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NOTE

Significantly higher levels of zinc and copper found in wild compared to hatchery-reared coho salmon smolts *Oncorhynchus kisutch*

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ABSTRACT: Wild and hatchery-reared coho salmon smolts *Oncorhynchus kisutch*, collected from Bingham Creek, Washington, USA, were examined for copper and zinc content. Using square wave voltametry, assays were made on eviscerated whole body samples. The results of the study indicated that zinc and copper levels were significantly lower in hatchery-reared smolts than in wild smolts of the same parentage. Mean zinc concentrations for wild and hatchery-reared smolts were, respectively, 16.79 and 11.50 µg g^{-1} g wet wt, 81.30 and 51.01 µg g^{-1} dry wt, and 0.16 and 0.111 µg mg^{-1} protein. Mean copper concentrations were 1.9 and 1.7 µg g^{-1} wet wt, 9.15 and 7.54 µg g^{-1} g dry wt, and 0.018 and 0.016 µg mg^{-1} protein. Differences in body burden were statistically significant ($p < 0.0001$) for both metals, except for copper on the µg mg^{-1} protein basis. The purpose of this note is to call attention to an apparent insufficiency of copper and zinc in hatchery-reared fish as compared to their wild counterparts. This study follows up on a project in which a similar condition was found to exist with respect to body content of selenium. The total work is part of a continuing effort to better evaluate immunocompetence in hatchery-reared fish.

KEY WORDS: Wild · Naturally reared · Coho smolts · Hatchery-reared · Copper · Zinc

Zinc is a clinically important trace element (Prasad 1979) that functions as a cofactor in metalloenzymes such as DNA and RNA polymerase (Vallee & Galdes 1984). Zinc also plays an important role in the immune system of vertebrates (Gershwin et al. 1985, Fraker et al. 1986) and participates in the inflammatory response as a component of superoxide dismutase.

Copper is an essential trace element that is found in cuproenzymes such as cytochrome c oxidase and superoxide dismutase. As a component of ceruloplasmin, copper plays a role in the absorption and transport of iron. Additionally, it is essential for skeletal development and for normal structure and function of the central nervous system (O'Dell 1976).

Evidence indicates that levels of some nutrients are deficient in hatchery-reared fish. The association between zinc deficiency and cataracts in rainbow trout *Salmo gairdneri* was described by Ketola (1979). In the early 1980s, a high prevalence of bilateral lenticular cataracts was observed among hatchery-reared chinook *Oncorhynchus tshawytscha* and coho *O. kisutch* salmon in Washington State (USA) and British Columbia (Canada). The condition was not observed in wild fish. The cataracts were likely caused by a functional zinc deficiency resulting from an interaction of zinc with high levels of calcium and/or phosphorus in the presence of phytic acid found in dietary fish meal (Hardy & Shearer 1985, Richardson et al. 1985). Felton et al. (1990) demonstrated that body content of selenium was lower in hatchery-reared coho salmon smolts and adults than in wild fish of the same age. This condition was not associated with clinical illness.

In the present study, body contents of zinc and copper were measured in wild and hatchery-reared coho salmon smolts that were derived from the same parentage and that were collected from a single site.

Materials and methods. The Washington Department of Fisheries operates a fish trap in association with the Simson Hatchery on Bingham Creek, a tributary of the Satsop River (Grays Harbor County, Washington, USA). When adult coho salmon migrate upstream in the fall, a portion of the fish is trapped and used to supply eggs for the Bingham Creek Hatchery. The remaining fish are allowed to pass above the trap to spawn naturally. Approximately 15 mo later the hatchery-reared smolts are released and allowed to migrate to the sea, along with naturally spawned smolts. The practices employed at this hatchery allow for comparison of wild and hatchery-reared smolts derived from the same parent stock.

