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Effects of salinity changes on zinc uptake and regulation by the decapod crustaceans *Palaemon elegans* and *Palaemonetes varians*

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ABSTRACT: The decapod crustaceans *Palaemon elegans* Rathke and *Palaemonetes varians* (Leach) regulate their body concentrations of zinc to an approximately constant level (ca 76 to 82 and 90 to 99 μg Zn g^{-1} dry wt respectively) when exposed to a wide range of dissolved Zn concentrations, until a threshold dissolved concentration when regulation breaks down and net accumulation of body Zn begins. This threshold concentration decreases with decrease in salinity, from 100 % SW to 50 % SW in the case of *P. elegans* (ca 92 to 27 μg Zn l^{-1} respectively) and from 100 % SW to 5 % SW in the case of *P. varians* (ca 191 to 19 μg Zn l^{-1} respectively), in correlation with increased rates of Zn uptake. *P. elegans* showed a further increased uptake rate in 25 % SW (1.054 ± 0.385 μg Zn g^{-1} d^{-1} in 25 % SW vs 0.735 ± 0.077 μg Zn g^{-1} d^{-1} in 100 % SW at 10 °C in 56.2 μg Zn l^{-1}), but had an atypical pattern of Zn regulation in raised Zn concentrations, probably as a result of atypically high Zn efflux caused by changes in water balance under osmotic stress. *P. varians* has a lower rate of Zn uptake than *P. elegans* under identical physico-chemical conditions (1.80 ± 0.61 vs 5.27 ± 3.67 μg Zn g^{-1} d^{-1} in 100 μg Zn l^{-1} in 50 % SW). Fifteen day acclimation of *P. varians* to either 50 or 25 % SW did not significantly change the subsequent mean rate of Zn uptake from 100 μg Zn l^{-1} in 25 % SW. The body Zn concentration of *P. varians* transferred to dilute media of 25 and 5 % SW increased initially as a low salinity response but decreased again after 2 to 10 d to be regulated at ca 95 μg Zn g^{-1}. It is concluded that Zn uptake and regulation in decapods are affected both by extrinsic physico-chemical factors and by intrinsic adaptations of the species concerned.

INTRODUCTION

As is typical for decapod crustaceans (Rainbow 1988), the littoral prawn *Palaemon elegans* regulates the total body concentration of zinc to a constant level over a wide range of dissolved Zn concentrations (White & Rainbow 1982, 1984a, b, Nugegoda & Rainbow 1987). Zn is taken into the body at a rate which increases with the concentration of dissolved Zn to which the animal is exposed; over the regulated exposure range Zn uptake is matched by increasing Zn excretion, thereby maintaining a constant body Zn concentration (White & Rainbow 1984a). Eventually Zn bioavailability exceeds a threshold and Zn excretion can no longer match Zn uptake, causing regulation breakdown and net Zn accumulation (Rainbow 1988).

Physico-chemical variables affect the uptake of Zn by the prawn (White & Rainbow 1984a, Nugegoda & Rainbow 1988). For example, at a given dissolved Zn concentration, a rise in temperature causes an increase in the uptake rate by *Palaemon elegans* (White & Rainbow 1984a). Increased Zn uptake in turn is correlated with a lowering of the threshold dissolved Zn concentration causing regulation breakdown in the prawn (Nugegoda & Rainbow 1987). Similarly the presence of the chelating agent EDTA in solution decreases the rate of Zn uptake by *P. elegans* and thereby increases the dissolved Zn concentrations causing regulation breakdown (Nugegoda & Rainbow 1988).

Changes in another physico-chemical variable, salinity, are known to affect the toxicity of heavy metals to marine and estuarine organisms (and also their uptake and accumulation), effects often differing between metals (Phillips 1980, McLusky et al. 1986). In the case of Zn and crustaceans, Jones (1975) showed that the toxicity of Zn to 6 species of marine and estuarine isopods increased as salinity decreased. McKenney &

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Neff (1979) found that the viability of larval decapods *Palaemonetes pugio* in elevated Zn concentrations was reduced by both the individual effects of salinity and temperature and by interactions between the 2 factors outside optimal salinity-temperature conditions. Bryant et al. (1985) found that the toxicity of Zn to the amphipod *Corophium volutator* increased as salinity decreased over the range 35 to 5 ppt.

This paper reports on the effect of changes in salinity on Zn uptake and regulation by 2 prawns – *Palaemon elegans* and *Palaemonetes varians*. *P. elegans* lives in intertidal rockpools frequently encountering salinity changes from 100 % to 50 % seawater (Morris & Taylor 1983). The related palaemonid *P. varians* is a salt marsh inhabitant of brackish waters of a lower salinity range and is never found in fully marine conditions (Smaldon 1979). The comparison is made to throw light on the relative importance of extrinsic physico-chemical factors (e.g. salinity changes) and intrinsic factors (e.g. adaptation to a specific osmotic range) on Zn uptake and regulation processes in decapod crustaceans. Parts of the study also examine the effects of salinity changes on Zn uptake in acclimated and non-acclimated prawns, and the effects of combined changes in salinity and temperature on Zn regulation in *P. varians*.

Results are presented to show that the breakdown in regulation of body Zn concentration occurs at lower dissolved Zn concentrations with decreased salinity in both prawns – over the range 100 % to 50 % SW for *Palaemon elegans*, and from 100 % to 5 % SW for *Palaemonetes varians*. In both cases the decreases in dissolved Zn concentrations corresponding to thresholds of regulation breakdown are correlated with increases in Zn uptake rate. The rate of Zn uptake by *P. elegans* under given conditions is higher than that of *P. varians* and correspondingly Zn regulation breaks down at lower dissolved Zn concentrations in *P. elegans*. It will be concluded that Zn uptake and regulation in decapods are affected both by extrinsic physico-chemical factors and by intrinsic adaptations of the species concerned.

MATERIALS AND METHODS

Collection and analysis

Palaemon elegans Rathke were collected from littoral rockpools near Millport, Isle of Cumbrae, Firth of Clyde, Scotland, and *Palaemonetes varians* (Leach) from a salt marsh at Tollesbury, Essex, England. The prawns were maintained in the laboratory at 10 °C (12:12 h light:dark regime, continuous aeration). *P. elegans* at 100 % SW (32 ppt salinity) and *P. varians* at 50 % SW in artificial seawater (Tropic Marin New [TMN], Tropicarium Buchschlag Dreieich, F. R. Germany). TMN was chosen as a reproducible medium, especially with respect to trace metals (2.5 μg Zn l^{-1}) and potential chelating agents. Before and during experiments prawns were kept individually in compartmented Perspex boxes and fed every 2 d with lamb's heart (77 μg Zn g^{-1}), a negligible dietary source of Zn, in separate feeding tanks (15 min) with clean TMN of appropriate salinity. Any prawns moulting in the 7 d before experiments were discarded.

For Zn analysis, prawns were individually frozen, then thawed, dried to constant weight at 60 °C and digested in conc. HNO_3 (Aristar grade, BDH Ltd) at 100 °C. Digests were made up to volume with double-distilled water and analysed for total Zn by flame atomic absorption spectrophotometry (Varian AA 375 spectrophotometer with background correction) and/or analysed for Zn-65 activity (as a measure of accumulated labelled Zn concentration) in a Nuclear Chicago gamma scintillation counter against standards of the same sample geometry. When live prawns were monitored for Zn-65 activity, the method of White & Rainbow (1984a) was used, all live counts being corrected against counts of the final acid digest of each prawn. No attempts were made to sex the prawns since White & Rainbow (1984b) showed that the sex of *Palaemon elegans* has no effect on rates of Zn uptake or loss.

Raw data of body Zn concentrations were normalized by log transformation before use of a priori and a posteriori analysis of variance (Sokal & Rohlf 1981). Regression lines were fitted by least squares.

Experimental details

Prawns were subjected to a stepwise decrease or increase in salinity over 1 to 3 d and acclimated for 3 to 5 d at the appropriate salinity (without added Zn) before the start of any experiment.

Experiment 1: Effect of salinity on Zn regulation. Series of 7 tanks with Zn concentrations of 2.5 (Control), 31.6, 56.2, 100, 178, 316 and 562 μg l^{-1} were maintained for 21 d at 10 °C, each tank with 12 prawns individually held in a compartmented Perspex box, in TMN (a) as 100, 75, 50 or 25 % SW for *Palaemon elegans* or (b) as 100, 75, 50, 25 or 5 % SW for *Palaemonetes varians*. Samples of prawns acclimated for 3 d to each salinity (without added Zn) were taken as Initials. Any dead prawns and moults were removed daily. After 21 d, surviving prawns were rinsed briefly in clean TMN of the experimental salinity and frozen for Zn analysis.

Experiment 2: *Palaemon elegans*. Effects of salinity on uptake of labelled Zn. *(a) Over 4 d at 56.2 μg Zn l^{-1}:* Experimental tanks were set up with 10 l of TMN of 100,

75, 50 or 25 % SW, each containing a concentration of 56.2 $\mu g\ l^{-1}$ total Zn labelled with 4 $\mu Ci\ l^{-1}$ Zn-65. In each tank, 12 prawns were held individually for 4 d at 10 °C. The uptake of labelled Zn in individual prawns was monitored by gamma counting of live specimens after 8, 23, 46, 54, 72 and 96 h, before prawns were frozen individually for analysis of total Zn and Zn-65 activity in digests. Prawns were fed the day before the start of the experiment and after 31 and 54 h. At the start of each experiment, 12 prawns acclimated to each salinity for 3 d (Initials) were taken. Control tanks of 100, 75, 50 and 25 % SW with 12 individually held prawns were also maintained for 4 d under the same conditions of salinity and temperature and fed similarly.

(b) Over 21 d at 100 μg Zn l^{-1}: Experimental tanks were set up with 15 l of TMN of 100 or 50 % SW, each with 100 $\mu g\ l^{-1}$ total Zn labelled with 20 $\mu Ci\ l^{-1}$ Zn-65. In each tank, 20 prawns were held individually for 21 d at 10 °C, being fed every other day. Uptake of labelled Zn in individual live prawns was monitored after 6, 12, 24, 31, 48 and 72 h and 4, 5, 8, 11, 14, 16, 18 and 21 d, before prawns were frozen individually for analysis for total Zn and Zn-65 in digests. Control tanks (without added Zn) with 15 individually held prawns fed similarly were also maintained in TMN of 100 and 50 % SW for 21 d under the same conditions. The medium in each tank was replaced on Days 4, 9 and 15 with TMN of the same salinity and labelled Zn concentration. Water samples of 5 ml from each experimental tank were taken periodically and counted to check for Zn loss from solution.

