Relative Contributions of Dietary and Waterborne Zinc in the Rainbow Trout, *Salmo gairdneri*

D. J. Spry, P. V. Hodson, and C. M. Wood

Great Lakes Fisheries Research Branch, Canada Center for Inland Waters, 867 Lakeshore Road, Burlington, Ont. L7R 4A6
and
Department of Biology, McMaster University, Hamilton, Ont. L8S 4K1

Spry, D. J., P. V. Hodson, and C. M. Wood. 1988. Relative contributions of dietary and waterborne zinc in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 45: 32–41.

Rainbow trout, *Salmo gairdneri*, were fed purified diets with zinc concentrations ranging from deficient to excessive (1, 90, 590 μg Zn·g^{-1}) and simultaneously exposed to a range of waterborne [Zn] (7, 39, 148, 529 μg Zn·L^{-1}). After 1 wk, fish fed the deficient diet, at ambient waterborne [Zn], had low plasma [Zn] which decreased further during the 16-wk experiment. Growth ceased after 12 wk; hematocrit and plasma protein were depressed. Both whole body [Zn] and body burden decreased by 16 wk, but most other elements were elevated. Increasing waterborne [Zn] alone increased plasma [Zn], whole body [Zn], and growth in a graded manner and normalized hematocrit, plasma protein, and other whole body elements. Increasing dietary [Zn] to 90 μg Zn·g^{-1} at ambient waterborne [Zn] prevented depression of plasma [Zn] and permitted normal growth and whole body [Zn]. Zinc uptake from water, probably across the gills, was independent of uptake from the diet since at any dietary [Zn], increasing the waterborne [Zn] resulted in increased whole body [Zn]. Even when dietary [Zn] was adequate, the waterborne contribution was as high as 57%, and 100% when the dietary [Zn] was deficient. There were no toxic effects on any of the variables measured.

Des truites arc-en-ciel, *Salmo gairdneri*, ont été soumises à un régime alimentaire purifié dont les concentratrions en zinc variaient d'insuffisantes à excessives (1, 90, 590 μg·g^{-1} de Zn) et simultanément exposées à des milieux (eau) de différentes [Zn] (7, 39, 148, 529 μg·L^{-1} de Zn). Au bout d'une semaine, la [Zn] plasmatique des poissons soumis au régime carencé et exposés à la [Zn] ambiante était faible et a continué à diminuer pendant les 16 sem de l'expérience. La croissance s'est arrêtée après 12 sem; l'hématocrite et le taux de protéine dans le plasma avaient diminué. La [Zn] corporelle et la charge corporelle en Zn ont diminué au cours des 16 semaines, mais celles de la plupart des autres éléments avaient augmenté. Le seul fait d'augmenter la [Zn] dans l'eau a entraîné une hausse graduelle de la [Zn] plasmatique, de la [Zn] corporelle et de la croissance ainsi que la normalisation de l'hématocrite, du taux de protéine dans le plasma et des concentrations des autres éléments corporels. L'augmentation de la [Zn] de la ration alimentaire à 90 μg·g^{-1} de Zn pour les poissons exposés à la [Zn] ambiante a permis d'éviter la baisse de la [Zn] plasmatique et d'obtenir une croissance et une [Zn] corporelle normales. La captation du zinc provenant du milieu, probablement à travers les branchies, était indépendante de la captation du zing présent dans l'alimentation, en effet, peu importe quelle était la [Zn] dans l'alimention de la [Zn] de l'eau se traduisait par une augmentation de la [Zn] corporelle. Même lorsque la [Zn] dans l'alimentation était appropriée, la contribution du milieu pouvait atteindre 57 %; elle était de 100 % lorsque la [Zn] dans l'alimentation était insuffisante. Les variables mesurées n'ont pas permis de déceler d'effets toxiques.

Received January 28, 1987
Accepted August 10, 1987
(J9115)

Reçu le 28 janvier 1987
Accepté le 10 août 1987

A dietary requirement for zinc has been documented in rainbow trout (*Salmo gairdneri*) based upon low growth, high mortality, cataracts, and fin erosion induced in trout fed a diet containing 1–4 μg Zn·g^{-1} (Ogino and Yang 1978; Wekell et al. 1983). Supplementing the diet to 15–30 μg·g^{-1} alleviated these symptoms. Diets containing high levels of calcium (and phosphate) and/or phytate (myoinositol hexaphosphate) increased the requirement for dietary zinc in freshwater salmonids, since they reduced zinc bioavailability (Ketola 1979; Hardy and Shearer 1985; Richardson et al. 1985). Poor growth, cataracts, and high mortality were reported, together with low plasma and whole body [Zn]. In contrast, high levels of dietary zinc appear fairly benign. Thus, Wekell et al. (1983) found no growth inhibition in fingerling trout over a dietary range of 440–1700 μg Zn·g^{-1}. Liver and whole blood [Zn] increased significantly over this range but in an attenuated fashion, whereas gill [Zn] increased almost linearly. No mortality or toxic symptoms occurred.

While dietary concentrations up to 1700 μg Zn·g^{-1} are well tolerated, waterborne concentrations of 1 μg Zn·mL^{-1} (i.e. 1000 μg ·L^{-1}) are well into the toxic range for rainbow trout (Spear 1981). Detrimental effects on ionoregulation, acid–base balance, and gas exchange have been documented (Skidmore 1970; Lewis and Lewis 1971; Sellers et al. 1975; Spry and Wood 1984, 1985). The maximum acceptable toxicant concentration based upon fry mortality for rainbow trout in hard water was 320–640 μg Zn·L^{-1} (Sinley et al. 1974). Even below this range, elevated waterborne [Zn] depressed growth in several

species due to appetite suppression and/or decreased conversion (Bengtsson 1974; Watson and McKeown 1976; Farmer et al. 1979).

Zinc can be accumulated directly from the water. Holcombe et al. (1979) exposed brook trout (*Salvelinus fontinalis*) for 24 wk to a waterborne [Zn] of 534 $\mu g \cdot L^{-1}$ and found threefold increases above controls in gill, kidney, and opercular bone [Zn], whereas brain and muscle were unaffected. Ration manipulation was used by Farmer et al. (1979) to study its effect upon zinc uptake from water. Freshwater-adapted Atlantic salmon (*Salmo salar*), fed a practical diet to satiation (dietary [Zn] unspecified), reached steady-state whole body concentrations after 50 d. These steady-state whole body [Zn] reflected waterborne [Zn] in a concentration-dependent fashion. Restriction of the ration to 2 or 3.5% body weight$\cdot d^{-1}$ resulted in continual accumulation over the 80-d exposure. The rate of accumulation was directly proportional to the waterborne [Zn].

Relative contributions of dietary versus waterborne zinc to zinc uptake have been assessed in marine fish. In plaice (*Pleuronectes platessa*), food was the major source for larvae and adults when waterborne [Zn] was low (15 $\mu g \cdot L^{-1}$), based upon ^{65}Zn accumulation (Pentreath 1973, 1976). However, in the same species, water contributed <10% at 100 $\mu g \cdot L^{-1}$ but increased to 50% at 600 $\mu g \cdot L^{-1}$ (Milner 1982), indicating that waterborne input can be significant. Willis and Sunda (1984) used a model food chain (*Chlamydomonas* ⟶ *Artemia* ⟶ fish) and a single waterborne [Zn] (0.21 μg free $Zn \cdot L^{-1}$) to estimate relative contributions in mosquito fish (*Gambusia affinis*) and spot (*Leiostomus xanthurus*). Food supplied 77% of the isotope load in mosquito fish after 120 d and 82% in spot after 30 d. Marine fish drink considerable amounts of the medium (~4–13% body weight$\cdot d^{-1}$) (Smith 1930; Shehadeh and Gordon 1969), and this could contribute half or more of the waterborne input. The remaining input from the water was suggested to be passive, being driven by the large gradient formed by the adsorption of zinc on the gill (Pentreath 1973).

