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Title: STRESS, OSMOREGULATION, AND THE HORMONE CORTISOL IN YEARLING COHO SALMON, ONCORHYNCHUS KISUTCH

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Carl B. Schreck

Freshwater (FW) and seawater (SW) acclimated yearling coho salmon, Oncorhynchus kisutch, were subjected to severe confinement stress in FW, SW, or a medium (1/3 SW) that was approximately isosmotic to the fish's blood. Chronic stress caused osmotic imbalances in FW and SW, but not in 1/3 SW. In SW, blood osmolarity and electrolyte concentrations increased, while in FW they generally decreased. Acclimation conditions (FW or SW) before stress influenced the severity and duration of the osmotic imbalance. Confinement stress greatly amplified the osmotic imbalance following transfer from FW to SW compared to that in unconfined fish whose water supply was switched from FW to SW. Plasma cortisol levels during stress were also affected by acclimation conditions and ambient salinity.

Plasma cortisol levels increased during acclimation to SW. Maxmimal concentrations of approximately 220 ng/ml occurred within 1.5 h after the water source was switched from FW to SW. After 21 d in SW, cortisol levels were still slightly elevated (23 ng/ml) compared to those in FW control fish (4 ng/ml).

Chronic treatment with cortisol lowered gill Na^+-K^+-ATPase levels in FW fish but did not affect plasma osmolarity, Na, K, Ca, or Mg levels in fish in FW or during acclimation to SW.

Thyroxine (T_4) and triiodothyronine (T_3) levels in plasma increased significantly after ambient water was switched from FW to SW. Maximal levels of T_3 (8.0 ng/ml) occurred within 12 h after the initial exposure to SW, followed by a return to FW basal levels (4.0 ng/ml) within 24 h. Plasma T_4 levels were higher than FW control levels (4.2 ng/ml) for at least 120 h after exposure to SW; peak levels (14.3 ng/ml) occurred at 12 and 72 h. Chronic treatment with cortisol significantly lowered plasma T_3 levels in FW and during acclimation to SW; but it had no significant effect on T_4 concentrations.

The metabolic clearance rate of corticosteroids determined after a single injection of ^3H-cortisol was higher in SW- than in FW-acclimated fish. Uptake and retention of corticosteroids in liver, gill filaments, and gall bladder bile was greater in SW than in FW fish. The stress of long-term (5 d), but not short-term (12 h), continuous confinement apparently increased the clearance rate of corticosteroids in both FW and SW fish. Chronic, but not acute, administration of exogenous cortisol at physiological levels appeared to increase the clearance rate of corticosteroids in FW fish.

STRESS, OSMOREGULATION, AND THE HORMONE CORTISOL IN YEARLING COHO SALMON, ONCORHYNCHUS KISUTCH

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STRESS, OSMOREGULATION, AND THE HORMONE CORTISOL IN YEARLING COHO SALMON, ONCORHYNCHUS KISUTCH

I. General Introduction

Coho salmon, Oncorhynchus kisutch, are a valuable economic resource in the Pacific Northwest. They are propagated in numerous hatcheries and the annual economic investment in this activity is enormous. The fish are commonly released from hatcheries as yearlings. Subsequently, the fish migrate downstream and eventually enter the ocean. Stress may be especially detrimental during this period of migration and seawater (SW) entry because the fish's capacity for physiological adaptation may be restricted (Schreck, 1981).

Putative theory regarding stress in fishes suggests that certain stimuli elicit general primary and secondary physiological responses that are mediated by the brain (Mazeaud et al., 1977). One primary response is the activation of the pituitary-interrenal axis, which results in the release of corticosteroid hormones into the blood (Donaldson, 1981). Numerous secondary responses typically follow the primary events; they may include osmotic, respiratory, and immunological changes (Mazeaud et al., 1977). The major purpose of the following research was to clarify certain aspects of the interrelation between the primary response of corticosteroid hormones and the secondary changes in osmotic status in the yearling coho salmon.

Cortisol is the primary corticosteroid hormone produced by the interrenal tissue of most teleostean species (Chester Jones et al., 1969). It has been implicated in the osmoregulatory processes of many euryhaline fishes. Cortisol reportedly promotes excretion of electrolytes in fish living in hypertonic media and conservation of electrolytes in hypotonic media (Maetz 1969a).

Initially, we measured osmoregulatory performance during stress in yearling coho salmon under various conditions of acclimation and ambient salinity. Osmoregulatory performance was correlated with the response of plasma cortisol levels during stress.

During the period before SW entry plasma cortisol concentration seems to increase in coho salmon (Specker and Schreck, 1982). We speculated that this change effectively prepares the fish for existence in SW, specifically by activating SW osmoregulatory mechanisms. We treated fish chronically with cortisol and monitored osmoregulatory peformance in freshwater (FW) and during acclimation to SW.

The concentrations of thyroid hormones in plasma increase before SW entry in coho salmon (Dickhoff et al., 1978). This increase is temporally coincident with the increase in plasma cortisol level observed by Specker and Schreck (1982). We hypothesized that the two phenomena may be functionally related. To test this we measured plasma thyroid hormone levels in fish that were chronically treated with cortisol.

Previous studies in the eel, Anguilla anguilla, have shown that both the secretion and clearance rates of cortisol change when the

fish are transferred from FW to SW (Leloup-Hatey, 1974; Henderson et al., 1974). We speculated that stress in fish may also affect both secretion and clearance rates. We estimated the clearance rate of corticosteroids in coho salmon that were acclimated to FW and SW and in fish that were subject to short-term or long-term stress. We also hypothesized that changes in secretion rate may affect the clearance rate of corticosteroids, and we tested this supposition by estimating clearance rates in fish after administration of exogenous cortisol.

II. INFLUENCE OF AMBIENT SALINITY ON OSMOREGULATORY PERFORMANCE AND CORTISOL CONCENTRATION DURING STRESS IN YEARLING COHO SALMON, ONCORHYNCHUS KISUTCH[1]

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Osmoregulatory dysfunction commonly occurs in teleostean fishes following severe physical stress (Mazeaud et al. 1977; Eddy 1981). Blood osmolarity and electrolyte concentrations are usually affected when fishes are handled or transported (Houston et al. 1971a,b; Hattingh and van Pletzen 1974; Fletcher 1975). Osmotic imbalances can also occur after cold shock (Stanley and Colby 1971), hypoxia (Kirk 1974), and exposure to certain environmental pollutants (Thomas et al. 1980).

The magnitude and direction of stress-induced osmotic imbalance (i.e., the net loss or gain of water and electrolytes) are at least partly dependent on the environmental salinity (Stanley and Colby 1971; Stevens 1972; Soivio and Oikari 1976). Fishes stressed in freshwater (FW) tend to gain water and lose electrolytes while the reverse occurs in a medium that is hyperosmotic to the fish's blood.

Osmotic imbalance during stress in FW can be partly alleviated in some cases by the addition of salt to the medium (Wedemeyer 1972; Miles et al. 1974; Tomasso et al. 1980). The presence of salt in the medium can also reduce mortality (Collins and Hulsey 1963; Hattingh et al. 1975; Long et al. 1977; Strange and Schreck 1978, 1980) and attenuate other physiological perturbations associated with stress, such as elevated levels of plasma glucose (Wedemeyer 1972) and cortisol (Strange and Schreck 1980). These results suggest that physiological compensation for osmotic imbalance is critical for the continued well-being of a fish after stress.

Cortisol, the primary corticosteroid hormone secreted by the interrenal tissue of most teleosts (Chester Jones et al. 1969), has been implicated in the osmoregulatory processes some of euryhaline fishes (Maetz 1969). Cortisol is also related to stress; its concentration in plasma increases dramatically in fishes after a wide variety of physical disturbances (Mazeaud et al. 1977; Donaldson 1981; Schreck 1981). It is possible that the rise in plasma cortisol concentration during stress may cause or amplify the concomitant osmotic imbalance (Henderson and Chester Jones 1967; Mayer and Maetz 1967; Lahlou and Giordan 1970; Umminger and Gist 1973). Conversely, changes in the internal concentration of electrolytes during stress may induce corticosteroid secretion independently of stress as shown by *in vitro* studies in the frog, *Rana temporaria* (Maser et al. 1982).

The objectives of this study were to determine if (1) stress induces osmoregulatory dysfunction in yearling coho salmon, *Oncorhynchus kisutch*, acclimated to FW or seawater (SW), (2) environmental salinity influences osmoregulatory performance during stress, and (3) the response of plasma cortisol level during stress is influenced by environmental salinity.

METHODS

Yearling coho salmon from Eagle Creek National Fish Hatchery (U.S. Fish and Wildlife Service) were transported to the Marine Science Center, Oregon State University in Newport, Oregon, during May 1981. On 22 May, half of the fish were placed in SW (daily salinity range =

23-30%) while the remainder were kept in FW. Fish were maintained in circular flow-through tanks (200 l, 0.9 m diameter) at 15-16° and fed daily with Oregon Moist Pellet diet until 1 d before the experiment.

On 12 June, groups of FW and SW acclimated fish (mean fork length = 15 cm, mean wet weight = 34 g) were netted and subjected to stress by continuous crowding and confinement in small (2 l) live-cages; four fish were placed in each live-cage. During confinement, both FW and SW acclimated fish were maintained with minimal disturbance in either FW, SW, or a 2:1 mixture of FW and SW (1/3 SW) which is approximately isosmotic to the fish's blood. Two replicate groups of four fish each were sampled from each of the six treatment conditions (FW-FW, FW-1/3 SW, FW-SW, SW-FW, SW-1/3 SW, SW-SW) at 1, 7, 24, and 48 h after confinement began. Undisturbed FW and SW acclimated fish were sampled once at the beginning of the experiment. For comparative purposes, we observed physiological changes in unconfined fish whose water supply was switched from FW to SW without disturbance (FW-SW Control) (see chapter III).

Fish were killed by a blow to the head; and their blood was sampled from the severed caudal artery in ammonium-heparinized capillary tubes. After centrifugation, the plasma was separated and stored at-20°C. Blood samples for hematocrit determination were collected in microhematocrit tubes, centrifuged, and measured immediately.

Cortisol concentration in plasma was measured by radioimmunoassay as described by Foster and Dunn (1974) and modified by Redding et al. (see Chapter III). Plasma osmolarity was measured with a vapor

pressure osmometer. Concentrations of electrolytes (Na, K, Ca, Mg) in plasma were determined with a flame atomic absorption spectrophotometer.

Statistical analyses of the data followed methods of Nie et al. (1975). Analysis of variance was followed by Duncan's Multiple Range Test where appropriate; The level of significance was $P < 0.05$. Data for plasma cortisol concentrations were transformed into natural log to increase homogeneity of variances; thus, these results are reported as geometric means.

RESULTS

General. Data from replicate groups were statistically similar; thus, they were pooled to form a single group. Continuous confinement stress in FW or SW significantly affected all measured variables (Figs. 1-4). In FW or SW, significant changes in osmotic status were usually evident within 1 h after confinement. Fish stressed in 1/3 SW showed relatively minor fluctuations in osmotic status.

Acclimation conditions before stress (FW or SW) had no significant effect on osmotic status during stress in 1/3 SW. Fish in the FW-SW group (i.e., fish acclimated to FW but stressed in SW) incurred high mortality; only two fish survived for 24 h. Severe osmotic dysfunction was evident in these two survivors. In the SW-FW group, all fish survived but showed signs of worsening osmotic imbalance 24 h after confinement. Fish in FW-FW and SW-SW groups survived and demonstrated clear trends toward complete physiological compensation for all measured variables by 48 h.

Figure 1. Plasma Na and K concentrations in freshwater (dark circles) and seawater (open circles) acclimated yearling coho salmon during continuous confinement stress in freshwater (dashed line), seawater (dashed-dotted line), or 1/3 seawater (solid line). Stars indicate values for unconfined control fish whose water supply was switched from freshwater to seawater. Values represent the mean of eight samples, except for stars ($\underline{N}$ = 10) or points marked with an asterisk ($\underline{N}$ = 2). Horizontal line directly above or below a point is the mean plus or minus the SE.

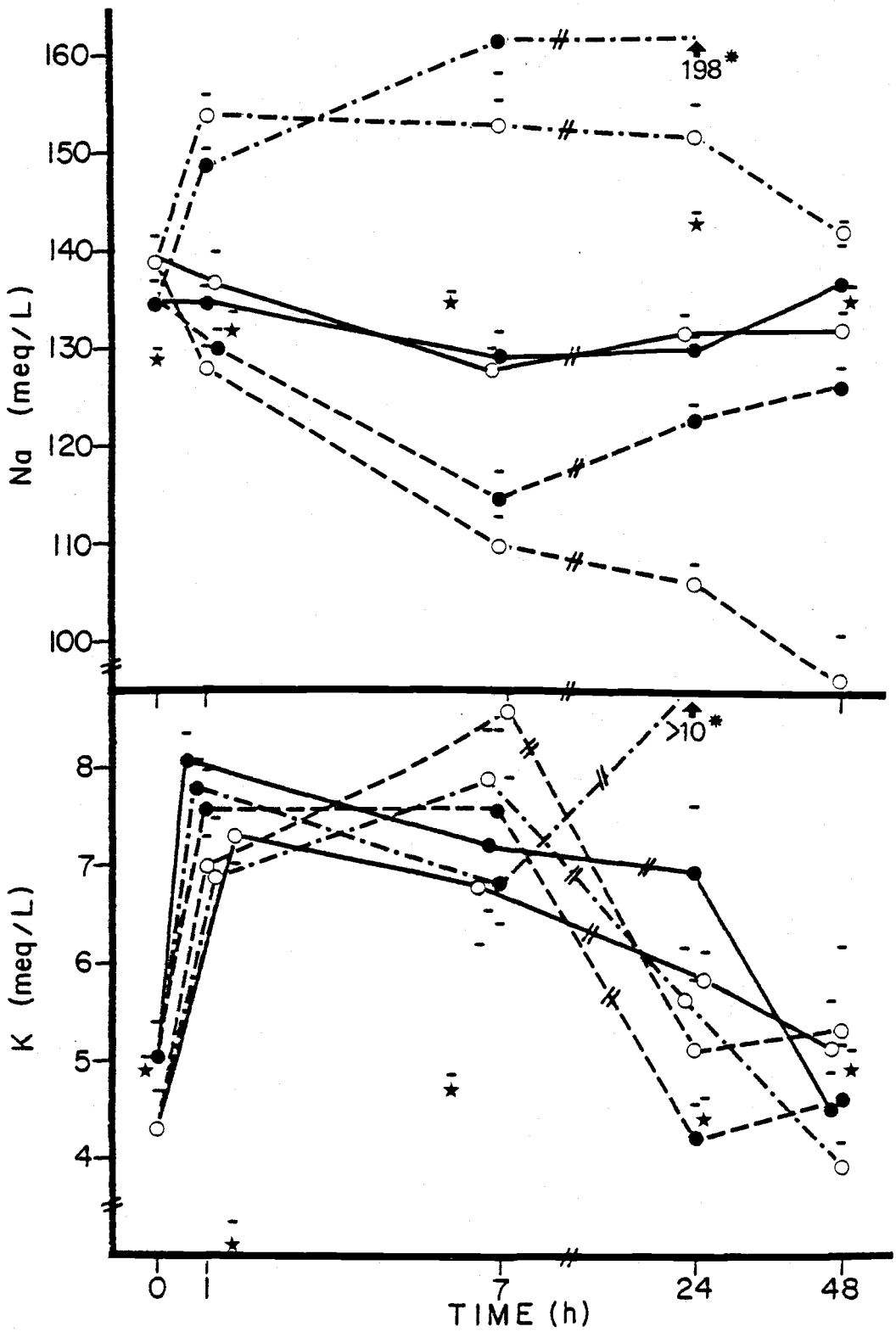
160
150
140
130
120
110
100
Na (meq/L)
198*
8
7
6
5
4
K (meq/L)
>10*
0
1
7
24
48
TIME (h)

Figure 1

Figure 2. Plasma Mg and Ca concentrations in yearling coho salmon during stress. Symbols are defined in Fig. 1.

