Stress Response of Coho Salmon (*Oncorhynchus kisutch*) Elicited by Cadmium and Copper and Potential Use of Cortisol as an Indicator of Stress[1]

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Exposure of juvenile coho salmon (*Oncorhynchus kisutch*) to copper (Cu) produced a marked, dose-dependent serum cortisol elevation. Treatment with cadmium (Cd) did not elicit a cortisol elevation, even in moribund fish. Stressing salmon with sublethal levels of Cu or handling plus close confinement resulted in "ideal" compensation (return to pre-stress levels) in cortisol titers. Salmon exposed to Cu had depressed serum chloride levels and reduced survival when challenged with salt water. Exposure to Cd did not influence serum chloride or the ability to tolerate salt water. Handling and close confinement produced the same cortisol elevation in controls as in Cu- or Cd-treated fish, but exposure to Cu reduced the ability of the fish to survive the stress of handling and confinement. Cortisol level and other characteristics of the General Adaptation Syndrome of stress should not be universally applied as indicators of stress in salmon.

Key words: stress, cortisol, heavy metals, coho salmon, *Oncorhynchus kisutch*, saltwater adaptation, stress resistance, stress indicator, cadmium, copper

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Quand des jeunes saumons coho (*Oncorhynchus kisutch*) sont exposés à du cuivre (Cu) il y a élévation marquée, dépendante de la dose, du cortisol sérique. Un traitement au cadmium (Cd) ne provoque pas d'élévation de cortisol, même chez les poissons moribonds. Le fait de soumettre les poissons à un stress par des doses sublétales de Cu ou par manipulation et maintien dans un espace restreint résulte en une compensation « idéale » (retour aux niveaux d'avant-stress) dans les titres de cortisol. Lorsque confrontés avec de l'eau salée, les saumons exposés au Cu accusent des niveaux abaissés de chlorure sérique et une survie amoindrie. L'exposition au Cd n'affecte pas le chlorure sérique ou l'aptitude à tolérer l'eau salée. La manipulation et le maintien en espace restreint produisent la même élévation de cortisol chez les témoins que chez les poissons traités au Cu ou au Cd, mais l'exposition au Cu diminue l'aptitude des poissons à survivre au stress causé par la manipulation et le maintien en espace restreint. On ne devrait pas utiliser universellement, comme indicateurs de stress chez les saumons, le niveau de cortisol et autres caractéristiques du syndrôme général d'adaptation du stress.

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HEAVY metals are widely distributed in aquatic systems and can affect fish populations by reducing growth, reproduction, or survival. One would suspect that metals would produce stress in fish, within the context of Selye (1950, 1973), particularly when death results. Hormones, primarily the corticosteroids, are believed to mediate the stress response by serving as a stimulus between the neural transducer and other factors of the General Adaptation Syndrome which consists of the physiological responses elicited by most stressing agents (Selye 1976; Mazeaud et al. 1977; Strange et al. 1977). Secondary stress responses such as changes in plasma

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and liver sugar levels, immunosuppression, and blood cell profile are thought to be in response to elevation in corticoid hormones and catecholamines. Acute exposure of sockeye salmon (*Oncorhynchus nerka*) to copper (Cu) produced a marked, dose-dependent elevation in corticoids monitored for 24 h (Donaldson and Dye 1975). Our objectives were to determine whether heavy metals produced a general corticoid stress response in fish, and, if so, to describe the dynamics of plasma cortisol levels over time to either acclimation (compensation) or exhaustion (death) — thereby characterizing potential response curves as outlined by Precht (1958). Our goal was to determine the feasibility of using blood cortisol levels as an indicator of heavy metal toxicity.

We administered Cu and cadmium (Cd) to juvenile coho salmon (*O. kisutch*). There is no a priori way of determining whether fish are stressed by sublethal concentrations of metals before they die. We used resistance to a second stress — either severe confinement and handling or survival in salt water — as a criterion for determining whether the fish had been stressed by the metals. We correlated these data with cortisol titers and speculated on a new hypothesis concerning corticoid elaboration and the stress response in fish.

