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Physiological responses to fluctuation in temperature or salinity in invertebrates. Adaptations of *Alpheus viridari* (Decapoda, Crustacea), *Terebellides parva* (Polychaeta) and *Golfinigia cylindrata* (Sipunculida) to the mangrove habitat

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Abstract The snapping shrimp *Alpheus viridari* (Armstrong, 1949), the polychaete *Terebellides parva* Solis-Weiss, Fauchald and Blankensteyn 1990, and the sipunculan *Golfingia cylindrata* (Keferstein, 1865) are commonly found in the same mangrove habitat, where they experience frequent, acute fluctuations in temperature and salinity. Ecological studies indicate a temporal variation, including occasional absence, in the distribution of both *G. cylindrata* and *T. parva*; this led us to examine the physiological adaptations of the three species (collected at Western Bay, Twin Cays, Belize in 1985, 1986 and 1988). Each was subjected to acute, repeated exposure to either control (35‰ S) and decreased (25‰ S) salinity or to control and increased (45‰ S) salinity. Ability to regulate water and ion content (g H_2O or µmol g^{-1} solute free dry wt) was examined. *A. viridari* behaved as a hyperosmotic conformer at decreased salinity but as an osmoconformer at increased salinity. Regardless of direction of salinity change, *A. viridari* regulated water content through change in Na^+, K^+, and Cl^- contents. In contrast, *G. cylindrata* behaved as an osmoconformer and did not demonstrate ability to regulate water content. *T. parva* behaved as an osmoconformer, showed incomplete regulation of water content via change in Na^+, K^+, and Cl^- contents but had limited survival following exposure to 45‰ S. Each species was also exposed to change in temperature. Species were subjected to acute, repeated exposure either to control (28 °C) and decreased (21 °C) temperature or to control and increased (35 °C) temperature. *A. viridari* regulated water and ion content under both experimental conditions. In contrast, *T. parva* did not regulate water and ion content under either experimental temperature. *G. cylindrata* did not regulate water and ion content during exposure to decreased temperature and did not survive exposure to increased temperature. For *A. viridari*, weight specific oxygen uptake rates (mg O_2 g^{-1} ash-free dry wt) were determined. Exposure to decreased salinity or to increased temperature resulted in a small sustained elevation in O_2 uptake. It is concluded that, unlike *A. viridari*, *T. parva* and *G. cylindrata* are only marginally adapted to withstand the salinity and temperature stresses, respectively, of the mangrove habitat. The inability of *T. parva* and *G. cylindrata* to fully adapt to extremes in the mangrove habitat could well explain the temporal variation seen in the distribution of these two species.

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Introduction

Offshore mangrove islands such as Twin Cays (Belize) are surrounded by normal salinity seawater (35‰ S) of about 28 °C. These islands consist of a very shallow "flood-plain" formed by the root-mat of the mangrove (mostly *Rhizophora mangle*). Any organism associated with this root-mat will be subject to variations in physico-chemical factors due to intense evaporation, torrential tropical squalls, and, along the margins, to runoff from shallow pools in the interior of the island. The specimens examined in the present study live in shallow water (<0.5 m) along the margin of the island. Recorded salinities range from 18.6 to 44.8‰ S, and water temperatures range from 20 to 36 °C (Kensley and Fauchald in preparation). The measured fluctuations are caused, minimally, by a combination of three independently varying features. The tropical sun baking down on the shallow, open pans in the interior of the island combines with the effects of the limited tidal range to create a very hot high-salinity runoff, draining across the margins of the islands at low tides. During heavy rainfalls both parameters drop, sometimes precipitously. The diur-

nal fluctuations are very much larger than the average annual ones and the change from hot brine to cold, low-salinity water may take less than 1 h. Moreover, thunderstorms occur in the late afternoon with great regularity during certain times of the year so that salinity and temperature changes are not only rapid, but frequently reverse after a 24-h period.

We examined the independent effects of salinity or temperature on three inhabitants of the flood-plain. *Alpheus viridari* (Decapoda, Crustacea), *Terebellides parva* (Polychaeta), and *Golfingia cylindrata* (Sipunculida) are all common; however, ecological studies indicate differences in the distribution of both *G. cylindrata* and *T. parva* over time, including occasional absence (Kensley and Fauchald in preparation).

The three species are most commonly found in distinctly different micro-habitats. An algal-mat, consisting almost exclusively of *Caulerpa verticillata* and covering the mangrove root-mat, creates a low "canopy" perhaps as much as 5 cm tall. The polychaete builds soft, collapsible tubes plastered to the fronds of the algae, often near the tips. The tubes are friable and are unlikely to protect the worms against changes in salinity or temperature. The sipunculan lives in tight-fitting burrows at or near the surface of the root-mat, presumably foraging with the introvert in the organic debris collecting on the surface and among the algal fronds. These burrows, although open at each end, could offer mechanical support to the inhabitant. The snapping shrimp live in burrows, easily the largest single kind of structure in the root-mat other than the roots themselves. These burrows appear to form nearly open galleries through the root-mat.

In the waters surrounding the nearby coral reef at Carrie Bow Cay, polychaetes were found to be distributed in habitats to which they were physiologically well adapted (Ferraris 1981). However, species also may occupy habitats to which they are only marginally adapted physiologically when that habitat can exclude competitive species (Connell 1961). Temporal variation in the distribution of *Terebellides parva* and *Golfingia cylindrata*, but not *Alpheus viridari*, raised the question of how well each of these species adapted to the extreme fluctuations found in temperature and salinity in the mangrove.

In habitats characterized by variation in salinity, the ability of species to regulate intra- and extracellular volume or to osmo- and ion regulate can be critical to successful occupation. Similarly, change in temperature potentially affects both osmo- and volume regulation through change in permeability. Both variation in temperature and salinity are known to affect O_2 uptake rate. Thus, when possible, we measured each of these physiological parameters to provide a more comprehensive picture of how these species respond to their environment. Previous studies have shown that response to fluctuating environmental conditions is not comparable to response to a simple change in an environmental variable (review: Davenport 1982), thus we attempted to simulate at least some of the daily acute fluctuations known to occur in this mangrove habitat.

Materials and methods

Collection and maintenance of invertebrates

Individuals were collected from a subtidal root-mat adjacent to *Rhizophora mangle* stands at Western Bay, Twin Cays, Belize, C.A. in spring or fall of 1985, 1986 and 1988. *Alpheus viridari* (Armstrong, 1949), *Terebellides parva* Solis-Weiss, Fauchald and Blankensteyn 1990, and *Golfingia cylindrata* (Keferstein, 1865) were found by screening portions of a *Caulerpa*, *Cladophora*, and *Halimeda* spp. algal-mat and portions of the root-mat below. Following collection, specimens were transported 1.5 km to the Carrie Bow Cay field station of the Smithsonian National Museum of Natural History where they were maintained under natural photoperiod conditions in running-seawater (28 °C; 35‰ S) for at least 7 d prior to experiments. Species were allowed to feed on detritus supplemented with commercial fish food. 48 h prior to experiments food was withdrawn.