There have been no directly comparable studies in freshwater, where drinking rates are known to be negligible (Shehadeh and Gordon 1969; Oduleye 1975). Stary et al. (1982) exposed guppies (*Lebistes reticulata*) to ^{65}Zn in either the water or in the diet. They concluded that diet was the more important factor. Pumpkinseed sunfish (*Lepomis gibbosus*), exposed to ^{65}Zn in either the diet or the water, accumulated ^{65}Zn faster from the diet, whether the diet was natural (snail) or purified (Merlini et al. 1976). In contrast, waterborne zinc was the main source for goldfish (*Carassius auratus*) (no details, Berg and Brazzelli, cited in Merlini et al. 1976).

It thus appears that in both freshwater and seawater fish there is a capacity to accumulate zinc through both routes, but the dietary source is usually the more important. However, the interactions between the two pathways appear highly complex and dependent upon the zinc concentrations in the two sources. It remains unclear whether the waterbourne route can replace the dietary route.

The aims of the present experiment were to (1) see if a dietary [Zn] deficiency similar to that previously reported could be induced in juvenile rainbow trout, and if so, whether trout could obtain sufficient zinc from the water to overcome this deficiency, (2) quantify uptake through the two pathways and to see if uptake through one route influenced uptake through the other, (3) look for toxic effects of higher dietary or waterborne zinc, and (4) see if metabolism of other elements (e.g. calcium) was altered by either dietary and/or waterborne zinc.

Materials and Methods

Experimental Design

Rainbow trout (*Salmo gairdneri*) fingerlings (3–4 g) were purchased from Spring Valley Trout Farm, Petersburg, Ont. They were held in flowing Burlington tap water dechlorinated by carbon filtration and sulphite addition. The photoperiod was 18 h light : 6 h dark, and ambient [Zn] was 7 ± 8 $\mu g \cdot L^{-1}$ (mean ± SD). Fish were held for 2 wk in a large holding tank and gradually changed from a commerical diet (~200 μg $Zn \cdot g^{-1}$, Martin Feed Mills, Elmira, Ont.) to the semipurified diet described below, and the temperature was raised from 11 to 15°C. After this initial acclimation, fish were transferred to the experimental battery which consisted of a grid of 30 tanks. Each tank was approximately a 30-cm cube holding 20–23 L of water. Water flowed continuously to the tanks from mixing chambers at 300 $mL \cdot min^{-1} \cdot tank^{-1}$, giving a 95% particle replacement time of 3–4 h (Sprague 1969). Tanks were gently aerated to ensure >90% saturation.

The experimental design was a complete block factorial consisting of 3 waterborne [Zn] levels (ambient plus 2 treatments) × 3 dietary [Zn] levels × 3 replicates (blocks) for a total of 27 tanks. To determine the effect of an even greater waterborne [Zn] on the experimental variables, particularly whether or not toxicity would occur, the remaining three tanks were used for three replicates of a further increase in the waterborne [Zn] to 529 $\mu g \cdot L^{-1}$ at the highest dietary [Zn] (590 $\mu g \cdot g^{-1}$). The experiment ran for 16 wk. The waterborne zinc (as $ZnSO_4 \cdot 7H_2O$) was metered by a peristaltic pump (Gilson minipulse) into the mixing cells of the diluter from three stock bottles. Daily measurements of waterborne [Zn] gave means ± SD of 7 ± 8 $\mu g \cdot L^{-1}$ for ambient and 39 ± 14, 148 ± 35, and 529 ± 85 $\mu g \cdot L^{-1}$ for the three elevated levels. Temperature was 15.3 ± 1.3°C. Weekly measurements of pH, dissolved oxygen, and conductivity were 8.16 ± 0.23, 8.8 ± 1.0 $mg \cdot L^{-1}$, and 281 ± 10 μS. Previously reported hardness and alkalinity values (as $CaCO_3$) were 135 ± 2 and 90 ± 4 $mg \cdot L^{-1}$ (Hodson et al. 1978).

On the basis of past experiments under the same conditions, some aggression and subsequent mortality was expected (Hodson et al. 1978). Small lengths of 1-in. inside diameter PVC pipe were added to provide shelter, and the tank lids were covered with black plastic on week 2 in an attempt to minimize this effect, but aggression persisted. Typically, fish showing severe signs of aggression did not feed. Opercles and fins became eroded until the fish was unable to propel itself or remain upright, and fungal infestation was common. Fish that rolled over were considered moribund and were recorded and sacrificed. The same criteria were used for removal of diseased fish (see Results).

Diet

The semipurified diet formulated by Dr. J. W. Hilton (University of Guelph) (Table 1) was calculated to provide all essential nutrients. Dietary [Zn] was nominally 0, 100 (practical level), and 700 μg $Zn \cdot g^{-1}$ (as sulphate). This was confirmed by dry ashing at 400°C and atomic absorption spectophotometry, and independently by neutron activation analysis, yielding measured [Zn] = 1, 90, and 590 μg $Zn \cdot g^{-1}$. The proximate composition was 40% protein, 15% lipid, 8% ash, and 9% moisture. All diets were steam-pelleted and crumbled to sizes readily acceptable to the fish over the course of the experiment.

TABLE 1. Formulation of basal diet fed to rainbow trout. The mineral premix provided the following (g·kg dry diet^{-1}): $CaHPO_4 \cdot 2H_2O$, 30; $CaCO_3$, 3; NaCl 15; K_2SO_4, 20; $MgSO_4$, 10; $FeSO_4 \cdot 7H_2O$, 0.7; $MnSO_4 \cdot H_2O$, 0.3; $CuSO_4 \cdot 5H_2O$, 0.16; KI, 0.015. The vitamin premix provided the following (mg·kg dry diet^{-1}, unless noted otherwise): thiamine, 10; riboflavin, 10; pantothenic acid, 10; niacin, 20; pyridoxine, 40; biotin, 0.5; folic acid, 20; vitamin B-12, 0.2; inositol, 500; ascorbic acid, 1000; choline chloride, 5500; retinyl palmitate, 7000 iu; cholecalciferol, 3000 iu; DL-α-tocopheryl acetate, 200 iu; menadione, 50; butylated hydroxytoluene (BHT), 25.

Ingredient	Concentration (%)
Egg albumin	40
Gelatin	10
Alpha-starch	10
Cerelose	10
Cellulose	5
Vitamin premix	2
Mineral premix	8
Fish oil	15

Fish were conditioned to the 90 μg Zn·g^{-1} diet for 1 wk prior to their placement in the test battery.

To begin the experiment, the rainbow trout fingerlings were added to the tanks in a stratified hierarchical fashion, 34 per tank. Small adjustments were necessary in a few tanks to standardize the initial starting weights. Trout were fed to satiation (cessation of feeding) three to four times daily (except prior to sampling, see below), decreasing to two to three times daily after 6 wk. Food consumption was monitored by feeding fish from preweighed aliquots of the diet. All diets were stored at −20°C when not in use. Fish weight was measured by batch weighing (netting all fish in the tank into a tared, water-filled bucket) once a week for the first 4 wk and then every other week for the remainder of the experiment. When weighing coincided with sampling, the sample was taken first and then the remaining fish in the tank were weighed and the weight of fish sampled added to the tank weight. Mean fish weight was calculated as the total fish weight divided by the number of fish per tank.

Sampling

For each sample day (weeks 0, 1, 2, 4, 8, and 16), 90 fish were sampled in random order, three from each tank. Food was withheld for 36–48 h prior to sampling. Fish were netted indiscriminately from the tank and blood samples collected in ammonium heparinized glass hematocrit tubes from the severed caudal peduncle. Hematocrit tubes were stored briefly on ice and then centrifuged. Hematocrit was read and plasma recovered from the tubes for later analysis for Na, Ca, Mg, and Zn (all by atomic absorption spectrophotometry on a Varian AA-1275) and, for week 16, total protein determined by refractometry on an American Optical TS meter (Alexander and Bell 1980). Fish weight and standard length were recorded and the general appearance of the fish noted for overt pathology, particularly cataracts and fin erosion, as these are symptomatic of zinc deficiency in trout (Ogino and Yang 1978). Fish were individually bagged and frozen at −20°C for whole body ion analysis.