Materials and Methods

Coho salmon eggs of the 1974 and 1975 year-classes obtained from Fall Creek Salmon Hatchery (Oregon) were incubated at 8–10°C. Fry and fingerlings were reared under natural light in well water at 12.3°C and fed Oregon moist pellets daily. Fish were yearlings during the tests and were 15–22 cm long. They were not fed during acute tests. Flow-through exposures were conducted in 1000-L, 1.54 m diam tanks with a gravity flow diluter delivering 12 L/min; 95% of the water in the tanks was replaced every 3.7 h. Details of the delivery system were described by Lorz and McPherson (1977). Background levels in the well water were less than 3.2 μg/L Cu and less than 1 μg/L Cd. Chemical characteristics of the well water were alkalinity — 76 mg/L as $CaCO_3$, hardness — 90 mg/L as $CaCO_3$, dissolved oxygen — 9.5 mg/L, ammonia — 0.154 mg/L, and pH — 7.03 (data from Lorz et al. 1978). Measured (flameless atomic absorption) concentrations of Cd and Cu used in tests are presented in Table 1. These analyses were run by the Environmental Protection Agency, Western Fish Toxicology Station. The tanks were exposed to natural light as well as to a simulated natural photoperiod provided by Vita-Lite fluorescent bulbs. Tanks were screened with black polyethylene to reduce disturbances.

During sampling, fish were captured as rapidly and with as little disturbance as possible, stunned by a blow to the head, and exsanguinated by severance of the caudal peduncle. It typically took <5 min to collect samples from the 10 fish from one tank. Serum, obtained by letting the blood clot at least 15 min and then centrifuging it, was stored at −15°C until analyzed. Cortisol was assayed in 25 μL of serum by using a competitive protein binding technique proposed by Murphy (1967) and modified to eliminate extraction by Strange and Schreck (1978). Corticoids determined by this procedure are 80% cortisol. Serum osmolarity was determined with a Wescor Model 5100 vapor pressure osmometer and chlorinity with a Corning Model 920 chloride meter.

Histological sections stained with hematoxylin–eosin were made from tissues of fish exposed to either Cd or Cu.

Statistical significance is judged on overlap of 95% confidence intervals (*t*-test).

TABLE 1. Nominal and measured concentrations of cadmium and copper used in acute tests with coho salmon.

Metal and date	Nominal (μg/L)	Measured concn (μg/L) Mean ± SE	*n*
Cadmium	0	0.27 0.09	12
June 21–July 2, 1976	4	4.46 0.28	12
	8	8.13 0.24	12
	12	10.67 0.21	12
Copper	0	0.8 0.30	5
Dec. 27–Jan. 6, 1977	15	13.8 0.13	5
	60	52.8 1.00	6
	90	76.1 0.75	6
Copper	0	1.37 0.43	3
Jan. 11–Jan. 18, 1977	140	94.1 11.04	4
	210	162.0 9.82	4

Experimental Design and Results

CADMIUM — CHRONIC TESTS

To determine sublethal effects of long-term exposure to Cd, we treated five replicated groups of about 350–420 fish each continuously, starting on February 26, 1976, with 0, 0.75. 1.5, 3.0, or 4.5 μg/L of Cd (as $CdCl_2$). After 48-d chronic exposure, on April 14, 1976, 15 fish were sampled from each of the 0 and 4.5 μg/L groups. Cortisol levels of the control and Cd-treated fish were not significantly different (Fig. 1).

We sampled fish at 0900 67 d after onset of the 0, 0.75, 1.5, 3.0, and 4.5 μg/L treatments. Cortisol levels did not differ between fish in the various treatments or from the concentrations found 1 mo earlier (Fig. 1).

We also suspended 10 fish each from the 0 and 4.5 μg/L groups in a 25- × 40-cm dip net immersed in a bucket of water from their original tanks to determine whether the Cd-treated fish elaborated cortisol due to this type of handling stress, as reported by Strange et al. (1977). Capture and 15-min confinement produced a fourfold elevation in cortisol titers in both the control and Cd-treated fish (Fig. 1).