Experimental protocol

Variation in temperature

Individuals ($n=6$ ind species^{-1}) were subjected to acute temperature changes consisting of four 24-h periods of control or experimental temperature at constant salinity. Thus, species were subjected to decreased or increased temperature for 24 h, returned to control temperature for 24 h, and the cycle repeated. Control temperature was 28 °C. Experimentally altered temperatures were either 7 °C lower (21 °C) or 7 °C higher (35 °C) than the control temperature. Salinity was 35‰ S.

Variation in salinity

Individuals ($n=6$ ind species^{-1}) were subjected to acute salinity changes consisting of four 24-h periods of control or experimental salinity at constant temperature. Thus, species were subjected to decreased or increased salinity for 24 h, returned to control salinity for 24 h, and the cycle repeated. Control salinity was 35‰ S; experimental salinities were either 10‰ lower (25‰ S) or 10‰ higher (45‰ S) (Table 1). Temperature was 28 °C.

Oxygen uptake

Weight specific oxygen uptake rates [mg O_2 g^{-1} ash-free dry wt (afdw) h^{-1} = g^{-1} afdw h^{-1}] of *Alpheus viridari* were measured during exposure to variation in temperature or salinity. In a given experiment, O_2 consumption rates of five or six pairs of *A. viridari* were measured simultaneously using specially designed chambered aquaria. Due to their small size, two *A. viridari* were placed in each chamber (volume ≈ 1 liter 0.2 g^{-1} afdw dry wt). Aquarium design allowed individual chambers to be either sealed and isolated or interconnected with each other and with the surrounding water bath. Chambers were flushed with fresh seawater of a given temperature and salinity and sealed. Aquarium design allowed a complete water change without handling the shrimp. 10 min after the chambers were sealed, O_2 concentrations were measured. Initial (≈ 5.0 to 5.6 mg O_2 l^{-1}) and final (≈ 4.5 to 5.3 mg O_2 l^{-1}) O_2 concentrations were measured by inserting a shielded self-stirring O_2 probe (YSI 5720; YSI Temperature/Salinity Compensated Dissolved Oxygen Meter 58) through a sealed port in the top of each chamber; values were corrected for background (≈ 0.06 mg O_2 l^{-1} h^{-1}). Temperature was recorded simultaneously. After determining each final O_2 concentration, a sample of seawater was removed and frozen for later analysis.

Measurements were made at the beginning and end of the first hour and at the beginning and end of the 24th hour of each 24-h cycle of experimental temperature or salinity manipulation. At the end of the 4-d experimental period, individual *A. viridari* were dried at 60 °C, frozen, and transported to Mt. Desert Island Biological Laboratory (MDIBL). There, each *A. viridari* was dried (60 °C) to constant weight (Cahn Automatic Electrobalance TA450; 0.1 mg), ashed (510 °C, 4 h), and the dry weight corrected for ash content (Ferraris 1981). The ash-free dry weight (mean ± SE) of each group of *A. viridari* tested ($n=5$ or 6 pairs group^{-1}) is reported with the results obtained for that group (see "Results"). *A. viridari* within a group did

Table 1 Osmolality, Na^+, K^+, and Cl^- ion concentrations of control (35‰ S) and experimental (25 or 45‰ S) salinities. Mean ± SE

	Salinity (‰)		
	35	25	45
mOsm kg^{-1} H_2O	1070 ± 5.2	771 ± 5.0	1416 ± 15.6
Na^+ mEq l^{-1}	499 ± 2.0	378 ± 4.5	677 ± 14.4
K^+ mEq l^{-1}	10.2 ± 0.22	7.4 ± 0.14	13.4 ± 0.48
Cl^- mEq l^{-1}	590 ± 2.2	434 ± 3.0	819 ± 4.4

not differ sufficiently in weight to allow determination of the relationship between metabolic rate and size.

Water and ion regulation

At specific times specimens were removed from aquaria and blotted on moistened filter paper. At the same time, we measured temperature and removed a sample of seawater for subsequent analysis (see "Analyses"). Entire organisms were then individually sealed in a preweighed 1.5-ml plastic tube, weighed, dried at 60 °C, frozen and stored until transported to MDIBL. At MDIBL, tissue samples were prepared for analysis by the reconstitution method (Ferraris and Schmidt-Nielsen 1982a). In summary, organisms were dried at 60 °C to constant weight. Each sample was then reconstituted by the addition of a known volume (50 to 500 µl) of deionized-distilled water, the tube sealed and heated in a water bath at 98 °C for 3 min. Samples were left undisturbed at 4 °C for 48 h to allow diffusion of ions and other osmotically active substances. Tube contents were mixed and centrifuged and analyses (see "Analyses") performed on the supernatant fluid. Values obtained were corrected for dilution (Ferraris and Schmidt-Nielsen 1982a). *Alpheus viridari* was subsequently ashed: the entire pellet of each sample was ashed (510 °C, 4 h), weighed, and the wet and dry weights corrected for ash content.

Calculations

Total body water content [g H_2O g^{-1} solute-free dry weight (sfdw) or g^{-1} solute-free ash-free dry weight (sfafdw)] was calculated after the method of Ferraris and Schmidt-Nielsen (1982a). Since the processes through which organisms regulate volume [regulatory volume decrease (RVD) and regulatory volume increase (RVI)] are associated both with a change in water content and with a decrease or increase in the amount or content of solutes, RVD and RVI change the specific weight of the cells. Calculation of g H_2O g^{-1} sfdw or sfafdw corrects the dry weight for the specific weight of the solutes based on specific gravity. Solute contents (µmol g^{-1} sfdw) were calculated by multiplying the solute concentration in tissue water (mEq l^{-1}) by the total body water content in g H_2O g^{-1} sfdw. Data are expressed as concentrations (mEq l^{-1}) when osmo- or ion regulation is discussed. However, to eliminate the masking affects of water, data are expressed as contents (g H_2O or µmol g^{-1} sfdw or sfafdw) when RVD and RVI are discussed.

Analyses

All tissue and seawater samples were analyzed for osmolality and ion concentrations at MDIBL using methods adapted for microliter volumes (Ferraris and Schmidt-Nielsen 1982a). Osmolality (mOsm kg^{-1} H_2O; Wescor 5100 B Vapor Pressure Osmometer), Na^+ and K^+ ion concentrations (mEq l^{-1}; Instrumentation Laboratories Flame Photometer Model 343) were determined on 5-µl samples. Cl^- ion concentration was measured by coulometric titration (Haake-Buchler Digital Chloridometer) on 10-µl samples.

Data were compared using analyses of variance followed by Tukey's Multiple Range Test for separation of significant means.

Results

Responses to salinity

Alpheus viridari responded to each salinity in a different manner. At control salinity (35‰ S), *A. viridari* was hypoosmotic to the medium (gradient ≈ 40 mOsm kg^{-1} H_2O; $P \leq 0.05$; Fig. 1 A). At 25‰ S, *A. viridari* maintained a larger but hyperosmotic gradient (≈ 100 mOsm kg^{-1} H_2O) across the body wall, whereas at 45‰ S this shrimp was isoosmotic to the seawater (Fig. 2 A). Regardless of direction of salinity change, Na^+ and Cl^- concentrations (mEq l^{-1}; Figs. 1 A and 2 A) followed a pattern similar to that of tissue osmolality. This was not the case with respect to K^+ (mEq l^{-1}), which varied but did not change significantly regardless of salinity.