Experiment 3: *Palaemonetes varians*. Uptake of labelled Zn at 10 °C in 50 % and 25 % SW. ***(a) After 15 d acclimation to 50 % SW:*** Two experimental tanks of 50 % and 25 % SW were prepared each with 100 $\mu g\ l^{-1}$ total Zn and 4 $\mu Ci\ l^{-1}$ Zn-65. Fifteen individually held *P. varians* acclimated for 15 d in 50 % SW were placed in each tank and held for 71 h at 10 °C, before being frozen for individual Zn analysis. Accumulation of labelled Zn in individual prawns was monitored by counting live specimens after 8, 23, 31, 46, 53 and 71 h. Prawns were fed before the start of the experiment and after 21 h. The concentrations of dissolved labelled Zn in the media were periodically monitored.

(b) After 15 d acclimation to appropriate salinity (50 % or 25 % SW): Expt 3a was repeated with *Palaemonetes varians* acclimated for 15 d to the same salinity (25 % or 50 % SW) to which the prawns would be exposed to 100 μg Zn l^{-1} for 71 h.

Experiment 4: *Palaemonetes varians*. Effect of acclimation to low salinities on total body Zn concentration. *P. varians* collected from Tollesbury were transported back to the laboratory in water of ca 16 ppt salinity, taken from the field site, and held overnight at 10 °C. Sixty prawns were placed in each of 3 tanks of 50, 25 and 10 % SW respectively. Prawns were fed every other day in feeding tanks of the appropriate salinity for 15 min. Six prawns from each salinity regime were frozen after 1, 2, 3, 4, 7, 10, 15 and 21 d for analysis of zinc.

Experiment 5: Regulation of Zn by *Palaemonetes varians* in 50 % SW at 15 °C. Seven tanks were prepared with Zn concentrations of 2.5 (Control), 31.6, 56.2, 100, 178, 316 and 562 μg Zn l^{-1} in 50 % SW. Twelve individually held *P. varians* were placed in each tank and maintained for 21 d at 15 °C ± 0.5 C°, being fed every other day in feeding tanks with clean 50 % SW at 15 °C for 15 min. Dead prawns and moults were removed daily. After 21 d, surviving prawns were individually frozen for Zn analysis.

RESULTS

Experiment 1: Effect of salinity on Zn regulation

Mean zinc concentrations initially in prawns acclimated to the appropriate salinity for 3 d (Initials), and mean Zn concentrations in control prawns held at the experimental salinity for 21 d (Controls) are shown in Table 1. In the case of *Palaemon elegans*, analysis of

Table 1. *Palaemon elegans* and *Palaemonetes varians*. Mean Zn concentrations ($\mu g\ g^{-1}$ ± 1 SD) of initial and control prawns from each experimental salinity at 10 °C, and in a sample (field) of *P. varians* immediately after field collection. (*P. elegans* were not exposed to 5 % SW). *n*: number of samples

Salinity (% SW)	Mean Zn concentration Initials (*n*)	Controls (*n*)
Palaemon elegans		
100	77.5 ± 9.3 (12)	81.6 ± 3.3 (11)
75	79.2 ± 10.6 (12)	80.2 ± 7.9 (11)
50	75.7 ± 7.4 (12)	76.1 ± 4.8 (12)
25	77.8 ± 5.9 (12)	76.9 ± 8.1 (7)
Palaemonetes varians		
100	93.8 ± 7.9 (12)	96.6 ± 7.4 (5)
75	94.8 ± 6.1 (12)	90.2 ± 9.6 (12)
50	89.6 ± 8.7 (12)	91.9 ± 7.6 (8)
25	104.9 ± 4.7 (12)	94.3 ± 10.2 (12)
5	134.0 ± 8.5 (12)	98.8 ± 4.3 (6)
Field (ca 50 % SW)	98.8 ± 5.8 (12)	

variance showed that in no case was there a significant difference ($p > 0.05$) between mean Zn concentrations of Initial and Control prawns at any salinity, and mean Zn concentrations did not differ significantly within the 4 sets of Initial prawns or within the 4 sets of Control prawns. Therefore exposure for 21 d to decreased salinities had no effect on the Zn concentration of *Palaemon elegans*.

For *Palaemonetes varians* (Table 1) in 100, 75 and 50 % SW mean Zn concentrations of Initials were not significantly different from those of Controls. However in 25 % and 5 % SW, mean Zn concentrations in Initials were significantly higher than in Controls. Initials in 25 % and 5 % SW also had significantly higher mean body Zn concentrations than all other Initials. This indicates an increase in body concentration within 3 d of transfer from 50 % SW as a response to low salinity in this species. After a further 21 d exposure to TMN at the 5 different salinities however, analysis of variance showed that the mean Zn concentrations of Control prawns in all salinities were not significantly different from each other. Therefore the elevation in body concentrations in the reduced salinities was no longer evident after 24 d, and is a short-term phenomenon.

Body Zn concentrations of *Palaemon elegans* and *Palaemonetes varians* exposed to the range of dissolved Zn concentrations in 100, 75, 50, 25 and 5 % SW (*P. varians* only) at 10 °C for 21 d are shown in Figs. 1 and 2. Prawns moulting before Day 19 of the experiments had Zn concentrations not significantly different from those of non-moulters and have been included in all analyses. Prawns moulting on Days 19 to 21 inclusive had raised body Zn concentrations in comparison with non-moulters and have been excluded.

The results are interpreted as described by Nugegoda & Rainbow (1987). Prawns are able to regulate total body Zn concentrations until a threshold exposure concentration is reached, when regulation breaks down and net accumulation of body Zn begins. After Nugegoda & Rainbow (1987), a regulated range for body Zn concentrations of the prawns at each experimental salinity is defined as the mean Zn concentration ± 1.96 standard deviations of Control prawns (log-transformed data) held for 21 d at the respective salinity (see Figs. 1 and 2). Prawns with total body concentrations greater than the defined upper regulation limit for each experiment were identified as non-regulators, showing further net accumulation of Zn. Table 2 shows the number of surviving non-regulators (excluding moulters on Days 19 to 21) as a fraction of total survivors at the end of 21 d in each exposure concentration at each experimental salinity.

For *Palaemon elegans*, an a posteriori comparison showed that in 100 % SW (Fig. 1a), there was no significant difference in mean prawn Zn concentrations up to the 178 μg Zn l^{-1} exposure, and these were significantly lower than the mean Zn concentrations in prawns exposed to higher concentrations. In 75 % SW (Fig. 1b), there was no significant difference in mean prawn concentrations up to the 100 μg Zn l^{-1} exposure. In 50 % SW (Fig. 1c) there was no significant difference between the mean Zn concentration of Control prawns and that of prawns exposed to 31.6 μg Zn l^{-1}, while mean concentrations of prawns in all higher exposures were significantly raised. However in 25 % SW (Fig. 1d), although the mean Zn concentration of prawns in the 178 μg Zn l^{-1} exposure was significantly higher than in other exposures, there was little identifiable breakdown in Zn regulation at higher external concentrations.

Thus breakdown in Zn regulation in *Palaemon elegans* occurs at lower external concentrations with decrease in salinity down to 50 % SW, and a corresponding increase in the percentage of non-regulators was identified at higher exposure concentrations (Table 2). Significant regressions could be plotted for total body Zn concentrations versus Zn exposure concentrations after regulation breakdown at salinities of 100, 75 and 50 % SW (Figs. 1a to c). Estimates of the exposure concentration corresponding to the point of regulation breakdown under the specific experimental conditions used are provided by the intersections of these regression lines with regulated prawn Zn concentrations, the starting concentrations for further net Zn accumulation. These regulated prawns concentrations (indicated on the figures) have been obtained by back transformation of log-transformed data and therefore differ in detail from the arithmetic means of control prawns as quoted in Table 1 which do not differ significantly with salinity regime (Table 1). For *P. elegans* the points of regulation breakdown are ca 92, 55 and 27 μg Zn l^{-1} in 100, 75 and 50 % SW respectively. Zn accumulation patterns are summarised comparatively in Fig. 3. The pattern of Zn accumulation by *P. elegans* at 25 % SW is clearly different from those at higher salinities with no clear pattern of Zn regulation breakdown in surviving prawns (Figs. 1d and 3).

In the case of *Palaemonetes varians* (Figs. 2 and 4), similar treatment of the data showed that there was little difference between the thresholds of Zn regulation breakdown in 100, 75 and 50 % SW at 10 °C, viz. 191, 183 and 146 μg Zn l^{-1} respectively. In these 3 highest salinities, only prawns exposed to 562 μg Zn l^{-1} for 21 d had mean Zn concentrations significantly higher than that of the Controls. However, in 25 % SW, prawns exposed to 178 μg Zn l^{-1} had a higher mean Zn concentration ($p < 0.05$) than that of Controls and 7/12 prawns were identified as non-regulators of total body Zn (Table 2). In 5 % SW, 86 % of prawns surviving 21 d exposure to 56.2 μg Zn l^{-1} were non-regulators. Few