Neutron Activation Analysis

Whole body element concentrations were determined by exposure of fish samples to thermal neutrons in the McMaster University reactor. Fish from the 16-wk sample were slit longitudinally to expose the gut and then freeze dried to determine water content. After the intestines were examined, and any food or fecal matter removed, the entire fish was sealed in 7-g polyethylene vials. For short-lived isotopes (I, Br, Mg, Cu, Na, V, K, Cl, Mn, Ca), the sample was irradiated in a neutron flux of 5×10^{12} n·cm^{-2}·s^{-1} for a 10-s irradiation. After a delay time of 120 s the samples were counted for 600 s using an APTEC hyperpure germanium detector (22% efficient with a 1.9 keV resolution at the 1332-keV ^{60}Co peak). Counts were accumulated on a Canberra multi channel analyzer model 40 or 90 equipped with internal live time correction and pile up rejection unit. Citrus leaf (NBS 1572) was used as the standard. For long-lived isotopes (Fe, Zn), the samples were loaded directly into the core (flux intensity 7.5×10^{12} n·cm^{-2}·s^{-1}) for 5–6 h. After a delay time of about 3 wk during which time the short- and medium-lived isotopes decayed, the samples were counted for a period of 900 s. Here the standard used was lobster hepatopancreas (TORT-1, National Research Council of Canada, Ottawa, Ont.). Different sample groups were standardized for variations in flux intensity by the use of flux wires.

Calculations and Statistics

Since the number of fish in each tank decreased due to sampling, mortality was expressed as a function of the total number of fish days accumulated per tank. Feed efficiency was the wet weight gain divided by the wet weight of feed given per tank. Feed intake (percentage of body weight per day) was calculated from the wet weight of feed given over the interval between fish weighings, the average of the weights at the two times, and the elapsed time. Condition factor was calculated as the wet weight (grams)/standard length^3 (centimetres) × 100. The apparent zinc retention (percentage) was calculated from the difference between the initial (week 0) and final (week 16) body burden (micrograms of zinc per fish) divided by the amount of zinc fed (dietary [Zn] × feed given), and expressed as a percentage. Estimates of the relative contribution of waterborne zinc to the final body zinc burden were arrived at by assuming that at ambient waterborne [Zn], uptake from the water was negligiable, and that the entire zinc load was from the diet. For each water level above ambient, the difference between the initial and final body burden (micrograms of zinc per fish) *minus* the same difference at ambient [Zn] for that dietary level was considered the absolute amount contributed by the water. In turn, this was expressed as a percentage of the total accumulated burden over the 16 wk for that dietary treatment.

The balanced part of the experiment (i.e. all the treatments exclusive of the high water × high diet) was analysed by two-way ANOVA having 3 dietary × 3 waterborne × 3 replicates, with 3 observations per cell for a total of 81 observations. Interaction terms between replicates and the two other factors were significant in only two instances. In all other cases, therefore, the pooled interaction variance was used to test the main effects. Where there were significant treatment effects, individual means were tested for significant differences by Peritz' *F* test (Harper 1984).

Results

Growth and Survival

Although fish were sampled at weeks 0, 1, 2, 4, 8, and 16, the trends shown over the course of the experiment were borne out by the results at week 16. Thus, except in rare instances,

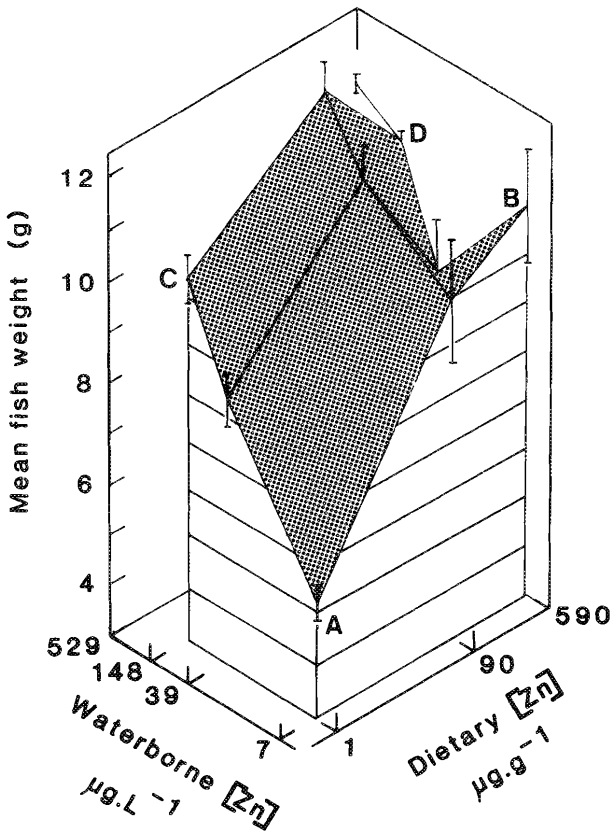

FIG. 1. Mean fish weight at the end of the experiment as a function of waterborne and dietary inputs. Values are means ± SEM (9), (3 fish from 3 different replicates). Letters ABCD are simply for orientation; see text for details.

only the week 0 (hereafter called the initial values) and week 16 values will be discussed.

The fish fed actively, but growth was less than on practical diets, or even casein-based semipurified diets (e.g. Hodson et al. 1980). Aggression may have been a factor here. There was no significant growth over the first 4 wk and no difference in growth among treatments over the first 6 wk. Thereafter, a gradual separation occurred, most clearly seen in the fish on the lowest dietary [Zn] (1 $\mu g \cdot g^{-1}$) at the lowest waterborne [Zn] (7 $\mu g \cdot L^{-1}$). These fish (hereafter called the deficient fish) had noticeably slower growth by week 8. The deficient fish continued to grow until week 12, when the mean weight per tank had risen from 3.20 ± 0.03 (3) to 5.49 ± 0.28 (3) g. No further growth occurred. An increase in zinc either from the diet alone (Fig. 1, edge AB) or from the water alone (Fig. 1, edge AC) provided for continued growth in a concentration-dependent fashion. By the end of the experiment, fish at the lowest treatment combination were less than half the size of the fish in the other treatments (Fig. 1), illustrating significant effects of both the water and dietary treatments. Based upon growth and mortality, there were no toxic effects of the high levels of either dietary or waterborne [Zn]. In fact, fish vigour appeared to increase with increasing waterborne [Zn].

Fish were fed about 2.5–4.0% of their body weight·d^{-1}. Intake tended to be lower at the highest dietary [Zn] (590 $\mu g \cdot g^{-1}$), perhaps reflecting a palatability effect. Over the last 2 wk of the experiment, deficient fish were fed more than any other treatment, and although the feed efficiency values were low for all treatments (range of means 19 ± 5 to 41 ± 9% (3)), fish in the deficient diet had an efficiency of zero. Despite the fact that fish in some treatments did not grow after 12 wk, there was no significant difference in condition factor among treatments (1.30 ± 0.04(9) to 1.35 ± 0.03 (9)), an effect that may have been masked by an apparently, but not significantly, higher water percentage in the deficient treatment (73.4 ± 0.5%) compared with the other treatments (means ranging from 70.0 ± 0.07 to 72.1 ± 0.6%).

Some disease and mortality occurred. Mortality from other than accidental causes was 7% (73/1020). Of this, 49% was due to nodular gill disease (Daoust and Ferguson 1985), aggression (26%), indeterminate causes (18%), and fungal infestation (7%). Disease mortality persisted despite formalin treatments (5-min immersions in 1:8000 dilution) in weeks 8 and 10. ANOVA indicated that disease was treatment related. There was a highly significant decrease in mortality with increasing waterborne [Zn]. Zinc-deficient fish seemed particularly susceptible to both nodular gill disease (most prevalent) and fungal infestation. There were only two incidents of gross cataracts, both in zinc-deficient fish. At the end of the experiment, all fish were examined for the presence or absence of nodules on the gill. There was clear evidence of the disease at all dietary treatments in fish at the lowest (i.e. ambient) waterborne [Zn]. At increased waterborne [Zn], however, very little evidence of the disease was found.