Alpheus viridari regulated total body water content (g H_2O g^{-1} sfafdw) with each exposure to 25‰ S (Fig. 1 B). Water content increased slightly during each exposure to decreased salinity and, within 1 h, returned to that found at 0 h. Water content did not change ($P \geq 0.05$) during either return to control salinity. Accompanying regulation of water content, Na^+ and Cl^- contents (µmol g^{-1} sfafdw) significantly decreased during each exposure to 25‰ S and increased during each return to 35‰ S (Fig. 1 B). K^+ con-

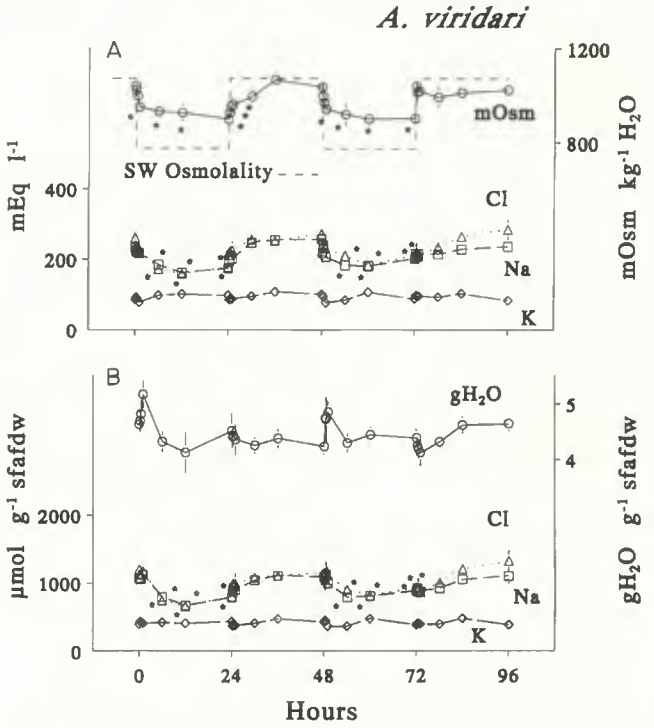


Fig. 1 *Alpheus viridari*. **A** Total tissue osmolality, Cl^-, Na^+, and K^+ ion concentrations (mOsm kg^{-1} H_2O or mEq l^{-1}) and **B** water, Cl^-, Na^+, and K^+ contents [g H_2O or µmol g^{-1} solute-free ash-free dry weight (sfafdw)] during repeated exposure to decreased osmolality (mOsm kg^{-1} H_2O) at constant temperature (28 °C). Mean ± SE; where an SE bar is not visible the data point symbol is larger than the SE bar; $n = 6$. (*SW* seawater.) * = significantly different ($P \leq 0.05$) from 0 h

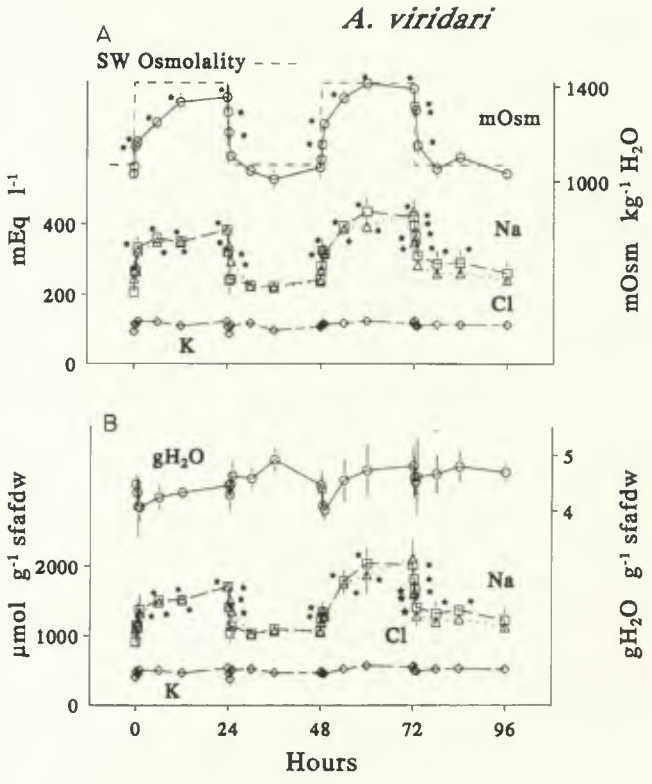


Fig. 2 *Alpheus viridari.* **A** Total tissue osmolality, Na^+, Cl^-, and K^+ ion concentrations (mOsm kg^{-1} H_2O or mEq l^{-1}) and **B** water, Na^+, K^+, and Cl^- contents [g H_2O or µmol g^{-1} solute-free ash-free dry weight (sfafdw)] during repeated exposure to increased osmolality (mOsm kg^{-1} H_2O) at constant temperature (28 °C). Mean ± SE; where an SE bar is not visible the data point symbol is larger than the SE bar; $n = 6$. (*SW* seawater.) * = significantly different ($P \leq 0.05$) from 0 h

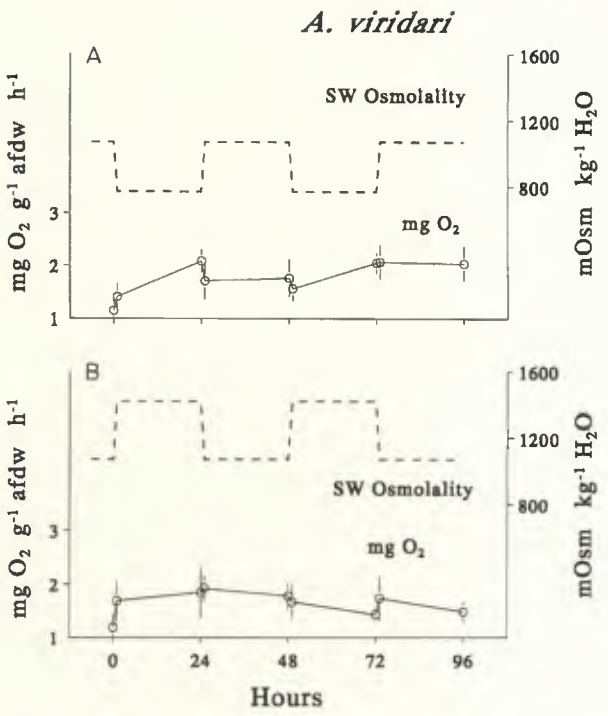


Fig. 3 *Alpheus viridari.* Oxygen uptake rate [mg O_2 g^{-1} ash-free dry wt (afdw) h^{-1}] during repeated exposure to **A** decreased osmolality (mass of *A. viridari* = 0.129 ± 0.029 g afdw) or **B** increased osmolality (mass of *A. viridari* = 0.195 ± 0.026 g afdw) at constant temperature (28 °C). Mean ± SE; where an SE bar is not visible the data point symbol is larger than the SE bar; $n = 6$. (*SW* seawater)

tent (µmol g^{-1} sfafdw) did not change ($P \geq 0.05$) during the experiment.