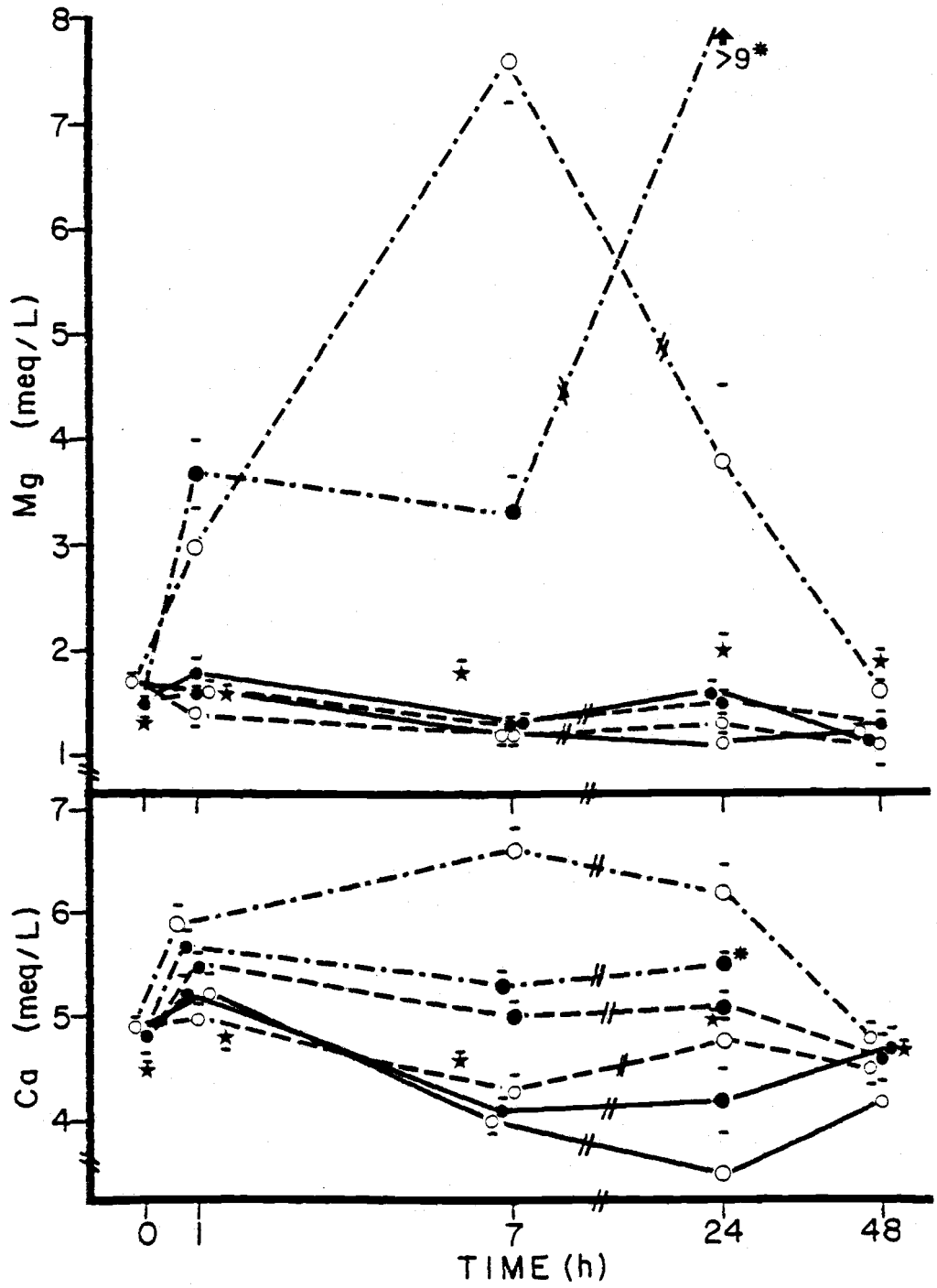

Figure 2

Figure 3. Plasma osmolarity and blood hematocrit levels in yearling coho salmon during stress. Symbols are defined in Fig. 1.

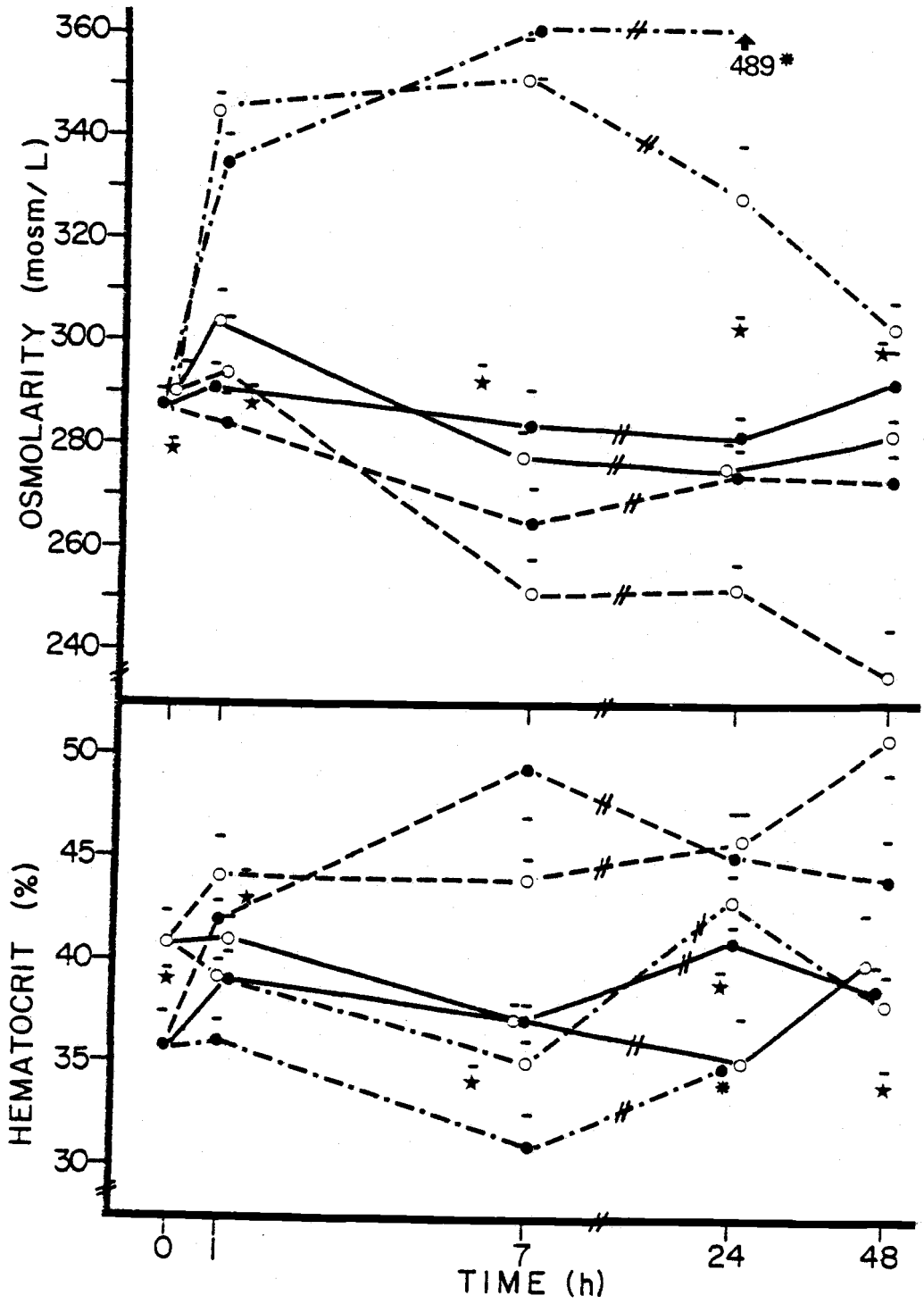
360
340
320
300
280
260
240
OSMOLARITY (mosm/L)
489*
50
45
40
35
30
HEMATOCRIT (%)
0
7
24
48
TIME (h)

Figure 3

Figure 4. Plasma cortisol concentrations in yearling coho salmon during stress. Symbols are defined in Fig. 1. Values represent the geometric mean of samples. Lines between points at 0 and 1 h were omitted for clarity.

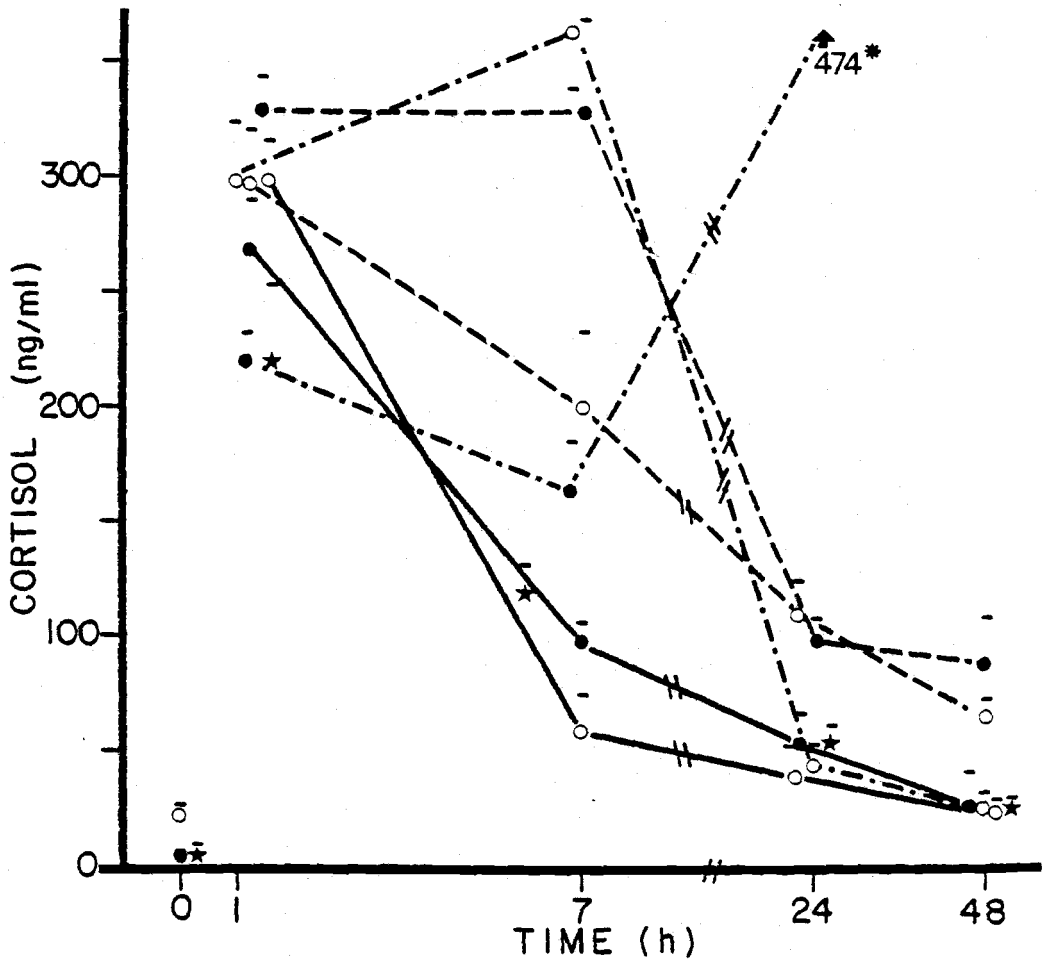

Figure 4

The magnitude of the osmotic perturbations was generally greater during stress in SW than in FW, regardless of acclimation conditions before stress. Confinement stress exacerbated the severity of the osmotic perturbation normally experienced by the fish when the ambient medium was changed from FW to SW.

Na. Results for plasma Na concentration conform to the general pattern described above (Fig. 1). Fish stressed in SW had significantly elevated Na levels at 1, 7, and 24 h; and in FW they had depressed levels at all times. No compensatory trend was evident in the SW-FW group. At 7 h, fish in the SW-1/3 SW group had lower Na levels than those in SW control fish at 0 h; but otherwise there was no significant effect during stress in 1/3 SW. Stress compounded the gain in plasma Na relative to that in the FW-SW Control group.

K. The response pattern of plasma K concentration was independent of the fish's acclimation state or the salinity during stress (Fig. 1). All groups showed a rapid increase in K levels and a return to basal concentration within 24 or 48 h, except for the FW-SW group. Plasma K response in the FW-SW Control group was opposite to that in the stressed fish in the FW-SW group at 1 h.

Mg. Fish stressed in SW had significantly higher plasma Mg levels than the fish stressed in FW or 1/3 SW at 1, 7, and 24 h after confinement (Fig. 2). Fish in the SW-FW or SW-1/3 SW groups had lower Mg levels at 7, 24, and 48 h than at 0 h. Mg levels in the FW-SW group were greatly augmented compared to those in the FW-SW Control group.

Ca. Plasma Ca levels increased in fish during stress in SW compared to controls at 0 h (Fig. 2). There was a significant increase in plasma Ca in the FW-FW group at 1 h and in the SW-FW group at 7 h. Fish in the SW-1/3 SW group had lower plasma Ca at 7, 24, and 48 h. Fish in the FW-1/3 SW group had lower Ca levels than those in the FW-FW group at 7 and 24 h. Plasma Ca in the SW-1/3 SW group was lower than that in the SW-FW group at 24 h. Ca levels in the FW-SW group were slightly augmented compared to those in the FW-SW Control group.

Osmolarity. The response pattern of plasma osmolarity was similar to that for Na (Fig. 3). No significant change occurred during stress in 1/3 SW. Plasma osmolarity was elevated at 1, 7, and 24 h in fish stressed in SW. Fish in the FW-FW group had significantly lower osmolarity at 7 h than that in FW controls at 0 h. Fish in the SW-FW group had lower plasma osmolarity at 7, 24, and 48 h and showed no compensating trend toward basal levels. Plasma osmolarity was greatly incrased by stress in the FW-SW group compared to that in the FW-SW Control group.

Hematocrit. Fish stressed in FW had significantly elevated hematocrit levels at all times, and in SW they were depressed at 7 h (Fig. 3). Hematocrit levels were higher than control levels for the FW-1/3 SW group but lower in the SW-1/3 SW group at 24 h. Fish acclimated to SW had significantly higher hematocrit levels than those in FW controls at 0 h.

Cortisol. Fish in all groups showed a rapid and significant elevation of plasma cortisol levels within 1 h (Fig. 4). By 24 h,

there was a clear trend toward basal levels; but cortisol was still significantly elevated at 48 h except in the SW-SW and SW-1/3 SW groups. At 1 h, all SW acclimated groups had similar plasma cortisol levels; but FW acclimated fish had significantly different responses, depending on the salinity during confinement. At 7 h, fish stressed in 1/3 SW had significantly lower cortisol levels than fish stressed in either FW or SW; cortisol levels were highest in the FW-FW and SW-SW groups and intermediate in the FW-SW and SW-FW groups. At 24 and 48 h, groups stressed in FW had higher cortisol levels than those stressed in 1/3 SW or those in the SW-SW group. Confinement stress in the FW-SW groups did not compound the response of cortisol observed in the FW-SW Control group until 24 h. Basal levels of cortisol at 0 h were higher in SW acclimated fish than in FW fish.

DISCUSSION

Confinement stress in yearling coho salmon caused osmoregulatory dysfunction in FW and SW; but in 1/3 SW, a medium that was approximately isosmotic to the fish's blood, stress did not induce an osmotic imbalance. The direction, magnitude, duration, and severity of the stress-induced osmotic imbalance were influenced by the ambient salinity during the stress and the acclimation state of the fish before stress. In SW, the stressed fish experienced a rapid increase in the concentration of plasma electrolytes while in FW the reverse was true, corroborating the conclusions of others (Stanley and Colby 1971; Stevens 1972).

The magnitude of change from basal levels in plasma osmolarity was generally greater in SW than in FW. Because the osmotic gradient between the blood and SW is about two times that between blood and FW, it is reasonable to predict that the magnitude of change in SW would be about twice that in FW; and the data for plasma osmolarity and Na roughly confirm this prediction. By the same logic, one would expect little change in osmotic status during stress when the medium is isosmotic to the blood. The maintenance of osmotic equilibrium during stress in 1/3 SW clearly supports this hypothesis. These results suggest that the salutary effects of salt treatment during handling or transportation of fish (e.g., Long et al. 1977) may be due to the attenuation of severe osmotic imbalance in the fish.

The duration and severity of the osmotic imbalance in FW and SW were affected by the fish's acclimation state before stress. Fish in the FW-SW group were incapable of tolerating the stressful conditions beyond 24 h; and the survivors at 24 h had extremely high osmotic levels. But, fish in the SW-SW group survived for the duration of the test and restored their internal osmolarity to basal levels. Similarly, fish in the SW-FW group showed a worsening osmotic status at 48 h while the FW-FW group showed a definite compensatory trend. Acclimation to FW or SW seems to activate different osmoregulatory mechanisms which are suitable for homeostatic control in FW or SW environments, but not both.

Normally, euryhaline fishes can make the transition from one mode of osmoregulation to the other with relatively minor fluctuations in osmotic status. However, the imposition of stress causes

osmoregulatory mechanisms to become less efficient, thus compounding the severity of the osmotic disturbance during the transition between modes. This is evident when contrasting results for the stressed fish in the FW-SW group with those of unconfined fish in the FW-SW Control group. These results corroborate those of Flagg (1981) who found a 10-50% reduction in survival rate after severe exercise and a 30-50% reduction in swimming stamina when yearling coho salmon were transferred directly from FW to SW.

In the context of fisheries management, it would seem prudent to avoid situations where physical disturbances, such as handling and transporting fish, are coincident with transfer to a medium whose salinity is incompatible to the acclimation state of the fish. Ideally, major osmotic disturbances during and after stress may be avoided or attenuated by transferring fish to a medium that is isosmotic to the fish's blood. For example, if the stress of transportation is severe, release of transported fish into a brackish estuarine environment, rather than into FW or SW, may have a salutory effect on the fish's health.