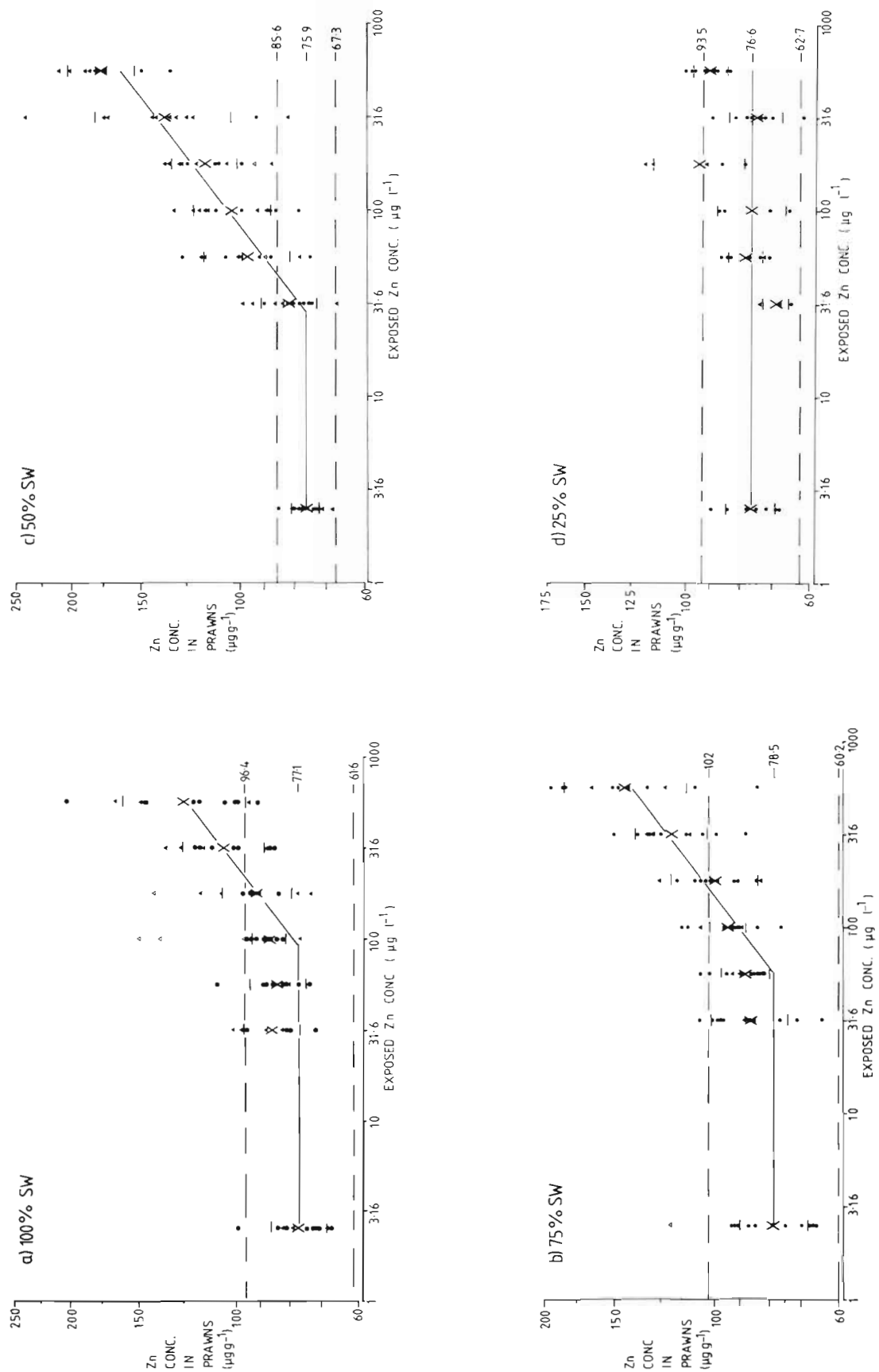

Fig. 1. *Palaemon elegans*. Body Zn concentrations (µg g^{-1}) of individual prawns (excluding prawns moulting on Days 19 to 21) surviving 21 d exposure to a range of dissolved Zn concentrations (µg l^{-1}) at 10 °C in (a) 100, (b) 75, (c) 50 and (d) 25 % SW. In each case, solid horizontal line is the mean Zn concentration of Control prawns (log-transformed data), dashed lines limit the regulated range (mean Zn concentration of Control prawns ± 1.96 SD using transformed data). Regression lines describing Zn accumulation after Zn regulation breakdown are (a) $\log Y = (0.257)\log X + 1.382$, $p = 0.02$, exposures ≥ 178 µg Zn l^{-1}; (b) $\log Y = (0.247)\log X + 1.465$, $p = 0.02$, exposures ≥ 100 µg Zn l^{-1}; (c) $\log Y = (0.255)\log X + 1.514$, $p = 0.008$, exposures ≥ 56.2 µg Zn l^{-1}. Symbols: (△) prawns that moulted between Days 19 and 21 inclusive; (▲) prawns that moulted before Day 19, (●) non-moulters. Short horizontal lines: sample means ($\overline{X}$) ± 1 SD. (Fig. 1a from Nugegoda & Rainbow [1987] with permission of Ophelia)

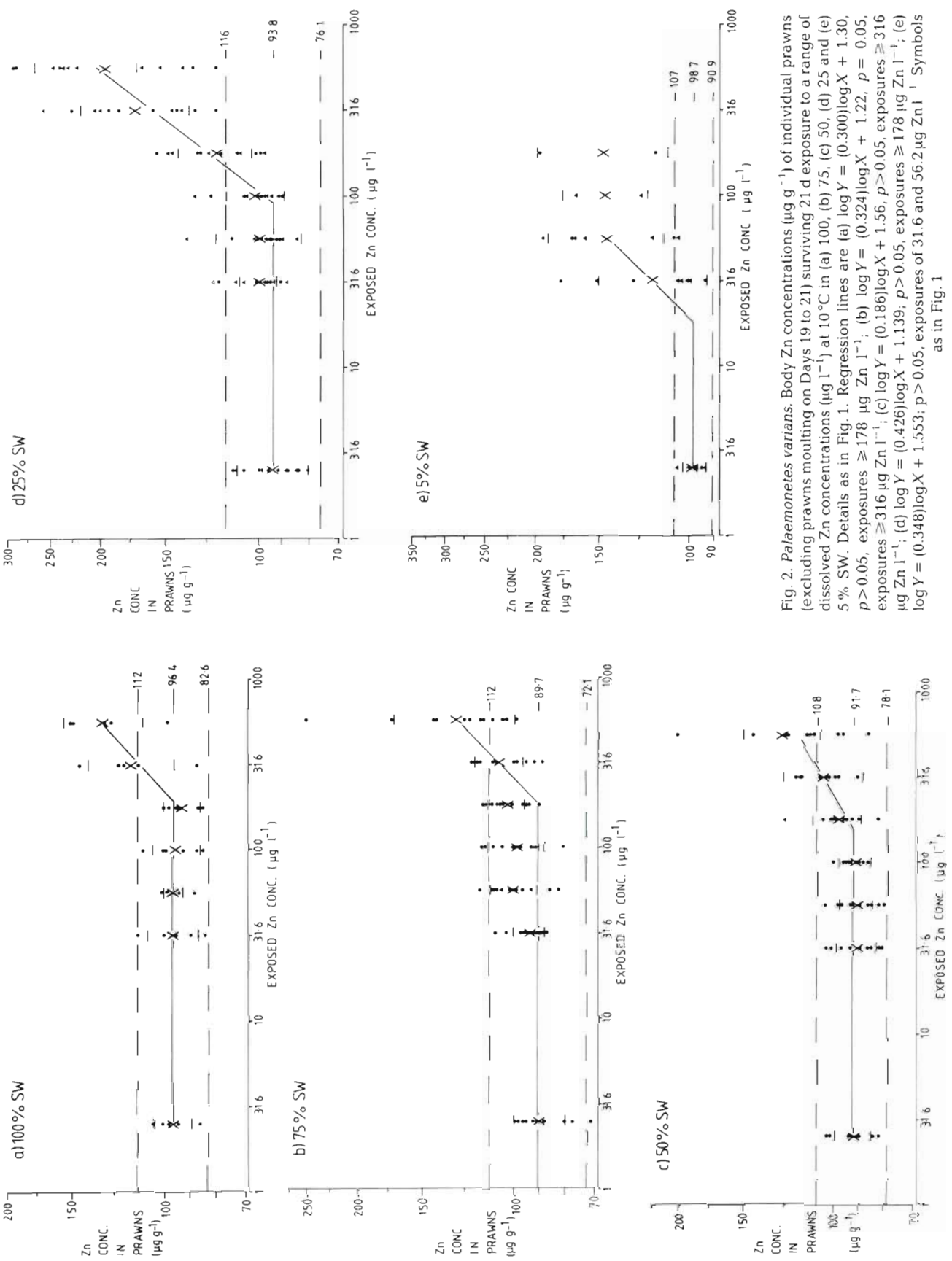

Fig. 2. *Palaemonetes varians*. Body Zn concentrations (µg g^{-1}) of individual prawns (excluding prawns moulting on Days 19 to 21) surviving 21 d exposure to a range of dissolved Zn concentrations (µg l^{-1}) at 10°C in (a) 100, (b) 75, (c) 50, (d) 25 and (e) 5 ‰ SW. Details as in Fig. 1. Regression lines are (a) $\log Y = (0.300)\log X + 1.30$, $p > 0.05$, exposures ≥ 178 µg Zn l^{-1}; (b) $\log Y = (0.324)\log X + 1.22$, $p = 0.05$, exposures ≥ 316 µg Zn l^{-1}; (c) $\log Y = (0.186)\log X + 1.56$, $p > 0.05$, exposures ≥ 316 µg Zn l^{-1}; (d) $\log Y = (0.426)\log X + 1.139$; $p > 0.05$, exposures ≥ 178 µg Zn l^{-1}; (e) $\log Y = (0.348)\log X + 1.553$; $p > 0.05$, exposures of 31.6 and 56.2 µg Zn l^{-1}. Symbols as in Fig. 1

Table 2. *Palaemon elegans* and *Palaemonetes varians*. Number of surviving prawns with total body Zn concentrations above the upper regulation limit as a fraction (%) of the total number of survivors (excluding those that moulted between Day 19 and 21) after 21 d in each Zn exposure in the experimental salinities at 10 °C

Zn exposure conc. ($\mu g\ l^{-1}$)	Number of non-regulators as a fraction (%) of total survivors				
	5 % SW	25 % SW	50 % SW	75 % SW	100 % SW
Palaemon elegans					
2.5 (control)		0/ 7 (0)	0/12 (0)	0/12 (0)	1/11 (9)
31.6		0/ 3 (0)	4/12 (33)	1/12 (8)	2/ 9 (22)
56.2		0/ 7 (0)	8/11 (73)	1/12 (8)	1/11 (9)
100		0/ 4 (0)	11/12 (92)	3/12 (25)	0/10 (0)
178		2/ 5 (40)	11/12 (92)	5/11 (42)	3/10 (30)
316		0/ 8 (0)	11/12 (92)	10/12 (83)	8/11 (82)
562		2/10 (20)	9/ 9 (100)	10/11 (91)	10/12 (83)
Palaemonetes varians					
2.5 (control)	0/6 (0)	0/12 (0)	0/ 8 (0)	0/12 (0)	0/ 5 (0)
31.6	2/8 (25)	1/11 (9)	0/ 9 (0)	0/12 (0)	1/ 5 (20)
56.2	6/7 (86)	1/12 (8)	0/ 9 (0)	0/12 (8)	0/ 5 (0)
100	2/2 (100)	2/12 (17)	0/ 9 (0)	2/12 (17)	0/ 6 (0)
178	2/2 (100)	7/12 (58)	1/10 (10)	2/12 (17)	0/ 6 (0)
316	1/1 (100)	12/12 (100)	3/ 9 (33)	4/12 (33)	3/ 5 (60)
562	N	12/12 (100)	6/10 (60)	8/11 (73)	5/ 5 (80)

