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Regulation of zinc concentration by *Palaemon elegans* (Crustacea: Decapoda): zinc flux and effects of temperature, zinc concentration and moulting

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ABSTRACT: The shrimp *Palaemon elegans* regulated body zinc concentrations when exposed to ambient zinc concentrations up to 100 μg Zn l^{-1}, regulation apparently being achieved by the rate of zinc loss varying to equal zinc uptake. Flux of zinc through the shrimp (followed using Zn-65 as a tracer) increased with temperature and external zinc concentration. The relation between zinc flux and external zinc concentration was linear in dissolved zinc concentrations between 10 and 42.5 μg Zn l^{-1}. Zinc flux did not vary with size (dry weight) of shrimps. Total body zinc consists of a number of component pools ('fast' and 'slow') exchanging at different rates, the pool sizes varying with the rate of zinc flux through the shrimp. The pools are therefore features of rate processes, not discrete physical entities. Moulting increased accumulation of labelled zinc from surrounding seawater.

INTRODUCTION

Several decapod crustaceans regulate total body zinc concentrations when exposed to elevated dissolved zinc levels (Bryan, 1964, 1966, 1967, 1968; Wright, 1976; Ray et al., 1980; White and Rainbow, 1982). Zinc regulation may therefore occur in all decapod crustaceans but little is known of the mechanisms involved.

Regulation of body zinc could occur by 2 methods. Firstly, zinc uptake could be restricted at the permeable interfaces between organism and environment such that only zinc required for growth and for replacing zinc lost in excretion and moulting would be taken up. The second method would involve little or no control of zinc uptake, the body zinc concentration being maintained by efficient zinc excretion, the rate of excretion equalling the rate of zinc uptake. These 2 mechanisms are not mutually exclusive and regulation could be achieved by a combination of the two.

The work presented here attempts to identify which mechanism is most important in zinc regulation in the natantian decapod *Palaemon elegans* Rathke. Experimental data are used to construct a preliminary model of zinc flux through the shrimp, and the effects of temperature and external dissolved zinc concentration on zinc flux through the shrimp are examined. This study also introduces an examination of the variability in the rate of zinc flux between individual shrimps and considers how moulting affects zinc flux rates. These aspects will be considered in more detail in a later paper (White and Rainbow, unpubl.).

MATERIAL AND METHODS

Palaemon elegans from Millport, Isle of Cumbrae, Firth of Clyde, UK, were acclimated to experimental temperatures (see below) for at least 3 d in the experimental medium, Tropic Marin New (TMN Tropicarium Buchschlag, Dreieich, F.R. Germany) before commencing any experiments. TMN was chosen because it provided a more reproducible medium than laboratory seawater, particularly with respect to levels of trace metals and dissolved organic matter which might chelate added metal. Moreover TMN prepared with fresh distilled water was confirmed to contain a lower original zinc concentration (2.5 μg Zn l^{-1}) than stored laboratory seawater, originally collected from British coastal waters.

Individual shrimp (or moults) taken for analysis were rinsed briefly in TMN and weighed wet (for later calculation of wet:dry weight ratios). Shrimp were

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dried to constant weight at 60 °C and digested in conc. nitric acid (Ultrar grade, Hopkin and Williams, Chadwell Heath, Essex, UK) at 100 °C. Digested samples were made up to volume and transferred to acid-washed glass vials for counting in a Nuclear Chicago 1185 gamma scintillation counter. Samples were counted against known standards of the same sample geometry after correcting for the background radiation count.

To measure Zn-65 activity in live shrimp, counting was performed in a moist glass vial with the tip of the shrimp's uropods just touching the bottom of the vial. This standardized position enabled geometric effects to be held constant. It was not possible to count shrimp in tubes filled with TMN as this allowed them to swim and alter the sample geometry. Counting was carried out for 1 min against known Zn-65 standards, the time being a compromise between achieving sufficient counts and stressing the shrimp. At the end of each experiment shrimp were counted whole to give a minimum count of 5,000 above background. This final figure was used to compare whole body counts with counts in digested samples. The digest count : whole body count ratio was less than 1 (0.4 to 0.8) and showed a significant increase with the dry weight of the shrimp, this relation being attributable to differences in the geometries of different sized shrimp and Zn-65 standards. The precise digest count : whole body count for each shrimp was used to calculate Zn-65 activity at each previous live count. Shrimp digests were finally analysed for total zinc using a Varian AA-375 series atomic absorption spectrophotometer fitted with a continuous deuterium background correction lamp.

TMN was dosed to required concentrations by addition of aliquots of a freshly prepared stock solution of Analar grade (B.D.H. Ltd.) $ZnCl_2$ incorporating Zn-65 tracer (Amersham UK Ltd.) as required with allowance for total zinc present in the tracer.

In all experiments a 12:12 light:dark regime was employed and the salinity was maintained at 33 ppt. Experimental solutions were aerated gently and covered to prevent evaporation or contamination. Samples of experimental solutions were taken throughout experiments to monitor Zn-65 activity.

EXPERIMENTAL DETAILS

Zinc flux through *Palaemon elegans*

An initial total of 40 *Palaemon elegans* were exposed for 10 d to 100 $\mu g\ l^{-1}$ of total zinc in 15 l of TMN which contained 10 $\mu Ci\ l^{-1}$ of Zn-65 as a tracer. After 10 d shrimp were transferred to another tank and further exposed, for 11 d, to 100 $\mu g\ l^{-1}$ total zinc with no added tracer. At intervals, 4 shrimp were taken and individually dried, weighed and digested, before analysis for both Zn-65 and total zinc. Shrimp were sampled after 4, 8, 19.5 and 46.5 h and 5, 10, 11, 12, 15 and 21 d. The experimental medium was changed on Days 5, 10 and 15 and sampled throughout the experiment to determine the concentration of Zn-65. Shrimp were fed, all together, every other day on lamb's heart after transfer to a separate tank for 15 min. Except during feeding, shrimp were maintained individually in acid-washed compartmental perspex boxes. The experimental temperature was 20 °C ± 2 C°.

Effect of temperature on zinc flux through shrimp

Groups of 8 shrimp were exposed to 100 $\mu g\ l^{-1}$ zinc with 10 $\mu Ci\ l^{-1}$ Zn-65 as a tracer at 5 °, 8 °, 15 ° or 20 °C in 1 l of TMN. Individual shrimp, one from each temperature regime, were sacrificed after 7, 24, 31, 48, 54, 72, 79 and 96 h and dried, weighed, digested, and analysed for both Zn-65 and total zinc.

Shrimp were not fed during the experiment and the TMN was not changed. Exposures were carried out in 2 l Pyrex beakers immersed in water baths at the appropriate temperatures, which did not vary from the declared values by more than ± 0.5 C°.

Shrimp had been acclimated to experimental temperatures by transferring them to 2 l of TMN at 10 °C and bringing this slowly to experimental temperatures using water baths. Maximum temperature change was 5 $C°\ d^{-1}$ and shrimp were held at the experimental temperatures for a further 2 d before commencing exposure to zinc.

Effect of the external zinc concentration on zinc flux through individual shrimp

Groups of 6 *Palaemon elegans* were exposed to 10, 20, 30, 40 or 50 $\mu g\ l^{-1}$ zinc, with zinc-65 as a tracer (specific activity, 0.1 $\mu Ci\ g^{-1}$ total zinc) for 72 h in 0.5 l of TMN. Shrimp were held separately in 0.8 l acid washed plastic beakers. Live shrimp were counted for Zn-65 at precisely 12 h intervals up to 72 h. After the 72 h count shrimp were dried, weighed and digested and analysed for Zn-65 and total zinc.