To see if Cd-exposure impaired the ability to adapt to seawater, we transferred 10 fish each from the 0 and 4.5 μg/L tanks to 0.61 m diam fiberglass tanks containing 120 L natural seawater (30‰ salinity) maintained at 10°C under simulated natural photoperiod. For controls, we transferred 10 fish each from the 0 and 4.5 μg/L groups to similar tanks containing water from their original tanks, and also transferred fish from the 4.5 μg/L treatment to a tank containing freshwater but

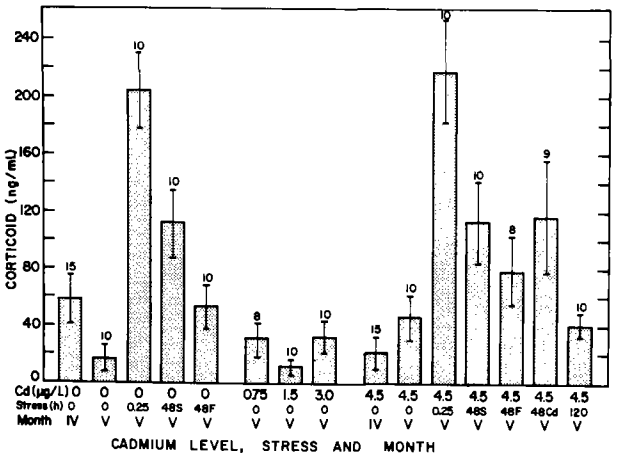

FIG. 1. Mean cortisol (corticoid) levels (±SE ⌶) in coho salmon exposed to 0, 0.75, 1.5, 3.0, and 4.5 µg/L cadmium since February 26, 1976 and sampled during April (IV) or May (V) (stress = 0) and after being (1) confined 0.25 h in a dip net suspended in a bucket (stress = 0.25), (2) transferred for 48 h to a smaller tank with static salt water (stress = 48S) or freshwater (stress = 48F) or water with cadmium from their original tank (stress = 48Cd), or (3) transferred for 120 h to a smaller tank with freshwater and no cadmium (stress = 120).

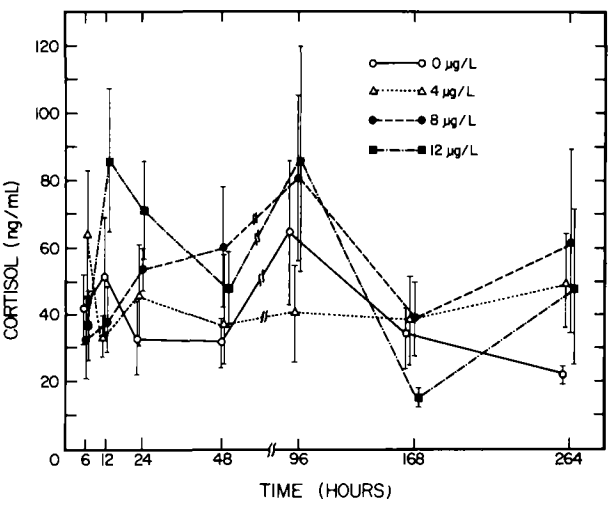

FIG. 2. Mean (±SE ⌶) cortisol levels in coho salmon exposed to various levels of cadmium for different lengths of time.

no Cd. After 48 h, mean cortisol levels of saltwater-challenged fish and those transferred to freshwater in both the 0 and 4.5 µg/L groups were similar and appeared intermediate between "basal" levels and those encountered after 15 min of stress (Fig. 1). The Cd-treated fish were fully able to adapt to seawater. However, these "intermediate" values did not differ significantly from "basal" levels.

We placed 10 fish from the 4.5 µg/L treatment into a 0.61-m tank supplied with flowing water but no Cd. These fish were sampled 120 h after transfer; mean cortisol levels had completely returned to basal values within this time after handling (Fig. 1).

Fish exposed to 4.5 µg/L Cd for 70 d did not differ histologically from controls in gonad, liver, head and tail kidney, and gill structure.

CADMIUM — ACUTE TESTS

To determine whether lethal levels of Cd affect cortisol level, we acclimated 35–47 coho salmon for 7 d in each of eight tanks. On June 21, 1976 duplicate tanks of fish were exposed starting at 0730 to 0, 4, 8, or 12 µg/L Cd as $CdCl_2$. Samples of fish were taken from alternate tanks of the same dosage at each sampling period to minimize stress due to disturbance. Ten fish were sampled from a tank at each dosage at 6, 12, 24, 48, 96, 168, and 264 h after the start of treatment.