The mechanism of osmoregulatory dysfunction during stress undoubtedly involves the gill epithelia, the major site of osmotic exchange with the external medium in teleostean fishes (Maetz 1974). During stress, the net transepithelial flux of Na and Cl changes in euryhaline fishes. In FW acclimated goldfish, Carassius auratus, handling caused a five-fold increase in Na and Cl efflux (Eddy and Bath 1979). In SW acclimated mullet, Mugil capito, and mummichog, Fundulus heteroclitus, stress induced a net decrease in Na and Cl

efflux (Pic 1978). Thus, stress-induced changes in the net flux of electrolytes across gill epithelia are bidirectional and dependent on external salinity, corresponding to the bidirectional shifts in the concentrations of plasma electrolytes found by us.

Comparing the response patterns of the measured osmotic variables, we found that plasma Na and osmolarity varied similarly, suggesting that Na concentration (in conjunction with that of its counterion, Cl) was a major determinant of plasma osmolarity during stress.

In all treatment groups, plasma K levels were elevated by stress and showed similar response patterns. Apparently, the internal dynamics of K during stress obviate the effects of the external environment. During physical disturbance, the pH balance of the fish's blood shifts toward the acidic because of CO_2 and lactate production (Heisler 1980). Such a shift may cause an increase of K in plasma due to a K^+–H^+ exchange mechanism between intra- and extra-cellular fluid (Turner et al. 1983). Fish in the FW-SW Control group showed a transient decrease in plasma K concentration opposite to that in stressed fish. Interestingly, Milne and Randall (1976) found a transient increase in blood pH in rainbow trout, Salmo gairdneri, 90 min after transfer from FW to SW; and our results suggest that this may have been related to a decrease in plasma K concentration.

There are few reports concerning the effects of stress on divalent electrolytes in fishes. Acute handling stress caused less than 5% variation in plasma Mg or Ca levels in northern pike, Esox lucius, in

FW or brackish water (Soivio and Oikari 1976). Houston et al. (1971a,b) also reported little change in Mg and Ca in brook trout, Salvelinus fontinalis, after acute stress in FW. In steelhead trout, Salmo gairdneri, Ca levels were significantly elevated for 24 h after acute handling stress in FW; but in coho salmon there was a tendency for Ca to decrease (Wedemeyer 1972). Our results indicated a small but significant decline in plasma Mg levels, persisting for at least 48 h in SW acclimated fish during stress in both FW and 1/3 SW. This suggests that the osmoregulatory mechanism for Mg in SW acclimated fish is inappropriate for the conservation of Mg during stress in media that is hypo-osmotic to SW. This conclusion generally applies to Ca regulation, too.

The regulation of plasma Mg and Ca in FW acclimated fish was not consistently affected during stress in FW or 1/3 SW, unlike the monovalent cations. However, there was a tendency for Ca levels to increase initially and, in 1/3 SW, to decrease below basal levels within 7 h after stress. Some of this variability may be associated with shifts in acid-base balance during stress, leading to rapid shifts in the bone-blood equilibrium for Ca as suggested by the work of Ruben and Bennett (1981).

In SW, both FW and SW acclimated fish had inadequate mechanisms to rapidly compensate for Mg or Ca loading during stress. The elevation of Mg and Ca in fish in the FW-SW Control group was significant but slight compared to the dramatic increase seen in stressed fish, especially for Mg. Generally, the uptake and excretion of divalent ions are thought to be functions of gut and kidney

tissues; however, Kirschner et al. (1974) concluded that the gill epithelium of rainbow trout is readily permeable to Mg. The concentration of Mg in SW is approximately 50 to 100 times greater than that in coho salmon plasma. Thus, relatively small changes in gill permeability during stess in SW may result in a large influx of Mg. The gill epithelium is also permeable to Ca (Pang et al. 1980) and potentially susceptible to Ca influx during stress in SW. However, the Ca gradient between SW and blood is much smaller (about 4 to 1) than that of Mg, so the magnitude of change expected for plasma Ca during stress in SW is less than that for Mg: our data support this contention. Furthermore, Ca balance in teleostean fishes is strictly regulated by elaborate hormonal (Pang et al. 1980) and pH-related (Ruben and Bennett 1981) mechanisms which tend to dampen stress-induced perturbations.

The results for blood hematocrit response are difficult to reconcile with the literature. Continuous stress in our study caused sustained elevations of hematocrit in FW. This may have been due to erythrocyte swelling (Soivio and Nikinmaa 1981) or general hemoconcentration from the redistribution of water internally (Mazeaud et al. 1977). However, our data for plasma electrolytes and and osmolarity suggest hemodilution rather than hemoconcentration during stress in FW. Others have found that hematocrit levels increased initially and then decreased for a prolonged time after acute stress in FW (Soivio and Oikari 1976; Casillas and Smith 1977; Beggs et al. 1980), consistent with the notion of general hemodilution after stress. During stress in SW, hematocrit levels were depressed at 7 h,

contrary to the results of Fletcher (1975) who also observed erythrocyte swelling in the winter flounder, Pseudopleuronectes americanus. In our study, electrolyte levels for fish stressed in SW suggest hemoconcentration; thus, we cannot conclude that lower hematocrit levels resulted from hemodilution.

Cortisol levels in plasma before and during confinement stress appeared to be significantly influenced by the ambient salinity. Before confinement, basal levels of plasma cortisol were significantly higher in SW acclimated fish than in FW fish, 23 and 4 ng/ml respectively. It is also likely that the secretion rate of cortisol is higher in SW since the metabolic clearance rate of corticosteroids is faster in SW acclimated fish (see Chapter V).

We found that in FW acclimated fish, the level of plasma cortisol was initially lower during stress in SW than that during stess in FW, similar to the results of Strange and Schreck (1980) for Oncorhynchus tshawytscha. But, confinement in SW for more than 7 h was lethal and seemed to drastically increase cortisol levels in FW acclimated fish. Cortisol levels in the FW-1/3 SW group, however, were attenuated throughout the experiment relative to the FW-FW group, and no mortality occurred.

The salinity-dependent response of cortisol at 1 h in FW acclimated fish may have been related to a direct inhibition of steroidogenesis in the interrenal tissue by Na, such as occurs in the frog (Maser et al. 1982). If so, the higher plasma Na levels in the FW-SW group might have inhibited cortisol secretion relative to the lower Na and higher cortisol levels in the FW-FW group. Such a control mechanism might

also explain the higher cortisol levels at 24 and 48 h in fish stressed in FW, since these fish had relatively low plasma Na levels.

The initial cortisol responses at 1 h in SW acclimated fish were independent of the media in which the fish were stressed; but within 7 h after confinement, cortisol levels were affected by ambient salinity. After 7 h of confinement, SW acclimated fish that were stressed in 1/3 SW had significantly lower cortisol levels than those in fish stressed in SW, analogously to the results for FW acclimated fish. We conclude that both the acclimation conditions and the ambient salinity during stress influence the response of plasma cortisol during stress.

Osmotic imbalance was not a prerequisite for elevated cortisol level during stress, although internal and external osmotic conditions seemed to modify the response. This was evident in those fish which were stressed in 1/3 SW; cortisol levels increased even though there was no significant osmotic imbalance. Furthermore, the most severe osmotic imbalances in SW (FW-SW group) or FW (SW-FW group) were correlated with intermediate cortisol levels at 7 h, again suggesting that osmotic status is not the primary determinant of plasma cortisol levels.

Conversely, plasma cortisol concentration is probably not a direct determinant of osmotic status in coho salmon, although results for other teleosts by Henderson and Chester Jones (1967) and Umminger and Gist (1973) suggest this possiblity. Chronic elevation of plasma cortisol concentration had little effect on osmoregulatory performance in FW or during acclimation to SW in yearling coho salmon (see Chapter

III). Osmotic perturbations during stress are probably directly affected by the release of catecholamines and concomitant changes in branchial permeability to water and electrolytes (Maetz 1974; Pic et al. 1974, 1975; Mazeaud and Mazeaud 1981).

The relation of cortisol and osmotic status during stress may center on energetic requirements. Cortisol has many important effects on the intermediary metabolism of teleosts (Chan and Woo 1978; Dave et al. 1979). During stress, cortisol may act to increase oxygen consumption (Chan and Woo 1978) and to maintain high concentrations of glucose in the blood (Leach and Taylor 1980), presumably to fuel the energetic requirements of the fish. Energetic requirements at rest and during exercise are greater in media that is either hypo- or hyperosmotic to the fish's blood than in media that is nearly isosmotic (Rao 1968; Farmer and Beamish 1969). Therefore, we speculate that during stress, cortisol levels incease initially to compensate for the energetic costs associated with severe exercise and potential osmotic imbalance. If osmoregulatory costs are minimized, as is the case in isosmotic media, then energetic homeostasis is facilitated; and cortisol secretion may be reduced concomitantly.

In summary, we found that the characteristics of stress-induced osmotic imbalances were influenced by the ambient salinity and by the acclimation state of the fish. Concomitant responses of plasma cortisol during stress were also affected by ambient salinity and may potentially be related to the energetic requirements of osmoregulation.

III. CORTISOL AND OSMOREGULATORY PERFORMANCE DURING SEAWATER ACCLIMATION IN YEARLING COHO SALMON, ONCORHYNCHUS KISUTCH[1]

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Running Title: Cortisol and Osmoregulation in Salmon

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Anadromous salmonid fishes undergo physiological changes, usually during the second year of life, preparatory to their transition from a freshwater (FW) to a marine environment. Cortisol, the primary corticosteroid hormone produced by the interrenal tissue of most teleostean species (Chester Jones et al., 1969), has been implicated in osmoregulatory processes in many euryhaline teleosts (reviewed by Maetz, 1969a; Johnson, 1973; Butler, 1973; Fontaine, 1975; Holmes and Pearce, 1979). Putative theory suggests that cortisol acts to promote excretion of electrolytes in fish living in hypertonic media and conservation of electrolytes in hypotonic media (Maetz 1969a). Cortisol appears to have more pronounced effects on osmoregulatory performance during acclimation and existence in hypertonic media but may play a role secondary to that of prolactin in hypotonic media (Bern, 1975).

Histological evidence suggests that activation of the pituitary-interrenal axis occurs during acclimation to hypertonic conditions in some teleosts (Chavin, 1956; Hanke et al., 1969; Olivereau, 1962). Direct measurement of corticosteroid concentrations in circulating blood indicates a rapid, but usually transient, increase after transfer from FW to hypertonic environments in several species of fish (Leloup-Hatey, 1964; Hirano, 1969; Ball et al., 1971; Forrest et al., 1973b; Porthe-Nibelle and Lahlou, 1974; Singley and Chavin, 1975; Hanson and Fleming, 1979; Ishioka, 1980; Strange and Schreck, 1980; Assem and Hanke, 1981).

During smoltification of anadromous salmonids, the developmental process preparatory to seawater (SW) entry (reviewed by Hoar, 1976;

Folmar and Dickhoff, 1980), pituitary-interrenal activity and plasma cortisol concentration appear to increase without external hyperosmotic stimulation (Fontaine and Hatey, 1954; Olivereau, 1962, 1975; McLeay, 1975; Komourdjian et al., 1976; Specker and Schreck, 1982).

Numerous studies have demonstrated the importance of cortisol with respect to osmoregulatory processes in various species of teleosts, primarily eels Anguilla sp. Cortisol influences water and electrolyte exchange in tissues of the gill, gut, kidney, and urinary bladder, concomitantly influencing the concentration of electrolytes in the blood and urine (Mayer et al., 1967; Chan et al., 1969; Pickford et al., 1970; Lahlou and Giordan, 1970; Hirano and Utida, 1971; Utida et al., 1972; Johnson, 1973; Ando, 1974; Porthe-Nibelle and Lahlou, 1975; Doneen, 1976; Gallis et al., 1979b; Assem and Hanke, 1981).

Cortisol also affects the activity of the transport enzyme Na^+-K^+-ATPase in the ion-transporting tissues of fish (Pickford et al., 1970; Epstein et al., 1971; Butler and Carmichael, 1972; Kamiya, 1972; Doyle and Epstein, 1972; Forrest et al., 1973a; Scheer and Langford, 1976; Gallis et al. 1979a). Na^+-K^+-ATPase may partly regulate osmoregulatory performance in teleosts (Maetz, 1969b); however, changes in the activity of this enzyme cannot account entirely for changes in electrolyte exchange in the eel, A. rostrata, during acclimation to SW (Forrest et al., 1973a).

Anadromous coho salmon, Oncorhynchus kisutch, are an important economic resource on the Pacific coast of North America and are intensively cultured in hatcheries before they are released as smolts.

Specker and Schreck (1982) demonstrated increased endogenous cortisol titers in coho salmon during smoltification, suggesting anticipatory changes in osmoregulatory mechanisms before the fish enter SW. The objectives of the present study were (1) to document changes in osmoregulatory performance and endogenous cortisol levels during SW acclimation in yearling coho salmon, and (2) to determine if augmentation of endogenous cortisol before and during SW acclimation affects osmoregulatory performance.

MATERIALS AND METHODS

Coho salmon from the 1980 brood year were transported from Eagle Creek National Fish Hatchery, Columbia River drainage, to the Marine Science Center, Oregon State University, Newport, Oregon, on 18 May 1981. The fish were maintained in FW (13-15°C) in large, circular, flow-through, holding tanks and fed daily with Oregon Moist Pellet diet.

On 13 June, fish 15.8 ± 0.9 cm in fork length ($\bar{x}$ ± SD) and weighing 39.7 ± 6.9 g were anesthetized with ethyl m-aminobenzoate methanesulfonate (MS-222®); then a Silastic capsule (1.0 cm length, 0.15 cm ID) filled with dry cortisol powder was implanted into the peritoneal cavity through a small incision on the ventral surface between the anus and the pelvic fins. Suturing was not necessary; wound closure was effective within 2 d. Preliminary studies showed that cortisol does not leach through the Silastic tubing at a rate sufficient to significantly elevate plasma cortisol levels in the fish. Therefore, one end of the capsule was left open, a condition

that resulted in a continous elevation of plasma cortisol levels for at least 30 d. Plasma cortisol titers for implanted fish were approximately 240 + 205 ng/ml ($\bar{x}$ + SD; range = 44-880) during the experiment. We used cortisol because it is the major corticosteroid present in the plasma of chinook salmon, O. tshawytscha (Hane and Robertson, 1959), and preliminary analyses in our laboratory confirmed this finding for coho salmon. Implants were used to avoid stress and pulsatile variation associated with multiple injections. The implantation procedure did not seem to stress the fish; most resumed feeding within 10 minutes after recovery from anesthesia. Sham-implanted control fish received an empty capsule; the resultant plasma cortisol titers in FW were similar to basal levels in unhandled control fish, 9 + 17 ng/ml ($\bar{x}$ + SD; range = 0-108). Unhandled controls, sham-implanted controls, and cortisol-implanted fish were transferred to six circular tanks (91 cm ID, 480 liters), two tanks per group and about 80-90 fish per tank.

Between 1000 and 1200 hours on 22 June (9 d post implantation) 30 fish from one tank of each control and treatment group were quickly netted, killed by a blow to the head, weighed, and measured. Blood was collected from the severed caudal artery in ammonium-heparinized capillary tubes, centrifuged, and separated; the plasma was stored at -20 °C. Blood samples for hematocrit determination were collected in microhematocrit tubes and centrifuged; percent packed cell volumes were determined immediately. Samples of gill filaments were taken for analysis of Na^+-K^+-ATPase activity.

In the remaining tanks, one for each treatment, the inflowing water supply was changed from FW to SW (12-15 °C, 26-33 ppt salinity, depending on the tidal stage); hereafter this event is called SW entry. Flow rate in all tanks was at least 20 liters/min; thus water exchange was rapid.

Subsequently, blood samples were obtained from 10 fish at 1.5, 6, 12, 24, 48, 72, and 120 h after SW entry in unhandled control fish; sham- and cortisol-implanted fish were sampled at 6, 24, 48, 72, and 120 h. Control and implanted fish in FW were sampled at 24 and 120 h. Fish held after the last sampling period appeared healthy and fed normally until 16 d post-implantation, after which extensive mortality occurred in cortisol-treated groups in both FW (80%) and SW (50%). Post-mortem analyses revealed extensive internal lesions and general deterioration of the viscera. A gram-positive bacterial infection, possibly Lactobaccilus sp., (K. Lannan, personal communication) was present in the cortisol-treated fish in both FW and SW. Control fish were unaffected by the disease. During this period FW temperature increased to 16-18 °C while SW temperature decreased to 9-15° C due to coastal upwelling.

To establish the effects of long-term SW acclimation on plasma cortisol levels we sampled fish that had been in SW for 21 d; control fish in FW were also sampled. These samples were taken on 12 June, when the average temperatures of FW and SW were similar.