In addition to the above repeatedly sampled shrimp, a further 6 individuals were exposed to a zinc concentration of 50 $\mu g\ l^{-1}$, with zinc-65 as a tracer, and were only counted at the end of the experiment in order to determine if handling had affected uptake of labelled zinc.

Terminology: To avoid ambiguity, a number of terms used throughout this study are defined as follows:

'Labelled zinc': zinc taken up during those stages of experiments where zinc-65 was added to TMN as a tracer for zinc. 'Accumulation': net increase in concentration of zinc, normally of labelled zinc. 'Uptake': input of zinc into the shrimp. 'Depuration': loss, by any means, of labelled zinc.

RESULTS

Experiment I: zinc flux through shrimp

Fig. 1 shows concentrations of labelled zinc in TMN throughout Experiment I. The decrease in concentration over the first 5 d of the experiment may in part be due to adsorption onto surfaces of the tank. There was little loss of labelled zinc however after changing the TMN on Day 5 and the declared concentration of 100 μg Zn l^{-1} will be used in discussing the data. It is assumed that the concentration of unlabelled zinc, at the same concentration of 100 μg Zn l^{-1}, showed similar negligible changes during Days 10 to 21. The concentration of labelled zinc in TMN on Days 10 to 21 measures amounts of labelled zinc lost from the shrimp and had a maximum value of 0.35 μg l^{-1} on Day 15. It was concluded that recycling of labelled zinc was negligible.

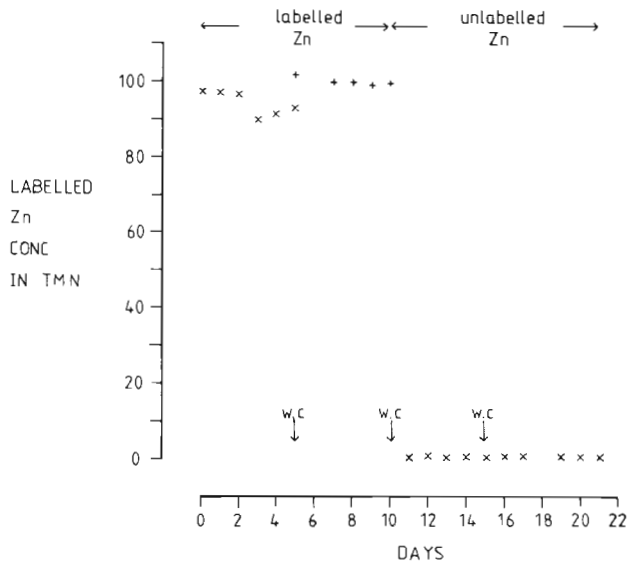

Fig. 1. Concentration of labelled zinc (μg l^{-1}) in Tropic Marin New (TMN) during Expt. I (nominal concentration 100 μg Zn l^{-1}; w.c: change of experimental water)

Total zinc concentrations in shrimp

Fig. 2 shows concentrations of total zinc in shrimp sampled throughout the experiment. Analysis of variance revealed no significant difference in mean concentrations at each sampling including initial control individuals (Day 0, 90.7 ± 6.1 μg Zn g^{-1}; $p \simeq 0.1$). Linear regression analysis showed no significant regression between time of exposure and zinc concentration ($p \simeq 0.2$). It can be concluded therefore that zinc concentrations of shrimp did not change during the experiment, and that shrimp regulate body zinc concentrations at this level of ambient dissolved zinc, in line with the conclusions of White and Rainbow (1982).

None of the shrimp died during the experiment, nor were there any moults. The lack of moults is probably due to the time of year the shrimp were collected, November, when the intermoult period in *Palaemon elegans* may be extended due to low temperatures (Hoglund, 1943).

Accumulation and loss of labelled zinc

If the total amount of zinc in the shrimp, which remains constant throughout the experiment, can be considered as a single homogeneous pool, the percentage of labelled zinc would be expected to rise and approach 100 %. This model (A) is shown in Fig. 3. Fig. 4, however, reveals that the uptake of labelled zinc by *Palaemon elegans* (as a percentage of total zinc), under these particular conditions, tends towards an asymptote of approximately 35 % of the total zinc content of the shrimp (later calculated to be 36 %). It appears therefore that the zinc content of shrimp is not a single homogeneous pool. In the light of these data, the next simplest model (B, Fig. 3) is one in which only a proportion of the total zinc content undergoes exchange with labelled zinc. With this model, if exposure to labelled zinc had been continued indefinitely the percentage of labelled zinc would have reached a maximum level, in this case approximately 35 % of the total zinc. At equilibrium, labelled zinc would flow only through this 'exchanging' pool, at a steady state.

Mathematically, Model B can be reduced to a simple differential equation

$$P_t = P_{ss} (1-e^{-kt}) \quad (1)$$

where: P_t = percentage of labelled zinc at time t; P_{ss} = percentage of labelled zinc when the exchanging pool is in a steady state, i.e. at the asymptote; k = rate constant in terms of the fraction of the pool moving per unit time.

Data for the accumulation of labelled zinc can be fitted to this equation by a maximum likelihood technique (Bliss, 1970). Calculations were performed on a CBM PET computer using a programme developed from mathematical and statistical techniques described in Sokal and Rohlf (1969) and Bliss (1970).

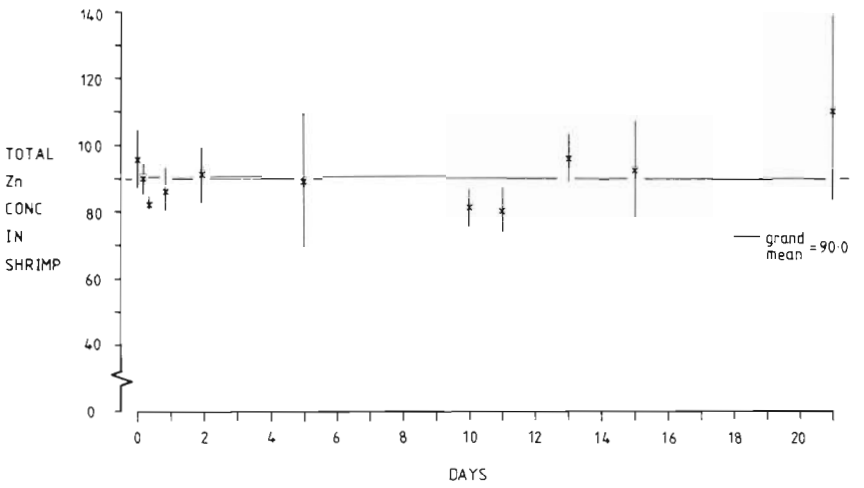

Fig. 2. *Palaemon elegans.* Mean total zinc concentration (μg g^{-1} dry weight, ± 1 SD; n = 4) in individuals exposed to 100 μg l^{-1} of labelled zinc for 10 d and then transferred to 100 μg l^{-1} of unlabelled zinc and exposed for a further 11 d (Expt. I)

