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Water and solute regulation in *Procephalothrix spiralis* Coe and *Clitellio arenarius* (Müller) III. Long-term acclimation to diluted seawaters and effect of putative neuroendocrine structures

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Abstract

When acutely transferred to diluted seawater (SW), *Procephalothrix spiralis* and *Clitellio arenarius* regulate water content (g H_2O/g solute free dry wt = s.f.d.w.) via loss of Na and Cl (μmoles/g s.f.d.w.). The present study extends these observations to a greater range of salinities and determines the effects of long-term, stepwise acclimation to diluted seawaters. Final exposure to a given experimental seawater (70, 50, 30, 15‰) was 48 hours. Osmolality (mOsm/kg H_2O) and Na, K, and Cl ion concentrations (mEq/l) were determined in total tissue water and in the extracellular fluid of *C. arenarius*. Extracellular volume was determined as the ^{14}C-polyethylene glycol space. Both species behaved as hyperosmotic conformers in diluted seawaters. However, reduction of the osmotic gradient between worm and medium occurred in *P. spiralis*, but not *C. arenarius*, in 30 and 15‰ SW. In both species, osmolality and Na, Cl, and K concentrations in total tissue water decreased with increased dilution of the SW. Water content increased with dilution of the medium but was lower than that which would be predicted based on approximation of the van 't Hoff relation. This indicated the occurrence of regulatory volume decrease (RVD). In *P. spiralis*, in 70 or 50‰ SW, RVD was accompanied by loss of Na and Cl contents. However, in 30 or 15‰ SW, Na and Cl contents increased and in worms in 15‰ SW K content decreased. The latter movements of Na, Cl and K are indicative of cellular hysteresis and were associated with decreased viability, indicating the lower limits of regulatory ability in this species. In comparison, RVD in *C. arenarius* occurred in all diluted seawaters and was accompanied by loss of Na and Cl contents. In *C. arenarius*, evidence for reduced viability was absent. Removal of the supra- and subesophageal ganglia of *C. arenarius* resulted in retention of water, Na and Cl (g H_2O or μmoles/g s.f.d.w.) in worms acclimated to 70‰ SW. Removal of the cerebral ganglia and cephalic glands of *P. spiralis* did not significantly influence regulation of water content.

Introduction

P. spiralis (Archinemertina) and *C. arenarius* (Oligochaeta) inhabit rocky intertidal areas on the northeast coast of North America. In this habitat, changes in the salinity of the surrounding seawaters can be of short duration, as with rainfall during low tide, or of long duration, as with the melting of intertidal ice sheets in protected bays. Both species are known to regulate water content (g H_2O/g s.f.d.w.) during short-term acute exposure to decreased salinity (Ferraris & Schmidt-Nielsen, 1982a, 1982b) as well as under fluctuating salinity conditions (Ferraris, 1984). The present study extends the existent observations on the responses of *P. spiralis* and *C. arenarius* to include long-term exposure and greater dillution of seawaters.

Ability to regulate extracellular, but not intracellu-

lar, water content during short-term hypoosmotic exposure as well as during exposure to fluctuating salinity conditions is correlated with the presence of the supra- and subesophageal ganglia in *C. arenarius* and the cerebral ganglia and cerebral organs in *Paranemertes peregrina* (Hoplonemertina) (Ferraris & Schmidt-Nielsen, 1982a; Ferraris, 1984, 1985a). Under the same osmotic conditions, the ability of *P. spiralis* to regulate intracellular water content is similarly independent of the presence of the cerebral ganglia and the cephalic glands (Ferraris & Schmidt-Nielsen, 1982b; Ferraris, 1984). (Regulation of total body water content in this species is primarily an intracellular phenomenon since the volume of the extracellular compartment is only about 5% of the total.) The putative neuroendocrine nature of cerebral ganglia (primary location of neurosecretory cells), cerebral organs, and cephalic glands as well as response of these structures, on a cytological level, to osmotic variation has been treated in detail elswhere (Drawert, 1968; Ferraris, 1979a, 1979b, 1979c, 1985b; Ferraris & Schmidt-Nielsen, 1982a). Additional questions addressed in the present study are (1) whether these neurosecretory or neuroglandular structures affect water content regulation in *P. spiralis* under more extreme conditions and (2) whether the observed influence of the cerebral ganglia on regulation of water content in *C. arenarius* persists following long-term stepwise acclimation.

Materials and methods

Collection and maintenance

Adult specimens of both species were collected from under intertidial rocks at Hulls Cove and Salsbury Cove, Maine. Animals were maintained in aquaria with recirculating natural, Frenchman Bay seawater (100% SW, Table 1) at 7 °C for at least one week before use.

Surgical procedures

Detailed descriptions of the ablation techniques used are provided in Ferraris & Schmidt-Nielsen (1982a, 1982b). A summary follows. Tissues of *P. spiralis* were ablated by transection of the animals with a microscalpel immediately posterior to the cerebral ganglia. This procedure effectively removes both the cerebral ganglia and the cephalic gland which lies immediately anterior to the ganglia. Tissues of *C. arenarius* were ablated by transection immediately behind the subesophageal ganglia which also removes the supraesophageal ganglia and the mouth. In other groups of worms of both species (sham-operated) wound healing, without removal of organs, was induced by incising the dorsal body wall in the cephalic region. Ablated and sham-operated worms were returned to aquaria for a 5-day recovery period prior to use in experiments. This is sufficient time for complete wound healing to occur following both types of surgery and for reestablishment of the mouth in ablated *C. arenarius*; it is insufficient for regeneration of the ganglia (Ferraris & Schmidt-Nielsen, 1982a, 1982b). Control worms were treated in an identical manner but were not operated upon.