On May 8, 1993, naturally reared coho salmon smolts (n = 20; average weight = 14.26 g) were netted from the Bingham Creek fish trap. On the same day, hatchery-reared (n = 20; average weight = 21.22 g) coho salmon smolts were netted from a rearing pond at the Bingham Creek Hatchery. The hatchery-reared fish had been fed a commercial diet (Bioproducts Stage II) containing an average of 87 ppm zinc and 7 ppm copper (D. Rolley, Bioproducts, 1935 NW Warrenton Dr., Warrenton, OR 97146, USA, pers. comm.).

The smolts were sacrificed by a blow to the head, weighed, eviscerated and placed in dry ice for return to the laboratory. The frozen carcasses were ground in an equal volume of glass-distilled water successively, using 2 separate homogenizers: the first, coarse ground homogenate, was prepared with an Osterizer; the second, a fine grind, was made with a Tel Mark Tissumizer.

Acid digestion was conducted according to the following protocol: A digestion mixture containing 2 g homogenate, 14 ml acid mixture (H_2SO_4/HNO_3, 4/10) and several glass beads were placed in a 100 ml Kjeldahl flask. The digestion was conducted at low to medium heat until all brown fumes disappeared and the white sulfite fumes appeared. Glass-distilled water was added to bring the final volume of the digestate to 10 ml. The digestate (1 ml) was diluted with 1 ml 0.1 M ammonium acetate (pH 4.5) and finely adjusted to pH 4.5 to 4.6 with NH_4OH and acetic acid. Glass-distilled water was used to create a final volume of 10 ml.

The digestate was assayed for total zinc and copper by the direct method of square wave voltametry (SWV) at a mercury electrode on a Princeton Applied Research Polarograph, Model 384B (Princeton, NJ, USA). Polarograph settings were as follows: initial potential −1.3 V vs Ag/AgCl, final potential −0.075 V vs Ag/AgCl, no conditional potential, bubbled with ultra pure nitrogen for 30 s, deposition time 60 s, scan rate 200 mV s^{-1}, equilibration time 5 s, 2 replications.

Calculations were made from acid-digested standards which were placed in the instrument's memory and compared against an appropriate acid blank. The acids used for digestion were reagent grade; the acids used for buffer and pH adjustment were ultra pure (Baker Chemical Co.). The test specimens were compared to dogfish powder standard Dorm-1 (National Research Council of Canada). The values obtained for the laboratory standard in digestion and assay procedures were 21.06 ± 1.7 ppm for zinc and 4.54 ± 0.2 ppm for copper. The published values for the Dorm-1 standard are: Zn = 21.3 ± 1.0 ppm; Cu = 5.22 ± 0.33 ppm (n = 3). The percent recovery for zinc and copper was 96 to 98.8 % and 85 to 87 %, respectively.

To rule out instrument errors and investigator bias, samples from wild and hatchery-reared fish were analyzed on an alternating basis and calculated to the same standards. The statistical test employed was a *t*-test from the software program MINITAB 8 (Minitab, Inc., Addison-Wesley and Benjamin/Cummings Co. 1992).

Results and discussion. Whole body concentrations of zinc in hatchery-reared fish were quite uniform, ranging from 9.6 to 13.8 µg g^{-1} (Table 1). By contrast, zinc concentrations in wild fish varied widely, ranging from a low of 10.1 to a high of 24.8 µg g^{-1}. The uniformity seen in hatchery-reared fish might reflect the fact that the fish were fed on a regular schedule and that they were subjected to essentially the same treatments from day to day. Body content of zinc was significantly lower in the hatchery-reared fish than in wild fish regardless of the type of tissue measurement used (Table 2), the mean values being 16.79 and 11.50 µg g^{-1} wet wt, 81.30 and 51.01 µg g^{-1} dry wt, and 0.1602 and 0.1110 µg mg^{-1} protein, respectively.

Copper concentrations were relatively uniform in both groups, ranging from 1.4 to 1.9 µg g^{-1} in hatchery-reared fish and from 1.5 to 2.1 µg g^{-1} in wild fish (Table 1). This observation may reflect the fact that copper is less commonly involved in metabolic processes and is thereby less subject to stress. Whole body copper content was higher in wild fish (mean = 1.9 µg g^{-1}) than in captive fish (mean = 1.7 µg g^{-1}) (Table 2). The observed difference was statistically significant.