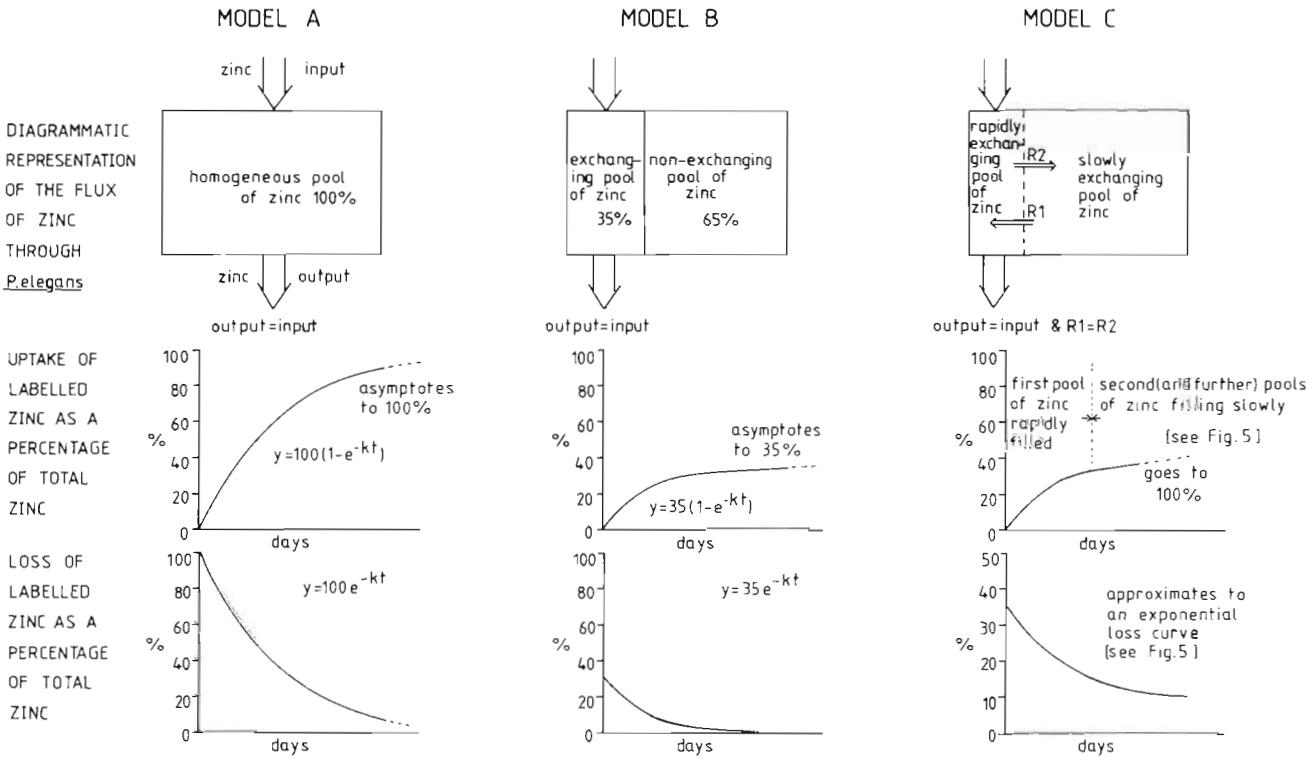

Fig. 3. Three possible models to describe zinc flux through *Palaemon elegans*. Equations describe plotted curves, where k = the rate constant; t = time

Fitting the data to Equation (1) gives an asymptote of 36 % and the accumulation of labelled zinc can be described by the equation:

$$P_t = 36.0\ (1-e^{-0.359t}) \quad (2)$$

The asymptotic curve produced is plotted in Fig. 4. The 95 % confidence limits for the asymptote are 27.2 to 47.7 % and show that the asymptote is significantly less than 100 %. It can be concluded therefore that not all the zinc in the shrimp is exchanged, confirming that Model A should be rejected.

The loss of labelled zinc during the depuration phase of the experiment (while exposed to 100 μg l^{-1} unlabelled zinc) is also shown in Fig. 4. If Model B holds, loss of labelled zinc can be described by a simple, exponential curve, i.e. there is a constant proportional loss in the amount of labelled zinc. The fitted curve (Fig. 4) was found by plotting $\log_e P_t$ against time which gave a best fit equation of:

$$P_t = 38.7\ e^{-0.124t} \quad (3)$$

This equation gives a biological half life of labelled zinc of 5.6 d.

As the concentration of total zinc does not change

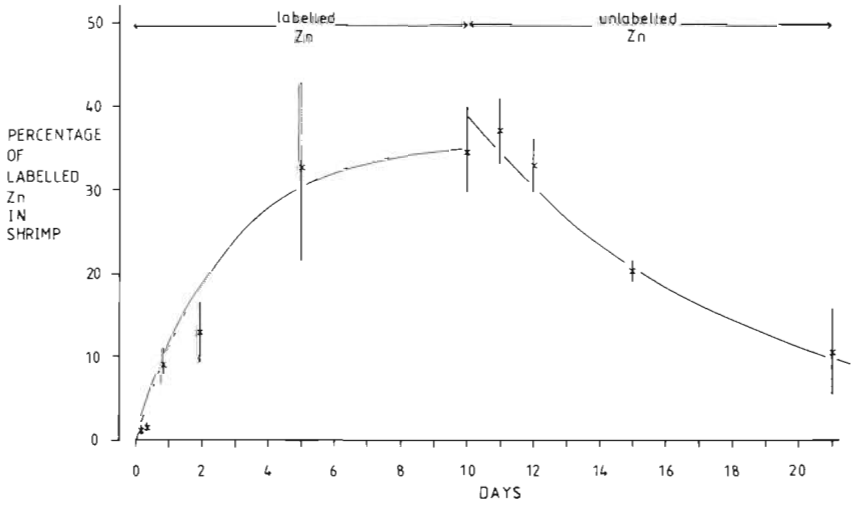

Fig. 4. *Palaemon elegans*. Uptake and loss of labelled zinc (percentage of total zinc) in individuals exposed to 100 μg l^{-1} of labelled zinc for 10 d and then transferred to 100 μg l^{-1} of unlabelled zinc and exposed for a further 11 d (Expt. I; means ± 1 SD; n = 4)

during the experiment, rates of uptake and loss of total zinc must be equal. The 2 rate constants (the slopes of logarithmic plots of the data) for uptake and loss of labelled zinc, 0.359 d^{-1} and 0.124 d^{-1}, respectively, are, however, significantly different ($p < 0.001$; F test: Sokal and Rohlf, 1969). It can be concluded therefore that labelled zinc was taken up at a faster rate than it was subsequently lost, indicating net accumulation of labelled zinc. This apparent anomaly between observed net accumulation of labelled zinc and simultaneous regulation of total zinc can be resolved if there is more than 1 pool of zinc undergoing zinc exchange and hence taking up labelled zinc. This suggests that Model B should be rejected and a further model be proposed.

The technique for resolving number and size of individual compartments or pools of this type (curve stripping) cannot, however, be used to resolve the individual components in this experiment, as a larger number of sampling points are required (Atkins, 1969; Shipley and Clark, 1972). It is therefore not possible to determine the size of, nor the rates of transfer between the 2 or more, pools of zinc that are apparently present in *Palaemon elegans*. One possible simple model (Fig. 3; C) may be used to illustrate why the measured rates of uptake and loss of labelled zinc differed (Fig. 4). Model C proposes 2 pools of zinc: 1 pool, undergoing rapid zinc exchange, is relatively quickly filled with labelled zinc thus producing the apparent asymptote, and 1 pool (in this case all other zinc) is filling at a proportionally slower rate. A consequence of Model C is that if exposure to labelled zinc had continued, the percentage of labelled zinc would have risen to 100 % of the total zinc concentration, i.e. would have resulted in total zinc exchange. Clearly equal rates of uptake and loss of labelled metal would only occur when all body zinc had been exchanged for labelled zinc.