Blood Variables

Hematocrits at week 16 (ranging from 30.2 ± 1.3 (9) to 36.1 ± 2.0% (9)) were similar to the week 0 value, except for the fish in the deficient treatment which were slightly anemic (25.9 ± 2.1% (9)) due to a significant diet effect. Plasma protein was also depressed in the deficient treatment (2.1 ± 1.0 g·100 mL^{-1} (9) compared with all other treatments (3.4 ± 0.7 (9) to 4.0 ± 0.8 (9)). There was a significant diet effect and a water–diet interaction. Plasma concentrations of major ions did not change over the course of the experiment. Final values (millimoles per litre) were as follows: Na, 148 ± 2 to 158 ± 2; Ca, 2.2 ± 0.3 to 2.6 ± 0.1; Mg, 1.0 ± 0.1 to 1.1 ± 0.1 (9).

In contrast, the effect of treatment upon plasma [Zn] was striking. There were significant water and diet effects, but no interaction. Initial values were 0.15 ± 0.004 mmol·L^{-1} (88) (9.57 ± 0.23 mg·L^{-1}). After only 1 wk of treatment, all of the fish on the deficient diet (Fig. 2A, edge AC) had significantly lower plasma [Zn]. Increasing the waterborne [Zn] increased the plasma [Zn], but still not to initial levels. Those on the intermediate diet were not different from initial values regardless of the waterborne concentrations. Fish on the high [Zn] diet were no different from the initial values at ambient waterborne concentrations, but as waterborne [Zn] increased, so did the plasma [Zn]. The lack of an interaction term between the diet and waterborne [Zn] confirmed that uptake via one route was independent of uptake via the other. The results at week 16 (Fig. 2B) were similar, except that at ambient waterborne [Zn] the plasma [Zn] from fish on the deficient diet had fallen to the point where it was scarcely detectable. Again, increases in waterborne [Zn] did permit some increases in plasma [Zn], but still not to initial levels. At higher dietary [Zn], there was generally a plateau around initial levels. Only at the highest waterborne and dietary level was there a significant increase above this plateau. Assuming the normal condition to be the medium diet and the ambient waterborne exposure, a decrement in dietary [Zn] from 90 to 1 μg Zn·L^{-1} under these water exposure conditions had a drastic effect on the plasma zinc status of

the fish. Increasing the dietary [Zn] from 90 to 590 μg Zn·L^{-1} caused no increase in plasma [Zn] (Fig. 2A, 2B, edge EB). Increasing the waterborne [Zn] from 7 to 148 μg Zn·L^{-1} had no effect on plasma [Zn] at higher dietary [Zn] (Fig. 2A, 2B, edge BD). This is indicative of plasma homeostatis over a broad range of dietary and waterborne [Zn].

Whole Body Elemental Analysis

Most of the whole body elements which were readily detectable using neutron activation analysis (Tables 2, 3) showed significant treatment effects. All the results are expressed on a dry weight basis. Since percent water was not significantly different across treatments, expressing results on a wet weight basis gave identical trends.

High waterborne [Zn] exposures had no effect on whole body elemental composition (other than zinc), further supporting the lack of any toxic effect on zinc on ion regulation, as seen previously with the plasma data. With the exception of whole body [Zn] (which showed a graded response) and [Cu] (which was unaffected), treatment differences were restricted to the deficient fish, and all showed virtually the same pattern of response to treatment, but with some variation in statistical significance.

Whole body [Ca] (Table 2) showed the treatment response most clearly. Elemental concentrations rose significantly, but only in the deficient fish. In contrast, the other treatments constituted a plateau which showed neither trends nor significant differences. This single cell caused significant treatment effects not only for dietary and waterborne [Zn] effects, but for the interaction as well. Similar significant responses were seen for Na, Mg, Br, and Fe. The elements V, K, and Cl showed similar elevations in deficient fish, but these were not significant. Whole body [Mn] was slightly different because in addition to elevations in whole body concentration seen in deficient fish, only the diet had a highly significant effect showing a decreasing whole body concentration as dietary [Zn] increased. Whole body [Cu] showed no treatment effects at all.

The generally higher elemental concentration in the deficient fish, compared with the other treatments at week 16, may have been an effect of fish size, since a scatter plot of whole body [Ca] versus fish weight irrespective of treatment (not shown) revealed an exponential decline with increasing size up to about 15 g. In fact, compared with initial values (Tables 2, 3), deficient fish had similar or lower concentrations for 8 of 11 elements. This again suggests a size effect. Only for Ca, Mn, and V were concentrations elevated significantly above initial values.

In contrast, whole body [Zn] (Fig. 3) reflected the loading to which the fish were subjected. Both diet and water were highly significant factors, and showed clear trends. There was no interaction between them, again indicating that zinc uptake by one route was independent of uptake via the other. The diet had by far the largest F value and was the major determinant of whole body [Zn] (Fig. 3). Fish on the deficient diet at ambient waterborne [Zn] (7 μg·L^{-1}) had the lowest whole body [Zn]. This did not increase when waterborne [Zn] was raised to 39 μg·L^{-1}, despite the fact that plasma [Zn] was higher (Fig. 2). Thus, plasma [Zn] under these conditions was more sensitive to loading than was whole body concentration. Only at a waterborne [Zn] = 148 μg Zn·L^{-1} was there an increased whole body concentration. At the other two dietary levels there were modest increases in the [Zn] as the waterborne [Zn] increased. Finally, at the highest dietary and the highest waterborne [Zn] there was a significant increase in whole body [Zn] to nearly double that of the week 0 value (week 0 = 1.41 ± 0.05 μmol·g^{-1} (15)). The deficient fish, in contrast, had whole body [Zn] of half the initial value. Unlike plasma [Zn] (Fig. 2B),

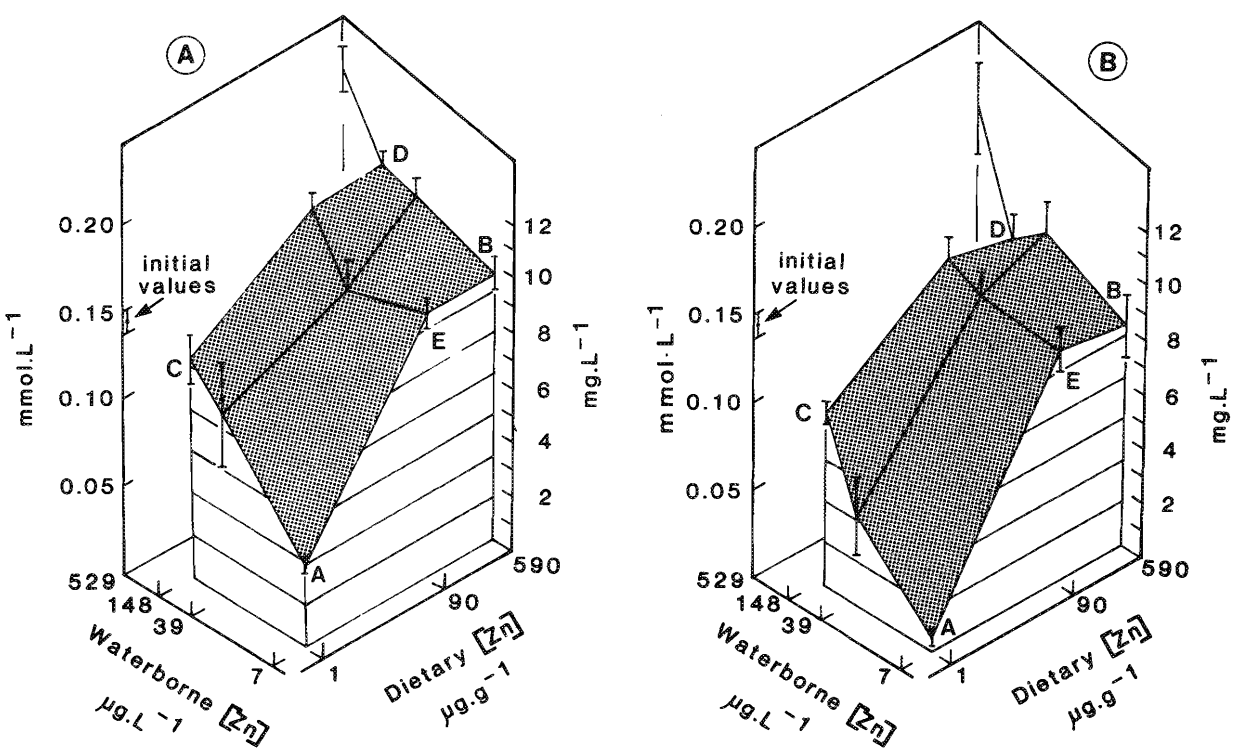

FIG. 2. Plasma [Zn] (mmol·L^{-1}) after (A) 1 wk of exposure and (B) 16 wk of exposure to various waterborne and dietary zinc input. Other details as in Fig. 1. Initial values (week 0) were 0.142 ± 0.004 mmol·L^{-1} (88).