No mortality occurred in the control or 4 µg/L groups. The average mortality for the 8 and 12 µg/L treatments through 144 h were 14.3 and 53.1%, respectively. The fish never behaved violently in response to the toxicant; instead, they appeared sedated before they died. No cortisol response was evident, even during periods of high mortality (Fig. 2). Osmolarity and chlorinity of the serum did not vary with treatment or duration of exposure (Lorz et al. 1978). Histological examination of fish of the 12 µg/L group revealed no discernible differences from controls after 120 h of Cd exposure.

COPPER — ACUTE TESTS

Sublethal and lethal concentrations of Cu were used to indicate whether there is a general lack of cortisol secretion due to heavy metal stress as indicated with Cd.

For sublethal tests, 80–144 yearling coho salmon per tank were acclimated for 7 d. The fish were exposed to 0, 15, 60, and 90 µg/L Cu (as $CuCl_2$) starting at 0730 on December 27, 1976. Samples of 10 fish were taken from each group at 2, 8, 24, 36, 78, and 170 h thereafter. No deaths occurred over the 170-h period of Cu treatment. However, toward the end of this exposure period, the fish in the 60- and 90-µg/L Cu concentrations appeared distressed; we suspect that some mortality would have occurred eventually. There was an immediate, dose-dependent increase in serum cortisol after Cu exposure began (Fig. 3A), followed by a return to more basal levels within 8–24 h. Thereafter, the cortisol titers of fish at the higher concentrations increased significantly and remained elevated until the end of the experiment.

We used resistance to a second stress as an index of reduced fitness. Two groups of 19 fish each were taken at 170 h from both the control and 90 µg/L treatment and placed in 17 cm diam perforated buckets immersed 8 cm in tanks with water similar to that in the experimental tanks. This process subjected the fish to severe stress involving handling and confinement. Blood from

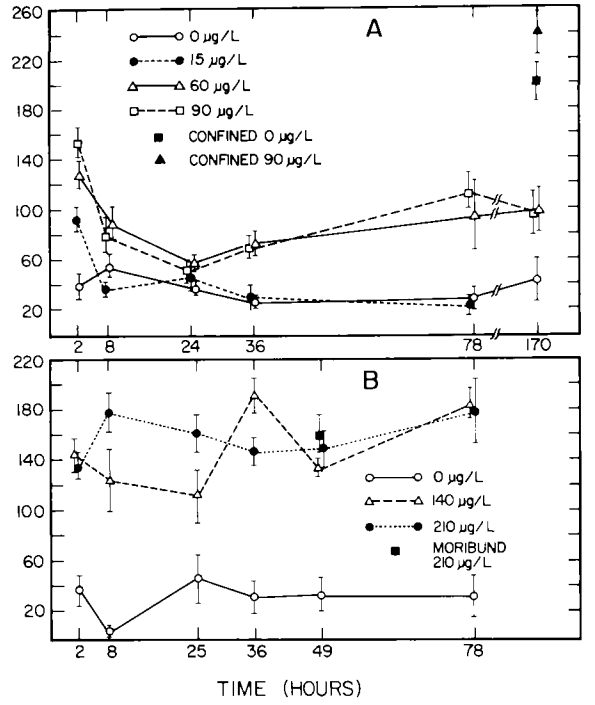

FIG. 3. Mean (±SE ⌶) cortisol levels in coho salmon exposed to (A) sublethal levels of copper for various lengths of time or subjected to confinement in a small live cage after exposure to 0 or 90 µg/L of copper, or (B) control or lethal levels of copper; square represents mean of fish sampled just before death.

The fish in one bucket from each treatment was sampled 1 h after confinement. Mortality was monitored for fish in the other buckets. Fish from each treatment were also transferred to tanks containing 120 L natural seawater. Fish from the 0 and 90 µg/L treatments confined for 1 h in the buckets exhibited similar and greatly increased cortisol levels due to the stress. Survival during confinement, however, was markedly different between the two groups. Fish receiving the Cu had a median survival time of about 12–15 h while the control group experienced no mortality even after 36 h of confinement. There was a dose-dependent relationship between Cu level and survival in seawater with the fish exposed to higher levels of Cu not able to adapt to the 30‰ salinity (Table 2). Survivorship in salt water appeared to be inversely correlated to cortisol level.