Experimental procedures

Control, ablated and sham-operated individuals of

Table 1. Seawater composition (mean ± S.E.).

Seawater (%)	Osmolality (mOsm)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
100	948 ± 1.25	461.3 ± 1.49	10.2 ± 0.05	522.6 ± 1.45
70	670 ± 1.32	334.3 ± 2.93	7.1 ± 0.07	370.2 ± 0.95
50	478 ± 0.85	235.5 ± 3.48	5.0 ± 0.07	272.5 ± 0.20
30	282 ± 0.65	134.3 ± 1.38	3.0 ± 0.08	155.0 ± 0.85
15	143 ± 0.48	70.9 ± 0.52	1.5 ± 0.03	79.5 ± 0.45

either species were simultaneously immersed in diluted seawaters in a stepwise manner so that exposure to 70, 50, 30, or 15% seawater (Table 1) lasted 48 hours. Following a 48 hour exposure, worms were either prepared for analysis or transferred to the next lower seawater concentration. Seawaters were prepared by dilution of 100% seawater with tap water. The water in aquaria was kept recirculating.

Animals were prepared for analysis by the reconstitution method as described in detail by Ferraris & Schmidt-Nielsen (1982a). In summary, entire worms (5–10 mg) were weighed to the nearest 0.001 mg (Cahn Automatic Electrobalance, Model 21), dried at 60 °C to constant weight, submerged in a small volume (50 µl) of deionized-distilled water and heated in a water bath at 98 °C for 3 minutes. Specimens were left undisturbed in small plastic tubes at 4 °C for about 48 hours to allow diffusion of ions and other osmotically active substances. Tube contents were then mixed and centrifuged and analyses performed on the supernatant. Values obtained were corrected for dilution (Ferraris & Schmidt-Nielsen, 1982a).

Analyses

Osmolality (mOsm/kg H_2O; Wescor 5100 B Vapor Pressure Osmometer) and Na and K ion concentrations (mEq/L; Instrumentation Laboratories, Model 343) were determined on duplicate 5 µl samples. Cl ion concentration (mEq/l) was measured by coulometric titration (Buchler-Cotlove Chloridometer) on single 10 µl samples.

Extracellular (PEG) space determination

^{14}C-polyethylene glycol M.W. 4000 (^{14}C-PEG) was used as a marker for extracellular fluid in both species; however, the techniques employed differed. PEG space was determined for *C. arenarius in vivo*, whereas, the same was determined for *P. spiralis* using an *in vitro* method. The very small size of *P. spiralis*, in combination with an acoelomate body plan, precluded obtaining sufficient extracellular fluid even by micropuncture methods to accurately assay for ^{14}C-PEG. The *in vivo* and *in vitro* methodologies and associated calculation of fractional extracellular space are described in detail in Ferraris & Schmidt-Nielsen (1982a and 1982b, respectively) and are summarized as follows:

In vivo

Animals were subjected to the same 48 hour stepwise acclimation to various seawaters. Individual *C. arenarius* received an intracoelomic and intravascular micropuncture injection of ^{14}C-PEG (0.01 µCi) in the appropriate seawater solution. The injected substance was allowed to equilibrate with the extracellular compartment for 2 hours. Worms were then weighed and a volume of mixed coelomic fluid and blood (extracellular fluid) was collected. Worms were then dried and reconstituted as above. ^{14}C-PEG concentration in extracellular fluids and in tissues were determined and the fractional extracellular space calculated. The fraction of the extracellular fluid was determined in a total of 40 worms stepwise acclimated to 100, 70, 50, 30 or 15% seawater.

In vitro

P. spiralis was subjected to experimental seawaters as above. Worms were sliced into 2 mm pieces and the tissue incubated in a ^{14}C-PEG solution for 2 hours. The incubation medium contained 100, 70, 50, 30, or 15% SW plus ^{14}C-PEG (0.25 µCi). Following equilibration, tissues were prepared for analysis by the reconstitution method. Tissue samples and incubation media were analyzed for ^{14}C-PEG concentration and the fractional extracellular space calculated. The fraction of the extracellular fluid was determined in a total of 79 worms stepwise acclimated to 100, 70, 50, 30 or 15% seawater.

Calculations

Total body water content [gram H_2O/gram solute free dry weight (g H_2O/g s.f.d.w.)] was calculated after the method of Schmidt-Nielsen *et al.* (1983). This method of determination of water content is more accurate than that based solely on dry weight. Since regulatory volume decrease (RVD) is associated both with a change in water content and with a decrease

in the amount of solutes, RVD results in a change in the specific weight of the cells. Calculation of g H_2O/g s.f.d.w. corrects the dry weight for the change in specific weight. The method, as used in the present study, has been described in detail (Ferraris & Schmidt-Nielsen, 1982a). Solute contents (μmoles/g s.f.d.w.) were calculated by multiplying the solute concentration in tissue water (μEq/ml) by the total tissue water content in g/g s.f.d.w.

Fractional extracellular fluid was calculated as fractional PEG space after the method of Ferraris & Schmidt-NIelsen (1982a, 1982b). Intracellular water was obtained by difference. Since the PEG space was not determined on all individuals in the present study we recognize a possible limitation in this method.

Data were compared using a one way analysis of variance followed by Student-Newman-Keuls Multiple Range test for separation of significant means. Data referred to as significantly different have a statistically significant difference at least at $P < 0.05$.