Cortisol was measured by a radioimmunoassay (RIA) modified from Foster and Dunn (1974) and validated for yearling coho salmon by us. In this technique, 10 µl of sample or standard is diluted with 200 µl

glutamate buffer (pH 3.3) in borosilicate glass test tubes. The mixture is heated in a water bath at 90-100° C for 15 min. After cooling to room temperature, 0.04 µCi of 1,2,6,7 ^{3}H-cortisol (New England Nuclear) in 500 µl phosphate buffer (pH 7.6) is added and mixed. Next, 100 µl of cortisol-specific antibody (Endocrine Science, Tarzana, CA) calculated to bind approximately 50% of the total radioactivity is added. The tubes are then vortexed gently and allowed to equilibrate at room temperature for 60 min. After 5 min in an ice bath, 200 µl of ice-cold 2.5% dextran-coated charcoal is added to each tube which is then vortexed gently and left in the ice bath for 5 min before centrifuging at 1000 g, 0 °C. Then 500 µl of the supernatant is transferred to 5 ml of an appropriate scintillation cocktail. The standard curve is transformed to log pg versus logit cpm; and the linear regression of the resulting line is used to calculate sample cortisol concentration.

Total counts, non-specific binding, blanks, and cortisol level of standard plasma samples were determined in each assay. Sensitivity of the assay was below 20 pg. Intra- and inter-assay coefficients of variation were both 5%. When samples of plasma were analyzed by RIA alone or by RIA after purification with thin layer chromatography (TLC), the ratio of average values obtained by the two methods was 0.98 (RIA/TLC:RIA) after correction for procedural losses. Cross-reactivity (measured at 50% displacement of bound ^{3}H-cortisol) with cortisone, the other major corticosteroid in teleostean fishes, was 7.0%. Recovery of added cortisol was 92% and dilution of standard plasma yielded results parallel to the standard curve.

Because plasma cortisol increases rapidly during stress (Strange et al., 1977), only the first 10 fish in any sample were utilized for cortisol determinations. Other measured variables showed no significant effects due to sampling stress.

Osmolarity was measured on a vapor pressure osmometer. Total concentrations of electrolytes (Na, K, Ca, Mg) in plasma were determined on a flame atomic absorbtion spectrophotometer; the proportion of these elments in free ionic form was not determined. Gill Na^+-K^+-ATPase activity was measured according to methods described by Johnson et al. (1977).

Statistical analyses of the data followed methods of Nie et al. (1975). Analysis of variance (in which we used a regression approach) on salinity, treatment, and time was followed by Duncan's Multiple Range Test where appropriate. Cortisol data were transformed into natural log to increase homogeneity of variance; thus these results are reported as geometric means. Pearson product moment correlations were calculated for all possible pairs of variables in each set of samples. Scatterplots and linear regression were used to analyze relations that appeared significant in discrete sets of samples and for pooled data from FW control groups and from SW control groups.

RESULTS

None of the measured variables differed significantly between sham-implanted and unhandled control groups; therefore data from these groups were pooled and treated as a single control.

Plasma cortisol levels increased rapidly in control fish after SW entry (Fig. 5). Maximum concentrations of about 220 ng/ml occurred within 1.5 h, followed by a rapid decline toward basal levels ($\bar{x}$ = 9 ng/ml). Cortisol titers were still slightly but significantly elevated ($\bar{x}$ = 23 ng/ml) after 21 d in SW. Plasma cortisol concentrations did not change in FW control fish; and they did not increase in cortisol-treated fish after SW entry.

Gill Na^+-K^+-ATPase activity was significantly lower in cortisol-treated fish in FW 9 d after implantation (Fig. 6). Cortisol treatment induced a 50% decrease in Na^+-K^+-ATPase, from 3.6 to 1.8 µmoles P_i/(mg protein•h).

Plasma osmolarity in control fish increased immediately after SW entry and was still elevated after 120 h (Table 1). Cortisol-treated fish tended to maintain higher osmolarity than control fish after 48 h. Plasma osmolarity in FW groups did not change significantly.

Plasma Na levels increased within 6 h after SW entry and remained elevated for at least 120 h relative to FW controls (Table 1). Cortisol treatment had no effect on plasma Na in SW or FW.

Plasma K levels were highly variable (Table 1). They were lower in SW than in FW control fish at 1.5 and 120 h. Cortisol treatment had no effect on plasma K levels in SW or FW.

Plasma Ca concentrations increased within 1.5 h after SW entry and remained elevated for at least 120 h (Table 1). Cortisol treatment had no effect on Ca levels in SW. Plasma Ca was higher in cortisol-treated fish in FW at 0 h but declined progressively thereafter to levels lower than those in control fish at 120 h.

Fig. 5. Plasma cortisol levels in yearling coho salmon in freshwater (FW) and during acclimation to seawater (SW) in yearling coho salmon. Cortisol values are the geometric mean, bars indicate ± SE, n = 20 unless shown by numeral. Values after 21 d indicated by "A". All values in SW fish were significantly ($P < 0.05$) higher than those in FW controls, except for that marked by "NS".

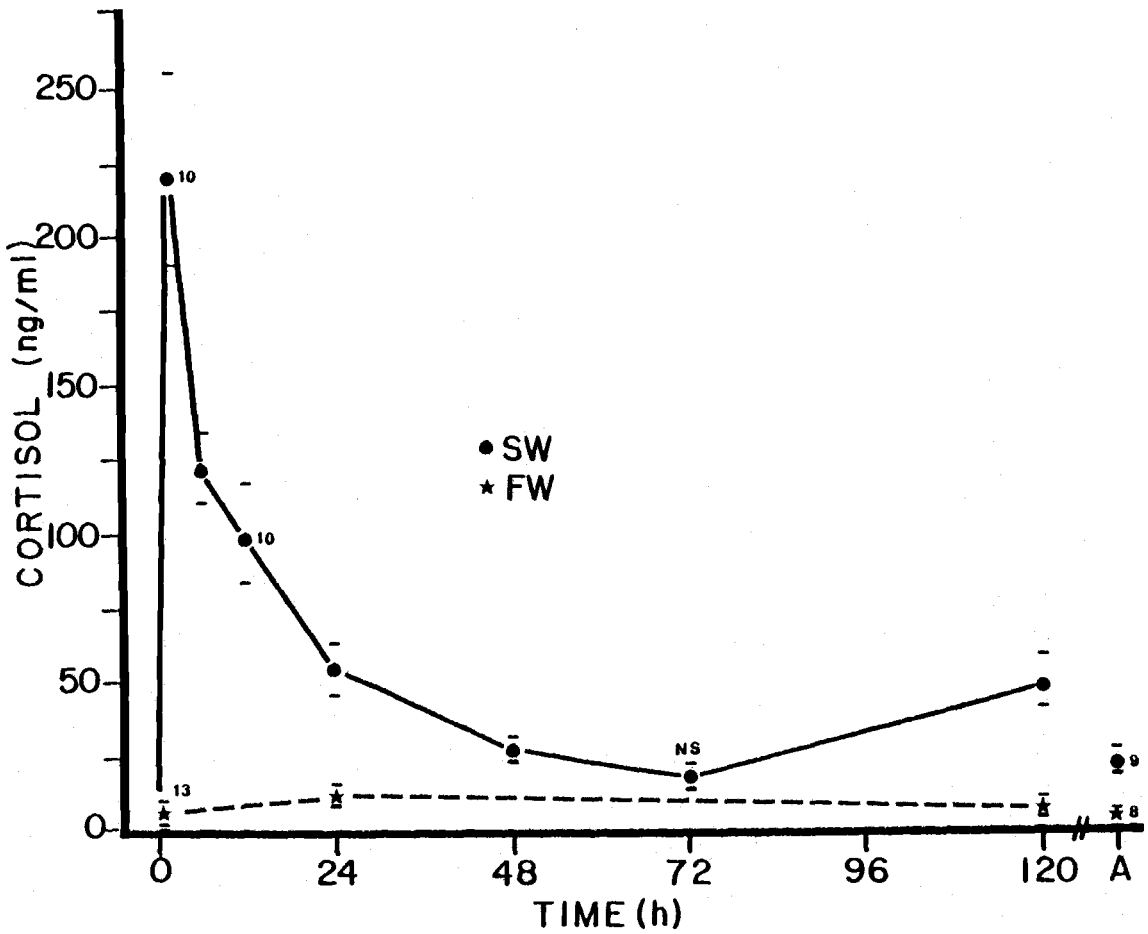
250
200
150
100
50
0
CORTISOL (ng/ml)
SW
FW
10
10
13
NS
9
8
0
24
48
72
96
120
A
TIME (h)

Figure 5

Fig. 6. Gill Na^+-K^+-ATPase activity in yearling coho salmon after 9 d treatment with cortisol (F) and in control fish (C) in freshwater. Units are in μmoles Pi/(mg protein • h). Values are the means, bars indicate SE, numerals indicate sample sizes. Difference between means is significant ($P < 0.01$).

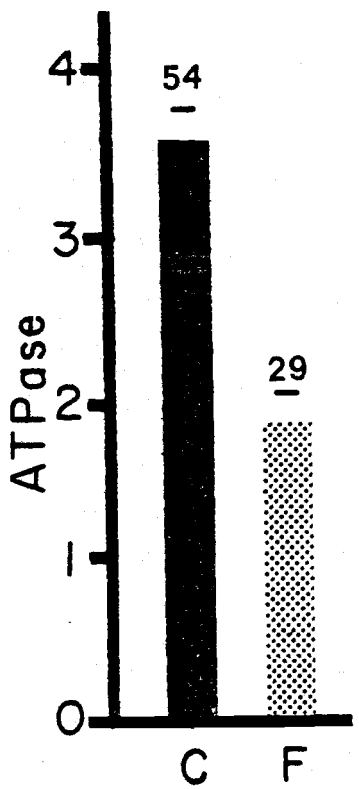

Figure 6

TABLE 1

Summary of mean ± S.E. (n) plasma osmolarity (mOsm/liter), Na, K, Ca, Mg (meq/liter) and blood hematocrit (%) for control and cortisol-treated fish in FW and during SW acclimation.

	Hours After SW Entry	FW		SW	
		Control	Cortisol	Control	Cortisol
Osmolarity	0	279 ± 1.0 (59)	281 ± 1.8 (30)	-	-
	1.5	-	-	288 ± 2.4 (10)[a]	-
	6	-	-	293 ± 2.7 (20)[a]	298 ± 3.1 (10)[a]
	12	-	-	299 ± 3.2 (10)[a]	-
	24	283 ± 2.1 (20)	276 ± 3.3 (10)	304 ± 2.3 (20)[a]	302 ± 4.7 (11)[a]
	48	-	-	299 ± 2.3 (20)[a]	303 ± 3.3 (10)[a]
	72	-	-	299 ± 3.4 (20)[a]	308 ± 8.3 (10)[a]
	120	281 ± 1.8 (20)	281 ± 2.1 (10)	288 ± 1.9 (20)[a]	300 ± 3.0 (10)[a,c]
Na	0	129 ± 0.8 (59)	130 ± 0.8 (30)	-	-
	1.5	-	-	132 ± 1.8 (10)	-
	6	-	-	135 ± 1.1 (20)[a]	137 ± 2.5 (10)
	12	-	-	141 ± 2.7 (10)[a]	-
	24	128 ± 1.2 (20)	128 ± 2.2 (10)	143 ± 1.0 (20)[a]	142 ± 1.4 (11)[a]
	48	-	-	140 ± 1.6 (19)[a]	137 ± 1.9 (10)
	72	-	-	140 ± 2.4 (20)[a]	145 ± 4.8 (10)[a]
	120	129 ± 1.1 (20)	125 ± 2.8 (10)	136 ± 1.0 (20)[a]	135 ± 1.8 (10)
K	0	4.9 ± 0.15 (59)	4.6 ± 0.18 (30)	-	-
	1.5	-	-	3.1 ± 0.24 (10)[a]	-
	6	-	-	4.7 ± 0.16 (20)	4.5 ± 0.28 (10)
	12	-	-	4.6 ± 0.21 (10)	-
	24	4.9 ± 0.18 (20)	5.3 ± 0.31 (10)	4.4 ± 0.21 (20)	3.9 ± 0.33 (11)
	48	-	-	4.9 ± 0.20 (20)	4.4 ± 0.29 (10)
	72	-	-	4.6 ± 0.19 (20)	5.0 ± 0.39 (10)
	120	4.9 ± 0.14 (20)	5.1 ± 0.35 (10)	4.0 ± 0.14 (20)[a]	4.3 ± 0.38 (10)

TABLE 1. Continued

	Hours After SW Entry	FW		SW	
		Control	Cortisol	Control	Cortisol
Ca	0	4.5 ± 0.04 (59)	4.7 ± 0.05 (30)[b]	-	-
	1.5	-	-	4.8 ± 0.10 (10)[a]	-
	6	-	-	4.6 ± 0.07 (20)	4.7 ± 0.17 (10)
	12	-	-	4.8 ± 0.09 (10)[a]	-
	24	4.3 ± 0.05 (20)	4.3 ± 0.18 (10)[a]	5.0 ± 0.06 (20)[a]	4.8 ± 0.06 (11)
	48	-	-	4.7 ± 0.09 (20)	4.7 ± 0.12 (10)
	72	-	-	4.7 ± 0.09 (20)	4.8 ± 0.13 (10)
	120	4.4 ± 0.05 (20)	3.7 ± 0.22 (10)[a,b]	4.7 ± 0.05 (20)	4.5 ± 0.19 (10)
Mg	0	1.3 ± 0.02 (59)	1.3 ± 0.03 (30)	-	-
	1.5	-	-	1.6 ± 0.06 (10)	-
	6	-	-	1.8 ± 0.08 (20)[a]	1.8 ± 0.18 (10)[a]
	12	-	-	1.8 ± 0.15 (10)[a]	-
	24	1.3 ± 0.04 (20)	1.2 ± 0.06 (10)	2.0 ± 0.14 (20)[a]	1.4 ± 0.08 (11)[c]
	48	-	-	1.9 ± 0.10 (20)[a]	1.6 ± 0.14 (10)
	72	-	-	1.6 ± 0.15 (20)	1.7 ± 0.16 (10)[a]
	120	1.3 ± 0.04 (20)	1.1 ± 0.09 (10)[a]	1.3 ± 0.05 (20)	1.4 ± 0.06 (10)
Hematocrit	0	39 ± 0.5 (59)	41 ± 0.8 (30)[b]	-	-
	1.5	-	-	43 ± 1.0 (10)[a]	-
	6	-	-	34 ± 0.9 (19)[a]	37 ± 1.1 (10)[a]
	12	-	-	35 ± 0.8 (10)[a]	-
	24	34 ± 0.5 (20)[a]	35 ± 1.3 (10)[a]	39 ± 0.6 (20)	39 ± 0.9 (11)
	48	-	-	34 ± 0.8 (20)[a]	35 ± 0.8 (10)[a]
	72	-	-	33 ± 0.7 (19)[a]	36 ± 1.1 (10)[a,c]
	120	35 ± 1.0 (8)[a]	37 ± 1.0 (10)[a]	35 ± 0.6 (20)[a]	38 ± 1.0 (10)[a,c]

[a]Significantly different from sample in FW at 0 h ($P < 0.05$).

[b]Significantly different from control ($P < 0.05$).

[c]Significantly different from control ($P < 0.01$).

Plasma Mg increased within 6 h after SW entry and remained elevated for at least 48 h (Table 1). At 24 h, Mg levels were lower in cortisol-treated fish in SW than in control fish.

Hematocrit levels decreased during the experiment in both FW and SW (Table 1). Cortisol elevated treatment hematocrit levels in FW at 0 h and in SW at 72 and 120 h.

Significant correlations were evident between osmolarity, Na, K, Ca, and Mg in most groups. Significant positive correlations were evident in many groups between plasma cortisol and K levels and between cortisol and hematocrit values. Significant negative correlations were frequently evident between weight (or length) and plasma K levels and osmolarity. Na^+-K^+-ATPase activity was not correlated to plasma electrolyte or cortisol concentrations in FW or SW.

DISCUSSION

Cortisol titers in plasma of yearling coho salmon increased rapidly after SW entry and then gradually returned to levels that were slightly but significantly higher than basal levels in FW. Chronic treatment with cortisol reduced Na^+-K^+-ATPase activity in gill tissue of FW fish; however, it had no significant effect on the fish's osmoregulatory performance in FW of after SW entry.

The rapid surge of plasma cortisol titers in coho salmon after SW entry corroborates similar findings in other teleosts (Hirano 1969; Forrest et al., 1973b; Porthe-Nibelle and Lahlou, 1974; Strange and

Schreck, 1980). However, contrary to most previous studies, cortisol levels were still slightly elevated after extended acclimation to SW.

Increased plasma cortisol titers during SW acclimation suggest possible involvement of this hormone in the induction of SW osmoregulatory mechanisms. Alternately, the stimulus of a new set of environmental conditions, here SW, may have induced a general stress response typical of acute physical disturbances (Donaldson, 1981; Schreck, 1981) with a concomitant increase in cortisol titer. Transition from a hyper- to a hypo-osmotic environment may also be considered a novel, and perhaps stressful, stimulus. Such a transition caused cortisol titers to increase in tilapia, Sarotherodon mossambicus (Assem and Hanke, 1981), and the red sea bream, Chrysophrys major (Ishioka, 1980), but had no such effect in eels (Hirano, 1969; Nishimura et al., 1976). Thus, for eels, environmental novelty would seem to be an insufficient explanation for the rise in plasma cortisol titers observed by Hirano (1969) and Forrest et al. (1973b) when the fish were transferred from FW to SW.