N: no survivors

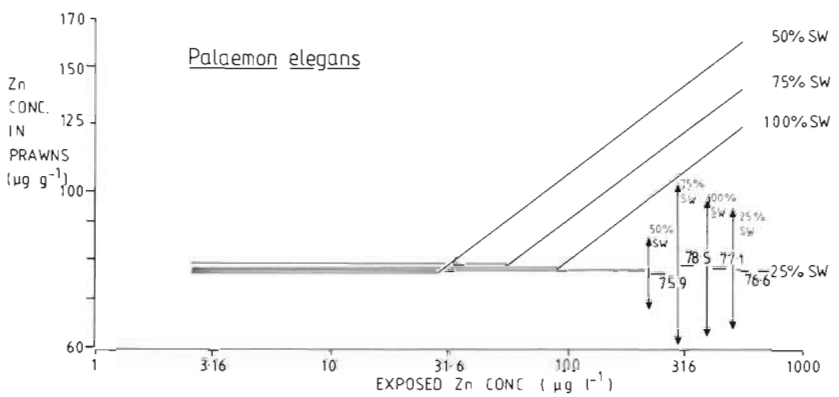

Fig. 3. *Palaemon elegans*. Summary of the effect of a range of dissolved Zn concentrations ($\mu g\ l^{-1}$) on body Zn concentrations ($\mu g\ g^{-1}$) of prawns surviving 21 d exposure in one of 4 salinities (100, 75, 50 or 25 % SW) at 10 °C (excluding prawns moulting on Days 19 to 21). Regression lines are from Fig. 1. Horizontal lines are mean Zn concentrations of Control prawns at each salinity drawn to the point of intersection with the regression line in the 100, 75 and 50 % SW experiments. Arrows indicate the regulated range (see text) at each salinity

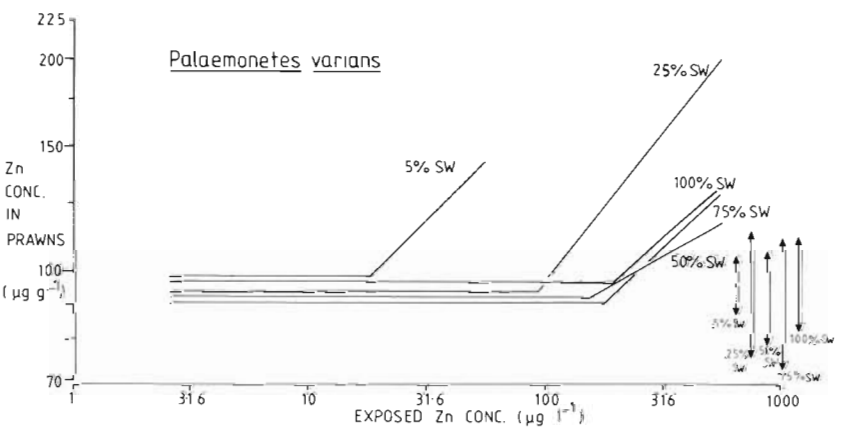

Fig. 4. *Palaemonetes varians*. Summary of the effect of a range of dissolved Zn concentrations ($\mu g\ l^{-1}$) on body Zn concentrations ($\mu g\ g^{-1}$) of prawns surviving 21 d exposure in salinities 100, 75, 50, 25 or 5 % SW at 10 °C (excluding prawns moulting on Days 19 to 21). Regression lines are from Fig. 2. Details as for Fig. 3

prawns survived exposure to higher concentrations at 5% SW and all were non-regulators. Thresholds of regulation breakdown in 25 and 5% SW were 90 and 19 μg Zn l^{-1} respectively. Therefore Zn regulation breakdown in *P. varians* clearly occurs at lower dissolved Zn concentrations with a decrease in salinity from 50% to 5% SW. There was less difference between dissolved Zn concentrations corresponding to the threshold of regulation breakdown over the salinity range 100 to 50% SW although the same trend with salinity was apparent.

Mortality and moulting

Mortalities of the 2 prawn species in the 5 experimental salinities are shown in Table 3.

In *Palaemon elegans*, mortality was low at salinities down to 50% SW At the highest exposure concentration of 562 μg Zn l^{-1}, 3 prawns died in 50% SW, 1 in 75% SW and 0 in 100% SW, indicating increased toxicity of Zn to *P. elegans* at decreased salinities. In 25% SW, 48% of the total number of experimental prawns died, including 5 of the 12 control prawns, a higher proportion than in the 2 highest Zn exposures. Therefore it appears that mortality of *P. elegans* increased in 25% SW even in the absence of high Zn concentrations. A large percentage of the mortalities occurred at or immediately after moulting (Table 3).

In *Palaemonetes varians*, there was high mortality in all Zn exposure concentrations in the 2 salinity extremes (i.e. 5 and 100% SW) and no or very low mortality in 25 and 75% SW Again many of the mortalities occurred during or immediately after moulting (Table 3).

In *Palaemon elegans* the proportion of moulters (Table 4) was high in 50 and 25% SW, suggesting that there is an increased frequency of moulting at lower salinities. Similarly in *Palaemonetes varians* a large proportion of the experimental prawns moulted in 25 and 5% SW (Table 4), while moulting was very infrequent in the higher salinities.

Experiment 2: *Palaemon elegans*. Effect of salinity on Zn uptake

The effects of different salinities on the rate of Zn uptake by *Palaemon elegans* were investigated at 2 exposure concentrations, (a) 56.2 μg Zn l^{-1} and (b) 100 μg Zn l^{-1}. Expt 2 (b) was extended to match the 21 d period used in the first experiment in an attempt to correlate rates of uptake in individual prawns with the presence/absence of regulation breakdown.

Table 3. *Palaemon elegans* and *Palaemonetes varians*. Mortality: no. of prawns that died as a fraction of the total exposed for up to 21 d in each Zn exposure concentration in each salinity regime, at 10°C. No. of prawns that died at, or immediately after, moulting is shown in brackets. (*P. elegans* were not exposed to 5% SW)

Zn exposure conc. (μg l^{-1})	Salinity (% SW) 5	25	50	75	100
Palaemon elegans					
2.5 (control)		5/12 (4)	0/12	0/12	0/12
31.6		9/12 (8)	0/12	0/12	0/12
56.2		5/12 (4)	1/12 (1)	0/12	0/12
100		8/12 (7)	0/12	0/12	0/12
178		7/12 (7)	0/12	1/12 (0)	0/12
316		4/12 (3)	0/12	0/12	0/12
562		2/12 (2)	3/12 (3)	1/12 (1)	0/12
Mortality (%)		40/84 (48%)	4/84 (5%)	2/84 (2%)	0
No. dying at moult (%)		36/40 (90%)	4/ 4 (100%)	1/ 2 (50%)	0
Palaemonetes varians					
2.5 (control)	6/12 (2)	0/12	4/12 (0)	0/12	7/12 (0)
31.6	4/12 (4)	0/12	3/12 (0)	0/12	7/12 (0)
56.2	5/12 (3)	0/12	3/12 (0)	0/12	7/12 (0)
100	10/12 (10)	0/12	3/12 (1)	0/12	6/12 (0)
178	10/12 (8)	0/12	2/12 (0)	0/12	6/12 (0)
316	11/12 (8)	0/12	3/12 (1)	0/12	7/12 (0)
562	12/12 (9)	0/12	2/12 (0)	1/12 (0)	7/12 (0)
Mortality %	58/84 (69%)	0	20/84 (24%)	1/84 (1%)	47/84 (56%)
No. dying at moult (%)	44/58 (76%)	0	2/20 (10%)	0	0

Table 4. *Palaemon elegans* and *Palaemonetes varians*. Moulting: no. of prawns (out of 12 in every case) that moulted during the 21 d in each Zn exposure concentration in each salinity regime, at 10 °C. (*P. elegans* were not exposed to 5 % SW)

Zn exposure conc. ($\mu g\ l^{-1}$)	Salinity (% SW) 5	25	50	75	100
Palaemon elegans					
2.5 (control)		4	7	1	3
31.6		10	5	3	6
56.2		5	8	5	1
100		7	7	4	4
178		10	8	2	7
316		3	11	4	5
562		2	9	3	4
Total		41 (49 %)	55 (65 %)	22 (26 %)	30 (36 %)
No. of moulters dying at moult (%)		36 (88 %)	4 (7 %)	1 (5 %)	0
Palaemonetes varians					
2.5 (control)	7	5	0	0	0
31.6	9	6	0	1	0
56.2	6	9	0	2	0
100	12	8	1	1	0
178	8	7	1	0	0
316	9	5	2	0	0
562	9	9	0	0	0
Total	60 (71 %)	49 (58 %)	4 (5 %)	4 (5 %)	0
No. of moulters dying at moult (%)	44 (73 %)	0	2 (50 %)	0	0

Expt 2a

Counting of water samples for Zn-65 confirmed that the dissolved Zn concentrations remained within 5 % of the declared value of 56.2 μg Zn l^{-1} in all tanks (25, 50, 75, 100 % SW) throughout the 4 d experiment.

Prawns were exposed to 56.2 $\mu g\ l^{-1}$ labelled Zn at each of the 4 salinities for 4 d at 10 °C. The accumulation of labelled Zn by individual prawns was followed over the 4 d period before prawns were analysed for total and labelled Zn.

Analysis of variance confirmed that there was no significant difference ($p > 0.05$) between any mean Zn body concentration of Initial prawns acclimated to each of the 4 salinities for 3 d, of Control prawns held at each of the 4 salinities for a further 4 d and of experimental

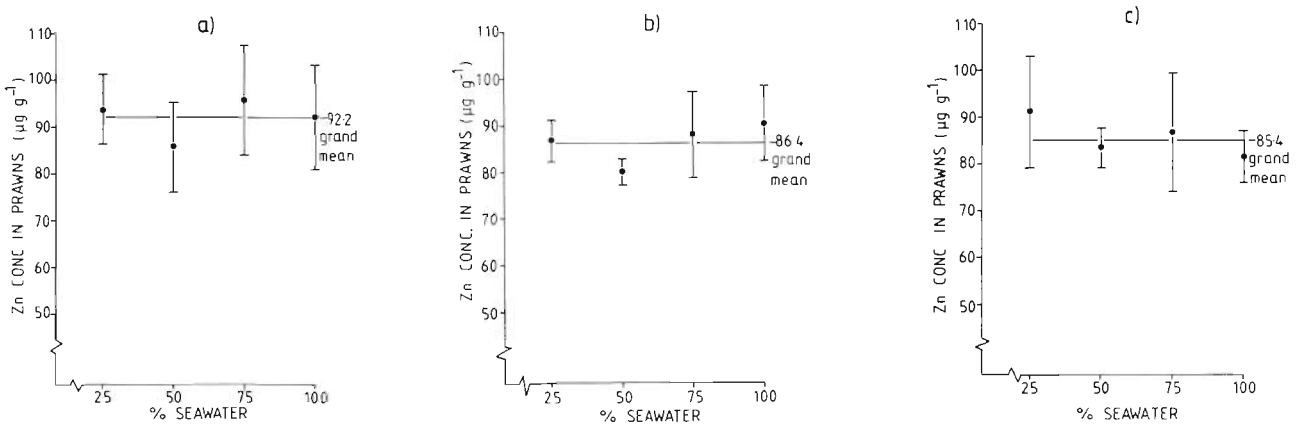

Fig. 5. *Palaemon elegans*. Mean total Zn concentrations ($\mu g\ g^{-1}$) of (a) initial prawns after 3 d acclimation to one of 4 salinities (% SW) at 10 °C, (b) Control prawns held for a further 4 d at one of 4 experimental salinities (% SW) at 10 °C, and (c) experimental prawns exposed to 56.2 μg labelled Zn l^{-1} for a further 4 d at one of 4 experimental salinities (% SW) at 10 °C. Horizontal lines denote grand means calculated for all 4 groups

prawns exposed to 56.2 µg Zn l^{-1} at each of the 4 salinities for the 4 d period (Fig. 5). Therefore it can be concluded that total body Zn concentrations of *Palaemon elegans* were regulated over the 4 d experimental period at all experimental salinities. Any nonregulator in the 50 % salinity (cf. Fig. 1c) would probably be undetectable, given the short exposure period, in which little if any net Zn accumulation would occur.