TABLE 2. Whole body macronutrient (Martell 1975) concentrations at week 16. Values are μmol·g dry weight^{-1}, means ± SEM (9), except mean weight where units are g and n = 3.

		Weight (g)	Na	Mg	Cl	K	Ca
7	1	5.3±0.3	176±7	48±2	150±8	253±8	685±29
	90	9.9±1.3	140±9	40±2	127±10	225±9	458±25
	590	10.9±1.2	139±7	39±2	127±7	236±10	473±21
39	1	8.3±0.5	148±5	39±2	133±6	232±10	502±19
	90	11.3±0.7	147±8	40±2	137±10	238±10	465±28
	590	8.3±1.1	153±7	41±2	140±6	264±11	525±26
148	1	10.4±0.4	142±5	39±2	127±7	225±9	481±16
	90	12.6±0.6	134±6	38±2	126±6	222±10	423±19
	590	10.7±0.2	145±7	37±2	134±7	233±11	490±30
529	590	11.4±0.1	139±7	36±2	133±7	237±11	450±19
Initial values (n = 15, except weight, where n = 30)		3.2±0.0	180±5	59±3	145±5	337±7	462±11

TABLE 3. Whole body micronutrient (Martell 1975; Br is not classed as a nutrient) element concentrations at week 16. Values are nmol·g dry weight^{-1}, means ± SEM (9).

		Zn	Fe	Cu	Mn	V	Br
7	1	586±20	956±75	26±13	233±14	29±3	85±8
	90	1163±44	546±33	64±12	158±22	17±2	56±5
	590	1526±75	561±42	39±9	116±9	15±3	56±5
39	1	592±68	625±39	50±11	168±16	18±3	57±3
	90	1293±41	609±63	70±18	146±15	16±2	56±7
	590	1709±127	548±46	61±20	107±12	18±4	61±9
148	1	1050±121	555±32	42±7	153±24	18±3	56±2
	90	1398±44	540±16	52±9	135±14	12±2	50±8
	590	1840±96	665±103	39±11	141±13	20±2	57±4
529	590	2737±219	568±35	24±9	139±7	16±2	58±4
Initial values (n = 15)		1409±48	781±91	114±20	148±7	6±2	109±9

whole body [Zn] (Fig. 3) showed no clear plateau region where its level was independent of dietary and waterborne [Zn].

Discussion

Effects on Variables other than Zinc

Growth on the zinc-adequate diets was less than expected, even for albumin-based diets (Ogino and Yang 1978; Wekell et al. 1983). Reasons for this are unknown, despite the fact that the diet was formulated to meet all known requirements. One possibility is that since the albumin was not heat treated, avidin may have rendered biotin less available. Low growth notwithstanding, all plasma variables were in the reported range for the species (Hille 1982). As well, all 11 measured whole body element concentrations fell within the ranges given by Shearer (1984) for the corresponding fish size.

There were clearly no toxic effects of exposure to high dietary and/or waterborne [Zn] based upon growth, mortality, major plasma ions, hematocrit, or plasma protein. Similarly, Wekell et al (1983) reported that dietary [Zn] as high as 1700 μg·g^{-1} had no toxic effect on growth and mortality. We also found no effects of high [Zn] upon whole body element concentrations, despite indications that high dietary [Zn] interfered with accumulation of copper in the liver of rainbow trout (Knox et al. 1984), and waterborne [Zn] = 800 μg·L^{-1} blocked net calcium uptake in soft water (Spry and Wood 1985).

Dietary restriction of zinc (1 μg·g^{-1}), on the other hand, rendered trout zinc deficient, using the criteria of cessation of growth, significant mortality, and decreased plasma and whole body [Zn]. Decreased plasma protein concentration and low hematocrit in zinc-deficient fish have not previously been reported and may signify reduced protein synthetic ability. Cataract formation seen in earlier studies of zinc deficiency (Ogino and Yang 1978; Ketola 1979) occurred in only two zinc-deficient fish in the present study and was not seen in the study of Wekell et al. (1983) using a similar diet. Additional dietary components such as high [Ca] and/or phytate which reduce the bioavailability of dietary zinc may be necessary for significant cataract formation (Richardson et al. 1985).

High mortality is always associated with zinc deficiency (Ogino and Yang 1978; Wekell et al. 1983), although Richardson et al. (1985) were the only ones to categorically state that this occurred in the absence of any identifiable pathogen. We found that mortality was generally restricted to deficient fish and was invariably associated with pathology such as fin erosion, fungal infection, and nodular gill disease. In part, this might be due to reduced immune function, which is common in zinc-deficient mammals (see Fraker et al. 1986). On the other hand, nodular gill disease was, after 16 wk, most

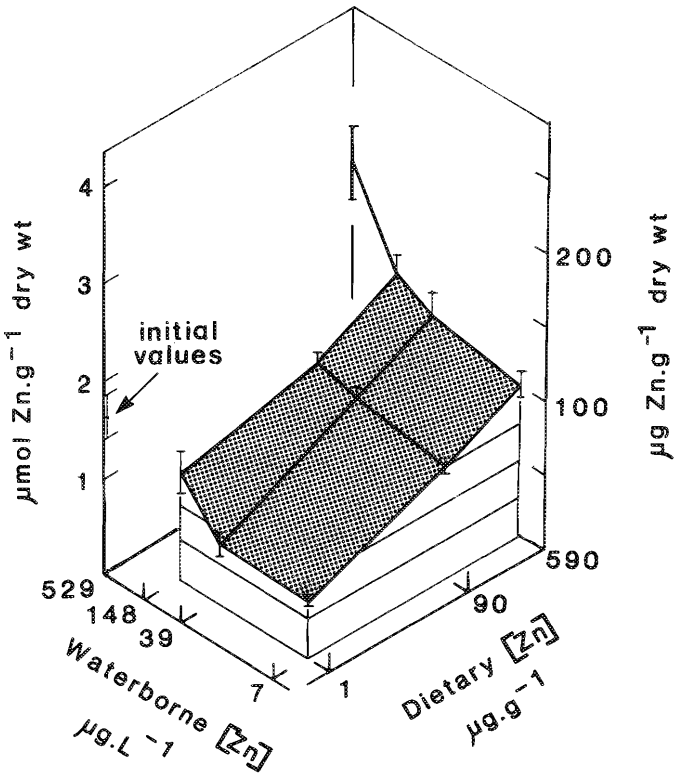

FIG. 3. Whole body [Zn] (μmol·g dry weight^{-1}) after 16 wk of treatment. Values are means ± SEM (9). Initital values (week 0) were 1.41 ± 0.05 μmol·g dry weight^{-1} (11).

prevalent in trout at ambient waterborne [Zn] regardless of dietary [Zn]. This suggests that elevated waterborne [Zn] itself may have had a prophylactic effect independent of the nutritional status of the fish, and warrants further study.

Concentration of all whole body elements (with the exception of copper) was elevated in deficient fish (Tables 2, 3). As noted earlier, this may have been a size effect, since these fish were significantly smaller than in other treatments, and negative correlations with size have been reported in wild fish (Wiener and Giesy 1979). Shearer (1984) however found positive correlations with size for most elements in laboratory-reared rainbow trout. Alternatively, a change in proximate body concentration may have accounted for the observed increases, especially for calcium, manganese, and vanadium where there was an elevation above initial values. Zinc-deficient fish had slightly higher ash weight and less lipid (Ogino and Yang 1978). Lipid has very low mineral concentrations, and high levels would tend to dilute elemental concentration (Shearer 1984).

