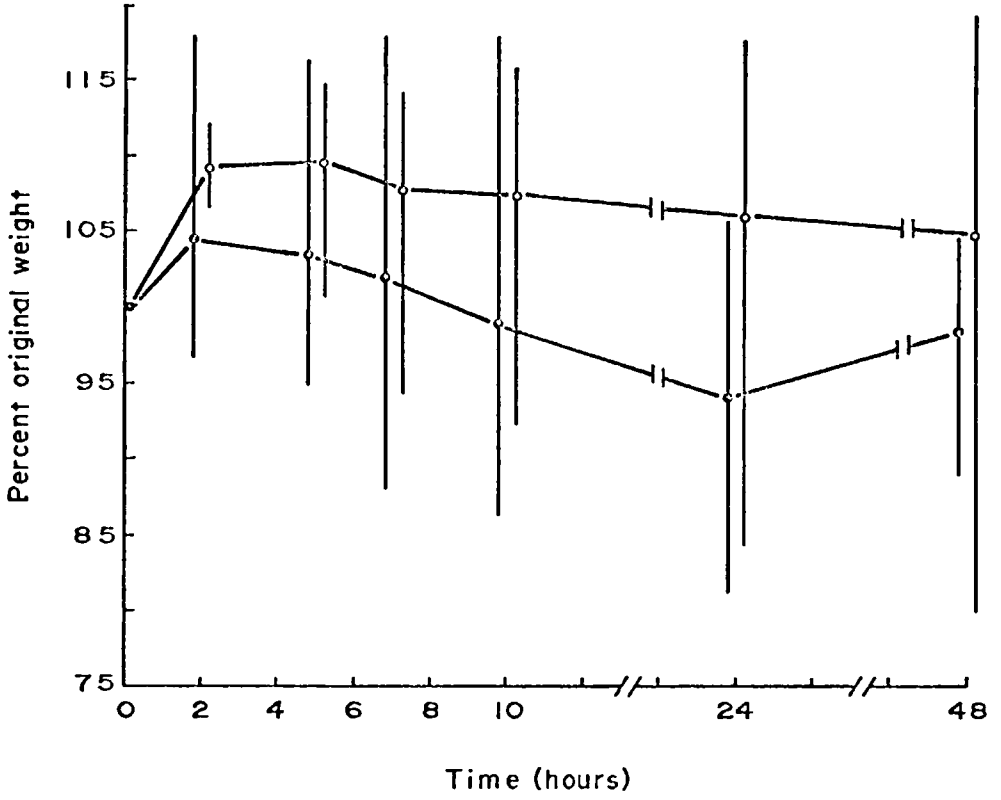

Figure 11. Weight changes in 80 percent sea water at 10°C.

Effects of Salinity on the Weight of the Worms at Moderate and Extreme Temperatures

If weight gains and losses were strictly a function of the salt content of the test solutions, one would expect that the worms would gain 20 percent and lose 16 percent of their weight in the hyposaline water (80 percent sea water) and the hypersaline water (116 percent sea water), respectively. In 80 percent sea water at 25° and 35°C (Figures 9 and 10) the means of Themiste, only, approach nearly the same percentage above the original weight as the percent salinity of the test solution was below normal sea water. Also, in 116 percent sea water, at 10°C only (Figure 8b), the means of both species approach the same percentage below the original weight as the percent salinity of the test solution was above the normal sea water.

In normal and dilute sea water, both species initially tend to keep the same weight and to gain weight, respectively, regardless of the temperature, and then tend to approach within ±5 percent of their original weight by 48 hours. At high salinities and at all temperatures both species initially lose weight but rarely return to within five percent of their original weight by 48 hours.

DISCUSSION

General Reactions to Salinity Changes

Since sipunculids are not able to regulate the osmotic pressure of their body fluids in any solution other than 100 percent sea water (Krogh, 1939; Hyman, 1959; Schmidt-Nielson and Laws, 1963), they must adjust to changes in salinity. Both Themiste lissum and Phascolosoma dentigerum react as expected. When placed into solutions which are 20 percent above and below normal sea water, the internal body fluid of each species requires less than five hours to reach the same osmoconcentration as the test solution. After this time the internal fluids remain in equilibrium with the test solution. After transfer from normal sea water to the higher salinity (116 percent sea water) there is usually an initial overshoot, so that the osmoconcentration of the body fluids is above that of the medium. It is not known why this occurs since the weight studies show no large water loss in 116 percent sea water. There is no corresponding undershoot when worms are transferred to the lower salinity.