To determine responses to lethal concentrations, we acclimated 110 yearling coho salmon per tank for 7 d before treatment with 0, 140, or 210 µg/L Cu as $CuCl_2$. Ten fish from each group were sampled at 2, 8, 25, 36, 49, and 78 h after onset of treatment. Also, three moribund fish were sampled after 49-h exposure to 210 µg/L to determine whether ensuing death altered cortisol levels. The median survival times were 60 and 48 h for the 140 and 210 µg/L groups, respectively. Exposure to 140 and 210 µg/L Cu resulted in similar, immediate elevations in cortisol levels (Fig. 3B). The

TABLE 2. Survival of yearling coho salmon exposed to copper in freshwater and their later survival after transfer to seawater (without copper).

Nominal Cu concn (µg/L)	Number of fish	Survival (%) after 190-h exposure	
		Freshwater	Seawater
Control	14	100	100
15	20	100	60
60	23	100	0[a]
90	18	100	0[a]

[a]All fish died after seawater exposures between 40 and 96 h.

TABLE 3. Serum osmolarity and chlorinity of coho salmon exposed to copper for various lengths of time (sample size, 5). SE in parentheses.

Exposure concn	Duration of exposure (h)	Osmolarity (mosmol)	Chlorinity (mEq/L)
Control	2	284.4 (1.176)	112.9 (1.26)
	8	286.6 (3.24)	120.0 (2.78)
	26	292.6 (1.69)	114.6 (0.53)
	36	295.9 (1.61)	114.0 (1.79)
	49	291.6 (2.19)	115.3 (1.69)
	78	285.6 (3.78)	113.4 (1.05)
140 µg/L	2	296.8 (4.25)	116.4 (1.07)
	8	292.9 (1.91)	111.9 (1.37)
	26	292.8 (5.19)	110.9 (1.37)
	36	292.6 (2.75)	94.4 (1.66)
	49	286.7 (2.93)	88.1 (1.58)
	78	293.5 (2.33)	84.5 (2.24)

increases in cortisol were similar to those among fish treated with 60 and 90 µg/L Cu (Fig. 3A). At 140 and 210 µg/L Cu, the cortisol levels remained high throughout the test period and did not differ from that of the three moribund fish. Cortisol levels in Cu-exposed fish were as high as titers produced by severe handling and confinement stresses as seen in Fig. 1 and 3A. Exposure to 140 µg/L produced no change in serum osmolarity, but chloride level decreased significantly after 26-h exposure (Table 3). Histological examination of controls and salmon exposed to 210 µg/L Cu for 25 or 49 h revealed normal livers in both groups. Kidneys of Cu-exposed fish had glomerular atrophy, cells with condensed pyknotic nuclei, and depletion of hematopoietic tissue. Gills of the treated fish exhibited slight epithelial necrosis and some hyperplasia and fusing of adjacent lamellae.

Discussion

The two metals appear to have completely different modes of action in eliciting a general stress response in coho salmon. Exposure to Cd caused death of yearling salmon without elevating their cortisol levels, while Cu exposure was followed by a large, dose-dependent cortisol response. We found in preliminary study that con-

current exposure of salmon to Cd plus Cu resulted in a cortisol response typical for the Cu component of the mixture. Acute exposure to mercury also appears to elevate cortisol (Lorz et al. 1978). The use of cortisol (and probably other clinical–chemical characteristics controlled by this steroid) as an index of the "health" of salmon thus depends on (1) the specific nature of the stress, (2) the severity (or concentration) of the stress, and (3) the length of time that the fish have been exposed to the stress. Hence, clinical chemistries such as cortisol determinations probably are not a good indicator of the general health of fish, for "normal" titers do not negate the possibility that fish are severely stressed.

Evaluation of resistance to a second stress is a useful means of assessing sublethal effects of the first stress. In addition, increased cortisol levels can be correlated with reduced resistance. For example. sublethal challenges of salmon with Cu reduced survival after confinement, and cortisol levels increased substantially. After application of Cu, the yearling salmon were capable of maintaining elevated cortisol levels for up to 170 h without evidence of interrenal exhaustion.