Model C is only 1 of a number of possible 2-compartment models described by Shipley and Clark (1972), which vary in number and location of sites of uptake and loss, but the number of possible models is infinite, increasing geometrically with the number of compartments.

Despite closely fitting the available data, the curves shown in Fig. 4 are in error as they are derived from Model B which has been shown to be incorrect. Fig. 5 illustrates the pattern of uptake and loss of zinc expected if Model C is correct. Curves of uptake and loss generated by the model (Fig. 5) are, however, good representations of the curves (Fig. 4) fitting the data of Experiment I and thus will be useful in further examining the data obtained. The initial rate of uptake of labelled zinc, which equals the overall flux of total zinc, is given by the tangent to the curve (Fig. 4) and equals P_{ss} (the asymptote) x k (the rate constant [see Equation 1]). The rate of exchange, 12.9 % of the total zinc per day is equivalent to 11.6 μg Zn g^{-1} dry weight d^{-1} in absolute terms. This large flux of zinc through the shrimp clearly shows that the regulation of zinc by *Palaemon elegans* demonstrated by White and Rainbow (1982), and confirmed here (Fig. 2), is not achieved by preventing the entry of zinc into the body.

As uptake of labelled zinc occurs in at least 2 pools, the measured percentage of labelled zinc in shrimp during the uptake phase of the experiment will include some labelled zinc in slower exchanging compartments. Therefore the calculated asymptote of 36 % will overestimate the size of the rapidly exchanging pool. The data do, however, appear to asymptote – suggesting that the rapidly exchanging pool has undergone (almost) complete exchange with labelled zinc and therefore accounts for a large proportion of the asymptotic value. This in turn suggests that at least two thirds of the zinc in *Palaemon elegans* is exchanged at a considerably slower rate under these experimental conditions.

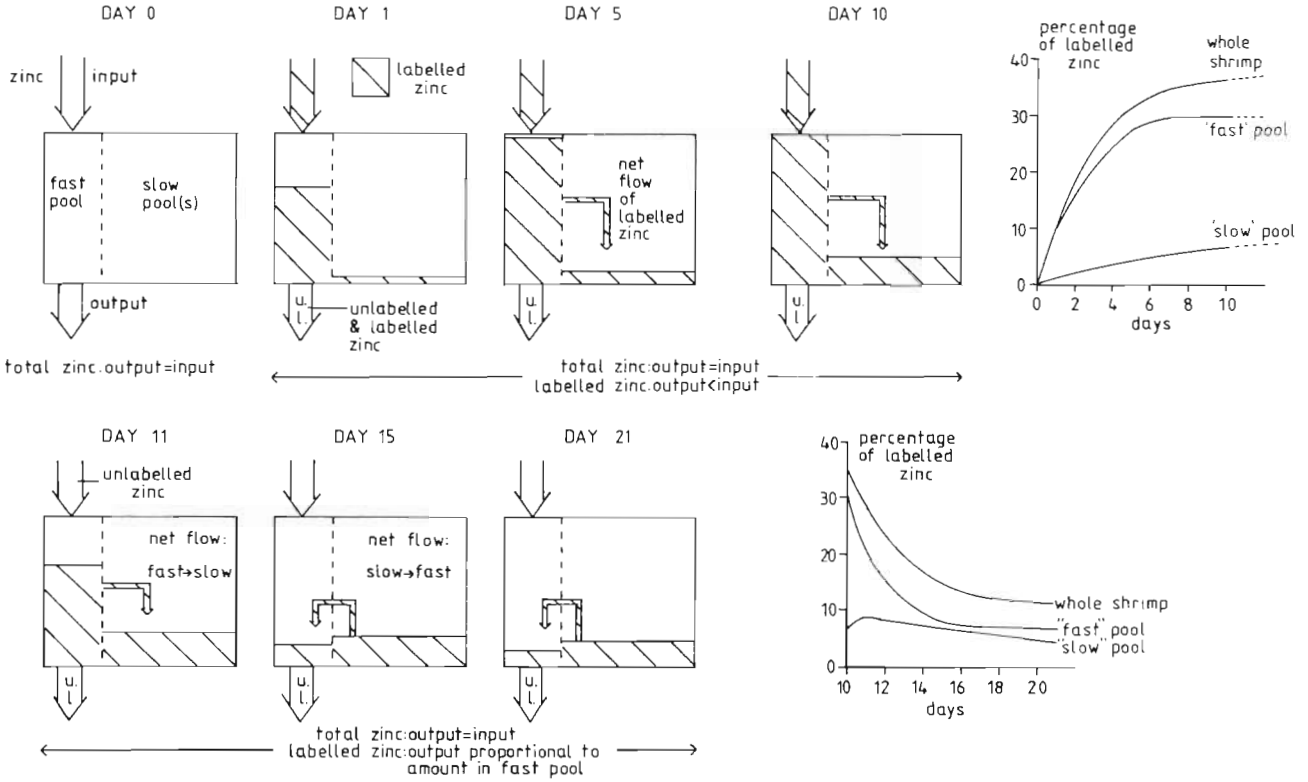

Fig. 5. *Palaemon elegans*. Modelling of the pattern of uptake and loss of labelled zinc during the 2 phases of Experiment I, assuming that body zinc consists of 2 pools of zinc which exchange at different rates, as shown in Fig. 3, Model C

Experiment II: Effect of temperature on zinc flux through the shrimp

Concentration of Zn-65 in experimental media

Samples of TMN taken from experimental tanks showed that at none of the 4 experimental temperatures did the Zn-65 actively differ from the declared value by more than 5 %.

Total zinc concentrations in shrimp

Total body zinc concentrations of individual shrimp exposed to 100 µg Zn l^{-1} for up to 96 h were analysed by a 2-way analysis of variance. There were no significant changes in zinc concentration with either time of exposure ($p \simeq 0.35$) or temperature ($p \simeq 0.25$), and it can again be concluded therefore that under these experimental conditions shrimp are regulating body zinc concentrations.

Uptake of labelled zinc

Fig. 6 shows the percentage of labelled zinc in individual shrimp sacrificed sequentially over 96 h at each of 4 experimental temperatures. Interpretation of these data is made difficult by the variability between individuals in rates of labelled zinc uptake. This variability cannot be explained by the effects of moulting since no shrimp moulted during the experiment. This variability is considered in Experiment III and further in White and Rainbow (unpubl.).

Fig. 6 does show though that the amount of labelled zinc accumulated, and therefore the rate of zinc flux increased with increasing temperature. The data for 5 and 15 °C significantly tended towards asymptotes (4.7 and 29.4 % of total zinc, respectively), and the calculated curves have been plotted. While the 20 °C data show some levelling off after 48 h, the data variability at both 20 and 8 °C prevents any significant fit being made to an asymptotic model. Even if the calculated asymptote overestimates somewhat the size of the 'fast' pool of zinc, it appears that its size varies with temperature, possibly due to increased zinc flux through the shrimp. This implies that the pools of zinc are not discrete physical entities but rather a function of rate processes within the shrimp.