Alpheus viridari regulated total body water content during exposure to increased salinity as well; throughout the experiment, water content (g H_2O g^{-1} sfafdw) underwent only minor variation ($P \geq 0.05$; Fig. 2 B). Change in water content was limited, as compared with the change expected of a true osmometer, primarily via rapid change in Na^+ and Cl^- contents. Na^+ and Cl^- contents (µmol g^{-1} sfafdw) increased ($P \leq 0.05$) with each exposure to 45‰ S and decreased with each return to 35‰ S (Fig. 2 B). K^+ content (µmol g^{-1} sfafdw) increased slightly with each exposure to 45‰ S (Fig. 2 B).

The direction of the salinity change had some affect on O_2 uptake in *Alpheus viridari*. O_2 uptake rate tended to increase with initial exposure to either decreased or increased salinity (Figs. 3 A, B) but was sustained only with decreased salinity.

When subjected to change in salinity, *Terebellides parva* responded differently from *Alpheus viridari*. The polychaete behaved as a hypoosmotic conformer in 35‰ S (gradient approximately 40 mOsm) but as an osmoconformer in both 25 and 45‰ S. During repeated exposure to decreased salinity, tissue osmolality (mOsm kg^{-1} H_2O), and Na^+, K^+, and Cl^- concentrations (mEq l^{-1}) essentially followed a square-wave pattern (Fig. 4 A). This was also true during first exposure to 45‰ S, however, *T. parva* did not survive the subsequent return to 35‰ S (data not shown).

In terms of volume regulation, *Terebellides parva* was able partially to regulate water content during exposure to decreased salinity. Following an initial increase in water content upon each change to 25‰ S, *T. parva* lost water (g H_2O g^{-1} sfdw; $P \leq 0.05$; Fig. 4 B). Water content decreased further with each return to 35‰ S. Accompanying regulation of water content, Na^+ and Cl^- contents (µmol g^{-1} sfdw) eventually decreased during each exposure to 25‰ S and increased during each return to 35‰ S ($P \leq 0.05$; Fig. 4 B). K^+ content (µmol g^{-1} sfdw) followed the same pattern but differences were not significant. As might be expected based upon this polychaete's survival, *T. parva* did not regulate total body water content during exposure to increased salinity.

Golfingia cylindrata demonstrated a third type of response in that it behaved as an osmoconformer at all salinities (Figs. 5 A and 6 A). In this sipunculan, total tissue os-

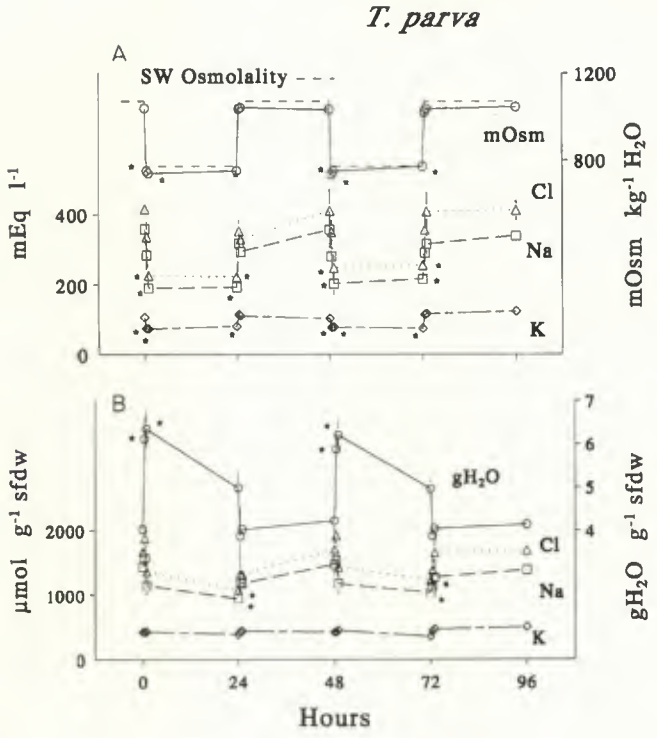


Fig. 4 *Terebellides parva.* **A** Total tissue osmolality, Cl^-, Na^+, and K^+ ion concentrations (mOsm kg^{-1} H_2O or mEq l^{-1}) and **B** water, Cl^-, Na^+, and K^+ contents [g H_2O or µmol g^{-1} solute-free dry weight (sfdw)] during repeated exposure to decreased osmolality (mOsm kg^{-1} H_2O) at constant temperature (28 °C). Mean ± SE; where an SE bar is not visible the data point symbol is larger than the SE bar; $n = 6$. (*SW* seawater.) * = significantly different ($P \leq 0.05$) from 0 h

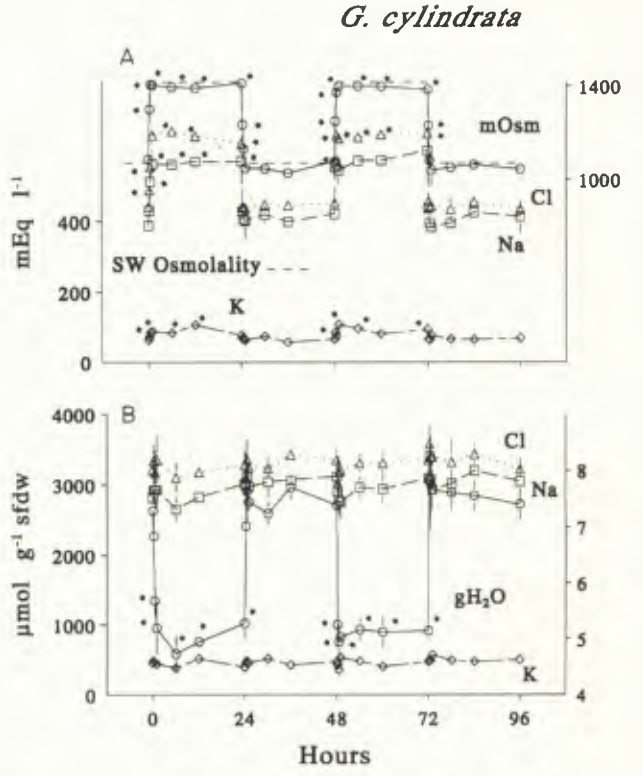
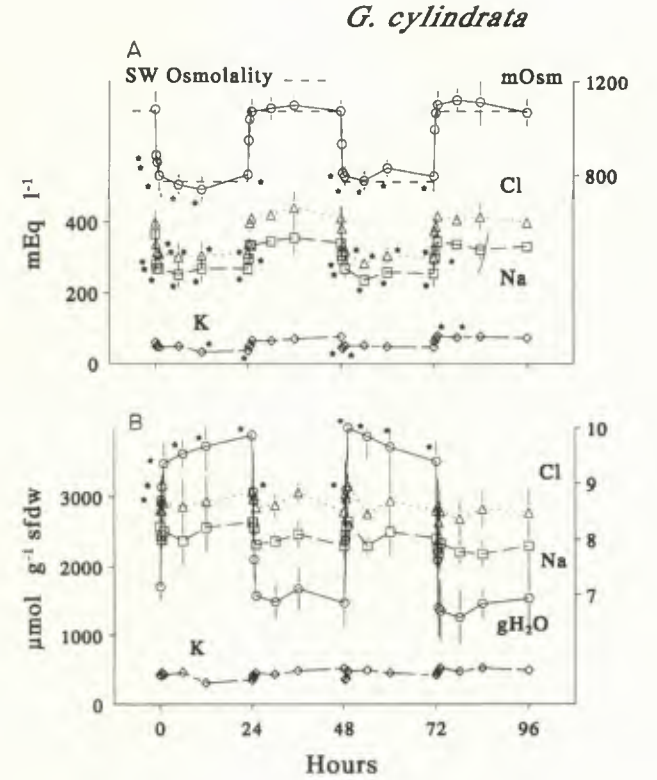