Extracellular and intracellular solute determination

Solute concentrations in the extracellular fluid of *C. arenarius* were determined by removal and analysis of a mixture of coelomic fluid and blood as described in Ferraris & Schmidt-Nielsen (1982a). Samples were analysed for Na, K and Cl ion concentrations (mEq/l). Extracellular solute concentrations were used in conjunction with the fractional PEG space to calculate extracellular ion content (μmoles/g s.f.d.w.). Total tissue ion content was measured and intracellular solutes (μmoles/g s.f.d.w.) were obtained by difference. In *P. spiralis*, sufficient extracellular fluid was not obtainable, even by micropuncture methods, to accurately assay for ion concentrations

Experimental protocol

The relation of the change in water content (V_2/V_1) to the simultaneously occurring change in tissue osmolality (π_1/π_2 was used to discern the occurrence of RVD during exposure to diluted seawaters. The V_1 and V_2 values are the water contents (g H_2O/g s.f.d.w.) of tissues (in this case entire worms) at the beginning and end of a given seawater exposure, respectively; π_1 and π_2 are the tissue water osmolalities (mOsm/kg H_20) of the same tissue for the beginning and end, respectively, of the same period (Ferraris, 1984). In a hypoosmotic medium, RVD may occur (1) while cells are swelling, as a swelling-limitation phase, and (2) subsequent to swelling, as a net volume loss. Both phases are accompanied by a decrease in solute content. Following acclimation to a hypoosmotic medium, if V_2/V_1 were less than π_1/π_2 water content would have changed less than would be expected given the change in tissue osmolality and the occurrence of RVD would be indicated.

Results

There were few consistent, significant differences among control, ablated and sham-operated animals. Hence, for clarity, all groups of a given species are referred to collectively as *C. arenarius* or as *P. spiralis*. Those differences among groups that occurred primarily involved the responses of *C. arenarius* in 70% seawater and *P. spiralis* in 30% seawater. All differences that were statistically significant ($P < 0.05$) are either reported in the text or appear in appropriate figures.

Osmolality and ion concentrations in total tissue water

C. arenarius was isoosmotic to the medium in 100% seawater but was significantly hyperosmotic to all other media (Fig. 1). The osmotic difference between worms and medium was approximately 45 mOsm in 70% SW whereas that in 15% SW was 85 mOsm.

P. spiralis was slightly hyperosmotic to 100% SW (Fig. 1). In experimental seawaters *P. spiralis* was significantly hyperosmotic to the medium maintaining an average difference of 75 mOsm in 70 and 50%