Rates of uptake of labelled Zn by individual prawns and the concentration of labelled Zn passively adsorbed onto the surface of each prawn were calculated using the model curve described by Nugegoda & Rainbow (1988).

Results are presented in Tables 5 and 6. The rates of Zn uptake showed great individual variability even within the same experimental salinity (Table 5). A log transformation was used to normalize the data and the mean rates of Zn uptake by *Palaemon elegans* in different salinities were compared by a posteriori analysis of variance. There was no significant difference between the mean rate of uptake ($p > 0.05$) in 100 % SW and 75 % SW but these rates were significantly lower ($p = 0.01$) than the rates of uptake in 50 and 25 % SW The rate of Zn uptake decreased with salinity and a significant ($p = 0.04$) linear regression could be plotted for the rate of Zn uptake versus salinity (as % SW) of the medium (Fig. 6a).

Table 5. *Palaemon elegans*. Mean rates of Zn uptake by prawns exposed to 56.2 µg labelled Zn l^{-1} in each experimental salinity for 4d at 10 °C

	25 % SW	50 % SW	75 % SW	100 % SW
Mean rate Zn uptake (µg g^{-1} d^{-1})	1.054	0.991	0.780	0.735
Standard deviation	0.385	0.156	0.185	0.077
n	9	12	11	9

The uptake of Zn at 56.2 µg Zn l^{-1} therefore is increased in 25 % SW in comparison with uptake rates at higher salinities, and an even greater increase in the rate of uptake could be expected at higher exposed Zn concentrations. Therefore in Expt 1, when *Palaemon elegans* was exposed to 178, 316 and 562 µg Zn l^{-1} in 25 % SW, the excess Zn expected to have been accumulated must necessarily have been lost within the 21 d period. Thus the absence of net Zn accumulation in prawns in the high exposure at 25 % SW was evidently not a result of prevention of entry of Zn into

Table 6. *Palaemon elegans*. Mean concentrations of labelled Zn estimated to be initially adsorbed onto the surface of prawns exposed to 56.2 µg labelled Zn l^{-1} in each experimental salinity for 4d at 10 °C

	25 % SW	50 % SW	75 % SW	100 % SW
Mean conc. adsorbed labelled Zn (µg g^{-1})	0.375	0.274	0.239	0.231
Standard deviation	0.093	0.052	0.054	0.098
n	9	12	11	9
Mean total Zn conc. (µg g^{-1})	91.0	83.5	86.7	81.4
Adsorbed conc. of labelled Zn as % total Zn conc.	0.41	0.33	0.28	0.28

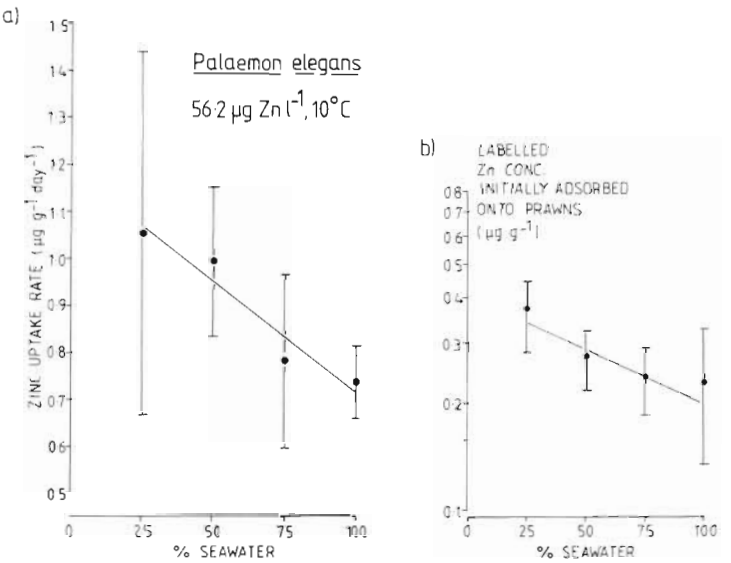

Fig. 6. *Palaemon elegans*. (a) Mean rates of Zn uptake (µg g^{-1} d^{-1}) and (b) mean labelled Zn concentrations (µg g^{-1}) initially adsorbed onto the body surface of prawns exposed to 56.2 µg labelled Zn l^{-1} for 4 d in one of 4 salinities at 10 °C. Vertical lines indicate ± 1 SD on either side of the means. (a) Regression line ($Y = -0.0048X + 1.881$, $p = 0.04$) describes the change of Zn uptake rate with change in salinity (as % SW); (b) regression line ($\log Y = -0.0031X + 0.390$, $p = 0.04$) describes the log-linear relation of the change in initially adsorbed labelled Zn concentration with change in salinity (as % SW)

the body but the result of an increased efflux of Zn in 25 % SW

The mean initial adsorption of labelled Zn also increased with decrease in salinity although there was great variability between individual prawns even within the same experimental salinity (Table 6). A significant inverse log-linear regression could be plotted of the concentration of Zn initially adsorbed versus the salinity of the medium (Fig. 6b).

Expt 2b

Counting of water samples for Zn-65 confirmed that dissolved Zn concentrations were maintained within 5 % of the declared concentration of 100 µg Zn l^{-1}.

The total Zn concentrations of individual prawns surviving exposure to 100 µg l^{-1} labelled Zn for 21 d in 100 and 50 % SW and in Control prawns held for 21 d in 100 and 50 % SW with no added Zn are shown in Fig. 7. The mean Zn concentrations of Initial prawns acclimated to either salinity for 3 d (also shown in Fig. 7) did not differ significantly from each other nor from mean Zn concentrations of Controls at the 2 salinities.

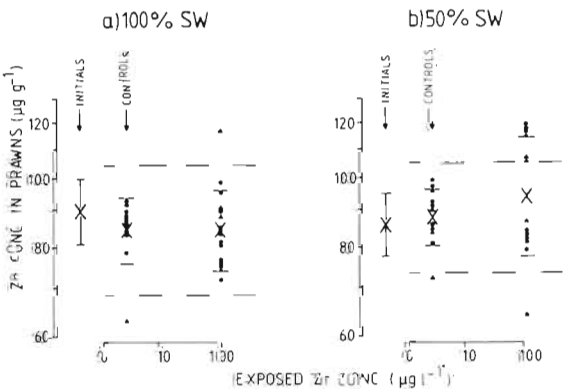

Fig. 7. *Palaemon elegans*. Total body Zn concentrations (µg g^{-1}) of Initial prawns (mean ± 1 SD), Control prawns, and prawns surviving exposure to 100 µg labelled Zn l^{-1} for 21 d in (a) 100 % SW, (b) 50 % SW at 10 °S. Symbols: (▲) prawns that moulted during the experiment; (●) non-moulters. Short horizontal lines: sample means ± 1 SD. The regulated range (mean zinc concentration ± 1.96 SD of Controls, transformed data) is limited by dashed lines

Limits of prawn Zn regulation were again defined as the Control mean Zn concentration ± 1.96 SD (log-transformed data) for each salinity, and all prawns with body concentrations greater than the upper regulation limit were identified as having shown further net accumulation (non-regulators). Five of 13 survivors showed further net accumulation of Zn when exposed to 100 µg Zn l^{-1} in 50 % SW while only 1/16 survivors did not regulate total body Zn concentrations at the same exposed concentration in 100 % SW (Fig. 7). This is supportive evidence for the conclusion reached earlier that *Palaemon elegans* shows increasing breakdown in Zn regulation at a defined exposure concentration at decreasing salinities, down to 50 % SW. Nevertheless the mean total Zn concentration of prawns exposed to elevated dissolved Zn in 100 and 50 % SW were not significantly different to that of the Controls in each salinity.

The concentration of labelled Zn in each prawn was plotted for all individuals as shown in the examples in Fig. 8. The data show a high accumulation of labelled

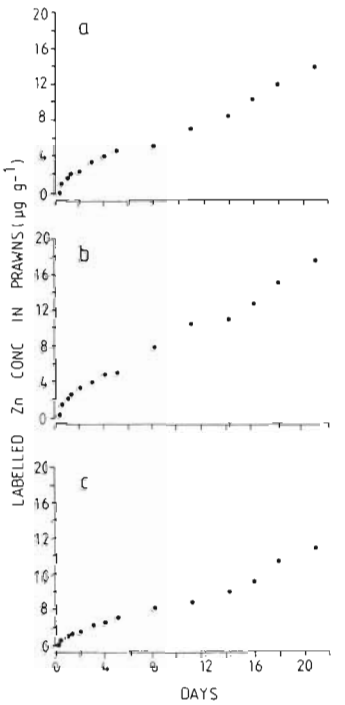

Fig. 8. *Palaemon elegans*. The accumulation of labelled Zn by 3 individual prawns (a, b, c) exposed to 100 µg labelled Zn l^{-1} in 100 % SW for 21 d at 10 °C

Zn with a tendency toward asymptotic values at ca 3 to 4 d. After about 4 d in all cases the accumulation of labelled Zn further increased above the apparent asymptote until the last sampling point (Day 21). In many cases the 6 h data point was low, probably indicative of as yet incomplete initial adsorption; 6 h data points were therefore excluded from all further analyses.

Curves were fitted to the data up to the 3 d data point (excluding the low 6 h point), using the model applied earlier (Nugegoda & Rainbow 1988), and individual rates of Zn uptake calculated. Mean uptake rates (± 1 SD) in *Palaemon elegans* exposed to 100 µg Zn l^{-1} at 10 °C were 5.27 ± 3.67 (n = 19) and 2.39 ± 0.84 (n = 14) µg Zn g^{-1} d^{-1} in 50 and 100 % SW respectively. The mean uptake rate by prawns in 50 % SW was signifi-

cantly greater than that of prawns in 100 % SW ($p = 0.006$) in spite of the great individual variability at each salinity.