The effect of temperature on zinc flux may be determined if it is assumed that net accumulation of labelled zinc is, at least initially, linear with time. The validity of this approximation will depend upon the curvature of the line describing the accumulation of

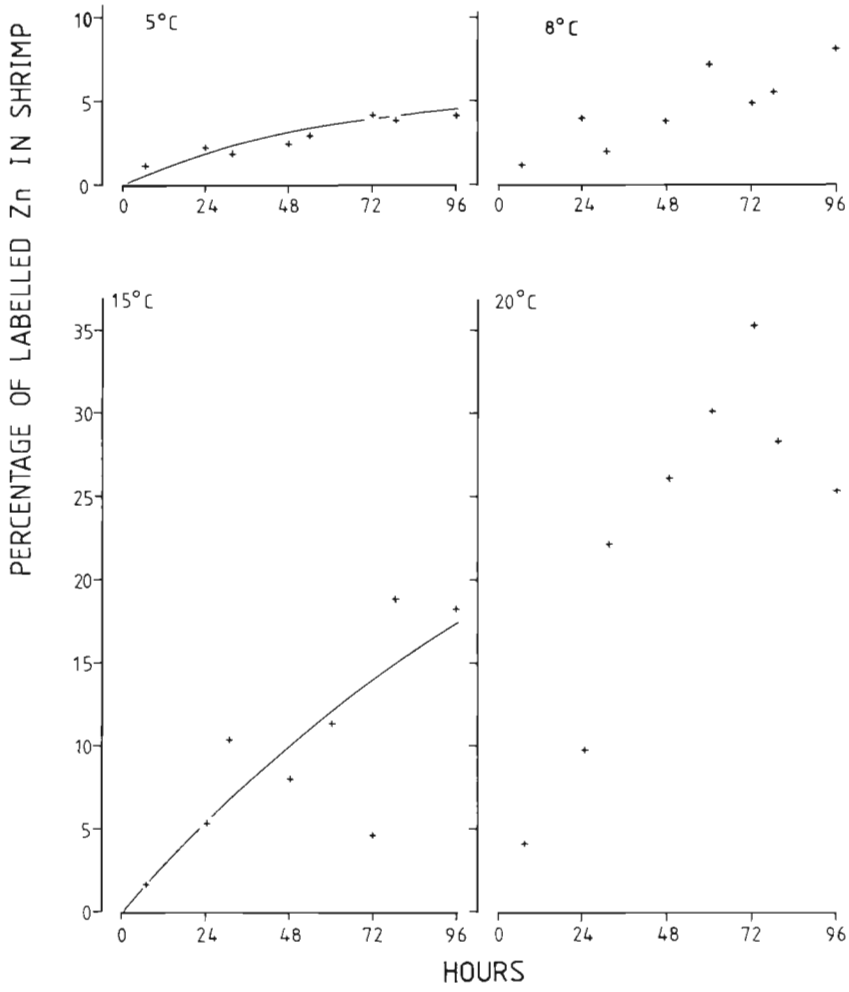

Fig. 6. *Palaemon elegans*. Effect of temperature on uptake of labelled zinc (percentage of total body zinc) in individuals. Groups of shrimp were exposed to 100 μg l^{-1} of labelled zinc at 1 of the 4 experimental temperatures (Expt. II). The 5 ° and 15 °C data tend towards asymptotes (see text); calculated curves are shown. The 8 ° and 20 °C data cannot be fitted adequately to asymptotic curves

labelled zinc. Fig. 7 shows mean rates of labelled zinc accumulation (as a percentage of the total zinc per hour) plotted against temperature, based on data up to 48 h (Fig. 6), but excluding 0 hour data points to avoid the effect of adsorption onto the cuticle (see later). It appears that the relation between temperature and rate of flux of zinc is logarithmic, with the rate of uptake (which equals the rate of loss) rising exponentially with temperature, doubling with every 4 C° increase.

Experiment III: Effect of the external zinc concentration on zinc flux through the shrimp

The 2 experiments reported thus far have only measured amounts of labelled zinc accumulated by individual shrimp at a single time. The assumption had been made that the rate of labelled zinc accumulation and sizes of any pools of zinc would, for any given set of experimental conditions, be equal for all individuals, or at least any individual variation would be insignificant.

By following labelled zinc accumulation in whole, live shrimp over a period it was possible to demonstrate individual variability and to determine the magnitude of the variation previously held to be insignificant. In addition to examining zinc flux over a range of dissolved zinc concentrations, this experiment also examined whether repeated counting of live individuals had any significant effect upon zinc flux rates through shrimp.

Zn-65 activity in experimental media

Samples of TMN taken from experimental beakers were confirmed to have Zn-65 concentrations within 5 % of the expected values over the range 10 to 40 μg Zn l^{-1}. The concentration of Zn-65 in the 2 sets of beakers containing a nominal 50 μg Zn l^{-1} was significantly less than expected (by approximately 16 %). This error may have been due to a faulty dosing pipette and the total zinc concentration in these beakers was recalculated to be 42.5 μg Zn l^{-1}.

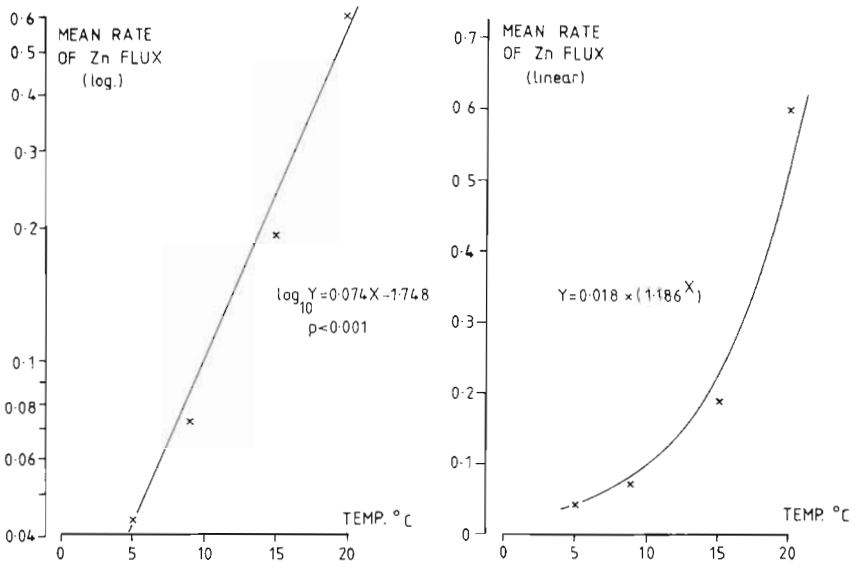

Fig. 7. *Palaemon elegans.* Logarithmic and linear plots of mean rates of zinc flux (percentage of total zinc in shrimp per hour) in individuals exposed to 100 µg l^{-1} labelled zinc at 4 experimental temperatures; Expt. II

Total zinc concentration in shrimp

Fig. 8 shows total zinc concentrations in repeatedly sampled shrimp after 3 d exposure to elevated zinc concentrations. Analysis of variance revealed no significant differences in mean total zinc concentrations in repeatedly counted shrimp at different external concentrations ($p \simeq 0.2$). Regression analysis showed no significant regression between total body zinc concentrations and external zinc concentrations ($p > 0.6$). A comparison of the repeatedly sampled shrimps exposed to 42.5 µg Zn l^{-1}, against those shrimps exposed to 42.5 µg Zn l^{-1} and only counted at the end of the experiment, exhibited no significant difference ($p \simeq 0.3$) in total body zinc concentrations. These results again fully support the thesis that *Palaemon elegans* regulates total body zinc concentration at the external concentrations under test.