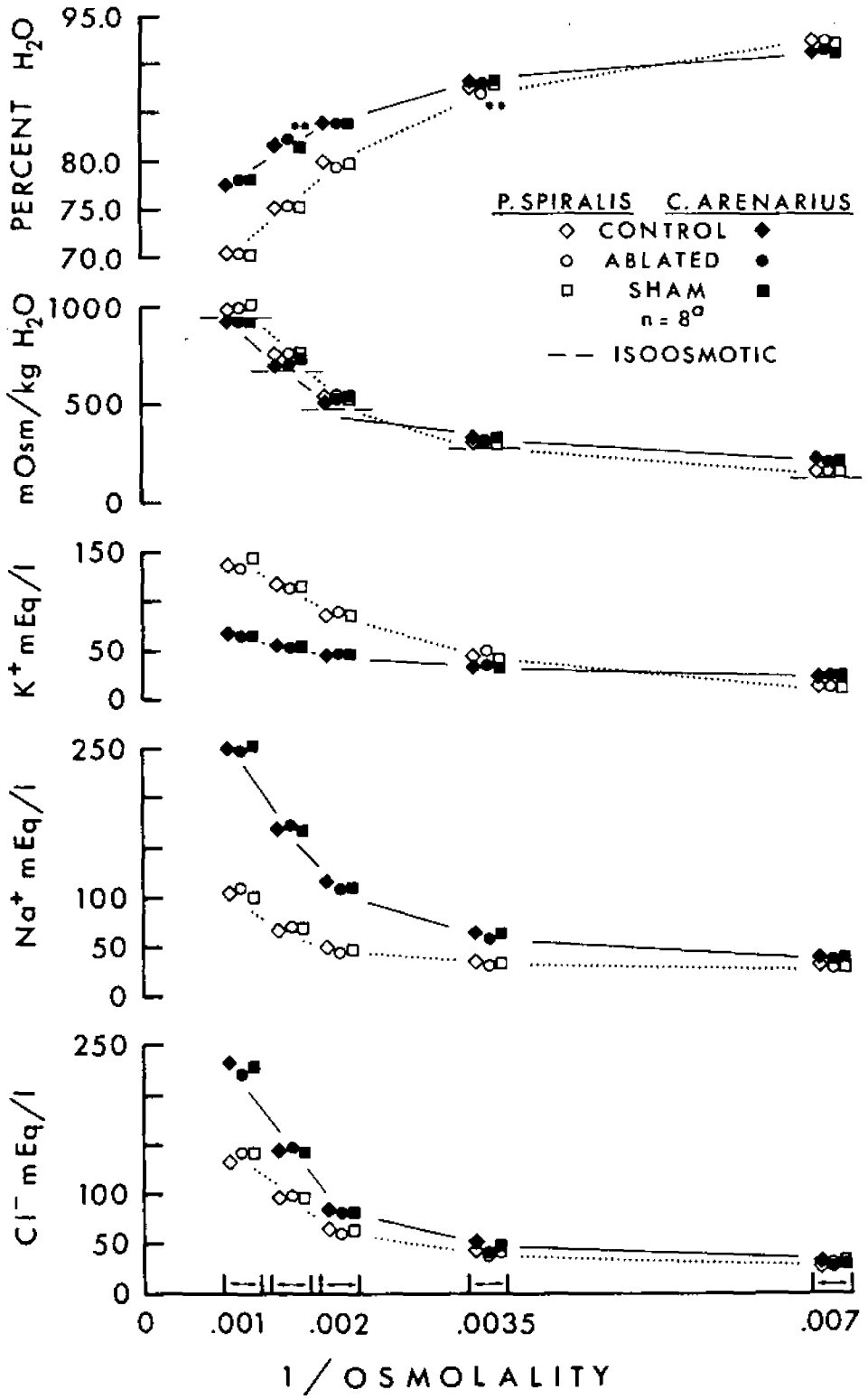

Fig. 1. Percent water, osmolality, and K, Na, and Cl concentrations [mean, S.E. (S.E.'s smaller than size of symbols)] in the total tissue water of control, ablated and sham-operated *P. spiralis* and *C. arenarius* after exposure to diluted seawaters. [a]n = 5 at 0.007 and n = 6 at 0.0015 for *P. spiralis* only. * Ablated animals significantly different ($P < 0.05$) from either control or sham-operated worms; **ablated worms significantly different from both control and sham-operated animals.

SW. When worms were in 30 or 15% SW, the gradient between worm and medium was only about 20 mOsm. Survival of this species in 15% SW was low (62.5%, n = 8); reduction in mobility and response to touch was obvious in worms in both 30 and 15% SW.

Percent water in *C. arenarius* increased significantly with each progressive dilution of the medium. There was an average overall increase of 13.4% (Fig. 1). Ablated *C. arenarius* were significantly higher in percent water than either control or sham-operated animals in 70% SW but differences among groups were not significant at any other seawater dilution.

P. spiralis also increased in percent water with decreasing seawater concentration (Fig. 1). However, in the case of this species, the overall increase in percent water from worms in 100% SW to those in 15% SW was 21.5%. Ablated worms were lower in percent water than other groups when worms were acclimated to 30% SW.

In *C. arenarius* the concentrations of all ions measured in total tissue water (Na, K and Cl mEq/l) decreased with the concentration of the medium (Fig. 1). Primary reduction was seen in Na and Cl concentration.

P. spiralis also successively decreased in tissue ion concentration (mEq/l) but primary reduction occurred with respect to K rather than Na reflecting an acoelomate body construction (Fig. 1).

Ion concentrations in extracellular fluid

Na concentrations (mEq/l) in the extracellular fluid of *C. arenarius* were either iso- or hyperionic to that of the medium (Table 2). In worms in 100, 30, and 15% SW, average fluid to medium ratios (F : M) were 1.04, 1.01, and 1.12, respectively. In worms in 70 and 50% SW, F : M ratios increased to 1.26 and 1.31, respectively. Extracellular K (mEq/l), however, was consistently hyperionic to the seawater. Fluid to medium ratios for K progressively increased (*i.e.*, 2.60, 3.28, 4.25, 5.39 and 10.52) with dilution of the medium. In contrast, extracellular Cl concentration was strongly hypoionic to full strength seawater

Table 2. Extracellular ion concentrations (mEq/l) in *C. arenarius* (mean ± S.E.) during exposure to diluted seawaters.

Seawater	Control	Ablated	Sham-operated
100%			
Na 473.6	492.9 ± 8.15 (5)	489.9 ± 15.93 (5)	492.6 ± 13.08
K 10.5	27.1 ± 0.88	26.3 ± 0.47	28.6 ± 2.48 (3)
Cl 513.9	364.5 ± 14.78 (8)	374.5 ± 17.68 (8)	372.8 ± 14.22 (8)
70%			
Na 322.0	403.2 ± 9.84	406.4 ± 5.23	412.1 ± 11.66 (3)
K 7.4	22.8 ± 1.96	26.8 ± 3.72	23.3 ± 1.29 (3)
Cl 372.2	327.4 ± 15.95	314.5 ± 16.39	300.7 ± 9.17
50%			
Na 239.3	291.2 ± 16.79	324.9 ± 18.79	306.9 ± 9.96
K 5.3	24.1 ± 1.63	22.4 ± 2.49	21.1 ± 0.90
Cl 274.5	225.3 ± 11.11	202.0 ± 2.75	233.4 ± 25.04
30%			
Na 139.6	142.9 ± 5.55	139.2 ± 6.99	141.9 ± 7.83
K 2.9	17.1 ± 2.54	16.4 ± 1.31	13.4 ± 0.34
Cl 159.9	162.5 ± 9.85	147.3 ± 12.35	158.5 ± 6.02
15%			
Na 72.4	81.3 ± 4.90	83.7 ± 4.34	77.2 ± 4.17
K 1.6	17.8 ± 3.56	16.0 ± 0.68	16.7 ± 2.58
Cl 79.3	75.9 ± 7.75	107.3 ± 14.90	83.8 ± 9.01

n = 4 except where otherwise indicated in parentheses.

(F:M = 0.72). With dilution of the medium Cl concentration became less hypoionic; F:M ratios equaled 0.84, 0.80 and 0.97 in 70, 50 and 30% SW, respectively. In worms in 15% SW, extracellular Cl concentration in control and sham-operated worms was isoionic to the medium (F:M = 1.01); an unusually high Cl concentration in ablated worms raised the average fluid to medium ratio to 1.12 in worms in this seawater. In spite of the high Cl concentration in ablated worms, differences in extracellular Cl concentration among the groups were not significant.

Comparative information on *P. spiralis* was not available due to the very small size of this species and the small volume of the extracellular compartment (approximately 5% of total body water content).