As would be expected from the data shown in Fig. 1c, a proportion of the prawns exposed to 100 µg Zn l^{-1} in 50 % SW for 21 d at 10 °C had suffered breakdown of regulation of body Zn concentration. [The accumulation of labelled Zn after the apparent asymptote at ca 3 d, as exemplified in Fig. 8, is further complicated in such prawns suffering Zn regulation breakdown (only in the 50 % SW experiment) because total body concentrations would have continuously increased after regulation breakdown. In regulators – all prawns in 100 % SW in Expt 2b (except 1 moulter) including those depicted in Fig. 8 – the increasing concentration of labelled Zn replaced original unlabelled Zn already present with no change in total Zn concentration.]

There was a significant difference ($p = 0.01$) between mean Zn uptake rates of regulators and non-regulators of the prawns exposed to 50 % SW in Expt 2b (Fig. 9). Again therefore there is a correlation between high Zn uptake rate and regulation breakdown.

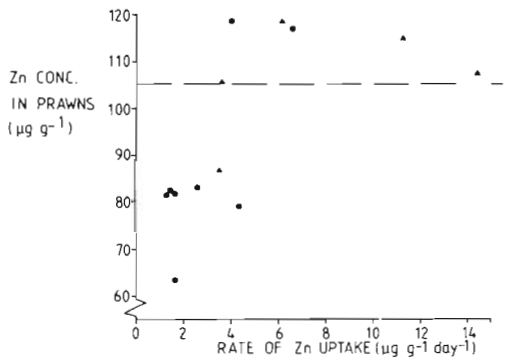

Fig. 9. *Palaemon elegans*. Total body Zn concentrations (µg g^{-1}) plotted against Zn uptake rates (µg g^{-1} d^{-1}) of individual prawns exposed to 100 µg Zn l^{-1} for 21 d in 50 % SW at 10 °C. Dashed line denotes the defined upper regulation limit and thereby separates regulators and non-regulators. Symbols: (▲) prawns that moulted between 3 and 19 d; (●) non-moulters

The mean labelled Zn concentration initially adsorbed onto the bodies of prawns exposed to 100 µg Zn l^{-1} 50 % SW was higher than that of prawns exposed to 100 µg Zn l^{-1} in 100 % SW (Table 7).

Table 8 provides data on mortality and moulting in Expt 2b. An increase in Zn uptake rate soon after moulting was clearly observed in all individuals that moulted in Expt 2b. Four of 6 prawns that showed breakdown in Zn regulation in 50 % SW were moulters, and the only non-regulator in 100 % SW similarly had moulted. Therefore at low salinities the added increase in the uptake rate after moulting resulted in prawns being unable to match excretion to uptake.

Table 7 *Palaemon elegans*. Concentrations of labelled Zn estimated as initially adsorbed onto the surface of prawns exposed to 100 µg labelled Zn l^{-1} for 21 d in each salinity regime at 10 °C

	Salinity	
	50 % SW	100 % SW
Mean conc. adsorbed labelled Zn (µg g^{-1})	0.457	0.210
Standard deviation	0.709	0.213
n	19	15

Experiment 3: *Palaemonetes varians*. Effect of salinity on Zn uptake

Palaemonetes varians were exposed to 100 µg l^{-1} labelled Zn at either 50 % or 25 % SW either after acclimation for 15 d to 50 % SW (Expt 3a) or after acclimation for 15 d to the appropriate salinity (50 % or 25 % SW) (Expt 3b).

Counting of labelled Zn in water samples showed that Zn concentrations remained within 7 % of the declared concentration of 100 µg Zn l^{-1} throughout the experiments.

Total Zn concentrations in prawns

Table 9 shows the mean total Zn concentrations of Initial prawns acclimated to 50 % SW for 15 d in Expt 3a, of Initial prawns acclimated to 50 % or 25 % SW in Expt 3b, and of Control prawns in both experiments held for 71 h at 50 % SW or 25 % SW. Mean total Zn concentrations in experimental prawns exposed to 100 µg labelled Zn l^{-1} for 71 h are also shown. Analysis of variance revealed no significant differences in the mean Zn concentrations of prawns in all tabulated groups.

Thus there was no change in regulated (Control) Zn concentration between prawns held at either 25 % or 50 % for 71 h after 15 d acclimation to 50 % SW (Table 9b). Similarly there was no change in regulated (Control) Zn concentration between prawns held at either 25 % or 50 % SW for up to 24 d (Table 9a). Similarly 71 h exposure to 100 µg Zn l^{-1} did not cause any change in prawn zinc concentration at 25 % SW or 50 % SW, any net accumulation of body Zn at 25 % SW (possible in a proportion of exposed *Palaemonetes varians* – see Fig. 2d) being insignificant in the time period. Pre-exposure of prawns to 15 d at 25 % or 50 % SW did not affect subsequent accumulation in 25 % SW i.e. accumulation history had no effect on Zn accumulation in 25 % SW (Table 9b).

Table 8. *Palaemon elegans*. Mortality and moulting. No. of Control and experimental prawns that died (%) and moulted (%) during exposure to 100 μg Zn l^{-1} in 100% SW or 50% SW for 21 d at 10°C. Last column shows no. of prawns that died at or immediately after moulting

Exposure		Mortality (%)	No. of moulters (%)	Deaths associated with moulting
100% SW	Control (21 d)	1/12 (8)	3/12 (25)	1
	Experimental (11 μg Zn l^{-1}, 21 d)	4/20 (20)	4/20 (20)	0
50% SW	Control (21 d)	1/12 (8)	5/12 (42)	1
	Experimental (100 μg Zn l^{-1}, 21 d)	7/20 (35)	5/20 (25)	0

Table 9. *Palaemonetes varians*. Expt 3: mean total body Zn concentrations (μg g^{-1} ± 1 SD) of Initial and Control prawns and experimental prawns exposed to 100 μg l^{-1} labelled Zn for 71 h after (a) acclimation to 50% SW only and (b) acclimation to the appropriate salinity, for 15 d. *n*: no. of samples

Acclimation medium (15 d)	Exposure medium (71 h)	Initials (*n*)	Controls (*n*)	Experimental (100 μg Zn l^{-1}) (*n*)
(a)				
50% SW	25% SW		100.3 ± 11.4 (15)	103.4 ± 10.4 (15)
		94.1 ± 1.07 (15)		
50% SW	50% SW		97.3 ± 10.8 (15)	103.1 ± 11.4 (15)
(b)				
25% SW	25% SW	96.1 ± 9.5 (12)	89.2 ± 10.3 (15)	103.0 ± 12.9 (15)
50% SW	50% SW	99.6 ± 10.2 (15)	93.6 ± 11.1 (15)	99.0 ± 9.3 (15)

Zinc uptake

Fig. 10 shows examples of the pattern of labelled Zn accumulation by individual *Palaemonetes varians* exposed for 71 h to 100 μg Zn l^{-1} at 50% SW, similar patterns being present when *P. varians* were exposed to 100 μg Zn l^{-1} at 25% SW. As for *Palaemon elegans*, model curves were fitted to the data for individual prawns (Nugegoda & Rainbow 1988). The fitted curves were significant ($p < 0.05$) in 92% of cases.

The rates of labelled Zn uptake in 100 μg Zn l^{-1} by *Palaemonetes varians* with 2 different acclimation histories are presented in Table 10. The mean uptake rate in 25% SW was significantly higher ($p < 0.05$) than that in 50% SW in both experiments. There was no significant difference between mean uptake rates by *P. varians* in 50% SW in both experiments: nor between mean uptake rates in 25% with or without acclimation to the salinity. It can be concluded that the rate of Zn uptake by *Palaemonetes varians* in 25% SW is unaffected by prior acclimation for 15 d to 50% SW as opposed to 25% SW.

There was a marked variability in the rate of Zn uptake in individual *Palaemonetes varians*. Uptake

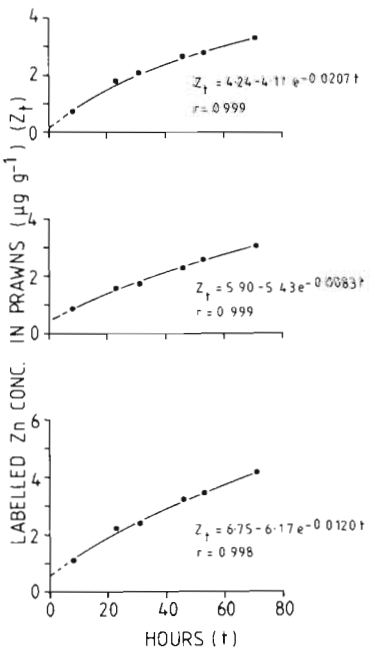

Fig. 10. *Palaemonetes varians*. Accumulation of labelled Zn (Z_t, μg g^{-1}) by 3 individual prawns exposed to 100 μg labelled Zn l^{-1} for up to 71 h (t) in 50% SW at 10°C after 15 d acclimation to 50% SW The fitted curves are significant at $p < 0.001$

Table 10. *Palaemonetes varians.* Mean rates of Zn uptake by individual prawns exposed to 100 μg l^{-1} labelled Zn for 71 h at 10 °C in either 50 % SW or 25 % SW after (a) acclimation for 15 d to 50 % SW and (b) acclimation for 15 d to either 50 % SW or 25 % SW as appropriate

Acclimation medium (15 d)	Exposure medium (71 h)	Mean rate Zn uptake (μg g^{-1} d^{-1})	SD	No.
(a)				
50 % SW	25 % SW	3.19	1.09	14
50 % SW	50 % SW	2.03	0.64	15
(b)				
25% SW	25 % SW	3.47	1.60	13
50 % SW	50 % SW	1.80	0.61	13

rates were not related to the dry weights of prawns in any exposure.

There was no significant difference between the mean Zn concentrations (μg g^{-1}) estimated as initially adsorbed onto the body of *Palaemonetes varians* in 50 % SW (0.464 ± 0.321, n = 15) and 25 % SW (0.321 ± 0.299, n = 14).

Experiment 4: *Palaemonetes varians.* Effect of acclimation to low salinities on total body Zn concentration

Results from the Expt 1 (Table 1) show that there is a transient rise in body Zn concentration of *Palaemonetes varians* transferred from 50 % SW to 25 or 5 % SW. Expt 4 was designed to delimit this concentration rise more narrowly.