Fig. 6 *Golfingia cylindrata.* **A** Total tissue osmolality, Cl^-, Na^+, and K^+ ion concentrations (mOsm kg^{-1} H_2O or mEq l^{-1}), and **B** Cl^-, Na^+, water, and K^+ contents [g H_2O or µmol g^{-1} solute-free ash-free dry weight (sfdw)] during repeated exposure to increased osmolality (mOsm kg^{-1} H_2O) at constant temperature (28 °C). Mean ± SE; where an SE bar is not visible the data point symbol is larger than the SE bar; $n = 6$. (*SW* seawater.) * = significantly different ($P \leq 0.05$) from 0 h



molality (mOsm kg^{-1} H_2O), and Na^+, K^+, and Cl^- concentrations (mEq l^{-1}) changed rapidly, often within 15 min of an experimental change in salinity.

Unlike *Alpheus viridari* or *Terebellides parva*, *Golfingia cylindrata* did not demonstrate regulation of water content during exposure to variation in salinity (Figs. 5 B and 6 B). Water content (g H_2O g^{-1} sfdw) changed inversely, in a square-wave pattern, with each seawater change. Na^+, K^+, and Cl^- contents (µmol g^{-1} sfdw) did not change significantly regardless of salinity.

Responses to temperature

In *Alpheus viridari*, exposure to 21 °C did not significantly affect total tissue osmolality (mOsm kg^{-1} H_2O), or Na^+, K^+, and Cl^- concentrations (mEq l^{-1}) (data not shown).

Fig. 5 *Golfingia cylindrata.* **A** Total tissue osmolality, Cl^-, Na^+, and K^+ ion concentrations (mOsm kg^{-1} H_2O or mEq l^{-1}) and **B** Cl^-, Na^+, water, and K^+ contents [g H_2O or µmol g^{-1} solute-free dry weight (sfdw)] during repeated exposure to decreased osmolality (mOsm kg^{-1} H_2O) at constant temperature (28 °C). Mean ± SE; where an SE bar is not visible the data point symbol is larger than the SE bar; $n = 6$. (*SW* seawater.) * = significantly different ($P \leq 0.05$) from 0 h

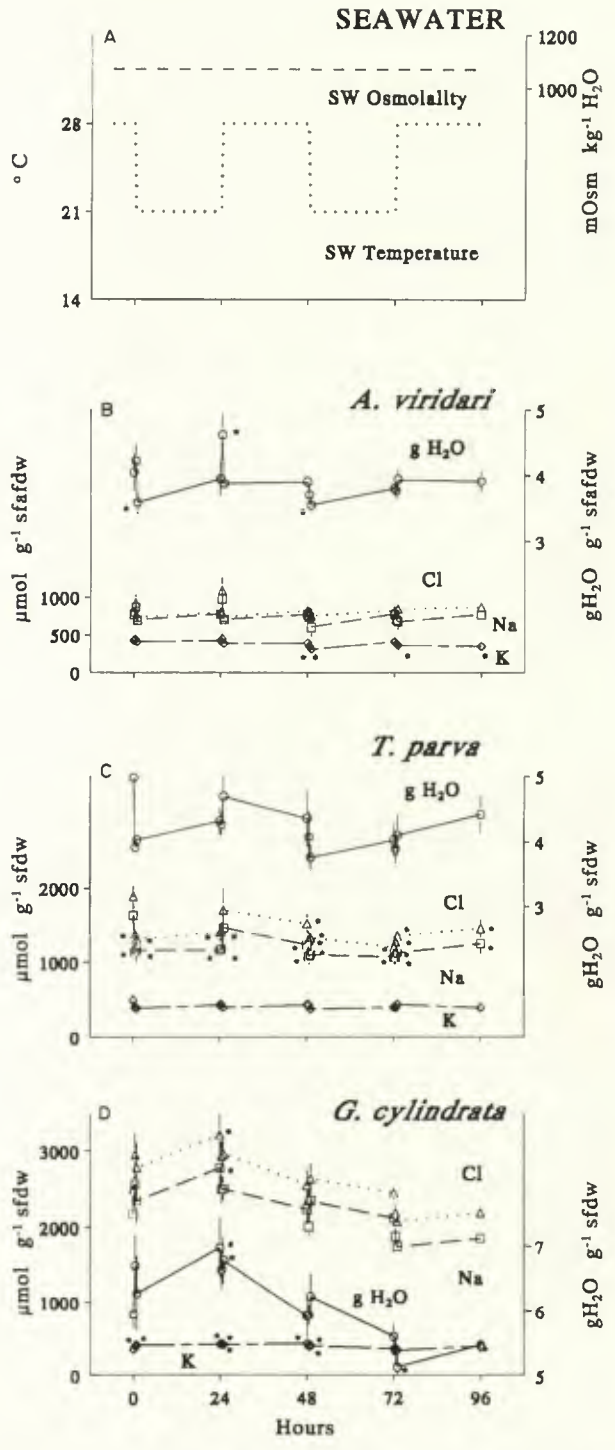


Fig. 7 *Alpheus viridari*, *Terebellides parva*, and *Golfingia cylindrata*. **A** Repeated exposure to decreased temperature (°C) at constant osmolality (mOsm kg^{-1} H_2O). Water, Cl^-, Na^+, and K^+ contents [g H_2O or µmol g^{-1} solute-free ash-free dry weight (sfafdw)]. **B** *A. viridari*. **C** *T. parva*. **D** *G. cylindrata*. Mean ± SE; where an SE bar is not visisble the data point symbol is larger than the SE bar; $n = 6$. (*SW* seawater.) * = significantly different ($P \leq 0.05$) from 0 h