Water and solute content

Water content (g H_2O/g s.f.d.w.) in *C. arenarius* increased significantly with each dilution of the medium (Fig. 2). Overall, total water content increased by an average of 7.7 g H_2O/g s.f.d.w. As also reflected by percent water, the amount of water in ablated worms was significantly higher than in in control or sham-operated worms after a 48 hour exposure to 70% SW.

P. spiralis similarily increased in water content with increased dilution (Fig. 2). However, while this species was initially significantly lower in water content than *C. arenarius*, by the end of the experiment *P. spiralis* had gained an average of 10.7 g H_2O/g s.f.d.w. and had surpassed the water content of *C. arenarius*. Ablated *P.spiralis* in 30% SW contained significantly less water than did either control or sham-operated animals.

In order to estimate the occurrence of regulatory volume decrease (cf. Ferraris & Schmidt-Nielsen, 1982a), the relative water contents (V_2/V_1) of worms in various seawaters were plotted against the relative osmolalities (π_1/π_2) of the same animals (Fig. 3). *C. arenarius*, in all experimental seawaters, demonstrated a lower water content than might be expected if these animals had behaved as true osmometers. Differences were significant ($P < 0.05$) in all seawaters except 70% and increased with decreasing osmolality. Similarly, *P. spiralis* had a lower water content than expected in all experimental seawaters ($P < 0.05$ except 70% SW). Differences between expected and demonstrated water contents were more pronounced in *C. arenarius* than in *P. spiralis*.

K content (μmoles/g s.f.d.w.) in *C. arenarius* did not decrease with acclimation to diluted seawaters (Fig. 2).

In *P. spiralis*, there was also no significant loss of K (μmoles/g s.f.d.w.) after stepwise acclimation to 70, 50 or 30% SW, however, K content dropped markedly after acclimation to 15% seawater (Fig. 2).

Na content (μmoles/g s.f.d.w.) (Fig. 2) in *C. arenarius* decreased with each dilution of the medium. Na content (μmoles/g s.f.d.w.) in ablated worms was higher ($P < 0.05$) than in control and sham-operated animmals in 70% seawater with no significant difference occurring in any other seawater concentration.

In *P. spiralis* there was a decrease ($P < 0.05$) in the Na content (μmoles/g s.f.d.w.)of all groups after exposure to 70% seawater; further reduction occurred in 50% SW (Fig. 2). Loss of Na, however, was followed by a significant Na gain in worms in 30% seawater and again in 15% seawater. Differences in Na content among the three groups were significant only in 30% SW.

For both species, a pattern similar to that which occurred in Na content was demonstrable with respect to Cl content (μmoles/g s.f.d.w.) (Fig. 2). In *C. arenarius* significant Cl (μmoles/g s.f.d.w.) was lost with each dilution of the medium except the last (15% SW). Ablated worms contained more Cl (μmoles/g s.f.d.w.) than sham-operated worms ($P < 0.05$) in 70% SW. In 30% seawater Cl content in ablated animals was only lower than that in control worms ($P < 0.05$).

In *P. spiralis*, Cl (μmoles/g s.f.d.w.) was lost in 70% SW and in 50% SW ($P < 0.05$) (Fig. 2). The amount of Cl in worms then progressively increased with acclimation to 30 and 15% seawater. Cl content was lower ($P < 0.05$) in ablated animals than in the control groups in 30% seawater with no significant differences occurring among groups in other seawaters.