Final levels of serum cortisol were the same between coho salmon merely stressed by confinement and those confined after cortisol titers had been substantially raised as a result of exposure to Cu. Also, acclimation to 4.5 μg/L Cd did not influence the dynamics of the stress response to another stress (handling). Complete compensation (return of cortisol to normal levels) following transfer of fish from one tank to a smaller tank required 48–120 h.

We attribute the different cortisol reactions between Cu- and Cd-treated fish to the possibility that Cu is more noxious than Cd. Adult Atlantic salmon (*Salmo salar*) actively avoided Cu (Sprague et al. 1965). We noticed that fish treated with Cu became restless and appeared to "fight" the toxicant, whereas Cd-exposed coho salmon simply appeared to be narcotized before death. Other investigators, however, noted hypersensitivity to external stimuli. often resulting in violent swimming movement at high concentrations of Cd in bluegill (*Lepomis macrochirus*) (Eaton 1974) and rainbow trout (*Salmo gairdneri*) (Kumada et al. 1973; White 1977). Cd at levels used here did not affect serum chloride levels, gill Na^+-K^+ ATPase, histology, saltwater adaptation, or downstream migration of smolts (Lorz et al. 1978), whereas Cu had a direct effect on all of these factors (Lorz and McPherson 1976, 1977). Elevated cortisol level is probably related either to creating the abnormal chloride and ATPase levels or to the process of trying to restore the values to normal, since corticoids have been implicated in electrolyte balance and gill ATPase activity (Epstein et al. 1971; Utida et al. 1972; Johnson 1973).

The dynamics of circulating cortisol levels in Cu-exposed fish depends on the concentration of the metal. The alarm phase of stress (Selye 1950) is characterized by an immediate increase in cortisol at all levels of Cu tested, albeit not for Cd-exposed fish. Similar results were noted in Cu-treated fish by Donaldson and Dye (1975). We presume that this early elevation of cortisol by Cu reflects the reaction of the fish to the recognition of the presence of a noxious or potentially harmful substance. This response lasted a few hours and was followed by the resistance phase. Cortisol levels returned almost to basal levels within 24 h at Cu concentrations up to 90 μg/L. Resistance is the phase of the stress response following the initial reaction (alarm) to the stressing agent and during which the organism tries to maintain or regain homeostatis. Resistance led to compensation at 15 μg/L Cu but to further resistance or perhaps eventual exhaustion (death) at 60 or 90 μg/L as seen by the second, gradual elevation in cortisol titers throughout the rest of the test period. The alarm, resistance, and exhaustion phases could not be readily distinguished at Cu concentrations of 140 μg/L Cu or above, for cortisol levels remained elevated and stable throughout the period of exposure, even at death. Although cortisol levels were increased in fish made moribund by Cu exposure, mean titers never reached those exhibited by handled and confined fish. If the fish were able to acclimate to the Cu (e.g. at 15 μg/L), their cortisol pattern followed a Precht (1958) type 2 shape, yielding "ideal" compensation. This pattern indicated that although the stress (Cu) was still present at the same severity, the cortisol levels of the fish returned to prestress conditions.

We believe, consequently, that some but not all stresses produce elevations of cortisol, and hence the mammalian concept of the General Adaptation Syndrome may not directly apply to fish. In anthropomorphic terms, it appears to us that stimuli that produce fright, discomfort, or pain in the fish elicit the General Adaptation Syndrome-type reactions. The practicality of using cortisol levels as a general index of environmental quality or fish health is thus limited. The variable response patterns and the fact that sublethal concentrations of a toxicant can result in "ideal" compensation further complicate this matter. Also, some toxicants (e.g. Cd) can be highly lethal yet produce no cortisol reaction. We suggest that physiological studies directed toward assessment of fish health or water quality should include determinations of response curves for various concentrations of the factors of interest over a substantial period of time. In addition, the meaning of the changes seen in the physiological characteristics, in terms of fitness of the fish, should be determined. For example, are the characteristics related to reduced chances for survival, growth, or reproduction? This work points out the fact that concepts based on mammalian physiology should not be universally applied to fish without vertification.

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