A reciprocal relationship between whole body [Zn] and [Fe] has been noted by some authors (Ogino and Yang 1978; Wekell et al. 1986). In the present study this only occurred in deficient fish over the first dietary interval (1–90 μg·g^{-1}). Thereafter, the two responses paralleled each other. Increases in waterborne [Zn] resulted in a flat response for [Fe] across all dietary levels. Moreover, the pattern was the same for most other elements. These results do not support a causal relationship between zinc and iron over a broad range of dietary zinc.

Effects on Plasma and Whole Body [Zn]

There are relatively few measurements of plasma [Zn] in trout. Although mammalian values are about 0.01–0.02 mmol·L^{-1} (Underwood 1977), plasma [Zn] in fish is, for reasons which are unclear, frequently 10-fold higher (Bettger et al. 1987) and shows a higher range, 0.18–0.37 (Knox et al. 1984) and 0.28 (Zeitoun et al. 1977) falling to 0.06 ± 0.03 mmol·L^{-1} in rainbow trout fasted for 7 d (Spry and Wood 1984). Migratory (nonfeeding) sockeye salmon (*Oncorhynchus nerka*) had initial values of ~0.37 mmol·L^{-1}, which fell 50% over a month during spawning (Fletcher et al. 1975). In the present study, both the initial value (week 0, 0.15 mmol·L^{-1}) and all treatments at week 16 (with the exception of deficient fish) compared well with literature values for fish.

Plasma [Zn] after only 1 wk was a sensitive indicator of zinc restriction (Fig. 2A) and was depressed to quite low levels well before there was a treatment effect on growth or diseases appeared. By the end of the experiment, it was obvious that in the deficient fish, plasma [Zn] was extremely low (Fig. 2B) and growth had ceased. In fact, plasma [Zn] was a good indicator of growth, since it approximately mirrored the mean fish weight at the end of the experiment. These results indicated a broad plateau of zinc input (waterborne [Zn] = 7–148 μg·L^{-1}, dietary [Zn] = 9–590 μg·g^{-1}) over which plasma [Zn] remained unaltered, suggesting considerable homeostasis which was only overridden at extremes of very high or very low input.

Whole body [Zn] was less responsive than plasma [Zn] to changes in waterborne and dietary levels (compare Fig. 3 with 2B), although it was clearly affected by both. In the most deficient fish (waterborne [Zn] = 7 μg·L^{-1}, dietary [Zn] = 1 μg·g^{-1}) which had stopped growing, whole body [Zn] was ~0.6 μmol·g dry weight^{-1}. A similar *concentration*, but greater absolute load, was seen at the next higher waterborne [Zn] (39 μg·L^{-1}) where the fish grew nearly twice as large (Fig. 1 and 3). This concentration is likely the critical whole body concentration below which fish will not grow. Interestingly, Ogino and Yang (1978) found 0.43 μmol Zn·g dry weight^{-1} in zinc-deficient trout, and a similar range was found by Wekell et al. (1986) for trout on a zinc-deficient diet.

As dietary [Zn] increased, whole body [Zn] also increased, but in a log-linear fashion, with the result that fish retained much less zinc than they were being fed (see below). For example, at ambient waterborne [Zn], a nearly sevenfold increase in dietary [Zn] only increased whole body [Zn] by ~31%. The interesting fact here again is that when a waterborne zinc concentration was imposed in addition to diet, there were smaller, but nearly linear increases in whole body [Zn]. Some isolation thus exists between the two routes of uptake.

Calculations of dietary zinc retention (Fig. 4) indicated four major points. First, deficient fish had negative retention, indicating that these fish actually had lower zinc body burdens at week 16 than they did at week 0. This probably occurred because intestinal loss of zinc, which is large in mammals (Spencer et al. 1980; Weigand and Kirchgessner 1978), exceeded absorptive capacity. Absorptive capacity in flounder was only about 20% of various $ZnCl_2$ loads (Shears and Fletcher 1983). Second, trout on the deficient diet accumulated much larger loads than could be accounted for by diet alone, even assuming complete absorption, thereby yielding apparent retention figures well over 100% (i.e. 292, 665%). Nevertheless, the absolute increase in zinc load in the fish was less than that seen at any of the higher dietary [Zn]'s. A stimulatory effect of waterborne [Zn] on apparent zinc retention was also seen at the higher dietary levels, but to a much lesser extent (values in the 2–18% range). Third, as dietary [Zn] increased, trout retained much less on a percentage basis, until at dietary [Zn] = 590 μg·g^{-1}, apparent retention from the diet was only about 2%.

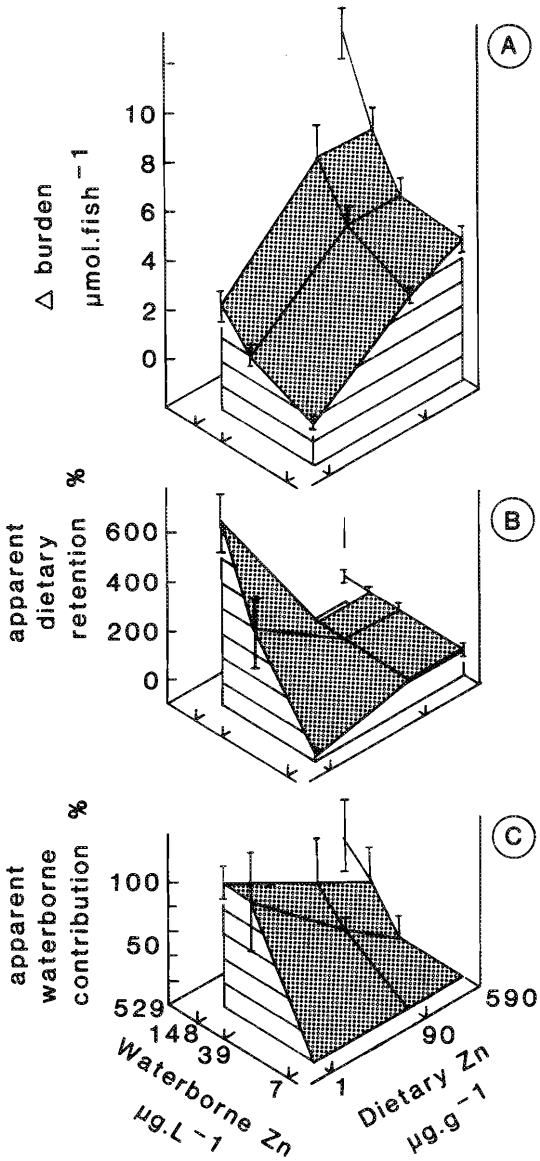

FIG. 4. (A) Change in body burden calculated as the difference in the means between the initital body burden (=0.99 μmol·fish^{-1} at week 0, n = 15) and that for ech treatment mean at 16 wk; (B) apparent dietary zinc retention; (C) apparent contribution by waterborne zinc to the total body burden. See text for details.

Fourth, at every dietary level, increases in waterborne [Zn] were associated with increased contributions from the waterborne source, with many values in the 31–57% range, even when dietary [Zn] was normal (90 μg·g^{-1}) or higher, and values of 100% when dietary [Zn] was deficient (1 μg·g^{-1}).

In view of this importance of zinc uptake from the water, by what route does it occur? One possibility is drinking of the medium, which might be especially important in fish on a low-zinc diet. Estimates of drinking rates for rainbow trout in freshwater range from nil (Shehadeh and Gordon 1969) to 1.43 μL·g^{-1}·h^{-1} (Lovegrove and Eddy 1982). Using the higher estimate, the highest waterborne [Zn] tested in fish on the deficient diet, 148 μg Zn·L^{-1}, would provide only 0.005 μg Zn·g^{-1}·d^{-1}. Food consumption of 3% body weight·d^{-1}, at dietary [Zn] = 1, 90, and 590 μg·g^{-1}, would give dietary inputs of 0.03, 2.7, and 17.7 μg Zn·d^{-1}. Even at the lowest dietary [Zn], the maximum input from imbibed water would only be 17% of the available dietary intake. Drinking therefore could only play a very small role in zinc uptake.