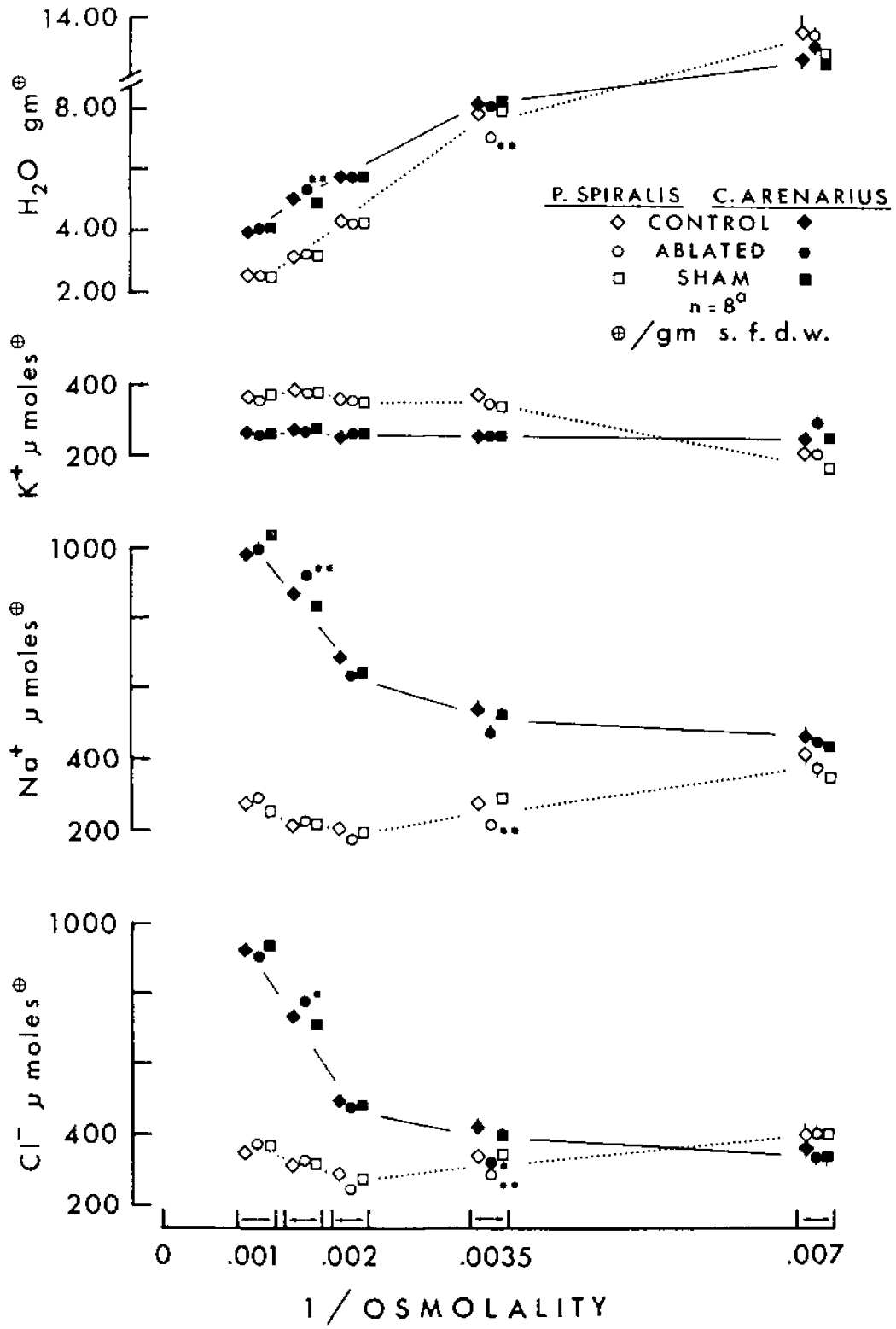

Fig. 2. Water (gram H_2O/gram solute free dry weight), K, Na, and Cl contents (μmoles/g solute free dry weight) (mean, S.E) in control, ablated and sham-operated *P. spiralis* and *C. arenarius* after exposure to diluted seawater. [a]n = 5 at 0.007 and n = 6 at 0.0015 for *P. spiralis* only.

* Ablated animals significantly different ($P < 0.05$) from either control or sham-operated worms; ** ablated worms significantly different from both control and sham-operated animals.

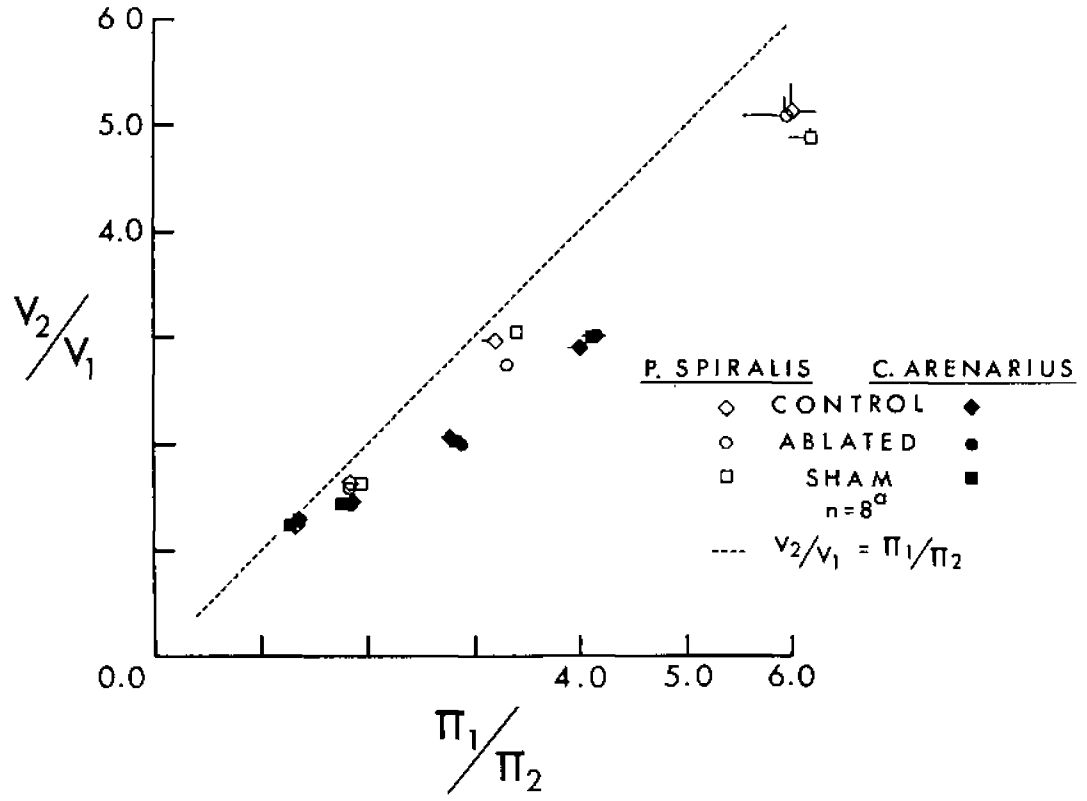

Fig. 3. Relative water content (V_2/V_1) (mean, S.E.) in control, ablated and sham-operated *P. spiralis* and *C. arenarius* in 100‰ and diluted seawaters compared with the relative osmolalities (π_1/π_2) (mean, S.E.) of the same animals. [a] n = 6 at π_1/π_2 about 1.3 and n = 5 at π_1/π_2 about 6.0 for *P. spiralis* only.

Extracellular (PEG) space and the water content of the extra- and intracellular compartments

In *C. arenarius*, the extracellular space, *i. e.* the fraction of the total tissue water in which ^{14}C-polythylene glycol was distributed, did not differ ($P < 0.05$) between control and ablated worms in any seawater concentration (Table 3). The mean value obtained in a given seawater was thus used in all calculations involving PEG space. Extracellular

Table 3. ^{14}C-PEG space as percent of total water content during exposure to diluted seawaters (mean ± S.E.).