The duration of the initial surge in cortisol titer after SW entry may have some significance to the fish's physiological readiness to enter SW. Longer periods of elevated cortisol may signify less efficient osmoregulatory performance and shorter periods may signify the converse. Similar rationale has been employed for transient increases in plasma Na level after SW entry in coho salmon (Clarke and Blackburn, 1977).

Cortisol treatment for 9 d in FW significantly reduced Na^+-K^+-ATPase activity in gill tissue by 50% compared with control

levels. This result is contrary to predictions based on previous work with the mummichog, Fundulus heteroclitus, (Pickford et al., 1970) and eels (Epstein et al., 1971; Kamiya, 1972: Doyle and Epstein, 1972; Forrest et al., 1973a). Although gill Na^+-K^+-ATPase activity was lower in cortisol-treated fish in the present study, the decrease was not reflected in the concentrations of plasma electrolytes or osmolarity. Nor was there any apparent correlation between enzyme activity and cortisol or electrolyte levels within groups. Thus, changes in Na^+-K^+-ATPase activity do not necessarily induce changes in osmoregulatory performance in yearling coho salmon in FW.

Hematocrit was consistently higher in cortisol-treated fish in both FW and SW relative to control fish. However, because osmotic status did not change after treatment with cortisol the effect on hematocrit was probably due to an increase in the number or size of circulating red blood cells rather than hemoconcentration. Significant positive correlations between cortisol titer and hematocrit level within several groups further supports the suggestion of a direct relationship.

Cortisol treatment did not induce significant and consistent changes in plasma electrolyte concentrations in either FW or SW; nor did it significantly affect plasma osmolarity except after 120 h in SW. Changes in plasma osmolarity and electrolyte concentrations after SW entry were similar to those reported by others (Conte et al., 1966; Miles and Smith, 1968; Folmar and Dickhoff, 1981). The general unresponsiveness of osmoregulatory performance to cortisol treatment is surprising, given the numerous reports of responsiveness in other

species, especially in SW (see reviews by Fontaine, 1975; Folmar and Dickhoff, 1980). Significant positive correlations between plasma cortisol and K concentrations did occur in several discrete groups of fish, but this result was probably indirect since chronic treatment with cortisol had no apparent effect on plasma K levels.

The general unresponsiveness of osmoregulatory performance to cortisol treatment in juvenile coho salmon may have several plausible explanations. First, in coho salmon, cortisol may not play a significant role in osmoregulatory processes as it does in some other species. In this case, the transient surge in endogenous cortisol after SW entry may be related to stress or other general metabolic requirements during acclimation to SW. Recently, Foskett et al. (1981) reported that cortisol treatment in FW acclimated tilapia significantly increased the number of chloride cells in the opercular membrane but had no effect on Cl secretion. Cortisol may function as an ionocyte chalone, promoting chloride cell proliferation as Conte (1979) postulated; but some factor other than cortisol may be required to activate ion transport mechanisms.

Secondly, the level of cortisol induced by the implanted capsules may have been inappropriate. Most investigators reporting significant effects by injection of cortisol have used pharmacological doses. The average titer resulting from cortisol treatment in the present experiment was approximately that experienced by the fish soon after SW entry, but considerably higher than that observed by Specker and Schreck (1982) during smoltification. The duration of the cortisol treatment (9 d) before SW entry was consistent with results by Forrest

et al. (1973a), which showed treatment for 10-14 d to be more effective than treatment for 2 d in A. rostrata. However, long-term chronic treatment of coho salmon in our experiments eventually resulted in physical deterioration of the fish and high mortality, similar to that observed by Robertson et al. (1963) in rainbow trout, Salmo gairdneri, that were chronically treated with cortisol.

Thirdly, the fish may have been at a developmental stage in which they were refractory to cortisol. Coho salmon revert to a parr-like condition if they remain in FW after smoltification (Hoar, 1976). Presumably, the sensitivity of osmoregulatory mechanisms to internal and external stimuli is optimal during the later stages of smoltification based on the correlation betwen thyroid hormone peaks and subsequent survival rates in SW as shown by Folmar and Dickhoff (1981). It is impossible to know precisely the developmental stage of the fish used in our experiment; but they had a silvery, smolt-like appearance and seemed to adapt readily to SW both behaviorally and physiologically. Similar experiments conducted at different times during the smoltification cycle would help elucidate this question.

Finally, a transient surge of cortisol immediately after SW entry, such as that experienced by control fish, may have been sufficient to induce all the physiological modifications necessary for osmoregulation in SW. In this case, chronic elevation of plasma cortisol may have been superfluous and eventually, as we observed, detrimental to the fish's well-being. Short-term (24 h) increases of plasma cortisol in FW eels caused a delayed and prolonged (7 d) increase in water transport across the gut, similar to changes

occurring naturally after SW entry (Hirano and Utida, 1971). When eels are transferred from FW to SW, cortisol is required to increase gill Na^+-K^+-ATPase activity initially but it is not required to maintain that higher activity (Scheer and Langford, 1976). To test the hypothesis that a transient surge of cortisol is sufficient for osmoregulation in SW, it would be necessary to conduct similar experiments with hypophysectomized or interrenalectomized animals, with and without replacement therapy with cortisol. Unfortunately, techniques for such surgical manipulations are unavailable for salmon at this time.

Our results confirm the general observation that endogenous cortisol titers increase rapidly during the initial phase of SW acclimation in euryhaline teleosts. However, they do not support the idea that cortisol directly influences osmoregulatory performance in coho salmon.

IV. EFFECTS OF CORTISOL ON THYROID HORMONE CONCENTRATIONS IN FRESHWATER AND DURING SEAWATER ACCLIMATION IN YEARLING COHO SALMON, ONCORHYNCHUS KISUTCH[1]

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Evidence for a functional relation between thyroid hormones and seawater (SW) acclimation in euryhaline teleostean fishes is suggestive but inconclusive. Teleosts inhabiting SW environments generally have higher thyroid activity than those in freshwater (FW) (Chavin, 1966). Levels of thyroid hormones in plasma of salmonid fishes may increase (Folmar and Dickhoff, 1979, 1981), decrease (Grau et al., 1980; Specker, 1980; Dickhoff et al., 1982), or remain unchanged (Milne and Leatherland, 1980a) during acclimation to SW. Administration of exogenous thyroxine (T_4) affects excretion of chloride (Baraduc, 1957), salinity preference (Baggerman, 1960), and the activity of gill NA^+-K^+-ATPase, an enzyme associated with ion-transporting membranes (Dickhoff et al., 1977), in salmonid fishes.

During smoltification of coho salmon, Oncorhynchus kisutch, the developmental process preparatory to SW entry (reviewed by Hoar, 1976; Folmar and Dickhoff, 1980), endogenous levels of thyroid hormones increase (Dickhoff et al., 1978; Grau et al., 1981), suggesting a possible role for thyroid hormones in the development of SW osmoregulatory capacity. Osmoregulatory performance in smolts of amago salmon, O. rhodurus, 24 h after transfer from FW to SW was most efficient immediately after a pronounced surge in thyroid activity (Nagahama et al., 1982). Further supporting this hypothesis is the finding that survival in SW was correlated to certain aspects of the T_4 surge during smoltification (Folmar and Dickhoff, 1981). However, work by Loretz et al. (1982) suggests that the T_4 surge may

not be the proximate stimulus for osmoregulatory changes observed in various tissues of coho salmon during smoltification.

Corticosteroid hormones, produced by the interrenal tissue of fishes, have also been implicated in smoltification (Specker and Schreck, 1982) and osmoregulatory function (Maetz, 1969). Consequently, the interrelation of corticosteroid and thyroid hormones is of interest with respect to smoltification and osmoregulation in fishes. Olivereau (1963, 1972) showed histologically that T_4 increased the activity of interrenal cells in the eel, Anguilla anguilla, and also reported a tendency for exogenously administered cortisol to reduce the size of pituitary thyrotropes. Thyroid epithelial cell size was reduced by adrenocortical extracts in goldfish, Carassius auratus (Chavin, 1956), and by exogenous cortisol in gonadectomized sockeye salmon, O. nerka (Van Overbeeke and McBride, 1971). Stimulatory effects of corticosteroids on thyroid activity have also been reported (Singh, 1969; Milne and Leatherland, 1980b).

The objectives of this research were (1) to document the responses of the thyroid hormones, T_4 and triiodothyronine (T_3), during acclimation to SW relative to changes in plasma cortisol level and osmoregulatory performance, and (2) to determine if administration of exogenous cortisol affects the levels of T_3 and T_4 in FW and during SW acclimation in yearling coho salmon.

MATERIALS AND METHODS

Experimental animals and methods used in this study were the same as those described by Redding et al. (see Chapter II). Briefly, yearling coho salmon in FW received intraperitoneal implants of cortisol 9 d before their water supply was changed from FW to SW (referred to as SW entry). Control fish received sham implants or were not handled at all. Samples of blood and gill tissue were taken at various times from 0 to 120 h after SW entry. Hematocrit level was determined immediately after sampling. Blood plasma was frozen and later analyzed for cortisol, osmolarity, Na, K, Ca, and Mg. Plasma concentrations of T_3 and T_4 were determined by radioimmunoassay according to methods described by Dickhoff et al. (1978).

Data were analyzed by analysis of variance and Duncan's Multiple Range Test where appropriate (Nie et al., 1975). Pearson product moment correlations were calculated for all variables with T_3 and T_4 in each discrete set of samples and for pooled samples from all SW or FW control groups.

RESULTS

Sham-implanted and unhandled control fish were not significantly different for any of the measured variables; therefore, data from these fish were pooled and considered as a single control group.

Plasma levels of T_3 and T_4 in the fish increased after SW entry (Figs. 7 and 8). Plasma T_3 levels were significantly ($P < 0.05$) elevated at 6 and 12 h after SW entry; the shape of the response curve

Fig. 7. Plasma triiodothyronine (T_3) concentrations in yearling coho salmon in freshwater (FW) and during seawater (SW) acclimation for fish treated chronically (starting 9 d before 0 h) with cortisol (F) and for untreated control fish (C). Values represent the mean, bars above or below points indicate SE, numerals indicate sample size.

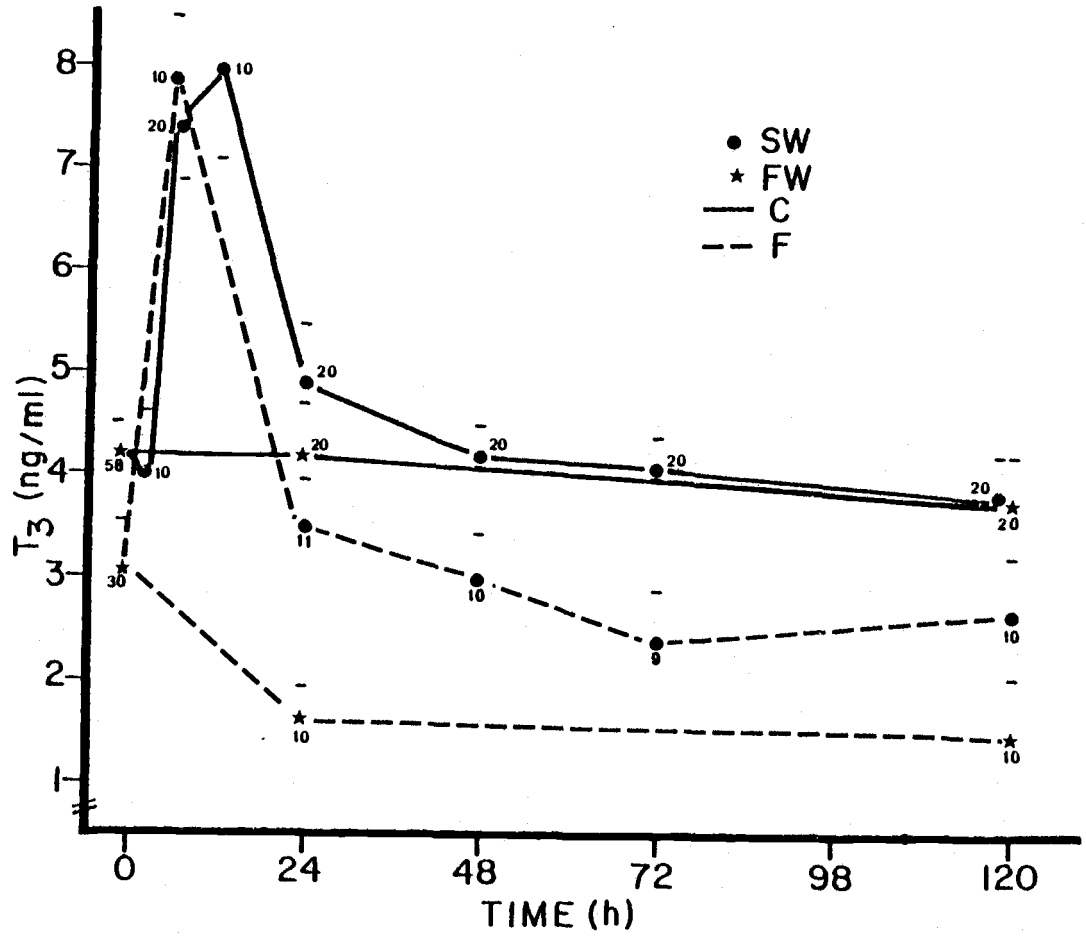

Figure 7

Fig. 8. Plasma thyroxine (T_4) concentrations in yearling coho salmon in freshwater (FW) and during seawater (SW) acclimation for fish treated chronically (starting 9 d before 0 h) with cortisol (F) and for untreated control fish (C). Values represent the mean, bars above or below points indicate SE, numerals indicate sample size.

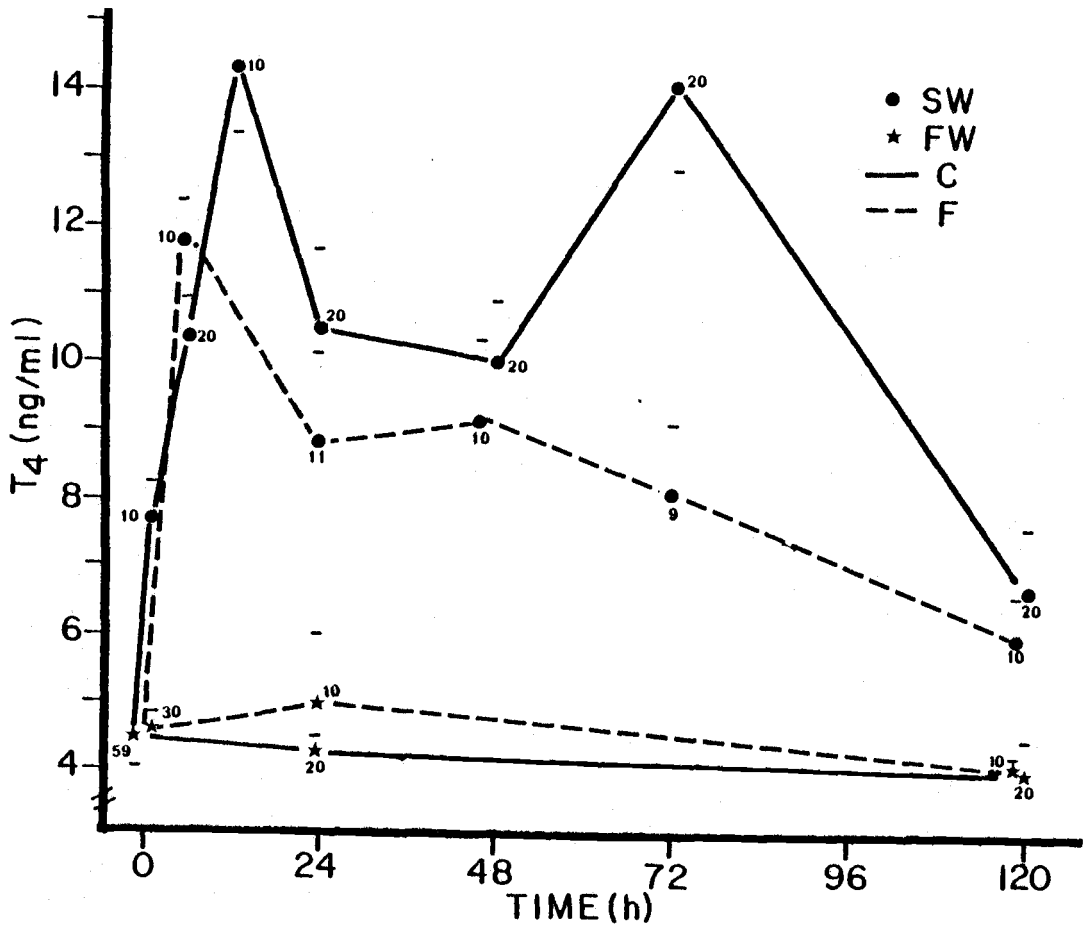

Figure 8

was unimodal. Plasma T_4 levels were significantly elevated within 1.5 h after SW entry and remained higher throughout the experiment. The shape of the T_4 response curve in SW control fish was distinctly bimodal, peak levels of about 14 ng/ml occurring at 12 and 72 h. In FW control fish neither T_3 nor T_4 levels changed significantly during the experiment.