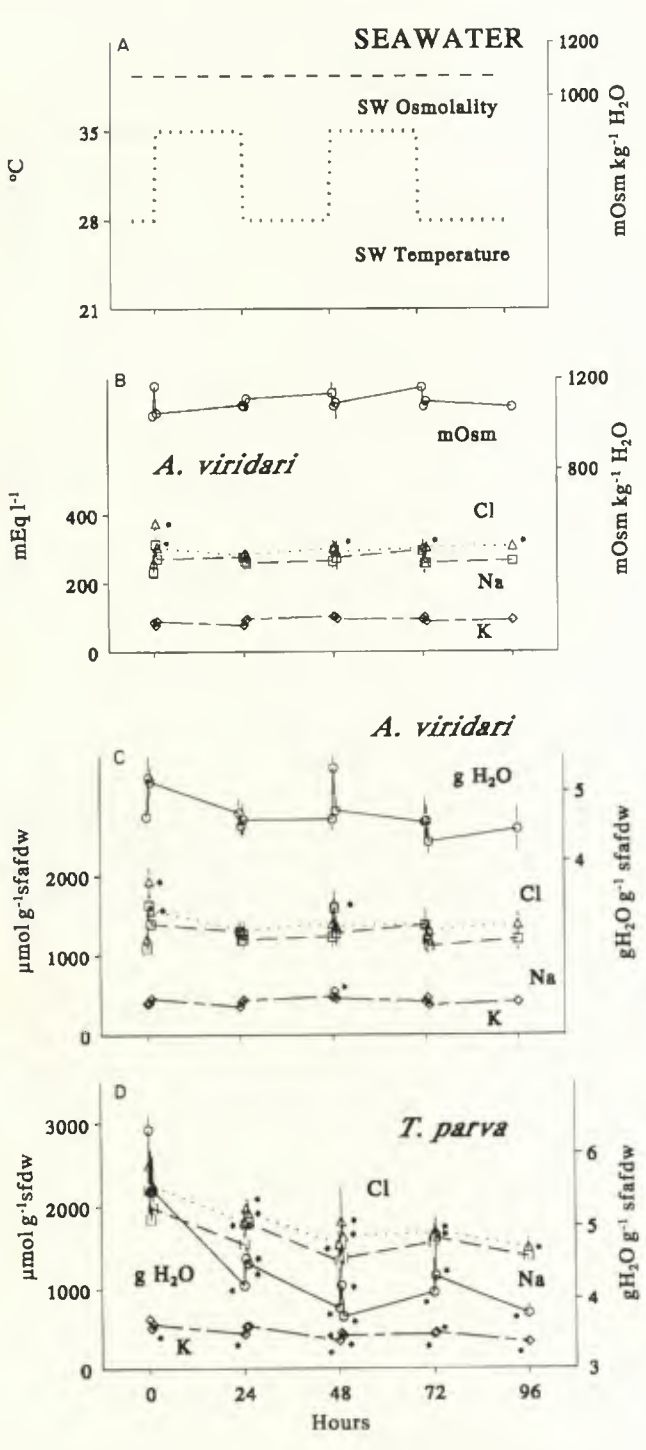


Fig. 8 *Alpheus viridari* and *Terebellides parva*. **A** Repeated exposure to inereased temperature (°C) at constant osmolality (mOsm kg^{-1} H_2O). **B** Total tissue osmolality, Cl^-. Na^+, and K^+ ion coneentrations (mOsm kg^{-1} H_2O or mEq l^{-1}). **C** *A. viridari* and **D** *T. parva* water, Cl^-, Na^+, and K^+ contents [g H_2O or µmol g^{-1} solute-free ash-free dry weight (sfafdw)]. Mean ± SE; where an SE bar is not visible the data point symbol is larger than the SE bar; $n = 6$. (*SW* seawater.) * = significantly different ($P \leq 0.05$) from 0 h

With each exposure to 21 °C total body water content (g H_2O g^{-1} sfafdw) decreased ($P \leq 0.05$); water content subsequently rose such that within 24 h it was not different ($P \geq 0.05$) from that found at 0 h (Fig. 7B). Na^+, K^+, and Cl^- contents (µmol g^{-1} sfafdw) followed the same pattern as water content, but statistically significant deviation from that at 0 h occurred only in K^+ content (Fig. 7B).

Exposure to increased temperature resulted in increased total tissue osmolality (mOsm kg^{-1} H_2O) and Na^+ and Cl^- concentrations (mEq l^{-1}); only Cl^- concentration differed significantly ($P \leq 0.05$; Fig. 8B). Zero hour levels were at least partially restored before shrimp were returned to 28 °C. K^+ concentration deviated slightly from that at 0 h but did not follow a particular pattern.

With exposure to increased temperature, total body water and ion contents in *Alpheus viridari* followed a pattern similar to that shown for osmolality. Water content (g H_2O g^{-1} sfafdw), and Na^+ and Cl^- contents (µmol g^{-1} sfafdw) increased ($P \leq 0.05$ for Cl^-) above values found at 0 h (Fig. 8C). Decrease of the same to 0-h amounts occurred before shrimp were returned to 28 °C. K^+ content (µmol g^{-1} sfafdw) followed a similar pattern (Fig. 8C).

Response of *Alpheus viridari* to variation in temperature also differed with respect to O_2 uptake. *A. viridari* exposed to decreased temperature essentially demonstrated a modified square-wave response; O_2 consumption varied directly ($P \leq 0.05$) with temperature (Fig. 9A). Response to the first exposure to decreased temperature was slower and of lesser amplitude than response to the second and there was an overshoot ($P \leq 0.05$) upon return of *A. viridari* to control temperature. In contrast, *A. viridari* exposed to increased temperature tended toward an elevated rate of O_2 uptake that was sustained throughout the experiment (Fig. 9B). When *Terebellides parva* was exposed to either decreased or to increased temperature there was no effect on total tissue osmolality (mOsm kg^{-1} H_2O), or Na^+, K^+, and Cl^- concentrations (mEq l^{-1}) ($P \geq 0.05$; data not shown). However, total body water content (g H_2O g^{-1} sfdw) decreased with exposure to either 21 or 35 °C ($P \leq 0.05$ at 35 °C; Figs. 7C and 8D). Polychaetes exposed to 21 °C recovered water content after return to control temperature (Fig. 7C), whereas those exposed to 35 °C did not (Fig. 8D). Na^+, K^+, and Cl^- contents (µmol g^{-1} sfdw) followed a pattern similar to water content.

In *Golfingia cylindrata*, repeated exposure to decreased temperature did not affect total tissue osmolality (mOsm kg^{-1} H_2O) or Na^+ and K^+ concentrations (mEq l^{-1}) ($P \geq 0.05$; data not shown). Cl^- concentration increased transiently 24 h after initial exposure to 21 °C. Total water content (g H_2O g^{-1} sfdw) in *G. cylindrata* increased with each exposure to 21 °C; the change was significant during the first exposure (Fig. 7D). Water content was not regulated and was restored only upon return to 28 °C. Na^+ and Cl^- contents (µmol g^{-1} sfdw) followed the same pattern as water content; each increased during exposure to 21 °C and subsequently decreased during return to 28 °C ($P \leq 0.05$; Fig. 7D).

Golfingia cylindrata did not demonstrate significant change in any measured parameter during exposure to 35 °C or subsequent return to 28 °C; however, this species did not survive beyond the 25th hour of the experiment (data not shown). For this sipunculan, it would appear that only brief exposures to elevated temperatures may be tolerated.