The other, far more likely, possibility is uptake across the gills. In acute exposures, the gill is always a site of high [Zn] (Joyner 1961; Hodson 1975; Bradley et al. 1985). Joyner's (1961) study further showed that esophageal wax plugs in brown bullheads (*Ictalurus nebulosus*) did not prevent accumulation of ^{65}Zn by several internal organs. Recently, we have shown that zinc is taken up from the water in a concentration-dependent fashion by intact adult trout, as well as by an isolated, perfused trout gill preparation (D.J. Spry and C. M. Wood, unpubl. data). Uptake by the intact trout was furthermore unaffected by esophageal ligation.

Our results clearly support homeostasis of internal zinc levels in the face of dietary and waterborne variation. For plasma, there was a broad region of regulation regardless of input, which was only depressed during extreme deprivation, and only elevated at very high dietary and waterborne input (Fig. 2A, 2B). Whole body [Zn] exhibited a rather less tightly controlled homeostasis in which there was no plateau region where concentration was independent of exposure. Perhaps "excess" whole body zinc was stored in a "sink" of lesser physiological importance (e.g. scales (Sauer and Watabe 1984) or bone). Nevertheless, over the same range where plasma showed a perfect plateau (Fig. 2B), whole body [Zn] exhibited less than two-fold variation in the face of 7- to 21-fold variation in the dietary and waterborne levels, respectively (Fig. 3). Indeed, on a whole body basis, apparent dietary retention was reduced to as low as 2% at high dietary [Zn] (Fig. 4). The true dietary retention would be even lower, since zinc was also entering from the water. This reduction may have been accomplished by decreased intestinal absorption, increased excretion, or both.

Studies on zinc accumulation in the field (cf. Bryan 1979; Giesy and Wiener 1977; Wiener and Giesy 1979; Roch et al. 1982; Saltes and Bailey 1984) support the concept of zinc homeostasis in fish, as has been well-documented in mammals (e.g. Cousins 1985). In laboratory exposures, whole body and tissue levels have risen, but only slightly (two- to threefold), when waterborne [Zn] has increased several hundred fold (Farmer et al. 1979; Holcombe et al. 1979). Steady-state concentrations which have been observed may in part be due to growth dilution (Spehar 1976; Farmer et al. 1979), although active excretion has been proposed (Pierson 1981). It is still not clear how closely whole body zinc is regulated, or whether this is effected by active or passive mechanisms which maximize net zinc uptake in deficiency and limit net uptake when excess zinc is present in the water or diet.

In summary, a zinc-deficient diet induced a zinc deficiency in trout when supplementary zinc was not available from the water. Elevations of waterborne [Zn] to 39 and 148 μg·L^{-1} partially corrected the deficiency but did not restore either plasma or whole body [Zn] to levels seen either initially or in fish raised for 16 wk on a zinc-adequate diet. At elevated waterborne [Zn], zinc was taken up from the water regardless of the dietary load. Waterborne contribution was up to 57% of the increased burden on zinc-adequate diets, despite the fact that dietary zinc was three orders of magnitude higher. At waterborne [Zn] most commonly encountered in the wild (<10 μg Zn·L^{-1}), waterborne contributions to whole body [Zn] are likely to be insignificant. Nonlethal waterborne [Zn] which may be encountered as a result of pollution (e.g. 39–529 μg·L^{-1}) may cause considerable uptake from the water. Elevation of waterborne [Zn] to even 500 μg·L^{-1} was not stressful to fingerling

trout based upon growth and mortality, and may have been prophylactic against waterborne pathogens. Plasma [Zn] was relatively constant over most of the range of zinc input, whereas whole body [Zn] reflected input from both sources, but in an attenuated fashion. Metabolism of other elements did not appear to be directly affected by zinc treatments, and there were no toxic effects.

Acknowledgments

We thank Dr. John Hilton, University of Guelph, for formulating the diet providing facilities to prepare it and for helpful comments. Dr. H. W. Ferguson, University of Guelph, is thanked for his diagnosis and consultation. Technical help was provided by G. Munger, B. Blunt, P. Spry, and C. Spry. Dr. S. Landsberger and Ms. A. Simsons of the McMaster Nuclear Reactor are thanked for advice with the neutron activation analysis. This study was funded by an NSERC strategic grant in environmental toxicology to C.M.W.

References

ALEXANDER, J. B., AND G. A. BELL. 1980. A comparison of five of the methods commonly used to measure protein concentrations in fish sera. J. Fish Biol. 16: 115–122.

BENGTSSON, B.-E. 1974. Effect of zinc on growth of the minnow *Phoxinus phoxinus*. Oikos 25: 370–373.

BETTGER, W. J., D. J. SPRY, K. A. COCKELL, C. Y. CHO, AND J. W. HILTON. 1987. The distribution of zinc and copper in plasma, erythrocytes and erythrocyte membranes of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 87C: 445–451.

BRADLEY, R. W., C. DUQUESNAY, AND J. B. SPRAGUE. 1985. Acclimation of rainbow trout to zinc: kinetics and mechanism of enhanced tolerance. J. Fish Biol. 27: 367–379.

BRYAN, G. W. 1979. Bioaccumulation of marine pollutants. Philos. Trans. R. Soc. Lond. B 286: 483–505.

COUSINS, R. J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol. Rev. 65: 238–309.

DAOUST, P.-Y., AND H. W. FERGUSON. 1985. Nodular gill disease: a unique form of proliferative gill disease in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. 8: 511–522.

FARMER, G. J., D. ASHFIELD, AND H. S. SAMANT. 1979. Effects of zinc on juvenile Atlantic salmon *Salmo salar*: acute toxicity, food intake, growth and bioaccumulation. Environ. Pollut. 19: 103–117.

FLETCHER, G. L., E. G. WATTS, M. J. KING. 1975. Copper, zinc, and total protein levels in the plasma of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 32: 78–82.

FRAKER, P. J., M. E. GERSHWIN, R. A. GOOD, AND A. PRASAD. 1986. Interrelationships between zinc and immune function. Fed. Proc. 45: 1474–1479.

GIESY, J. P. JR., AND J. G. WIENER. 1977. Frequency distributions of trace metal concentrations in five freshwater fishes. Trans. Am. Fish. Soc. 106: 393–403.

HARDY, R. W., AND K. D. SHEARER. 1985. Effect of dietary calcium phosphate and zinc supplementation on whole body zinc concentration of rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 42: 181–184.

HARPER, J. F. 1984. Peritz' *F* test: BASIC program of a robust multiple comparison test for statistical analysis of all differences among group means. Comput. Biol. Med. 14: 437–445.

HILLE, S. 1982. A literature review of the blood chemistry of rainbow trout, *Salmo gairdneri* Rich. J. Fish Biol. 20: 535–569.

HODSON, P. V. 1975. Zinc uptake by Atlantic salmon (*Salmo salar*) exposed to a lethal concentration of zinc at 3, 11, and 19°C. J. Fish. Res. Board Can. 32: 2552–2556.

HODSON, P. V., B. R. BLUNT, AND D. J. SPRY. 1978. Chronic toxicity of waterborne and dietary lead to rainbow trout (*Salmo gairdneri*) in Lake Ontario water. Water Res. 12: 869–878.

HODSON, P. V., J. W. HILTON, B. R. BLUNT, AND J. S. SLINGER. 1980. Effects of dietary ascorbic acid on chronic lead toxicity to young rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 37: 170–176.

HOLCOMBE, G. W., D. A. BENOIT, AND E. N. LEONARD. 1979. Long-term effects of zinc exposures on brook trout (*Salvelinus fontinalis*). Trans. Am. Fish. Soc. 108: 76–87.

JOYNER, T. 1961. Exchange of zinc with environmental solutions by the brown bullhead. Trans. Am. Fish. Soc. 90: 444–448.