Seawater (‰)	*C. arenarius*			*P. spiralis* (n = 16)
	Control (n = 4)	Ablated (n = 4)	Control + ablated (n = 8)	
100	*46.58 ± 3.41	47.41 ± 3.83	47.01 ± 2.38	4.09 ± 0.390
70	40.33 ± 2.89	41.79 ± 3.53	41.06 ± 2.13	3.50 ± 0.259
50	35.91 ± 4.95	33.87 ± 4.53	34.89 ± 3.13	4.26 ± 0.471
30	46.08 ± 1.11	50.24 ± 2.86	48.16 ± 1.62	5.07 ± 0.461
15	50.89 ± 7.12	54.17 ± 4.15	52.53 ± 3.86	8.74 ± 0.696 (n = 15)

*Values encompassed by the same line or lines on the same level are not significantly different ($P < 0.05$).

space decreased with dilution of the medium until mean PEG space in 50% SW was significantly less than that in full strength seawater. However, with further dilution the extracellular space increased. Mean values in 30 and 15% SW were greater than in 50% SW ($P < 0.05$) although not significantly different from results obtained in full strength seawater. The increase in the PEG space in 30 and 15% SW may indicate PEG entry into cells since calculation of intracellular solute content (μmoles/g s.f.d.w.) at these salinities would result in negative values.

In *P. spiralis* the PEG space tended to follow a pattern similar to that observed in *C. arenarius*. Extracellular space decreased with exposure to 70% SW and then increased with further dilution of the medium (Table 3). Exposure to 15% SW resulted in an increase in the extracellular space which was significantly higher than all other values obtained. Results obtained in 30 and 15% SW probably indicate leakage of PEG into cells.

The extracellular water content (g H_2O/g s.f.d.w.) of *C. arenarius* increased in diluted seawaters, however, hydration (V_2/V_1) in this compartment was consistently less than what might be expected based on changes in tissue osmolality (π_1/π_2). Apparent RVD in this compartment was accompanied by loss of extracellular Na and Cl (μmoles/g s.f.d.w.).

Intracellular water content rose with each dilution of the medium. Intracellular hydration (V_2/V_1) was not different from what might be expected of a true osmometer in 70% seawater but was less than expected (π_1/π_2) at all other seawater concentrations. Apparent RVD in this compartment was accompanied by loss of intracellular Na and Cl but not K (μmoles/g s.f.d.w.).

Based on the distribution of ^{14}C-PEG, regulation of total body water content in *P. spiralis* primarily reflects events occurring in the intracellular compartment. Apparent RVD was accompanied by loss of Na and Cl (μmoles/g s.f.d.w.) during acclimation to 70 and 50% SW.

Discussion

The responses of control *C. arenarius* and *P. spiralis* will be discussed first. This will be followed by treatment of the effects of ablation.

Control and sham-operated worms

Osmolality and ion concentrations in extracellular fluid and total tissue water

Both *P. spiralis* and *C. arenarius* were approximately isoosmotic with the full strength seawater in which they were maintained but behaved as hyperosmotic conformers with long-term, stepwise acclimation to diluted media. Similar results have been observed in other marine invertebrates (review: Oglesby, 1978) as well as when *P. spiralis* and *C. arenarius* are subjected to short-term, acute exposure to 70 or 50% SW (Ferraris & Schmidt-Nielsen, 1982a, 1982b). However, in present study, *P. spiralis* and *C. arenarius* differed in their response to the more reduced salinities (30 and 15% SW). *C. arenarius* became more hyperosmotic to the medium whereas in *P. spiralis* the difference between tissue and medium osmolality decreased. Thus, while *P. spiralis* was initially somewhat hyperosmotic to *C. arenarius*, the reverse was true when worms were acclimated to the more diluted seawaters. This appeared to be primarily due to a greater influx of water into *P. spiralis* which was evident in worms in 30% SW and continued with acclimation to 15% SW. The appearance and behavior of *P. spiralis* in these salinities indicated highly reduced viability and limited survival (particularly in 15% SW). In contrast *C. arenarius* did not demonstrate extensive reduction in motility although the animals were visibly swollen. *C. arenarius* also survived exposure to 15% SW for several weeks (observations terminated) while *P. spiralis* did not survive more than 24 hours beyond the termination of the experiment. The ability of *C. arenarius* to tolerate a wider range of salinities than *P. spiralis* is compatible with differences in their distribution in the rocky intertidal. Although both species are abundant under intertidal rocks, *C. arenarius* is also commonly found at more elevated locations, *e.g.* rock crevices where exposure to osmotic variation is potentially more extreme than that occurring due to seepage under rocks. In this habitat, the lower water permeability found in *C.*

arenarius would be of advantage (Ferraris & Schmidt-Nielsen, 1982a, 1982b). Although comparative information is limited, other marine oligochaetes are also known to demonstrate significant osmo- or volume regulatory ability. In comparison with *C. arēnarius*, *Enchytraeus albidus* maintains a stronger osmotic gradient when immersed in seawater below 15‰ S and *Marionina arenarius* is a stronger volume regulator under both hypo- and hyperosmotic conditions (Schöne, 1971; Lasserre, 1975).