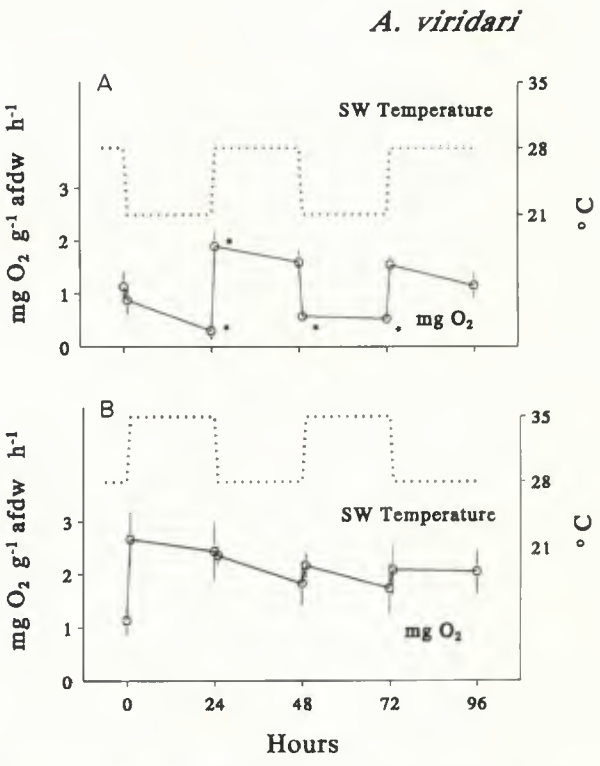


Fig. 9 *Alpheus viridari*. Oxygen uptake rate [mg O_2 g^{-1} ash-free dry wt (afdw) h^{-1}] during repeated exposure to **A** decreased temperature (mass of *A. viridari* = 0.211 ± 0.023 g afdw) or **B** increased temperature (mass of *A. viridari* = 0.256 ± 0.026 g afdw). Mean ± SE; where an SE bar is not visible the data point symbol is larger than the SE bar; $n = 5$. (*SW* seawater.) * = significantly different ($P \leq 0.05$) from 0 h

Discussion

Responses to salinity

The behavior of *Alpheus viridari* during repeated change in salinity was quite different depending on the direction of the salinity change. At 35‰ S control salinity *Alpheus viridari* maintained a small hypoosmotic gradient to the seawater. This has been found previously in osmoconforming marine invertebrates, although most species tend to be hyperosmotic to the medium as was found for *A. viridari* at 25‰ S (review: Oglesby 1978; Ferraris and Schmidt-Nielsen 1982a, b). No osmotic gradient was maintained by this shrimp at increased salinity.

Alpheus viridari regulated water content efficiently regardless of the direction of the salinity change and demonstrated both limitation and recovery phases of RVD and

RVI. Since *A. viridari* exposed to 45‰S did not osmoregulate to the same degree as those exposed to 25‰S, regulation of water content in 45‰S necessitated greater solute adjustment. In each salinity, Na^+ and Cl^- were the primary solutes accompanying regulation of water content, so it can be assumed that part of the observed regulation involved the extracellular compartment. Some portion of the Na^+ and Cl^- may also be derived intracellularly since marine invertebrates commonly have substantial intracellular Na^+ and Cl^- concentrations (e.g. *Limulus polyphemus*, Dragolovich and Pierce 1992). With regard to K^+, particularly when *A. viridari* was exposed to 45‰S, regulation of water content presumably was intracellular. The use of inorganic ions during a swelling or shrinkage limitation phase as well as during a subsequent volume recovery phase is found in other marine organisms. Under fluctuating salinity conditions Na^+ and Cl^-, but not K^+ are used for RVD and RVI in *Procephalothrix spiralis* (Nemertina) and *Clitellio arenarius* (Oligochaeta) (Ferraris 1984). K^+ acts as an osmolyte during RVD in *Carcinus maenas* (Decapoda) and *Glycera dibranchiata* (Polychaeta) (Kevers et al. 1979a, b; Costa and Pierce 1983) and during RVI in *Platichthys flesus* (Teleostei) (Vislie 1980). Na^+ and Cl^- are used during RVI in *Limulus polyphemus* (Merostomata) (Dragolovich and Pierce 1992).

Crustaceans, such as *Crangon vulgaris* (Natantia) and *Onisimus glacialis* (Amphipoda), show an increase in O_2 consumption with increase in osmotic gradient across the body wall (Hagerman 1970; Aarset and Aunaas 1990). Similarly, *Alpheus viridari* showed a small elevation in O_2 uptake rate during exposure to decreased salinity coinciding with a small increase in osmotic gradient. However, *A. viridari* did not show a change in O_2 uptake during exposure to increased salinity where the corresponding decrease in osmotic gradient would be expected to reduce O_2 uptake. Others have calculated the energy required for osmoregulation in crustacea and have found it to account for only a minor percentage of the change in O_2 consumption (e.g. Dalla Via 1987). Correlations between change in external osmolality and O_2 consumption may have some other basis. Working with *Callinectes sapidus* (Decapoda), Gilles (1973) suggests that catabolism of free amino acids accounts for an increase in O_2 consumption during cell volume regulation; such a mechanism would apply during RVD and could apply to *A. viridari* at decreased salinity. Similarly, protein catabolism as a source of amino nitrogen during RVI (Hawkins and Hilbish 1992) could account for an increase in O_2 consumption during exposure to hypersaline media as occurs, for example, in *O. glacialis* (Aarset and Aunaas 1990). Volume regulatory response was not measured for *O. glacialis*, but from the results presented here it can be seen that *A. viridari* undergoes RVI without an accompanying change in O_2 uptake.

Our results show that *Terebellides parva* also responded differently depending on whether exposure was to 25 or to 45‰S. In either seawater, *T. parva* behaved as an osmoconformer and tissue ion concentrations changed to approximately the same degree regardless of the direction of the salinity change. However, there was only limited survival of *T. parva* following exposure to 45‰S. *Arenicola marina* (Polychaeta) was also shown to be a strict osmoconformer when salinity was fluctuated between 100 and 30% seawater (Shumway and Davenport 1977). The ability to resist change in tissue osmolality is a function of permeability and extrarenal ion regulation, whereas the ability to limit change in intracellular water content is a function of intracellular solute regulation. Not surprisingly, because polychaetes and sipunculans are soft-bodied, the rate and degree that tissue osmolality changed in *T. parva* upon change in external osmolality was similar to that seen in *Golfingia cylindrata* but not that in *Alpheus viridari*. However, in soft-bodied marine worms, the lack of the cuticular barriers to water and solute movement found in crustaceans does not necessarily constitute an ultimate disadvantage at increased salinity as it would appear to in *T. parva*. As was seen, the response of *G. cylindrata* under the same conditions was quite different.

With regard to volume regulation, *Terebellides parva* did not limit initial change in water content in either salinity, but this polychaete did undergo subsequent water loss or RVD in 25‰S. In contrast, *A. viridari* limited water change and underwent subsequent RVD or RVI depending on salinity. In *T. parva* at low salinity, regulation of water content was not complete as it was in *A. viridari*, but *T. parva* similarly utilizes Na^+, Cl^-, and possibly K^+ as solute for short-term regulation of water content. Other polychaetes, such as *Neanthes succinea* and *Mercierella enigmatica*, also have been shown to lose intracellular inorganic ions, concurrent with RVD (Freel et al. 1973; Skaer 1974). While intracellular inorganic ion content was not measured in *Arenicola marina*, this worm appears to regulate water content via tissue free amino acids when salinities fluctuate between 100 and 30% seawater (Shumway and Davenport 1977).