Uptake of labelled zinc by individual shrimp

Fig. 9 shows uptake of labelled zinc (as µg Zn g^{-1} dry wt) by repeatedly sampled shrimp exposed to 10 to 42.5 µg l^{-1} of labelled zinc for 72 h. There are 2 apparent phases of labelled zinc uptake, an initial rapid uptake between 0 and 12 h, followed by a phase where uptake is approximately linear. Initial uptake is thought to be due to adsorption onto the cuticle, probably as a result of rapid isotopic exchange with non-labelled zinc already loosely occupying a fixed number of binding sites on the external surface. Between 12 and 72 h, uptake of labelled zinc appears to be approximately linear with time (except where modified by moulting) in all 5 experimental exposures. Data from the 2 earlier experiments have, however, shown that labelled zinc uptake tends to asymptote as the fast pool of zinc fills. Presumably, therefore, under

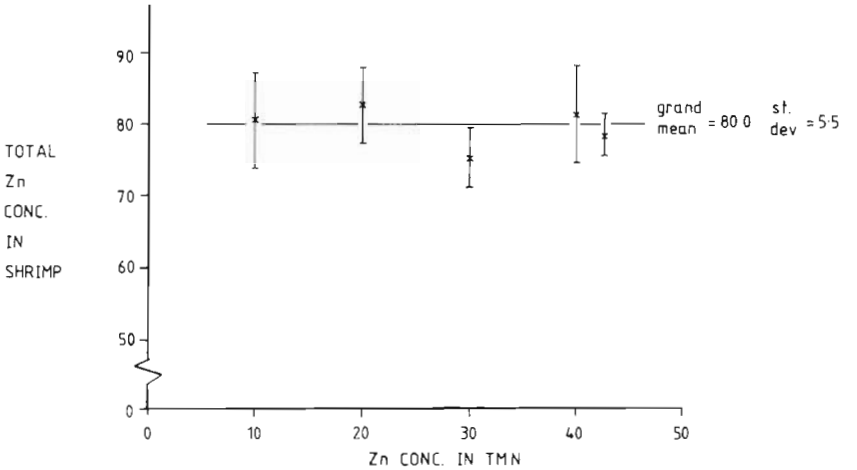

Fig. 8. *Palaemon elegans.* Mean total zinc concentration (µg g^{-1} dr. wt, ± 1 SD, n = 6) in individuals exposed to 1 concentration between 10 and 42.5 µg l^{-1} of labelled zinc, for 3 d in TMN (Expt. III)

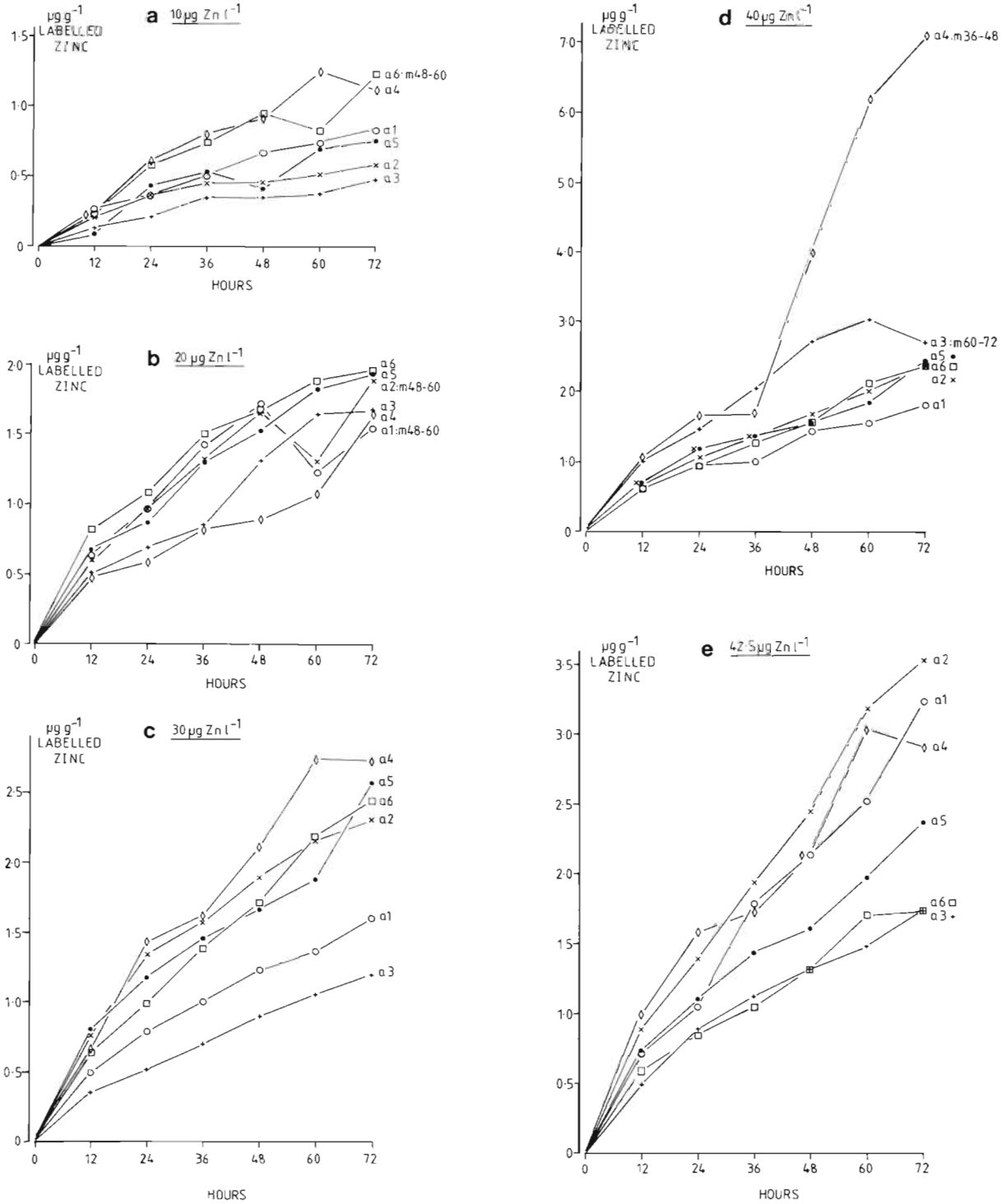

Fig. 9. *Palaemon elegans*. Uptake of labelled zinc (µg g^{-1} dr. wt.) by individuals exposed to (a) 10 µg l^{-1} labelled zinc; (b) 20 µg l^{-1}; (c) 30 µg l^{-1}; (d) 40 µg l^{-1}; (e) 42.5 µg l^{-1} of labelled zinc for up to 72 h at 15 °C (Expt. III). a: animal number referred to in text; m: shrimp moulted during experiment and time interval when moulting occurred (h). Note change of scale in (d)

the experimental conditions used, there has been insufficient labelled zinc uptake in any of the shrimp to cause any marked curvature towards an asymptote.