Fig. 11 shows the mean total body Zn concentrations

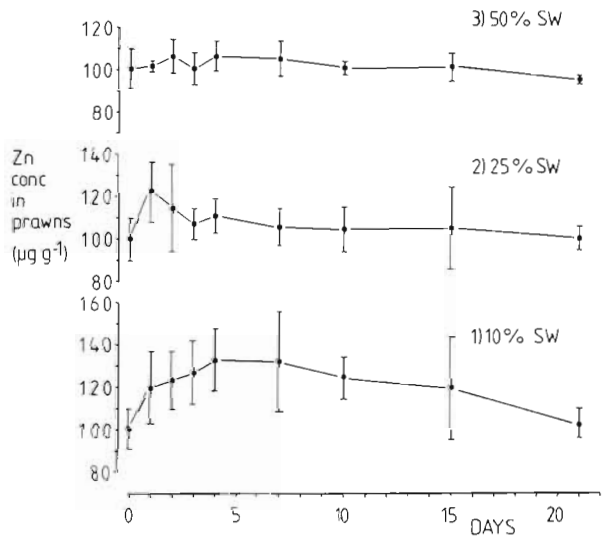

Fig. 11. *Palaemonetes varians.* Mean total body Zn concentrations (μg g^{-1} ± 1 SD) in prawns sampled periodically throughout 21 d acclimation in (1) 10 %, (2) 25 % and (3) 50 % SW. (Number of samples = 6 in each case)

in *Palaemonetes varians* held in 50, 25 and 10 % SW with no added Zn for up to 21 d, after being held in 50 % SW since collection. Mean Zn concentration (± 1 SD) of the Initials (Day 0 for all salinities) was 99.6 ± 10.2 μg g^{-1}.

In 10 % SW there was a rise in total body Zn concentration of *Palaemonetes varians* over the first 4 d, followed by a slow decrease. The mean Zn concentrations in prawns from Day 2 to Day 4 were significantly higher than that of Initial prawns (and of prawns in 50 % SW at any sampling point). After 21 d the mean Zn concentration of the prawns did not differ significantly from that of Initial prawns.

In 25 % SW a marked increase in body Zn concentration was evident after acclimation for 1 d and the mean body concentrations subsequently showed a trend towards decreasing concentration. Mean body Zn concentration did not change significantly during the 21 d acclimation in 50 % SW. Analysis of variance showed no significant difference between the mean Zn concentrations of prawns after 21 d acclimation to the 3 salinities.

Experiment 5: *Palaemonetes varians.* Regulation of body Zn in 50 % SW at 15 °C

Total body Zn concentrations of *Palaemonetes varians* surviving 21 d exposure to a range of dissolved Zn concentrations in 50 % SW at the elevated temperature of 15 °C are shown in Fig. 12. The mean Zn concentration in Initial prawns (103.9 ± 9.3 μg g^{-1}, n = 10) was not significantly different from that of Controls (103.3 ± 6.2 μg g^{-1}, n = 6). However, mean Zn concentrations in Initials and Controls from this experiment were significantly higher than mean Zn concentrations of Initials and Controls from the same experiment carried out at 10 °C (Fig. 2c and Table 1). Both experiments were performed simultaneously on prawns from the same stock, and Initial and Control prawns had not

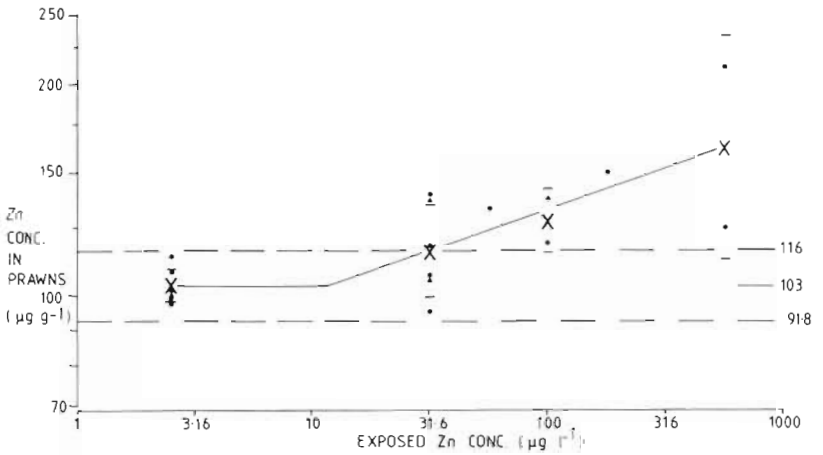

Fig. 12. *Palaemonetes varians*. Total body Zn concentrations (µg g^{-1}) of prawns surviving 21 d exposure to the range of dissolved Zn concentrations (µg l^{-1}) in 50 % SW at 15 °C. Details as for Fig. 1. Regression line ($\log Y = (0.117)\log X + 1.890$; $p > 0.05$) describes net Zn accumulation after Zn regulation breakdown (exposures ≥ 31.6 µg Zn l^{-1})

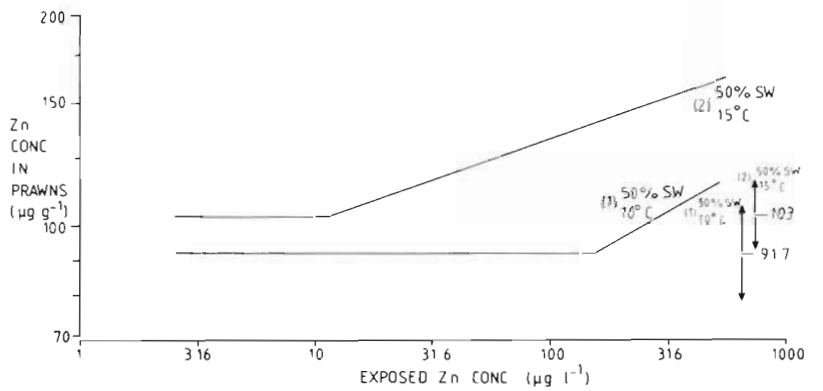

Fig. 13. *Palaemonetes varians*. Comparison of Zn regulation and further net Zn accumulation by prawns exposed to a range of dissolved Zn concentrations (µg l^{-1}) in 50 % SW at (1) 10 °C and (2) 15 °C. Regression lines are from Figs. 2c and 12. Details as for Fig. 3

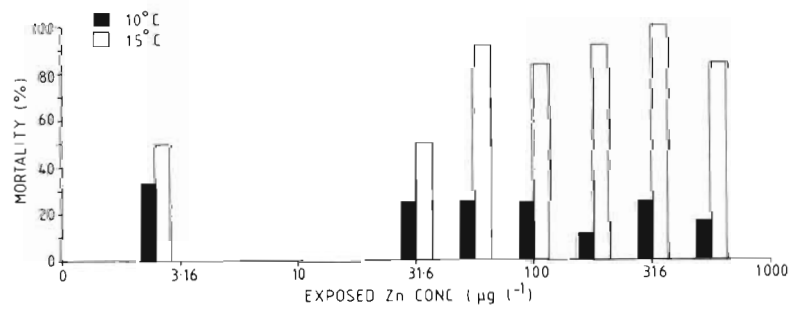

Fig. 14. *Palaemonetes varians*. Mortality: no. of prawns that died during the 21 d exposure as a percentage of the total number of prawns exposed to the range of dissolved Zn concentrations in 50 % SW at 10 ° and 15 °C

been exposed to elevated dissolved Zn concentrations. This therefore shows that body Zn in *P. varians* is regulated at a higher concentration at 15 °C than at 10 °C.

Fig. 13 compares Zn accumulation patterns at 10 °C and 15 °C and clearly shows that regulation breakdown occurs at a lower dissolved Zn exposure at the higher temperature.

Survival was very low in *Palaemonetes varians* exposed to elevated dissolved Zn concentrations at 15 °C. The mortality in each exposure has been plotted as a percentage in Fig. 14. Dissolved concentrations greater than 31.6 µg Zn l^{-1} were more toxic to *Palaemonetes varians* in 50 % at 15 °C than at 10 °C, probably as a result of the inability of prawns to regulate total body Zn concentrations.

DISCUSSION

It is known that *Palaemon elegans* is able to regulate the total body concentration of zinc over a range of dissolved Zn exposures in 100 % seawater; when a threshold concentration of dissolved Zn is reached, regulation breaks down, and net Zn accumulation begins (White & Rainbow 1982, 1984a, b, Nugegoda & Rainbow 1987, 1988). This study confirms that a similar pattern of Zn regulation below a threshold exposure is present when *P. elegans* is exposed to a range of dissolved concentrations in 75 and 50 % SW. There is no change in level of the regulated Zn concentration with decreasing salinity, but the threshold Zn concentration at which regulation breaks down does decrease as salinity is reduced to 50 % SW.

Similarly *Palaemonetes varians* regulates the body Zn concentration to a constant level over a wide range of dissolved Zn concentrations in 100 % SW until a threshold exposure is reached, regulation breaks down and net accumulation of body Zn begins. Similar accumulation patterns are seen for *P. varians* at salinities ranging through 100, 75, 50, 25 and 5 % SW. The Zn concentration equivalent to the threshold of regulation breakdown decreases with reduction in salinity. There is a relatively small change over the salinity range 100 % to 50 % SW. As for *Palaemon elegans* there is no change in the regulated body Zn concentration of *P. varians* with decreasing salinity.

The rate of Zn uptake increases with decreasing salinity in both palaemonid species. In *Palaemon elegans* at least, regulation is brought about by the matching of the rate of Zn excretion to the rate of Zn uptake, this latter being affected by physico-chemical changes such as temperature (White & Rainbow 1984a, Nugegoda & Rainbow 1987) and the presence of the Zn chelating agent EDTA (Nugegoda & Rainbow 1988). The results of this study can be interpreted by assuming that as the salinity is decreased (to 50 % SW in the case of *P. elegans,* to 5 % SW in *Palaemonetes varians*), the rate of Zn uptake in the prawns is increased so that the maximum rate of Zn excretion is exceeded at lower dissolved Zn exposures. Similar interpretations explain for *P. elegans:* (1) the lowering of the dissolved Zn concentration corresponding to regulation breakdown with increased temperature (Nugegoda & Rainbow 1987) as Zn uptake increases (White & Rainbow 1984a); (2) the raising of the regulation breakdown exposure threshold in the presence of EDTA which reduces Zn uptake (Nugegoda & Rainbow 1988).

The mechanism whereby changes in salinity affect heavy metal accumulation is not clear. Phillips (1976) suggested that effects of salinity may be due to changes in the ratio of major ions to heavy metals. Bryan & Hummerstone (1973) suggested that the salinity effects on manganese uptake could be related to calcium availability and Wright (1977) considered that the uptake of cadmium by the shore crab *Carcinus maenas* might be related to the ambient calcium availability. The interpretation favoured here is that a decrease in salinity increases the proportion of the free uncomplexed metal ion in solution, this ion being the most bioavailable species of the metal for uptake by aquatic biota (Sunda et al. 1978, Engel & Fowler 1979, Zamuda & Sunda 1982, Florence 1983, Nugegoda & Rainbow, 1988).

Mantoura et al. (1978) modelled the increase in concentration of Zn^{2+} over other species of Zn (e.g. Zn-Cl complexes) in seawater down the salinity gradient of an estuary. This model proposes a percentage increase of about 25 % in the Zn^{2+} species with a decrease in salinity from 32 ppt (47 % of zinc as Zn^{2+}) to 15 ppt. Therefore if the concentration of Zn^{2+} is the deciding factor in Zn uptake, a decrease in salinity would be expected to cause an increase in Zn uptake by an aquatic organism.