The results of weight studies on these sipunculids are somewhat different from those of Gross (1954). He found that Dendrostomum zostericolum in hypo- or hyperosmotic sea

water gained or lost weight continuously for 24 hours before tapering off and beginning to return toward normal. It took four days for worms of this species to approach to within five percent of the original weight. The largest changes in weight of Themiste and Phascolosoma occurred within 10 hours of transfer to the different salinities. By 24 hours, in most cases, the worms were again approaching their original weights. Both Themiste and Phascolosoma are much smaller worms than D. zostericolum and this may account for their ability to accommodate more rapidly.

After transfer to the hypersaline water (116 percent sea water) the worms lose weight rapidly for the first five hours. This is probably due to diffusion of water from the less concentrated coelomic fluid to the external medium. After the worms reach osmotic equilibrium with the test solution at about five hours, they begin to gain weight at a very slow rate. By 48 hours most of the mean weights were less than but still within 15 percent of the initial weight. This slow return toward the initial weight in both hyper- and hypoosmotic sea water may be due to a readjustment of the internal hydrostatic pressure toward the optimum for each worm. Presumably after the initial gain or loss of water following transfers to hypo- or hyperosmotic sea water, the internal hydrostatic pressure increases or decreases, respectively. When the animals have

reached osmotic equilibrium, then there may be an exchange of salts and water with the external medium until an optimum hydrostatic pressure is achieved. There is apparently a fairly wide range of internal hydrostatic pressure within which sipunculids may function (Zuckerlandl, 1950) and this may account for the slow return toward original weight of these worms.

The initial gains and losses of weight appear to occur at the same rate. This would seem to be counter to Adolph's (1937) observation that water diffuses into the worms more quickly than it diffuses out.

When worms are transferred to hyposaline water (80 percent sea water) they gain weight quickly. The osmoconcentration of the coelomic fluid drops rapidly and then, by five hours, it reaches an equilibrium with the external medium. However, at this time the osmoconcentrations of the coelomic fluid are always slightly greater than those of the test solutions. In fact, the mean osmoconcentrations never approached those of the test solution before 12 hours. After the coelomic fluid concentration approximates that of the test solution, the worms lose weight and in nearly all cases, the mean weights are within five percent of the original weight by 48 hours. A possible mechanism which will explain this phenomenon has been proposed by Virkar (1963, 1965, 1966). When the worms are placed in hypoosmotic

sea water they may release free amino acids from the body wall into the coelom to help restore the osmotic balance until the water gain can be regulated by the nephridia and some of the water is lost. This same release of free amino acids from the tissues to the body fluids has been observed in polychaetes in hypoosmotic conditions (Clark, 1968). Stephens and Virkar (1966) found that the brittle star, Ophiactis arenosa, would increase the rate of uptake of glycine or valine from the external medium in response to a decrease in salinity. These excess free amino acids are osmotically active particles and may cause the coelomic fluid to be slightly hyperosmotic to the medium. Perhaps after a longer period of observation than was used here, the internal osmoconcentration would become isosmotic with the external medium as some of the free amino acids are excreted or re-absorbed by the body wall. An additional effect at this salinity is seen at all three test temperatures; namely that the range of variation is greater than at any other salinity.

General Effects of Temperature on Osmoconcentration and Weight

The only differences in response that can be ascribed to temperature occurred at 10°C in both hypersaline and hyposaline water. The rates at which worms of both species reached osmotic equilibrium with the test solutions were

considerably slower at 10°C than at the two higher temperatures. The major effect of temperature on the weight changes occurred at 25°C in all three salinities. In both species the variation is greatest at this temperature throughout the entire 48 hour period.