The slope of the calculated regression equations for labelled zinc accumulation against time represents the rate of labelled zinc uptake which equals the rate of zinc flux through the shrimp, and all references will now be made in terms of zinc flux. (For shrimp that

moulted during the experiment only data prior to the moult being shed and consisting of at least 3 points, were included in the calculation of regression equations.) Fig. 10 shows mean rates of zinc flux through shrimp plotted against external zinc concentration

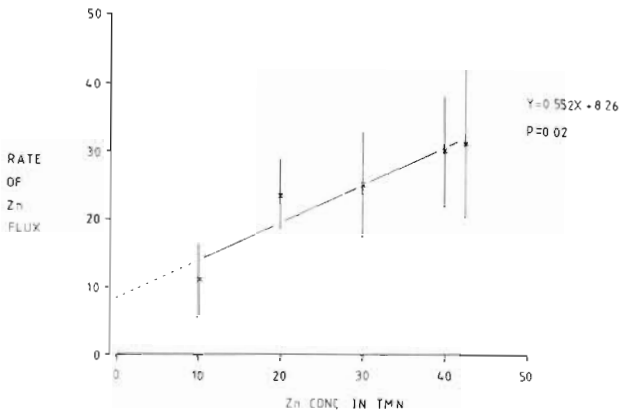

Fig. 10. *Palaemon elegans*. Mean rates of zinc flux (ng g^{-1} dr. wt. h^{-1}, ± 1 SD, n = 6) through individuals exposed to 1 concentration between 10 and 42.5 g l^{-1} of labelled zinc in Tropic Marin New (TMN) at 15 °C (Expt. III)

between which there is a significant relation ($p \simeq 0.02$). Since it is technically difficult to measure uptake of labelled zinc at even lower zinc concentrations it is of interest to consider what might be the situation by extrapolation of the plotted line (Fig. 10) back to the y-axis. The extrapolated line does not pass through the origin but gives with a calculated rate of zinc flux of 8.26 ng Zn g^{-1} h^{-1} at 0 µg Zn l^{-1}. This suggests that at very low concentrations there may be active uptake of zinc by the shrimp from the environment. This estimate is, however, made with some error and confidence limits need to be established. The 95 % confidence limits for the rate of zinc flux are 8.26 ± 11.05 ng Zn g^{-1} h^{-1}. As the confidence limits include 0 ng g^{-1} h^{-1}, it is not possible from these data to determine conclusively if there is active uptake of zinc by the shrimp or if there is a minimum finite concentration of dissolved zinc at which zinc is exchanged between the shrimp and the surrounding seawater.

It is clear from Fig. 9 that there is considerable variation in the rate of labelled zinc uptake between individuals. This variation could not be attributed to sex nor to total body zinc concentration (plots of percentage labelled zinc against time show similar variation in the rates of zinc flux). The size of the shrimp also had no apparent effect on zinc flux rates, as at no zinc concentration was there any significant correlation ($p > 0.05$) between labelled zinc uptake and shrimp dry weight. This is confirmed in a more extensive study of variability of zinc flux between individuals (White and Rainbow, unpubl.).

Initial uptake of labelled zinc by individual shrimp

The regression equations calculated for the 12 to 72 h periods shown in Fig. 9 may be extrapolated back to the intercept on the y-axis (Time 0), to give an estimate of the initial immediate uptake of labelled zinc which probably represents isotopic exchange with zinc adsorbed onto the cuticle. This initial uptake of labelled zinc showed some variation with the external zinc concentration (Fig. 11). It appears that initial

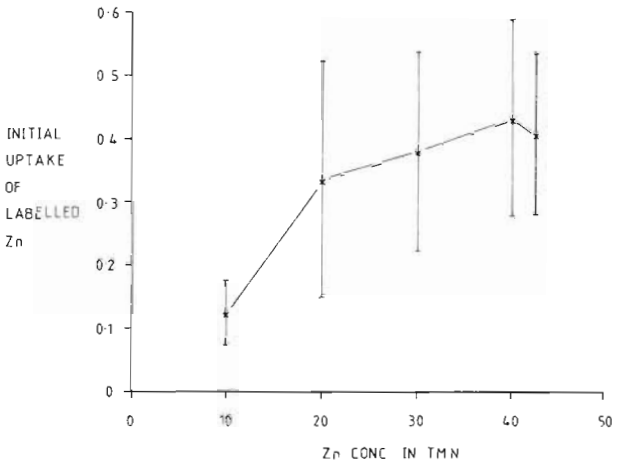

Fig. 11. *Palaemon elegans*. Initial uptake of labelled zinc (µg g^{-1} dr. wt., mean ± 1 SD, n = 6) by individuals exposed to one concentration between 10 and 42.5 µg l^{-1} of labelled zinc in Tropic Marin New (TMN) at 15 °C (Expt. III)

uptake of zinc increases to a plateau and analysis of variance showed no significant differences in the means at 20 to 42.5 µg Zn l^{-1} ($p \simeq 0.2$). This does not however necessarily imply that at 10 µg l^{-1} the available adsorption sites on the cuticle are never filled. It is more likely that at this concentration the exchange with non-labelled zinc is slower and is not completed within 12 h.

Effect of moulting on uptake of labelled zinc

Another feature of Fig. 9 is the marked effect of moulting upon labelled zinc uptake. At 10 and 20 µg Zn l^{-1}, shrimp which moulted showed an immediate drop in the amount of labelled zinc which subsequently increased at a rate apparently greater than that prior to moulting. Of the 2 *Palaemon elegans* that moulted in the 40 µg Zn l^{-1} exposure (Fig. 9d) 1 (Individual 3) showed a small drop in the amount of labelled zinc while the other (Individual 4) revealed a marked increase with a rate of labelled zinc accumulation approximately 4 times greater than non-moulted shrimp.

Differences in the pattern of labelled zinc uptake, for moulted individuals, are probably due to the interplay of 2 factors: external zinc concentration and time elapsed between the moult being shed and subsequent counting, remembering that labelled zinc uptake rates are proportional to the external concentration. Thus the rate of zinc uptake is relatively slow in the 10 and 20 μg Zn l^{-1} exposures and the first counting after moulting shows a large percentage drop in labelled zinc, indicating loss of a comparatively large initial component of labelled zinc associated with the cuticle. In the 40 μg Zn l^{-1} exposure the rate of labelled zinc accumulation is greater and the observed difference in the pattern of uptake in the 2 individuals at this concentration is probably due to differences in the amount of time passed between moulting and subsequent counting. As the precise time between moulting and subsequent counting is not known, no attempt can be made to calculate the amount of labelled zinc lost with the moult.

As the amount of total zinc in the shrimp does not change irrespective of moulting, the rapid uptake of labelled zinc may represent an increased flux of zinc, presumably only temporary, through the whole shrimp or replacement of unlabelled zinc lost with the moult by labelled zinc, though both may occur together to some extent.

Effect of repeated counting on uptake of labelled zinc

Since the initial rate of labelled zinc uptake cannot be determined precisely for those shrimp counted once at the end of the experiment ('single sample'), comparison with repeatedly sampled shrimp was made on the basis of the labelled zinc concentration in the shrimp.

There was no significant difference in the mean labelled zinc concentration in the 2 groups at the end of the experiment (single sample; $\bar{x} = 2.32$, $s = 0.94$); repeatedly sampled; $\bar{x} = 2.75$, $s = 0.72$; $p \simeq 0.4$). It can be concluded therefore that any stress caused by repeated sampling of individual had no effect upon the rate of labelled zinc uptake.

DISCUSSION

In experiments where *Palaemon elegans* were exposed to dissolved zinc concentrations, ranging from 10 to 100 μg Zn l^{-1}, for up to 21 d, there is no evidence for an increase in shrimp total zinc concentrations. These data support the conclusion of White and Rainbow (1982) that *P. elegans* regulates body zinc concentrations when exposed to elevated dissolved zinc concentrations, at least within certain limits. Similar regulation of zinc has been reported in a number of decapod crustaceans and may be characteristic of the group as a whole (Bryan, 1964, 1966, 1968, 1976; Ray et al., 1980).