Chronic treatment with cortisol significantly ($P < 0.01$) decreased T_3 levels in FW and during acclimation to SW (Fig. 7). In FW, T_3 levels were reduced by cortisol at all sampling times. After SW entry, cortisol treatment did not prevent the rapid surge of T_3 evident in untreated control fish; however, T_3 levels were consistently lower in cortisol-treated fish after 24 h in SW ($P < 0.05$ at 48 and 72 h). In cortisol-treated fish in FW, T_3 concentrations were lower ($P < 0.05$) at 24 and 120 h than at 0 h.

Cortisol treatment did not significantly affect plasma T_4 in FW or SW except at 72 h after SW entry when T_4 levels were lower ($P < 0.01$) in cortisol-treated fish (Fig. 8). Cortisol treatment appeared to negate the bimodal response of T_4 during SW acclimation.

Significant ($P < 0.05$) positive correlations were observed in several sets of samples between weight (or length) and T_3 and T_4. Significant negative correlations were evident between plasma K^+ concentration and T_3 and T_4 titers in some samples.

DISCUSSION

Circulating levels of T_3 and T_4 in plasma increased rapidly after SW entry in yearling coho salmon. Plasma T_3 levels returned to basal

value within 24 h after SW entry, but T_4 levels remained elevated for at least 5 d. Chronic treatment with cortisol significantly reduced plasma T_3 levels in FW and after SW entry, but it had little effect on T_4 levels.

Increased concentrations of plasma T_3 and T_4 after SW entry in this study corroborate the earlier findings of Folmar and Dickhoff (1979, 1981) but contradict the results of others (Grau et al., 1980; Milne and Leatherland, 1980a; Specker, 1980; Dickhoff et al., 1982). Strikingly, the bimodal response of T_4 and unimodal response of T_3 after SW entry in our study were similar to those reported by Folmar and Dickhoff (1979, 1981) although the relative timing of the peaks differed between studies. Thyroid hormone responses during acclimation to SW are highly variable (W.W. Dickhoff, personal communication). Juvenile coho salmon were used in the published research cited above; thus, it is likely that differences in experimental conditions, developmental state of the fish, or both, account for the discrepant findings between studies. For example, stress associated with certain experimental procedures may influence T_4 levels (Brown et al., 1978). Lunar periodicity may also affect endogenous T_4 levels in some cases (Grau et al., 1981).

A positive correlation frequently was found between fish size and thyroid hormone levels, corroborating previous results for coho salmon (Folmar and Dickhoff, 1981) and rainbow trout Salmo gairdneri (Brown et al., 1978). Larger fish also tended to have lower plasma K concentrations (see Chapter III). Together these relationships to body

size may account for the observed negative correlation between thyroid hormone and plasma K^+ levels in several sets of samples.

Plasma T_3 and T_4 levels were positively correlated ($P < 0.01$) in a pooled sample from all SW control groups, but not in a pooled sampled from all FW control groups. The correlation may have been evident in SW but not in FW because of the generally higher levels of T_4 and T_3 in SW. The positive relation between T_4 and T_3 in SW is consistent with the findings of Folmar and Dickhoff (1981).

Cortisol treatment in juvenile coho salmon clearly decreased the levels of circulating T_3 in FW and during SW acclimation, while having little effect on T_4 levels. We cannot exlain the apparent decrease in plasma T_3 concentrations in FW cortisol-treated fish between 0 and 24 h. These results generally support the theory that products of the pituitary-interrenal axis inhibit thyroid hormone production in fishes (Chavin, 1956; Olivereau, 1972; Van Overbeeke and McBride, 1971; Leatherland and Lam, 1971). The nature of this inhibition in coho salmon is unclear because only T_3, and not T_4, was affected. In rainbow trout, injection of cortisol tended to decrease T_3 levels by 30% (non-significant) while simultaneously raising T_4 levels by 78% (Milne and Leatherland, 1980b). Perhaps the mechanism by which cortisol induced lower T_3 levels involves a more rapid clearance of T_3 from the blood or a slower conversion of T_4 to T_3.

Cortisol treatment did not prevent a rapid and maximal response by T_3 after SW entry (Fig. 7). Thus the mechanism by which T_3 levels are stimulated by SW remained functional despite elevated cortisol levels.

Functional interactions may occur between cortisol and thyroid hormones during the fish's acclimation to SW. After SW entry there was a rapid increase in plasma cortisol levels (see Chapter III) coincident with surges in T_3 and T_4 levels. Interrenal activity in the eel was stimulated by exogenous T_4 (Olivereau, 1963). Jaffe (1981) showed that corticosterone production in response to adrenocorticotropin was enhanced after treatment with T_3 in the frog, Rana catesbeiana. Thyroid hormones may also affect the matabolic clearance of corticosteroids in mammals (Schriefers, 1967). Conversely, corticosteroid hormones seem to affect the level of thyroid hormones as discussed above, although there is no consensus about the nature of this relation.

Regarding the possible osmoregulatory function of thyroid hormones, our results suggest that T_3 has no direct role because the cortisol-induced decrease in T_3 levels had no apparent effect on osmoregulatory performance in FW or during SW acclimation (see Chapter III).

In summary, our results support the notion that thyroid hormones are involved in the process of SW acclimation, although the direct involvement of T_3 in osmoregulatory function seems unlikely. Importantly, cortisol may have a direct role with respect to thyroid hormone metabolism in yearling coho salmon.

V. METABOLIC CLEARANCE OF CORTICOSTEROIDS IN YEARLING COHO SALMON, ONCORHYNCHUS KISUTCH, IN FRESHWATER AND SEAWATER AND AFTER STRESS[1]

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Running Title: Clearance of Corticosteroids in Salmon

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Corticosteroid hormones have diverse functions in teleosts. They affect osmoregulatory performance (Maetz, 1969) and intermediary metabolism (Chan and Woo, 1978) in some species. Corticosteroids have also been implicated in physiological changes attendant to sexual maturation (Robertson et al., 1961; Sundararaj and Goswami, 1966; Terkatin-Shimony et al., 1980) and environmental stress (Mazeaud et al., 1977; Donaldson, 1981; Schreck, 1981).

Measurement of endogenous corticosteroid levels in plasma yields only partial information on hormone dynamics. An equally important parameter of hormone dynamics is the metabolic clearance rate (MCR), defined as the volume of plasma irreversibly cleared of hormone per unit of time (Tait and Burstein, 1964).

Changes in the MCR of cortisol during sexual maturation and subsequent senescence in sockeye salmon, Oncorhynchus nerka were reported by Donaldson and Fagerlund (1968, 1970). Gonadectomy of adult sockeye salmon decreased the MCR of cortisol during the period of normal sexual maturation (Donaldson and Fagerlund, 1970) and replacement therapy with androgens in males or estrogens in females, stimulated MCR relative to gonadectomized control fish (Donaldson and Fagerlund, 1969; Fagerlund and Donaldson 1969). Leloup-Hatey (1974) and Henderson et al. (1974) observed differences in the MCR of cortisol between eels, Anguilla anguilla, acclimated to freshwater (FW) and to seawater (SW). Similar differences were reported for adult sockeye salmon acclimated to FW and to SW (Donaldson and Dye, 1970).

In coho salmon, Oncorhynchus kisutch, the concentration of endogenous corticosteroids in plasma appears to increase during smoltification (Specker and Schreck, 1982) and during acclimation to SW (see Chapter III). The MCR of corticosteroids may change concomitantly during smoltification and SW acclimation, perhaps as a cause or result of changing plasma cortisol titers. It is reasonable to suppose that the activity of enzymes responsible for steroid catobolism is affected directly or indirectly by changes in plasma steroid levels (Schriefers, 1967). Conversely, changes in the MCR of steroids may alter plasma steroid titers.

Stress associated with physical disturbances can cause a rapid and sustained elevation of plasma corticosteroid titers (Donaldson, 1981; Schreck, 1981). Again, one might expect to observe concomitant changes in the MCR of corticosteroids during stress as a cause or result of changing plasma corticosteroid titers.

The distribution and clearance of corticosteroids in various tissues appear to differ between gonadectomized and intact sexually mature sockeye salmon (Donaldson and Fagerlund, 1972). Higher corticosteroid uptake rates in tissues of mature salmon correspond to a faster MCR in plasma compared with those in gonadectomized fish. Thus, if acclimation to SW or stress alter the MCR of corticosteroids, one might also expect to see changes in the corticosteroid dynamics of tissues.

The objectives of the present study were to determine (1) the MCR of ^{3}H-cortisol and its metabolites (total-^{3}H) in plasma of

yearling coho salmon acclimated to FW and SW (hereafter termed FW and SW fish), (2) if the uptake and retention of total-^{3}H in various tissues is the same in FW and SW fish, (3) if short-term or long-term stress alters the clearance of total-^{3}H from plasma in FW and SW fish, and (4) if acute or chronic administration of exogenous cortisol alters the clearance rate of total-^{3}H from plasma.

MATERIALS AND METHODS

Experimental animals. Juvenile coho salmon were obtained from several sources. In experiments conducted from December 1980 to April 1981, we used fish resulting from a cross of Big Creek and Sol Duc stocks (BC-SD) or fish from the Alsea River stock (Oregon Department of Fish and Wildlife). In 1982, fish originated from the Eagle Creek National Fish Hatchery (U.S. Fish and Wildlife Service). All fish were reared in FW (11-12°C) at Smith Farm Hatchery, Oregon State University. During the experiments, fish were maintained in small flow-through tanks (100 liters, 0.6 m diameter) unless noted otherwise. Fish were fed daily with Oregon Moist Pellet diet until 1 d before the experiments.

General Methods. To determine MCR of total-^{3}H, fish were anesthetized with 50 mg/liter ethyl m-aminobenzoate methanesulfonate (MS-222) and injected intracardially with 5μCi (17 ng) of 1,2,6,7 ^{3}H-cortisol (New England Nuclear), the purity of which was checked by thin layer chromatography according to Quesenberry et al. (1965). The ^{3}H-cortisol was delivered in 25-50 μl of 5% ethanol:saline,

including 100 ng of unlabeled cortisol. At various times after injection, fish were killed by a blow to the head and their blood was sampled from the severed caudal artery in heparinized capillary tubes. Blood samples were immediately centrifuged and 20 µl of plasma was solubilized with 200 µl of Protosol® (New England Nuclear) in glass scintillation vials. Ten milliliters of Neutralizer® cocktail (Research Products International) were added to each vial and mixed. Total radioactivity was measured and expressed as a percentage of the injected dose per milliliter of plasma after data from individual fish were standardized to 25 g body weight.

Tissue samples were placed in pre-weighed vials, weighed, solubilized with 1 ml of Protosol®, mixed with 10 ml Neutralizer® cocktail, measured for total radioactivity, and corrected for quenching by the addition of an internal standard (10,000 dpm). Results from tissue samples were expressed as a percentage of the injected dose and as a proportion of radioactivity present in plasma (tissue/plasma ratio = dpm per gram of tissue/dpm per milliliter plasma).

We injected ^{3}H-cortisol because cortisol is the major corticosteroid in the blood of most teleostean fishes (Chester Jones et al., 1969) and preliminary analyses in our laboratory confirmed this finding for coho salmon. Cortisol is irreversibly converted to cortisone in adult sockeye salmon (Donaldson and Fagerlund, 1968). In the eel, cortisol and cortisone together comprise more than 70% of the total radioactivity present in dichloromethane-extracted plasma for at least 6 h after a single injection of ^{3}H-cortisol (Leloup-Hatey,

1976). Unpublished results from our laboratory corroborate this finding for yearling coho salmon. Donaldson and Fagerlund (1968) found little difference in the amount of radioactivity present in extracted versus unextracted plasma. Therefore, we infer that calculations of MCR based on total-^{3}H in unextracted plasma are representative of general corticosteroid MCR--i.e., the MCR of cortisol plus its major metabolite, cortisone. The MCR for cortisol alone would be substantially faster than that for total-^{3}H.

Statistics. The MCR of total-^{3}H is inversely proportional to the area (A) under the curve of total-^{3}H versus time, so that MCR = 100/A when radioactivity is expressed as a percentage of the injected dose per milliliter of plasma. We calculated A, MCR, and their corresponding standard error terms and degrees of freedom as described by Normand and Fortier (1970). When only part of a clearance curve is available, MCR cannot be calculated. However, when treatment groups within a single experiment are compared, the half-life ($T_{1/2}$) of a hormone may provide a reasonable index of the relative metabolic clearance during the specific time period considered (Nugent et al., 1961). Therefore, in experiments with three sample times the $T_{1/2}$ of total-^{3}H was calculated according to Normand and Fortier (1970). For experiments with only two sample times we used analysis of variance to compare levels of radioactivity between treatment groups at each time. Results for MCR and $T_{1/2}$ in plasma and total-^{3}H uptake in tissues were compared by a t'-test, assuming unequal variances (Snedecor and Cochran, 1981).

Data for plasma cortisol levels were transformed to natural log to increase homogeneity of variances and compared by analysis of variance and Student's t-test.

EXPERIMENTAL DESIGN AND RESULTS

Clearance in FW and SW. On 11 December 1980 we conducted an experiment to determine MCR of total-^{3}H in FW fish (Alsea, mean wet weight $[\overline{W}]$ = 14 g) at Smith Farm Hatchery. Blood samples were taken from 10 fish at 0.2, 0.5, 1, 2, 4, 8, 12, and 24 h after injection with ^{3}H-cortisol.

We also transferred FW fish (BC-SD) to the Marine Science Center, Oregon State University, Newport, Oregon and subsequently maintained them for 24 d in FW or SW (28-33 ppt) flow-through tanks (100 liters, 0.9 m diameter) at 11-13°C. On 27 April we sampled blood from 10 FW and SW fish ($\overline{W}$ = 23 g) at 0.2, 0.5, 1, 4, 11, 19, and 24 h after injection with ^{3}H-cortisol. We also took samples (about 100 mg) of liver, gill filament, and anterior gut tissue, and the entire gall bladder with bile at 4, 11, and 24 h.

The MCR of total-^{3}H in FW fish after injection of ^{3}H-cortisol was significantly lower in April than in December (Table 2). The MCR in SW fish was significantly higher than that in FW fish in April (Table 2, Fig. 9).

The amount of total-^{3}H present after injection with ^{3}H-cortisol declined with time in liver and gill tissue and increased with time in the gall bladder of both FW and SW fish (Table 3). Total-^{3}H in gut tissue declined with time in SW fish but remained high for at least

Table 2. Metabolic clearance rate of total-^{3}H after injection of ^{3}H-cortisol in yearling coho salmon acclimated to freshwater and seawater.

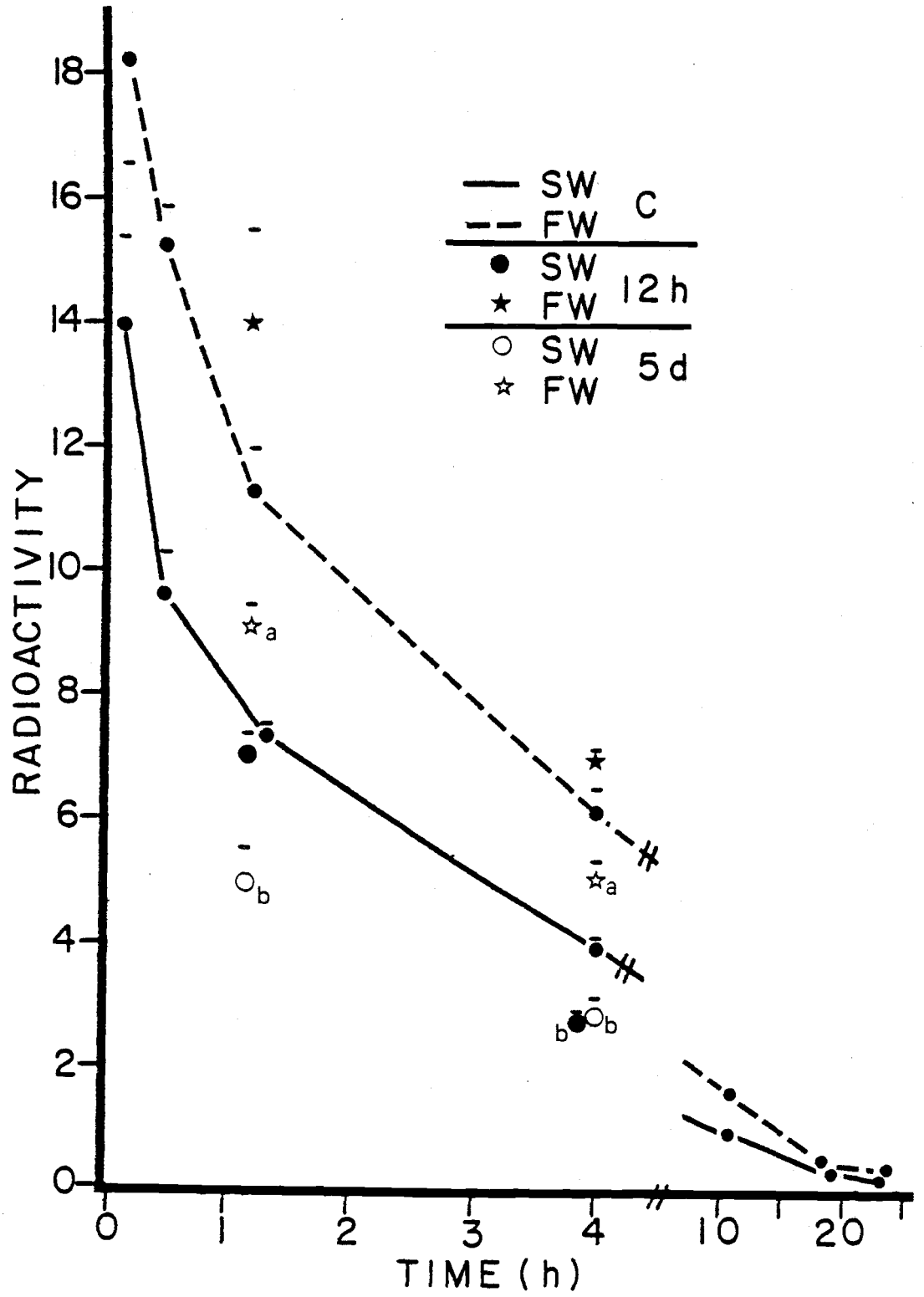
Acclimation and month[a]	Metabolic clearance rate (ml plasma/h per 25 g body weight)		
	Mean	SE	Degrees of freedom
Freshwater			
December	1.46	0.04	49
April	1.33[b]	0.04	20
Seawater			
April	2.11[c]	0.06	31

[a] December fish from Alsea stock, April fish from Big Creek-Sol Duck cross.