In both *P. spiralis* and *C. arenarius*, as the osmolality of the medium decreased, Na, K, and Cl concentrations (mEq/l) in total tissue water decreased. In *C. arenarius* extracellular K and Na concentrations were regulated hyperionic while Cl was hypoionic to the seawater until worms were acclimated to 30% SW. Once *C. arenarius* was acclimated to 30 or 15% SW there was no evidence for regulation of extracellular Na or Cl concentration. This was, however, without apparent deleterious effect. Thus, it is not surprising that this species is frequently found in brackish water having salinities down to approximately 11‰ S (cf. Pfannkuche, 1980).

Comparative information on extracellular ion concentrations in *P. spiralis* is not available. With respect to ions in total tissue water this species superficially appeared to stabilize Na and Cl (but not K) concentrations with dilution of the medium beyond 50% SW. These observations are, however, more consistent with leakage of Na, Cl and water into the cells and inability to maintain cellular integrity.

Water and solute content

Both worms gained water with dilution of the medium but both species also demonstrated an ability to regulate water content. When relative water contents (V_2/V_1) were compared with the relative changes in tissue osmolality (π_1/π_2) experienced by the same worms (Fig. 3) then it was apparent that both animals contained less water than if they behaved as true osmometers.

Based on calculation of the distribution of water and solutes, *C. arenarius* regulated both intra- and extracellular water content via loss of Na and Cl, but not K (μmoles/g s.f.d.w.) regardless of salinity. In *C. arenarius* acclimated to 30 and 15% SW the difference between predicted and measured water content was pronounced (Fig. 3). Data obtained on extracellular space determination in worms acclimated to these seawaters indicated a possible change in the permeability of cells to polyethylene glycol. However, under the same salinity conditions, *C. arenarius* continued to maintain a significant difference between the osmotic concentration of the tissues and that of the medium. There was also no evidence of cellular hysteresis with respect to movement of solutes with acclimation to decreased salnities. Thus, the volume regulatory ability of this species may be considered significant at low seawater concentrations.

In *P. spiralis* acclimated to 70 and 50% SW, regulatory volume decrease involved intracellular loss of Na and Cl but not K (μmoles/g s.f.d.w.). RVD via intracellular loss of Na and Cl is found in a variety of marine invertebrates (Freel *et al.*, 1973; Freel, 1978; Ferraris & Schmidt-Nielsen, 1982a; Warren & Pierce, 1982; Ferraris, 1984, 1985a). When *P. spiralis* is acutely exposed to 70% SW there is intracellular Na and Cl loss that is associated with RVD (Ferraris & Schmidt-Nielsen, 1982b). However, when this species is acutely exposed to 50% SW there is an intracellular K loss that occurs in the absence of regulatory volume decrease (Ferraris & Schmidt-Nielsen, 1982b). The results of the present study demonstrate that *P. spiralis* can regulate water content in salinities as low as 50% SW when exposure occurs gradually. However, the limits of RVD in *P. spiralis* appear to be in the vicinity of 30% SW even when acclimation is gradual as in the present study. Thus, in contrast with *C. arenarius*, when *P. spiralis* was acclimated to 30 or 15% SW, this species showed evidence of PEG entry into cells that was coupled with cellular hysteresis (indicated by the increased amount of Na and Cl but decreased K in worms).

Ablated worms

C. arenarius lacking supra- and subesophageal ganglia retained more water, Na and Cl (gH_2O or μmoles/g s.f.d.w.) than did control groups after 48 hours in 70% seawater. These results are similar to those obtained with exposure periods of shorter du-

ration (Ferraris & Schmidt-Nielsen, 1982a; Ferraris, 1984). However, in the present study there was no significant difference among the groups in 50% SW. This is in contrast to the response of these worms to acute exposure to 50% SW where ablated animals maintain greater water and solute contents (Ferraris & Schmidt-Nielsen, 1982a). The results obtained in the present study and those in parallel work (Ferraris & Schmidt-Nielsen, 1982a) are consistent with ablation interfering with the onset and time course of extracellular volume regulation (*e.g.* increased urine production) rather than impairment of the mechanism itself. For example, with acute exposure to 50% SW, although ablated worms consistently contained more water than control groups, they also rid themselves of somewhat more water than controls after the onset of regulation (Ferraris & Schmidt-Nielsen, 1982a). Thus, with the longer and more gradual acclimation periods used in the present study, ablated *C. arenarius* no longer retained more water and solutes than the control groups.

In the present study, ablated *P. spiralis* contained less water than did control groups when acclimated to 30% seawater. However, viability was sufficiently poor in this species at this salinity that these results cannot be considered biologically significant. A lack of effect of removal of the cerebral ganglia and cephalic glands on intracellular volume regulation in this species has also been demonstrated during acute exposure to reduced salinities as well as during exposure to fluctuating salinities (Ferraris & Schmidt-Nielsen, 1982b; Ferraris, 1984). Similar results were obtained with *Paranemertes peregrina* (Ferraris, 1985a). Ablation of the cerebral ganglia and cerebral organs in *P. peregrina* has no affect on intracellular volume regulation. However, in a manner similar to that found in *C. arenarius*, these putative neuroendocrine structures do affect the ability of *P. peregrina* to regulate extracellular volume under anisoosmotic conditions (Ferraris, 1985a). Other species that, like *P. peregrina*, have a large (at least 25% of the total) extracellular fluid volume have also been examined for an effect of removal of the cerebral ganglia and cerebral organs. In each case (*Lineus ruber, L. viridis, Amphiporus lactifloreus*) volume regulation has been impaired or eliminated by ablation of one or both of these structures (Lechenault, 1965a, 1965b; Varndell, 1981). Unfortunately, the distribution of water and solutes between the intra- and extracellular compartments was not examined. For a complete review of the relevant literature the reader is referred to Ferraris (1984, 1985a, 1985b).

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