When exposed to elevated zinc concentrations, the crab *Carcinus maenas* shows an initial increase in the body zinc concentration but after about 2 wk, it stabilizes and remains constant at this slightly elevated level (Bryan, 1966). This acclimation period in *C. maenas* may be due to a delay in increasing the rate of zinc excretion to balance the rate of uptake. An acclimation phase is not apparent in *Pandalus montagui* (Ray et al., 1980) nor in *Palaemon elegans,* suggesting that they respond more rapidly to changes in rates of zinc uptake.

It is not possible with the data available to construct a complete model for the flux of zinc through *Palaemon elegans,* but some of the elements from which one could be constructed can be identified. The accumulation of labelled zinc asymptotes to a fraction of total body zinc, indicating that zinc within the shrimp cannot be considered as a single homogeneous pool undergoing equal rates of exchange with zinc from the external environment. It appears that body zinc can be modelled as a number of pools undergoing exchange at different rates, the sizes of these pools not being fixed. At rapid rates of zinc flux, due to high external zinc concentrations or raised temperatures, a larger proportion of total body zinc appears to be labile than when the rate of zinc flux is low. Thus it seems that these pools of zinc are not discrete physical entities but rather a feature of rate processes within the shrimp, so that at high rates of zinc flux, labelled zinc is rapidly incorporated into more pools of zinc than when the rate of zinc flux is low.

A number of other studies have examined the flux of zinc through marine crustaceans using compartmental models, including those of Fowler et al. (1971), Heyraud and Fowler (1973), Small and Fowler (1973) and Small et al. (1973). These studies used Zn-65 to follow uptake and loss of zinc, but the accumulation of the isotope has not been expressed as a percentage of the total body zinc but rather as the concentration factor, i.e. the concentration of zinc-65 in the test animal divided by the concentration of zinc-65 in the seawater. As total zinc concentrations are not reported in the studies listed above, it is not possible to determine if these species are, like *Palaemon elegans,* regulating body zinc concentrations. Fowler et al. (1975) report that in the amphipod *Gammarus locusta* the concentration factor of Zn-65 levels off in continued exposure to labelled zinc indicating that an equilibrium is reached between rates of uptake and loss of Zn-65. Without data for total zinc concentrations, however, it is not

clear if this levelling off represents an equilibrium at raised body zinc levels, or total zinc exchange between labelled and non-labelled zinc (total zinc concentration remaining constant), or as in *P. elegans* an apparent asymptote caused by a proportion of the total regulated zinc content undergoing rapid exchange. Renfro et al. (1975) compared the concentration factors of Zn-65 and total zinc in *Carcinus maenas* and a benthic shrimp *Lysmata seticaudata* after 96 d exposure to labelled zinc. Although net accumulation of Zn-65 appeared to cease or slow down before the end of the experiment, the concentration factors for Zn-65 were less than the concentration factors for total zinc, indicating that isotopic equilibrium had not been attained. This suggests that there are pools of zinc within these 2 decapods exchanging only slowly with zinc taken up from the surrounding seawater in agreement with the data for *P. elegans.*

Fowler et al (1971), Heyraud and Fowler (1973), Small et al. (1973) and Fowler et al. (1975) all report that the loss of previously accumulated Zn-65 crustaceans is not exponential with time and can be described by the loss of Zn-65, at different rates, from at least 2 zinc compartments. The rate of Zn-65 loss from the 'slowest' of these compartments varied between a biological half life of 76 d in *Gammarus locusta* (Fowler et al., 1975) and 140 d in *Euphausia pacifica* (Fowler et al., 1971) though these rates may vary with the rate of stable zinc flux. Due to the low number of sampling points it was not possible to resolve the loss of labelled zinc by *Palaemon elegans* into a number of components, the data being adequately described by a single exponential term loss equation.

Zinc flux through *Palaemon elegans* does not proceed at a uniform rate, varying with dissolved zinc concentrations and temperature. There is a linear relation between zinc flux and external zinc concentration, at least between 10 and 42.5 μg Zn l^{-1}. It may be that this relation is linear over the whole range of concentrations at which *P. elegans* regulates zinc.

Extrapolating the data from Experiment II to find the rate of zinc exchange and therefore zinc flux at 0 μg Zn l^{-1} gives a rate of zinc exchange of 8.26 ng Zn g^{-1}, suggesting that there may be some active uptake of zinc. Owing to variation about the regression line, however, the confidence limits for the intersection of the extrapolated line with y-axis (Fig. 10) include zero, i.e. the rate of zinc flux at 0 μg Zn l^{-1} is not significantly different from 0 μg Zn g^{-1} d^{-1} ($p \simeq 0.1$). It is interesting to note, however, that Bryan (1966) reports some evidence for active transport of zinc in *Carcinus maenas* and *Homarus vulgaris.*

As total body zinc concentrations of *Palaemon elegans* remain constant independently of the rate of zinc uptake, at least up to 100 μg Zn l^{-1}, it follows that the rates of zinc loss and uptake are equal. Although not equated to rates of zinc-65 uptake, losses of Zn-65 under a range of zinc concentrations have been measured in the crab *Pachygrapsus marmoratus,* and found to be proportional to the external zinc concentration (Heyraud and Fowler, 1973) indicating that zinc flux is related to the external zinc concentration.

Temperature has a marked effect on zinc flux through *Palaemon elegans,* doubling with every 4 C° increase. Increased flux at higher temperature may be due to increases in metabolic rate which secondarily bring zinc across the body surfaces and/or to increases in simple physical transport processes across membranes. It seems unlikely that increased metabolic activity can account for all of the observed increase in zinc flux (2 fold per 4 C° increase) as respiration rates in crustaceans only increase by approximately 2 to 3 fold per 10 C° increase (Wolvekamp and Waterman, 1960). An alternative is that increased zinc flux at higher temperatures is related to an increase in the ventilation rate. As, however, ventilation volume in decapods increases linearly with temperature (Wolvekamp and Waterman, 1960) again this alone cannot account for the observed increase in zinc flux.

Rates of zinc flux through *Palaemon elegans* vary with both temperature and external zinc concentration, the highest rate of zinc exchange measured being 14.4 % of the total zinc content of the shrimp per day, or in absolute terms 11.9 μg Zn g^{-1} d^{-1} (Experiment II, 100 μg Zn l^{-1} at 20 °C). As total body zinc concentrations are regulated despite such high rates of zinc uptake there must be an efficient system of excretion in *P. elegans* to clear excess zinc. In the lobster *Homarus vulgaris* urine zinc concentrations increased with external zinc concentrations, and urine may therefore be an important route of zinc excretion though losses across the body surface may also be appreciable (Bryan, 1964). In *Carcinus maenas* major losses of zinc occur across the gills, being increasingly important in seawater with high zinc concentrations and hence during rapid zinc loss, but urinary excretion does occur and is important at low zinc concentrations (Bryan, 1966). Although no attempt has been made in this study to examine how excess zinc is lost by *P. elegans* (except due to moulting), other data (White and Rainbow, unpubl.) suggest that the gills may be a major site of loss.

Zinc flux through *Palaemon elegans* is clearly modified by moulting which increases the uptake of labelled zinc from solution. It is difficult to determine precisely the effect of moulting on labelled zinc uptake (and hence total zinc flux) as it does not appear to be discrete, increased uptake continuing for some time after moulting. It is not clear from the data if this

increased uptake represents replacement of zinc lost with the moult or if zinc flux is generally increased, perhaps due to increased permeability of the cuticle.

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