[b] Significantly different from FW fish in December ($P < 0.05$).

[c] Significantly different from FW fish in April ($P < 0.001$).

Fig. 9. Total-^{3}H activity of plasma versus time in yearling coho salmon acclimated to freshwater (FW) or seawater (SW) after injection of ^{3}H-cortisol. Fish were unconfined (C) or subject to short-term (12 h) or long-term (5 d) continuous confinement stress before injection. Units are in % dose/ml plasma per 25 g body weight. Values represent the mean, bars directly above or below points are the SE (after 10 h, the SE were too small to show), n = 8-10; letters indicate significant difference from control: a = $\underline{P} < 0.05$; b = $\underline{P} < 0.01$.


SW
FW
C
SW
FW
12 h
SW
FW
5 d
RADIOACTIVITY
TIME (h)
0
2
4
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8
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14
16
18
0
1
2
3
4
10
20
a
b
a
b
b
b

Figure 9

Table 3. Total-^{3}H radioactivity and tissue/plasma ratio (T/P) of tissues after injection of ^{3}H-cortisol in yearling coho salmon acclimated to freshwater (FW) and seawater (SW).

Time after injection (h)	Acclimation	Liver		Gut		Gill		Gall bladder	
		% dose/g	T/P	% dose/g	T/P	% dose/g	T/P	% dose/dg	T/P
1	FW	11.9 ± 1.3[a]	0.97 ± 0.09[b]	6.7 ± 0.6	0.55 ± 0.05	4.7 ± 0.5	0.39 ± 0.04	8.5 ± 2.9	5 ± 1(9)
	SW	12.1 ± 1.2	1.58 ± 0.17[d]	5.6 ± 0.6	0.72 ± 0.07[c]	4.2 ± 0.5	0.53 ± 0.05[c]	9.5 ± 2.3	13 ± 3(7)[c]
11	FW	4.7 ± 0.4	2.87 ± 0.28	4.9 ± 0.9	3.02 ± 0.52	0.3 ± 0.02	0.18 ± 0.01	44.1 ± 2.4	278 ± 21(8)
	SW	4.1 ± 0.6	4.43 ± 0.60[c]	2.9 ± 0.3[c]	3.11 ± 0.26	0.4 ± 0.07	0.39 ± 0.05[d]	43.8 ± 3.7	537 ± 104(6)[c]
24	FW	2.7 ± 0.4	10.77 ± 2.05	6.9 ± 0.8	26.52 ± 3.20	0.1 ± 0.01	0.42 ± 0.03	51.5 ± 1.6	1977 ± 116(9)
	SW	2.4 ± 0.7	16.43 ± 5.00	1.2 ± 0.1[d]	7.73 ± 0.96[d]	0.1 ± 0.01	0.72 ± 0.06[d]	44.1 ± 5.5	2746 ± 345(8)[c]

[a] Mean ± SE, n = 10 unless otherwise indicated in parentheses, units = % dose/g (or dg) tissue per 25 g body weight.

[b] Mean ± SE, T/P = dpm per g tissue/dpm per ml plasma.

[c] Significantly different from FW control ($P < 0.05$).

[d] Significantly different from FW control ($P < 0.01$).

24 h in FW fish. The difference in total-^{3}H level in gut tissue between FW and SW fish was significant at 11 and 24 h after injection.

The tissue/plasma ratio of total-^{3}H increased in liver, gut, and gall bladder but remained low in gill tissue (Table 3). The SW fish had consistently higher ratios than did FW fish for liver, gill, and gall bladder. The ratios for gut tissue in SW fish were higher at 1 h but lower at 24 h than those in FW fish.

Short-and Long-term Stress. To test the possibility that stress alters the clearance dynamics of corticosteroids, we subjected both FW and SW fish ($\overline{W}$ = 23 g) to severe stress (i.e., netting, crowding, and continuous confinement in live-cages [five fish per 2 liter cage]) for 12 h or 5 d before injecting them with ^{3}H-cortisol. After injection, fish were returned to their respective live-cages and sampled 1 and 4 h later. We conducted this experiment simultaneously with the previously described experiment on 27 April; thus, unconfined FW and SW fish served as controls.

Short-term (12 h) confinement stress did not significantly affect the amount of total-^{3}H in plasma compared with that in unconfined control fish, except in SW at 4 h after injection (Fig. 9); 30% of the fish were moribund in this SW group by 4 h.

Long-term (5 d) confinement stress caused 60% mortality in SW fish before they were injected. At 1 and 4 h after injection the total-^{3}H in plasma was significantly lower in fish subjected to long-term stress than in controls, in both FW and SW (Fig. 9).

Plasma cortisol levels. To determine the effect of handling and injection stress on endogenous cortisol titers, we injected

intracardially 110 ng of unlabeled cortisol into FW and SW fish (Eagle Creek, $\overline{W}$ = 33 g), confined as above (12 h and 5 d) and unconfined, on 22 June 1981. Plasma samples were taken at 0, 0.5, 1, 4 and 11 h after injection for unconfined control groups and at 1 and 4 h for confined groups. Plasma cortisol titers were determined by a radioimmunoassay described by Foster and Dunn (1974) and modified by Redding et al. (see Chapter III).

Unconfined control fish in SW had higher plasma cortisol levels at 4 h and lower levels at 11 h after injection than did control fish in FW (Table 4). Confinement for 12 h before injection in FW fish significantly augmented plasma cortisol levels 1 and 4 h after injection, relative to those in unconfined control fish in FW. Confinement for 12 h in SW fish caused 50% mortality before injection and augmented the concentration of cortisol 1 h after injection compared with control fish in SW. Long-term confinement in FW for 5 d before injection tended to inhibit the response of cortisol after injection at 1 h and 4 h. Long-term confinement in SW caused 55% mortality before injection; however, all the remaining fish survived until 4 h after injection despite having higher cortisol levels than those in control fish. There was no consistent correlation between the concentration of cortisol and the amount of radioactivity present in plasma after injection with ^{3}H-cortisol.

Acute and chronic treatment with exogenous cortisol. To determine whether acute treatment with cortisol alters the clearance of total-^{3}H, we injected FW fish (Eagle Creek, $\overline{W}$ = 57 g) with ^{3}H-cortisol plus various amounts of unlabeled cortisol, yielding

Table 4. Plasma cortisol levels (ng/ml, geometric mean + SE; n in parentheses) in yearling coho salmon, acclimated to freshwater (FW) or seawater (SW), after injection with saline in unconfined control fish and in fish subject to confinement stress for 12 h or 5 d before injection.

Acclimation and duration of stress	Hours after injection				
	0	0.5	1	4	11
Freshwater					
Control	4 + 2(8)	368 + 18(10)	324 + 57(10)	91 + 14(9)	120 + 24(10)
12 h	-	-	483 + 46(10)[a]	256 + 59(10)[b]	-
5 d	-	-	166 + 29(6)[a]	73 + 21(7)	-
Seawater					
Control	23 + 4(9)[d]	348 + 16(10)	295 + 28(10)	142 + 14(8)[c]	41 + 7(10)[c]
12 h[e]	-	-	489 + 73(7)[a]	251 + 166(3)	-
5 d[f]	-	-	-	234 + 41(9)[a]	-

[a] Significantly different from control ($P < 0.05$); [b] ($P < 0.01$).

[c] Significantly different from FW control ($P < 0.05$); [d] ($P < 0.01$).

[e] 50% mortality before injection; surviving fish sampled at 1 and 4 h.

[f] 55% mortality before injection; all surviving fish sampled at 4 h.

total acute cortisol doses (ng) of 17 (control), 170 (low) or 17,000 (high). Blood samples for determination of radioactivity were taken from seven fish of each group at 1, 4, and 8 h after injection on 27 July 1982.

High amounts of exogenous cortisol (17,000 ng) administered simultaneously with an injection of ^{3}H-cortisol significantly increased the $T_{1/2}$ of total-^{3}H in plasma (Table 5). Low doses of cortisol (170 ng) did not influence $T_{1/2}$ of total-^{3}H.

To determine the effects of chronic cortisol treatment on plasma clearance of total-^{3}H we implanted small, cortisol-filled silastic capsules into the peritoneal cavity of fish 5 d before injection with ^{3}H-cortisol, according to Redding et al. (see Chapter III). Empty capsules were implanted in control fish. Cortisol-implanted fish had higher plasma cortisol concentrations ($\overline{X}$ = 260 ng/ml) than sham-implanted fish (37 ng/ml). On 26 July 1982, six fish (Eagle Creek, $\overline{W}$ = 38 g) from each group were sampled at 1, 4, and 8 h after injection. In a separate experiment on 9 August 1982 six fish (Eagle Creek $\overline{W}$ = 46 g) from each group were sampled at 0.5, 1, and 6 h after injection.

Chronic treatment with exogenous cortisol significantly reduced the $T_{1/2}$ of total-^{3}H (Table 6). Total-^{3}H concentration if plasma was elevated in cortisol-treated fish for at least 1 h after injection with ^{3}H-cortisol, but thereafter it was lower than that in controls.

DISCUSSION

The clearance of corticosteroids from plasma of yearling coho salmon was faster in SW than in FW fish, and it appeared to be faster

Table 5. Radioactivity and half-life ($T_{1/2}$) of total-^3H in plasma after injection with ^3H-cortisol and unlabeled cortisol in yearling coho salmon acclimated to freshwater.

Time after injection (h) and $T_{1/2}$ (h)	Total amount of cortisol injected (ng)		
	17 (Control)	170 (Low)	17,000 (High)
1	14.5 ± 0.77[a]	13.6 ± 0.59	10.7 ± 0.44[b]
4	4.8 ± 0.23	5.4 ± 0.26	5.0 ± 0.28
8	1.8 ± 0.14	2.2 ± 0.48	2.5 ± 0.26
$T_{1/2}$	2.3 ± 0.11	2.5 ± 0.22	3.2 ± 0.28[b]

[a] Mean ± SE, n = 7, units = % dose/ml plasma per 25 g body weight.

[b] Significantly different from control ($P < 0.01$).

Table 6. Radioactivity and half-life ($T_{1/2}$) of total-^{3}H in plasma after injection with ^{3}H-cortisol in yearling coho salmon acclimated to freshwater and chronically treated for 5 d with cortisol.

Date, time after injection (h), and $T_{1/2}$ (h)	Control	Cortisol
26 July		
1	9.8 ± 0.65[a]	13.4 ± 0.58
4	5.2 ± 0.31	3.9 ± 0.62
8	2.3 ± 0.39	0.9 ± 0.10
$T_{1/2}$	3.2 ± 0.35	1.8 ± 0.11[b]
9 August		
0.5	17.9 ± 1.58	25.3 ± 2.06
1	13.9 ± 0.92	17.0 ± 0.94
6	3.3 ± 0.29	2.1 ± 0.16
$T_{1/2}$	2.3 ± 0.15	1.6 ± 0.07[b]

[a] Mean ± SE, n = 6, units = % dose/ml per 25 g body weight.

[b] Significantly different from control group ($P < 0.001$).

in fish subject to long-term stress than in those subject to short-term stress or in unconfined fish. Faster clearance from plasma corresponded with greater uptake and retnetion of radioactivity in the liver, gill, and gall bladder of SW fish. Chronic, but not acute, administration of cortisol at physiological levels apparently increased the clearance rate of corticosteroids.

The MCR of total-^{3}H in SW increased by 58% relative to that in FW fish. Assuming that changes in the MCR of total-^{3}H are representative of changes in the MCR of authentic cortisol, our results corroborate those of others who found 40-85% higher MCR of cortisol in SW fish than in FW fish (Donaldson and Dye 1970; Leloup-Hatey, 1974; Henderson et al., 1974).

Putative theory suggests that cortisol plays an important role in osmoregulatory function in SW but is relatively less important in FW for euryhaline fishes such as the eel and salmon (Maetz, 1969). If the relative importance of cortisol for osmoregulation of other physiological functions changes when the fish migrates from FW to SW, then it seems reasonable that the dynamics (i.e., secretion rate and MCR) of this steroid might also change. Our data do not allow realistic estimates of cortisol secretion rate for coho salmon because they are based on measurements of total-^{3}H, not authentic cortisol. However, if, as our data suggest, the MCR of cortisol is higher in SW than in FW, and because basal levels of cortisol in SW and FW average about 23 and 4 ng/ml, respectively (Table 4), then secretion rate would appear to be substantially higher in SW than in FW, since secretion rate equals the MCR multiplied by the basal cortisol level

under steady-state conditions (Tait and Burstein, 1964). This conclusion is consistent with the higher secretion rate of cortisol found in SW than in FW eels (Leloup-Hatey, 1974; Henderson et al., 1974).

The small but significant difference in the MCR of corticosteroids between December and April in FW fish may have reflected developmental changes associated with the parr-smolt transformation of coho salmon. However, we cannot exclude the possibility that differences in the genetic origins of the fish or the experimental environments contributed to the observed difference in the MCR in FW. Unpublished results from our laboratory suggest that the MCR of corticosteroids actually increases during April relative to the MCR in preceding months.

Radioactivity accumulated in the gall bladder after injection of ^{3}H-cortisol in both FW and SW coho salmon. This result was expected, since the bile is a major site for the excretion of catabolized steroids in fish (Idler and Truscott, 1972). Higher tissue/plasma ratios in the gall bladder of SW fish relative to those in FW fish support the conclusion of higher MCR of corticosteroids in SW fish.

Total-^{3}H in liver tissue declined with time but the liver tissue/plasma ratio increased. Furthermore, the ratios in liver were higher in SW than in FW fish. These findings suggest that uptake and retention of corticosteroids occurred in the liver and that the capacity for uptake and retention was enhanced in SW.

Total-^{3}H in gill filaments declined with time and gill tissue/plasma ratios remained low, indicating that gill tissue had a

relatively low capacity for retention of corticosteroids. However, gill tissue of SW fish showed consistently higher capacity for uptake and retention of corticosteroids than did gill tissue of FW fish. This observation tends to support the notions that cortisol is relatively more important for existence in SW than in FW and that gill tissue is a site for corticosteroid action. Goodman and Butler (1972) failed to demonstrate differential uptake of ^{3}H-cortisol in gill tissue from FW and SW eels; however, their procedures were substantially different from ours, since their SW fish were held in FW after injection.

The differences we observed in the radioactivity of gut tissue in FW and SW fish may have been due to differential bile secretion rates. Food content in the intestines was markedly higher in FW than in SW fish. Greater bile secretion into the intestine induced by higher food content could easily account for the continuously high level of radioactivity in the gut of FW fish. However, it is also plausible that the gut tissue itself accumulated more radioactivity in FW than in SW fish.

Short-term confinement stress in FW before injection of ^{3}H-cortisol had no apparent effect on the clearance of corticosteroids from plasma in FW fish, although the stress significantly augmented the level of plasma cortisol after injection. Short-term stress before injection seemed to severely compound the stress normally asociated with injection in SW fish, augmenting cortisol level as in FW but also causing some fish to die within 4 h. There was also a significant decline in total-^{3}H of plasma 4 h after injection, but not

after 1 h. This observation may have indicated a faster clearance of corticosteroids after short-term stress in SW fish; however, with only two sample points such a conclusion is equivocal. Short-term stress for 4 h in adult guinea pigs significantly increased the MCR of cortisol (Manin and Delost, 1981).