Golfingia cylindrata behaved in a similar manner regardless of the direction of the salinity change. *G. cylindrata* demonstrated osmoconformity under each salinity condition but did not show evidence of ability to regulate water content at all. This species essentially tolerated large, repeated changes in water content without apparent deleterious effect. Unlike *Alpheus viridari*, *Golfingia cylindrata* showed no ability to limit swelling or shrinking in an initial limitation phase via change in solute content. Unlike both *A. viridari* and *Terebellides parva* there was no demonstration of subsequent RVD or RVI either. Instead, *G. cylindrata* showed itself to be extremely tolerant and able to survive, unlike the similarly soft-bodied *T. parva*, in the apparent absence of regulation. Other sipunculans show similar tolerance as well as lack of osmo- and volume regulatory ability over a wide range of salinities (Adolf 1936; Virkar 1966), but they do not occupy similarly variable habitats.

Responses to temperature

Exposure of *Alpheus viridari* to elevated temperatures had an effect on total tissue ion concentrations, whereas expo-

sure to decreased temperature did not. Both Na^+ and Cl^- concentrations increased transiently during exposure to high temperature thereby reducing the osmotic gradient across the body wall. This is in contrast to the findings of Burton (review: 1986), who showed that, under isoosmotic conditions, Na and Cl concentrations decreased with increasing temperature in the majority of crustaceans examined. Exceptions included species of *Gammarus*, *Crangon*, *Goniopsis*, and *Ucides* and were not restricted to temperate or tropical organisms (Burton 1986, Table 1 therein).

In *Alpheus viridari*, exposure to either a decrease or an increase in temperature resulted in an effect on water content; water content varied directly with temperature. Water, Na^+, and Cl^- contents initially changed, presumably due to passive leak. Subsequent regulation appeared to involve either recovery of solutes and water or elimination of excess Na^+, Cl^-, K^+, and water from both intra- and extracellular compartments. When *A. viridari* is in 35‰S, whole organism osmolality is lower than that of the external medium. If increased temperature causes an increase in ion permeability, as indicated by increased ion concentrations, this would account for the behavior of water and solutes when *A. viridari* is exposed to an increase in temperature. If a decrease in temperature caused a decrease in ion permeability without change in extrarenal ion extrusion, one would expect to see a decrease in tissue ion concentrations. Since tissue ion concentrations were unchanged, decreased temperature may result only in isoosmotic loss at the antennal gland. Although the mechanism is unknown, the effect is transient since *A. viridari* regulates water and solute content back to control amounts while still exposed to decreased temperature.

Response of *Alpheus viridari* to variation in temperature also differed with respect to O_2 uptake. When *A. viridari* was repeatedly exposed to decreased temperature, O_2 uptake varied directly with each temperature change. In contrast, exposure to increased temperature resulted in a sustained, elevated rate of O_2 uptake. For reasons discussed above, the latter increased uptake rate is not likely to be related to the observed temperature-induced disruption of Na^+ and Cl^- concentrations and subsequent requirement for ion regulation. Marine invertebrates are varied in their response to temperature, as assessed by O_2 consumption. Unlike *A. viridari*, some show correlation between temperature insensitivity and occupation of thermally variable environments (review: Newell 1973; Ferraris 1981), whereas others show no particular correlation between temperature sensitivity and habitat temperature (Mangum 1972; Newell 1973).

Like *Alpheus viridari*, the tissues of *Terebellides parva* are hypoosmotic to 35‰S seawater. Both species lose water and inorganic ions at decreased temperature, perhaps through isoosmotic fluid loss at the nephridia. However, unlike *A. viridari*, *T. parva* exposed to 21 °C recovered control water content only when returned to control temperature. *T. parva* appeared to be less tolerant of exposure to elevated temperatures. During repeated exposure to high temperature there was a slow sustained leak of water, Na^+, K^+, and Cl^-. It is reasonable to assume that survival would be questionable if this condition were prolonged.

Golfingia cylindrata showed only a transient increase in Cl^- ion concentration when exposed to decreased temperature. As noted above, an increase in inorganic ion concentration with decrease in temperature is not uncommon (review: Burton 1986). This sipunculan also seemed to undergo passive increase in water and solute contents at decreased temperature without apparent regulation and without apparent deleterious affect. However, results were quite different when the temperature was increased, and survival was limited.

Throughout the experiments, we encountered *Alpheus viridari* bearing embryo masses. During each of the experiments described, we observed that the embryos remained viable as indicated by continued movement within egg cases (unpublished observations). These observations suggest that *A. viridari* successfully is able to reproduce in the fluctuating environment of the mangrove habitat.

Terebellides parva demonstrates an intolerance of both high salinity and high temperature; these are conditions that are likely to co-occur in the mangrove habitat. Unlike shrimp, which carry embryo masses externally attached to appendages, reproductive or gravid individuals of *T. parva* were not obvious and were not observed. Hence, it is not known if the *T. parva* sampled were members of a successfully reproducing population or if the mangrove population is maintained through recruitment from offshore populations. Similarly, it is unknown if the *Golfingia cylindrata* in the present study successfully reproduce in the mangrove habitat. It would appear, however, that the elevated temperature intolerance of this sipunculan would be limiting. Populations of both *T. parva* and *G. cylindrata* may be renewed by migration as are populations of *Nereis diversicolor* (Polychaeta) in the Tamar estuary. Smith (1955, 1956, 1964) showed that the adults of this nereid occurred at salinities too low to support early larval development.

The ability to occupy the mangrove habitat, considering the range of responses to temperature and salinity observed in the present study, could be related to the microhabitat of each of the three species. Living in open burrows, *Alpheus viridari* must be capable of reacting rapidly and efficiently to changes in temperature and salinity. The shrimp are not protected by the micro-habitat from these changes, not least since *A. viridari* maintain an active aeration current through their burrows. The polychaete lives in an environment where the water movement could be slowed mechanically by the presence of the algal-mat, as does the sipunculan which lives within the root-mat. The algal-mat is loosely constructed and both of these substrates are very porous; a significant buffering effect, while possible, is not likely considering the dramatic temperature or salinity changes that occur within minutes in the surrounding seawater.

The different mechanisms used by these three species also in part reflect phylogenetically imposed structural constraints among members of three different phyla. Regardless of whether populations of *Terebellides parva* and

Golfingia cylindrata are reproducing in situ or are maintained through outside recruitment, the adults of the worms are only marginally adapted to withstand the salinity or temperature extremes, respectively, of the mangrove habitat. Hence, their ability to take advantage of this habitat, other than transiently, will be dependent on the extent of genetic variation within populations. Whether populations that live in habitats where temperature and salinity vary less are, in turn, less able to withstand extremes in these parameters remains to be investigated. In the case of each of these species, we will examine whether a given organism is able to withstand the extremes of the mangrove habitat because of an ability to express certain temperature- or salinity-induced genes having a protective function (Ferraris 1993).

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