KETOLA, H. G. 1979. Influence of dietary zinc on cataracts in rainbow trout (*Salmo gairdneri*). J. Nutr. 109: 965–969.

KNOX, D., C. B. COWLEY, AND J. W. ADRON. 1984. Effects of dietary zinc intake upon copper metabolism in rainbow trout (*Salmo gairdneri*). Aquaculture 40: 199–207.

LEWIS, S. D., AND W. M. LEWIS. 1971. The effect of zinc and copper in the osmolality of blood serum of the channel catfish, *Ictalurus punctatus* Rafinesque, and golden shiner, *Notemigonus crysoleucas* Mitchill. Trans. Am. Fish. Soc. 100: 639–643.

LOVEGROVE, S. M., AND B. EDDY. 1982. Uptake and accumulation of zinc in juvenile rainbow trout *Salmo gairdneri*. Environ. Biol. Fishes 7: 285–289.

MARTELL, A. E. 1975. The influence of natural and synthetic ligands on the transport and function of metal ions in the environment. Pure Appl. Chem. 44: 81–113.

MERLINI, M., G. POZZI, A. BRAZZELLI, AND A. BERG. 1976. The transfer of ^{65}Zn from natural and synthetic foods to a freshwater fish, p. 226–229. *In* C. E. Cushing [ed.] Radioecology and energy resources. Ecol. Soc. Am. Publ. 1, Dowdon, Hutchinson and Ross, Stroudsburg, PA.

MILNER, N. J. 1982. The accumulation of zinc by O-group plaice, *Pleuronectes platessa* (L.), from high concentrations in sea water and food. J. Fish Biol. 21: 325–336.

ODULEYE, S. O. 1975. The effects of calcium on water balance of the brown trout *Salmo trutta*. J. Exp. Biol. 63: 343–356.

OGINO, C., AND G.-Y. YANG. 1978. Requirement of rainbow trout for dietary zinc. Bull. Jpn. Soc. Sci. Fish. 44: 1015–1018.

PENTREATH, R. J. 1973. The accumulation and retention of ^{65}Zn and ^{54}Mn by the plaice, *Pleuronectes platessa* L. J. Exp. Mar. Biol. Ecol. 12: 1–18.

1976. Some further studies on the accumulation and retention of ^{65}Zn and ^{54}Mn by the plaice, *Pleuronectes platessa* L. J. Exp. Mar. Biol. Ecol. 21: 179–189.

PIERSON, K. B. 1981. Effects of chronic zinc exposure on the growth, sexual maturity, reproduction, and bioaccumulation of the guppy, *Poecilia reticulata*. Can. J. Fish. Aquat. Sci. 38: 23–31.

RICHARDSON, N. L., D. A. HIGGS, R. M. BEAMES, AND J. R. MCBRIDE. 1985. Influence of dietary calcium, phosphorus, zinc and sodium phytate on cataract incidence, growth and histopathology in juvenile chinook salmon (*Oncorhynchus tshawytscha*). J. Nutr. 115: 553–567.

ROCH, M., J. A. MCCARTER, A. T. MATHESON, M. J. R. CLARK, AND R. W. OLAFSON. 1982. Hepatic metallothionein in rainbow trout (*Salmo gairdneri*) as an indicator of metal pollution in the Campbell River system. Can. J. Fish. Aquat. Sci. 39: 1596–1601.

SALTES, J. G., AND G. C. BAILEY. 1984. Use of fish gill and liver tissue to monitor zinc pollution. Bull. Environ. Contamin. Toxicol. 32: 233–237.

SAUER, G. R., AND N. WATABE. 1984. Zinc uptake and its effect on calcification in the scales of the mummichog, *Fundulus heteroclitus*. Aquat. Toxicol. 5: 51–66.

SELLERS, C. M. JR., A. G. HEHTH, AND M. L. BASS. 1975. The effect of sublethal concentrations of copper and zinc on ventilatory activity, blood oxygen, and pH in rainbow trout (*Salmo gairdneri*). Water Res. 9: 401–408.

SHEARER, K. D. 1984. Changes in elemental composition of hatchery-reared rainbow trout, *Salmo gairdneri*, associated with growth and reproduction. Can. J. Fish. Aquat. Sci. 41: 1592–1600.

SHEARS, M. A., AND G. L. FLETCHER. 1983. Regulation of Zn^{2+} uptake from the gastrointestinal tract of a marine teleost, the winter flounder (*Pseudopleuronectes americanus*). Can. J. Fish. Aquat. Sci. 40 (Suppl. 2): 197–205.

SHEHADEH, Z. H., AND M. S. GORDON. 1969. The role of the intestine in salinity adaptation of the rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol. 30: 397–418.

SINLEY, J. R., J. P. GOETTL JR., AND P. H. DAVIES. 1974. The effects of zinc on rainbow trout (*Salmo gairdneri*) in hard and soft water. Bull. Environ. Contamin. Toxicol. 12: 193–201.

SKIDMORE, J. F. 1970. Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. J. Exp. Biol. 52: 481–494.

SMITH, H. W. 1930. The absorption and excretion of water and salts by marine teleosts. Am. J. Physiol. 93: 480–505.

SPEAR, P. A. 1981. Zinc in the aquatic environment: chemistry, distribution, and toxicology. National Research Council of Canada, Environmental Secretariat Publ. No. 17589, Publications NRCC, Ottawa, Ont. K1A 0R6.

SPEHAR, R. L. 1976. Cadmium and zinc toxicity to flagfish, *Jordanella floridae*. J. Fish. Res. Board Can. 33: 1939–1945.

SPENCER, H., C. A. GATZA, L. KRAMER, AND D. OSIS. 1980. Zinc metabolism in human beings, p. 105–119. *In* J. O. Nriagu [ed.] Zinc in the environment. II: Health effects. Wiley-Interscience, Toronto, Ont.

SPRAGUE, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793–821.

SPRY, D. J., AND C. M. WOOD. 1984. Acid–base, plasma ion and blood gas changes in rainbow trout during short term toxic zinc exposure. J. Comp. Physiol. B 154: 149–158.

1985. Ion flux rates, acid–base status, and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. Can. J. Fish. Aquat. Sci. 42: 1332–1341.

STARY, J., K. KRATZER, B. HAVLIK, J. PRASILOVA, AND J. HANUSOVA. 1982. The cumulation of zinc and cadmium in fish (*Poecilia reticulata*). Int. J. Environ. Anal. Chem. 11: 117–120.

UNDERWOOD, E. J. 1977. Trace elements in human and animal nutrition. 4th ed. Academic Press, New York, NY. 545 p.

WATSON, T. A., AND B. A. MCKEOWN. 1976. The effect of sublethal concentrations of zinc on growth and plasma glucose levels in rainbow trout, *Salmo gairdneri* (Richardson). J. Wildl. Dis. 12: 263–270.

WEIGAND, E., AND M. KIRCHGESSNER. 1978. Homeostatic adjustments in zinc digestion to widely varying dietary zinc intake. Nutr. Metab. 22: 101–112.

WEKELL, J. C., K. D. SHEARER, AND E. J. GAUGLITZ JR. 1986. Zinc supplementation of trout diets: tissue indicators of body zinc status. Prog. Fish-Cult. 48: 205–212.

WEKELL, J. C., K. D. SHEARER, AND C. R. HOULE. 1983. High zinc supplementation of rainbow trout diets. Prog. Fish-Cult. 45: 144–147.

WIENER, J. G., AND J. P. GIESY, JR. 1979. Concentrations of Cd, Cu, Mn, Pb, and Zn in fishes in a highly organic softwater pond. J. Fish. Res. Board Can. 36: 270–279.

WILLIS, J. N., AND W. G. SUNDA. 1984. Relative contributions of food and water in the accumulation of zinc by two species of marine fish. Mar. Biol. 80: 273–279.

ZEITOUN, I. H., L. D. HUGHES, AND D. E. ULLREY. 1977. Effect of shock exposures of chlorine on the plasma electrolyte concentrations of adult rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 34: 1034–1039.