Results obtained with *Palaemon elegans* at 25 % SW are at first sight inconsistent with the above interpretation of regulation breakdown. It has been confirmed that the uptake of Zn by *Palaemon elegans* was further raised as the salinity decreased to 25 % SW, in line with interpretations of increased bioavailability of Zn in reduced salinities, but there was no expected regulation breakdown as at higher salinities. The conclusion drawn earlier was that the observed rise in Zn uptake in 25 % SW must have been matched by increased Zn excretion, necessarily to a level not possible at comparable Zn exposures in higher salinities.

Palaemon elegans lives in littoral rockpools and Morris & Taylor (1983) have presented physico-chemical data for pools of the Isle of Cumbrae, Firth of Clyde, from which the experimental prawns were collected. Salinities of 16 ppt (50 % SW) were not unusual in the rockpools but no salinities below 15 ppt were recorded over a period of 1 yr (Morris & Taylor 1983). *P. elegans* may therefore be adapted to cope with osmotic problems down to 50 % SW (see also Ramirez de Isla Hernandez & Taylor 1985) but a salinity of 25 % SW may place the prawns under considerable osmotic stress. Taylor et al. (1985) for example showed that haemolymph calcium concentrations drop sharply in *P. elegans* in salinities lower than 10 ppt (31 % SW), indicating impairment of normal physiological and ion-regulatory processes. In more dilute media there seems to be a marked decline in the regulation of calcium and chloride ions, and ion regulatory and osmoregulatory mechanisms increasingly break down (Panikkar 1941, Ramirez de Isla Hernandez & Taylor 1985, Taylor et al. 1985). The observed increased mortality in 25 % SW supports this argument. Such increased mortality was

apparently not an effect of the increased toxicity of Zn in 25 % SW since 5/12 prawns died in the Controls, this proportion being greater than the proportion of prawns (2/12) that died in the highest exposure Zn concentration of 562 μg Zn l^{-1}

Urine production by the antennary glands in palaemonids is increased at low salinities (Panikkar 1941, Parry 1955, Mantel & Farmer 1983). It appears that in this very hypo-osmotic medium of 25 % SW, *Palaemon elegans* undergoes a considerably raised osmotic uptake of water, balanced by a correspondingly large efflux of urine. The efflux of urine (isoosmotic with the haemolymph) would consist of water and dissolved salts (salt loss being compensated by increased active uptake of sodium ions in the gills) and the urine may act as an exit route for the excess Zn taken up. Whether or not the urine is routinely a major route for Zn removal in *P. elegans* as in the lobster *Homarus gammarus (vulgaris)* (Bryan 1964), the rate of Zn efflux from prawns in 25 % SW even in high Zn concentrations appears to be equal to or greater than Zn uptake.

The details of the decrease in Zn concentrations at the thresholds of regulation breakdown with reduction in salinity are different in *Palaemonetes varians* reflecting intrinsic differences between the 2 prawns. *P. varians* lives in brackish salt marsh pools and drainage channels. It is not found in full strength seawater in Britain (Parry 1955, Smaldon 1979) and can survive a lower range of salinities than *Palaemon elegans*. *P. varians* can therefore thrive in 25 % SW and shows a more typical pattern of regulation and regulation breakdown at this salinity than does *P. elegans*. Parry (1955) showed that the rate of urine production in *P. varians* increased ca 6 times with changes in salinity from 50 % to 25 % SW, but presumably any increased Zn loss in the urine is inadequate to cope with the increased influx of Zn in exposed concentrations above 100 μg Zn l^{-1} in 25 % SW. The experiments involving *P. varians* at 5 % SW are more indicative of a prawn under osmotic stress with poor survivorship.

Adapted to a lower salinity range, *Palaemonetes varians* can be expected to have a more impermeable exoskeleton than *Palaemon elegans* (Mantel & Farmer 1983). Indeed under the same conditions (100 μg Zn l^{-1} in 50 % SW), the Zn uptake rate of *P. varians* (1.80 ± 0.61 μg Zn $g^{-1} d^{-1}$) is significantly lower than that of *P. elegans* (5.27 ± 3.67 μg Zn $g^{-1} d^{-1}$). Thus the rate of Zn uptake and pattern of Zn accumulation by the prawns at a particular salinity are a feature both of the extrinsic physico-chemical environment and of the intrinsic adaptations of the species concerned.

This study has confirmed earlier findings for *Palaemon elegans* (White & Rainbow 1984a, b, Nugegoda & Rainbow 1988) that there is considerable intraspecific variability in individual Zn uptake rates and correspondingly in individual thresholds of regulation breakdown. The same variability is applicable to *Palaemonetes varians*. Moreover, at least for *Palaemon elegans*, individual prawns with high rates of Zn uptake are more likely to show regulation breakdown, although maximum rates of Zn excretion will not necessarily vary between individual prawns in the same pattern.

In addition to intraspecific variability that may be genetically based (White & Rainbow 1984b), variability in Zn uptake rates is also associated with the moulting cycle. Moulting causes a transient (up to 3 d) increase in the total body Zn concentration of *Palaemon elegans* before regulatory mechanisms cause a return to a constant level (see also White & Rainbow 1984a, b, Nugegoda & Rainbow 1987, 1988). Many of the observed mortalities in experiments with both species were associated with moulting, presumably as a result of the toxic effects of such extra rapidly accumulated Zn concentrations.

The pattern of accumulation of labelled Zn by individual prawns of both species showed a tendency toward an asymptotic value (after ca 3 to 4 d in *Palaemon elegans* exposed to 100 μg Zn l^{-1} in both 100 and 50 % SW), beyond which accumulation continued to increase. The results indicate that Zn in the prawns is composed of at least 2 pools of Zn as predicted by the model of White & Rainbow (1984a) for *P. elegans*. The apparent asymptote seen at ca 4 d is probably due to the rapidly exchanging pool of Zn (termed the 'fast pool') within the prawn, being almost totally exchanged with labelled Zn taken up from the medium. The uptake of labelled Zn into the pool or pools of Zn that exchange less rapidly with the environment (termed the 'slow pool[s]') could continue throughout the 21 d and predictably further if the experiment was not terminated, until 100 % of the Zn within the prawn was exchanged for labelled Zn. The slow pool would reach an asymptote only when all Zn within the prawn is labelled. This model is shown diagrammatically in Fig. 15.

There is an initial adsorption of labelled Zn onto the body of prawns placed in labelled media. This adsorption can be interpreted as having 2 components: (1) instantaneous adsorption onto unsaturated Zn binding sites on the cuticle surface, (2) passive isotopic exchange of unlabelled Zn already loosely bound onto cuticular surface with labelled Zn in the medium. The second process may be incomplete in many of the experimental prawns in this study after only 6 h and account for the apparently low 6 h count of labelled Zn.

Regulated body Zn concentrations in both *Palaemon elegans* and *Palaemonetes varians* showed no long-term differences at different salinities. However at

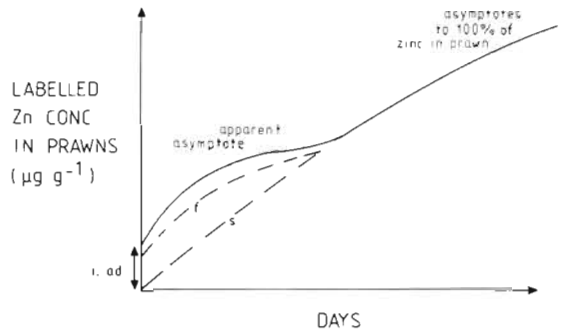

Fig. 15. Diagrammatic representation of the uptake of labelled Zn into prawns assuming that body Zn consists of 2 pools exchanging at different rates. f: uptake of labelled Zn into the 'fast' pool; s: uptake of labelled Zn into the 'slow' pool; i.ad.: labelled Zn initially adsorbed onto prawns

salinities of 25 % SW and below, *P. varians* showed a transient increase in body Zn concentration, which lasted less than a week at 10 °C in 25 % SW but longer in lower salinities, in all cases being absent after 24 d. In contrast, temperature increases cause a permanent rise in regulated Zn concentration in *P. elegans* (Nugegoda & Rainbow 1987) and indeed *P. varians,* exposed to a range of salinities at 15 °C, regulated body Zn to a higher level than at 10 °C.

Such changes in regulated body Zn concentrations probably reflect metabolic needs (White & Rainbow 1985, Rainbow 1988). Possible explanations for the initial increase in body Zn levels in *Palaemonetes varians* transferred to low salinity media include a temporary increase in haemocyanin levels, following the hypothesis that haemocyanin serves as a store for free amino acids required at high salinity as intracellular osmolytes (Mangum 1983), and that Zn is an essential component stabilising the quaternary structure of haemocyanin (Martin et al. 1977). The lack of such an effect in *Palaemon elegans* may be a species difference related to the salinity range inhabited, and/or a result of the masking of a low short-term increase in haemocyanin concentration in the prawns at 25‰ SW by the marked individual variability in prawn haemocyanin concentrations (Taylor et al. 1985).

Alternatively the initial increase in body Zn concentrations in *Palaemonetes varians* in low salinities may result from an increased requirement for the Zn-containing enzyme carbonic anhydrase. Henry & Cameron (1982) and Henry (1984) showed that the concentration of the Zn-containing enzyme carbonic anhydrase increased in gills of the blue crab *Callinectes sapidus* acclimated to low environmental salinities, in response to the associated greater active uptake on ions. Body concentrations of Zn associated with carbonic anhydrase either do not rise significantly in *Palaemon elegans* at low salinities, or any such rise is accommodated by changes in the internal disposition of body Zn. In addition, in strong osmoregulators like *Palaemonetes* spp., the rate of salt loss across the body surface appears to be reduced with short-term exposure to more dilute media (Mantel & Farmer 1983). Whether the initial increase in body Zn concentration is related to such an ion-regulatory phenomenon remains to be shown.

Prior acclimation to either 50 % or 25 % SW did not affect the subsequent rate of Zn uptake by *Palaemonetes varians* in 25 % SW. This result suggests that differences in Zn uptake between decapod species result from permanent intrinsic differences as opposed to temporary effects entrained by recent environmental history.

Zn was more toxic to *Palaemonetes varians* in 50 % SW at 15 °C than at 20 °C, and the threshold concentrations for Zn regulation breakdown also showed an inverse relation with temperature, as for *Palaemon elegans* (White & Rainbow 1984a, Nugegoda & Rainbow 1987). For both prawns therefore, reduced salinity and raised temperatures increase Zn bioavailability, enhancing Zn uptake and promoting the possible accumulation of lethal body Zn concentrations.

In summary therefore Zn uptake and regulation by decapods are affected by extrinsic physico-chemical factors such as changes in salinity and temperature, and by intrinsic features of the species themselves, related presumably to their adaptations to the physico-chemical characteristics of their typical habitats.

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