Comparison of Themiste and Phascolosoma

There are no significant differences in osmotic concentration or weight responses between the two species in that both react as expected to the salinity changes. The temperature changes seem generally to cause the same reactions in both species. The significant difference between Themiste and Phascolosoma is in the greater sensitivity of Themiste to 35°C as was shown in the weight studies; and the greatest number of deaths of Themiste occurred at 35°C in the hypersaline water. These results coincide with those of Taylor (1964), although he used temperatures up to 42°C for short periods of time and in these experiments lower temperatures are applied over a longer period of time. In both cases, Themiste is less able to survive the high temperatures than Phascolosoma. The only mortalities among Phascolosoma occurred at 10°C and in all salinities only after the animals had been kept in the stock aquarium for over a month. Phascolosoma dentigerum is a tropical form with an Indo-Pacific distribution (Fisher 1952). This may account for the sensitivity

of these worms at 10°C during stress conditions such as starvation. Themiste lissum is known only from intertidal areas of the Gulf of California. The most closely related species is Themiste schmitti found on the lee side of Vieja Island, Peru (Fisher 1952). The average yearly water temperature in this area is 14° to 17°C.

The high temperature did cause some sluggishness in the reactions of both species. Vinogradova and Dzhamusova (1963) found a deterioration in excitability of the retractor muscles of Phascolosoma margaritaceaum after prolonged exposure to high temperatures.

Conclusions

Apart from the points mentioned above no major differences are found between these two species and those studied by Gross (1954), Adolph (1937), Oglesby (1968), and Kamenoto and Larson (1964). Many of the studies which have been made on the effects of osmotic stress on sipunculids were done in hypoosmotic sea water only, probably because dilute sea water is more readily encountered by sipunculids in temperate regions with heavier precipitation and greater fresh water runoff. However, I feel that hyperosmotic conditions are more likely to occur at Puerto Penasco due to the heat and the drying influence of the winds. Moreover, from observations of the sensitivity of Themiste to the higher salinity, this would seem to be an

important factor in determining the distribution of this species in the intertidal zone. This may be why this species is not often found in the areas exposed for the longest periods during a tidal excursion, i.e., the barnacle-covered rocks.

Temperature is undoubtedly a very important factor in the distribution of these two species. Hutchins (1947) found that high summer temperatures were important factors in the distribution of tropical and subtropical intertidal animals. This is seen in the extreme sensitivity of Themiste to long exposures to 35°C. This degree of exposure probably occurs only rarely at Puerto Penasco since the water seldom reaches this temperature and the air temperature at night is generally much cooler. Southward (1958) found that the temperatures in an intertidal area were generally within the tolerance limits of the animals. Even occasional extreme temperatures would have little, if any, effect on the adult animals.

Further work is needed in determining more exactly the distribution of these two species in the intertidal zone at Puerto Penasco. Careful studies of the temperature and salinity of the microhabitat would be most enlightening, especially since little is known of the salinity variations during the tidal excursions. Studies should be made of the life history of these two species particularly in

determining how and when these worms form their burrows. Additional work is needed on the mechanisms involved in attaining osmotic equilibrium with the environment. Elucidation of the exchange of fluids between the animal and its environment should include not only measurements of changes in osmotic pressure but also the determination of hydrostatic pressure under similar experimental conditions and studies of active transport, excretion and digestion, all of which influence the exchange of ions between organism and environment.

SUMMARY

1. The osmotic concentrations of the coelomic fluid of two species of sipunculid worms, Phascolosoma dentigerum and Themiste lissum, were determined after the worms were exposed to 80, 100 and 116 percent sea water and to temperatures of 10°, 25° and 35°C. No differences in the rate or degree of osmotic change were noted between species. The time for the osmotic pressure of the coelomic fluid of both species to reach equilibrium is greatest at the low temperature.

2. Under the same conditions, the changes in weight over a period of 48 hours of both species were determined. No significant differences were found between the two species, although in all three salinities the range of variation in weight was greatest at 25°C.

3. There is a difference in mortality of the two species after extended exposure to experimental conditions. Themiste is more sensitive to 35°C and Phascolosoma, under stress, is more sensitive to 10°C. These responses to temperature probably explain differences in the distribution of the two species in the field.

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