There seems to be no relationship between body weight and the concentration of zinc or copper found in either the hatchery-reared or wild fish (Table 1).

The observed differences in overall zinc and copper content seem paradoxical. Fish in the hatchery were fed to satiation with a commercial diet containing 87 ppm of zinc and 7 ppm of copper. Wild fish, on the other hand, were forced to forage and compete for naturally occurring food sources. It is possible that the added zinc and copper for coho salmon is inadequate and that this inadequacy accounted for the discrepancy noted between wild and cultured fish. It is also possible that the dietary additions accurately reflect zinc and copper needs of juvenile coho salmon, but that stress factors associated with hatchery rearing lead to a functional disparity between wild and captive fish.

The higher levels of zinc and copper in wild fish may improve their metabolism and enhance the efficiency of several host defense mechanisms (e.g. detoxification system, immune system, inflammatory response). To examine this hypothesis, feeding trials are currently

Table 1. *Oncorhynchus kisutch*. Comparison of whole body zinc and copper concentrations in hatchery-reared and wild coho salmon of the same parentage. Concentrations expressed in μg g^{-1} wet wt of homogenized eviscerated carcass tissue. Samples arranged according to increasing body weight

Sample no.	Hatchery-reared coho: Body wt (g)	Zinc (μg g^{-1})	Copper (μg g^{-1})	Wild coho: Body wt (g)	Zinc (μg g^{-1})	Copper (μg g^{-1})
1	10.3	12.6	1.60	10.6	16.76	2.08
2	12.7	12.5	1.74	10.7	22.40	2.08
3	14.7	12.1	1.60	10.8	14.30	1.88
4	16.1	13.8	1.70	10.9	12.17	2.03
5	17.1	11.1	1.72	11.4	22.71	1.70
6	17.6	11.6	1.65	11.5	20.70	2.13
7	18.6	11.1	1.71	11.6	16.97	1.76
8	20.4	11.3	1.80	11.9	16.74	1.90
9	21.6	12.5	1.61	12.4	14.10	1.94
10	21.8	10.6	1.65	12.4	14.40	2.02
11	21.8	11.7	1.80	13.7	22.94	1.62
12	22.1	10.4	1.84	14.1	24.76	1.54
13	23.3	12.2	1.65	14.4	11.20	1.91
14	24.1	10.0	1.70	14.5	17.91	2.01
15	26.1	9.6	1.74	15.0	17.60	2.00
16	26.3	10.0	1.43	15.4	10.10	2.08
17	26.6	11.1	1.72	15.7	14.22	1.88
18	26.6	10.0	1.75	16.1	12.00	2.05
19	27.3	13.2	1.85	19.8	19.14	1.52
20	29.4	11.7	1.87	46.4	13.70	2.05

Table 2. *Oncorhynchus kisutch*. Zinc and copper concentrations (means and standard deviations) in eviscerated whole body samples of hatchery-reared versus naturally reared coho salmon smolts

Sample	Zinc concentration: μg g^{-1} wet wt	μg g^{-1} dry wt	μg mg^{-1} prot.	Copper concentration: μg g^{-1} wet wt	μg g^{-1} dry wt	μg mg^{-1} prot.
Hatchery-reared (n = 20)						
Mean	11.50	51.01	0.1110	1.705	7.54	0.0165
SD	1.12	7.39	0.0163	0.103	0.67	0.0023
SEM	0.25	1.7	0.0036	0.023	0.15	0.0005
Wild (n = 20)						
Mean	16.79	81.3	0.1602	1.908	9.15	0.0185
SD	4.35	25.0	0.0412	0.186	1.00	0.0039
SEM	0.97	5.6	0.0092	0.042	0.22	0.0008
p	< 0.0001	< 0.0001	< 0.0001	< 0.0002	< 0.0001	= 0.057

being conducted in which the body content (Zn, Cu) of hatchery-reared fish will be elevated to the same level as that found in wild fish.

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