Interestingly, the apparent clearance of corticosteroids after short-term stress in SW fish was greater at 4 h than in control fish, even though several fish were moribund at the time of sampling. Moribundity itself did not seem to impair corticosteroid clearance, contrary to earlier reports for sockeye salmon (Idler et al., 1963) and Atlantic cod, Gadus morhua (Idler and Freeman, 1965).

Long-term confinement stress before injection significantly increased the clearance of corticosteroids in both FW and SW fish at 1 and 4 h after injection. In FW, long-term stress also appeared to dampen the increase in plasma cortisol levels after injection, suggesting a more rapid clearance or a diminished secretion rate of cortisol. In SW acclimated fish, long-term confinement induced over 50% mortality before clearance of total-^{3}H was assessed; thus, data from these groups are biased. Possibly, the more resilient survivors had selectively higher clearance rates for corticosteroids than those fish that died. These resilient survivors appeared healthy 4 h after injection despite having higher plasma cortisol levels than control fish, and at a time when fish subject to short-term stress were becoming moribund.

Why does the clearance of corticosteroids increase concomitantly with SW acclimation and long-term stress? Our results show that

chronic treatment with exogenous cortisol lowered the $T_{1/2}$ of corticosteroids. Previous studies with gonadectomized adult sockeye salmon failed to demonstrate significant changes in cortisol metabolism after injection with cortisol (Donaldson and Fagerlund, 1969; Fagerlund and Donaldson, 1969). However, results similar to ours have been reported for testosterone metabolism in man after chronic administration of exogenous testosterone (Southern et al., 1968). It is possible that sustained elevation of circulating corticosteroids directly induces de novo synthesis of catabolizing enzymes in the liver and other tissues (Schriefers, 1967). Alternately, corticosteroids may act indirectly by affecting the hepatic hexose monophosphate shunt (Willmer and Foster, 1965), thereby affecting the supply of NADPH, which is an important cofactor for the reduction and deactivation of corticosteroids (Herbst et al., 1960). During SW acclimation (see Chapter III) and during stress (Schreck, 1981), the concentration of plasma cortisol in yearling salmon increases and remains elevated for an extended period relative to the rapidly transient surge after acute stress (Strange et al., 1978). Assuming that the observed decrease in $T_{1/2}$ after chronic treatment with exogenous cortisol reflected higher MCR of corticosteroids, then chronic elevation of endogenous cortisol level, such as that which occurs during chronic stress and SW acclimation, may induce a faster clearance rate for corticosteroids. These results may partly explain why sexually mature adult salmon have both higher plasma cortisol levels and higher MCR for cortisol than those in immature fish, as shown by Donaldson and Fagerlund (1970).

Thyroid hormones may also be involved in the increased MCR of corticosteroids during SW acclimation. In mammals, treatment with exogenous thyroid hormones can increase the MCR of corticosteroids (Schriefers, 1967). Since the concentrations of thyroid hormones may increase during SW acclimation in coho salmon (see Chapter IV) it is possible that the MCR of corticosteroids might increase concomitantly.

Acute increases in plasma cortisol due to stress or exogenous treatment did not appear to significantly affect the clearance of corticosteroids except when massive doses were delivered. At very high doses of exogenous cortisol (17,000 ng) the mechanism of corticosteroid clearance may have become saturated, resulting in a slower apparent rate of clearance for total-^{3}H. The lack of effects caused by acute cortisol doses at low physiological levels supports the contention that the differences we observed between FW and SW fish and between confined and unconfined fish were not caused by relatively small differences in the circulating levels of cortisol.

In summary, we found that (1) the clearance of corticosteorids from plasma was faster in SW than in FW fish, (2) the uptake and retention of corticosteroids in the liver, gill filaments, and gall bladder were greater in SW than in FW fish, (3) fish subject to long-term confinement stress had more rapid clearance of corticosteroids than did unconfined control fish or fish subject to short-term stress, and (4) chronic, but not acute, administration of cortisol at physiological levels increased the clearance rate of corticosteroids.

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APPENDICES

Appendix I. EFFECTS OF EXPOSURE TO SUSPENDED SOLIDS ON STEELHEAD TROUT (*SALMO GAIRDNERI*) AND COHO SALMON (*ONCORHYNCHUS KISUTCH*)[1]

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Running Title: Effects of Suspended Solids on Salmonids

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Abstract

Yearling coho salmon, Oncorhynchus kisutch, and steelhead trout, Salmo gairdneri, were exposed to high (2-3 g/l) and low (0.5 g/l) concentrations of suspended topsoil for 7-8 d. Exposure to high levels of suspended solids temporarily elevated plasma cortisol concentrations, indicating that such exposure may have been stressful to the fish. Elevated ambient temperature did not exacerbate the effects of exposure to suspended topsoil. Osmoregulatory performance in freshwater and after transfer to seawater was unaffected by exposure to suspended topsoil; however, the fish's resistance to a water-borne pathogen was reduced.

Inorganic particulate material enters aquatic environments as a result of many natural causes (e.g., erosion, landslides) and human activities (e.g., road construction, logging, agriculture). Particulate material in aquatic ecosystems may affect fishes directly, via exposure to the suspended solids, or indirectly, by altering components of the ecosystem such as substrate composition. Literature on the effects of inorganic particulate material on aquatic ecosystems and fishes has been extensively reviewed by others (Cordone and Kelly 1961; European Inland Fisheries Advisory Commission 1965; Stern and Stickle 1978; Iwamoto et al. 1978; Muncy et al. 1979). Most research regarding direct effects on fishes has determined only the mortality rates after exposure to variable concentrations of suspended solids (Wallen 1951; Herbert and Merkins 1961; Rodgers 1969; LeGore and Des Voigne 1973; Auld and Shubel 1978; Peddicord and McFarland 1978). In most species tested mortality rates increased only after long-term exposure to extremely high levels of suspended solids. Other researchers have observed growth rates (Buck et al. 1956; Swenson and Matson 1976) and behavior (Noggle 1978; Gardner 1981; Grandall and Swenson 1982; Whitman et al. 1982) in relation to the concentrations of suspended solids.

Exposure to suspended solids may also induce sublethal physiological effects in fishes. Sherk et al. (1975) and Neuman et al. (1975) measured hematological and respiratory responses after exposure to suspended solids in several estuarine species. Horkel and Pearson (1976) assessed respiratory responses in Lepomis cyanellus. Noggle (1978) evaluated some physiological responses in salmonids.

These studies suggest that physiological changes may occur in fishes after exposure to suspended solids; and these changes may be indicative of sublethal stress.

Putative theory regarding stress in fishes suggests that certain primary and secondary physiological responses occur during stress. In particular, the pituitary-interrenal axis is activated during stress, resulting in the release of corticosteroid hormones into the blood (Mazeaud et al. 1977; Donaldson 1981). As such, the concentration of cortisol in blood plasma seems to be a general indicator of stress in fishes, elevated levels indicating that the fish is stressed (Schreck and Lorz 1978). Implicit in the concept of stress is a reduction in the performance capacity (i.e., the ability to perform necessary functions) of the fish (Schreck 1981).

Exposure to suspended solids may be stressful to a fish if the respiratory epithelia of the gill is damaged or made inefficient by clogging or excessive mucous production.

During summer months, the ambient water temperature in undisturbed coastal streams of Oregon seldom exceeds 15°C; but, in watersheds subject to clearcut logging the ambient temperature in streams may exceed 20°C (Brown and Krygier 1970). Elevated ambient temperature can cause sublethal stress in salmonids (Houston 1971; Wedemeyer 1973). Simultaneous imposition of stress from different sources may have additive effects on physiological responses and performance capacity (Schreck 1981). Therefore, the combination of elevated ambient temperature and high concentration of suspended solids may be especially detrimental to fish.

Stress can induce osmoregulatory dysfunction in salmonid fishes (see Chapter I). Osmoregulatory performance is especially important during the transition from freshwater to seawater environments in anadromous fishes. If exposure to suspended solids causes damage to the gill or generally stresses the fish, osmoregulatory performance may be affected.

Stress can also suppress the immune system in fishes, thus increasing their susceptibility to pathogens (Ellis 1981).

The purpose of this study was to determine whether exposure to suspended solids is stressful to yearling coho salmon, Oncorhynchus kisutch, and steelhead trout, Salmo gairdneri. Specifically, our objectives were to determine if exposure to suspended topsoil (1) elicits certain physiological responses typical of stress in salmonid fishes, (2) damages gill epithelia, (3) changes physiological performance when ambient temperature is elevated, (4) changes osmoregulatory performance when fish are transferred to 75% seawater, and (5) increases the mortality rate after exposure to a pathogen.

METHODS

Steelhead trout were obtained from either the Alsea or Marion Forks Hatcheries of the Oregon Department of Fish and Wildlife (ODFW). Coho salmon were obtained from the Fall Creek or Sandy Hatcheries (ODFW). Fish were maintained before the experiments at Oregon State University, Smith Farm Hatchery.

We constructed an apparatus to control the concentration of suspended solids in a flow-through system. Essentially, the apparatus

consisted of a hopper, a vibrating tray (Eirez®), a 100 l mixing chamber, and a 20 l dilution chamber. Sieved and dried sandy loam topsoil (44% < 75 μ diameter) from an excavation near Corvallis, OR flowed from the hopper onto the vibrating tray, and from there into the mixing chamber. Delivery rate of dry soil to the mixer was adjusted by altering the vibratory amplitude of the tray. The concentration of suspended solids in the mixer depended on the delivery rate of dry soil and the flow rate of water into the mixer.

A fraction of the water in the mixer flowed into the dilution chamber where it was diluted according to a known ratio. Water from either the mixer or the dilutor flowed into the experimental tanks, allowing for two experimental concentrations of suspended solids. Tanks were 0.6 m in diameter and inflow rate was 2 l/min. Solids were kept in suspension in the test tanks by means of recirculating pumps and aerators. Fish in control tanks received a constant flow of well-water.

The concentration of suspended solids in Oregon coastal streams seldom exceeds 0.5 g/l under normal conditions; however, during logging operations the concentrations may occasionally exceed 7 g/l (Brown and Krygier 1971). We evaluated the effects produced by two concentrations of suspended solids, high (about 2-3 g/l) and low (about 0.5 g/l). We measured the concentration of suspended solids once daily in our experiments by evaporating water samples and weighing the residue. The reported values in this study are the range of all daily samples.

Water quality was similar in control and treatment tanks (temperature = 12.5-13.5°C, dissolved oxygen = 8-10 mg/l, pH = 7.0, 7.1, conductivity = 222-225 mmohs, alkalinity = 60-80 mg $CaCO_3$/l).

Fish were acclimated to the experimental aquaria for 7-10 d prior to the imposition of treatment. During sampling fish were killed by a blow to the head and plasma samples were obtained by severing the fish's caudal artery and collecting the blood in ammonium-heparinized capillary tubes; after centrifugation the samples were stored at -15°C. We used a competitive-protein-binding assay to measure cortisol as described by Strange and Schreck (1978). Plasma sodium concentration was measured on a flame photometer; and plasma osmolality was measured on a vapor pressure osmometer. Whole blood was collected in a microhematocrit tube, centrifuged, and to determine packed cell volume. Samples of gill tissue were fixed in Bouin's solution, imbedded in parafin, sectioned, mounted on slides, and then stained with hematoxylin and eosin.

Experiments involving pathogenic organisms were conducted at the ODFW Fish Disease Laboratory in Corvallis.

Hematological data were analyzed by analysis of variance and the means were compared by Student's *t*-test where appropriate. Some data for cortisol concentrations were transformed logarithmically to increase homogeneity among sample variances. Results from the disease challenge experiment were analyzed by a Chi-square test.

EXPERIMENTAL DESIGN AND RESULTS

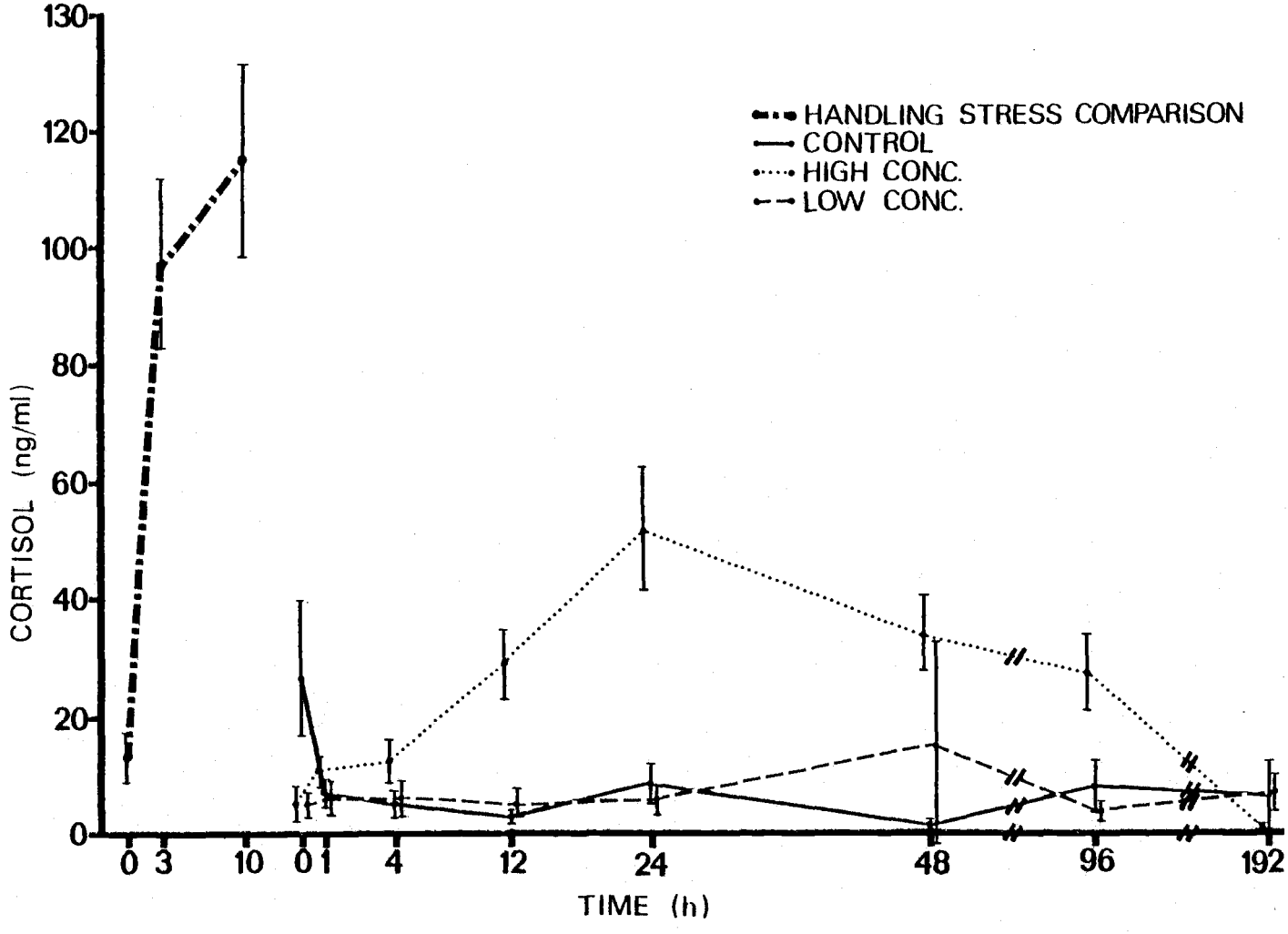
Physiological responses - steelhead trout. To assess the effects of suspended solids on yearling steelhead trout (mean fork length (FL) = 13.7 cm), we exposed fish to high (1.7-2.7 g/l), low (0.4-0.6 g/l), and control (0.0 g/l) concentrations of suspended topsoil. Fish were offered normal food rations of Oregon Moist Pellet diet during the exposure period. Two replicate tanks were maintained for each treatment group. Beginning at 0800, 30 November 1978, blood samples were obtained from six fish in each tank after 0, 1, 4, 12, 24, 48, 96, and 192 h exposure to treatment conditions, and plasma cortisol level was determined later. To ascertain, for comparative purposes, how a severe stress would affect the level of cortisol in the fish's blood, we subjected fish to handling and continuous crowding in small live-cages.

No mortality occurred during the 192 h experiment or during the 10 d after the treatment was stopped. Mean plasma cortisol concentrations in fish exposed to suspended topsoil were similar between replicate groups; thus the results were pooled within treatment groups. Fish exposed to high levels of suspended topsoil showed a highly significant ($P < 0.01$) elevation in cortisol between 12 and 48 h (Fig. 1). By 192 h, cortisol concentrations in fish exposed to high suspended solids had returned to a level near that of the control group.

The maximum concentration of cortisol for fish exposed to high suspended solids was approximately 40% of that shown by severely

Appendix I

Figure 1. Plasma cortisol concentration (mean ± SE) in juvenile steelhead trout subjected to handling and crowding or exposed to high (1.7-2.7 g/l), low (0.4-0.6 g/l), and control (0.0 g/l) concentrations of suspended topsoil. n = 12.



Appendix I
Figure 1

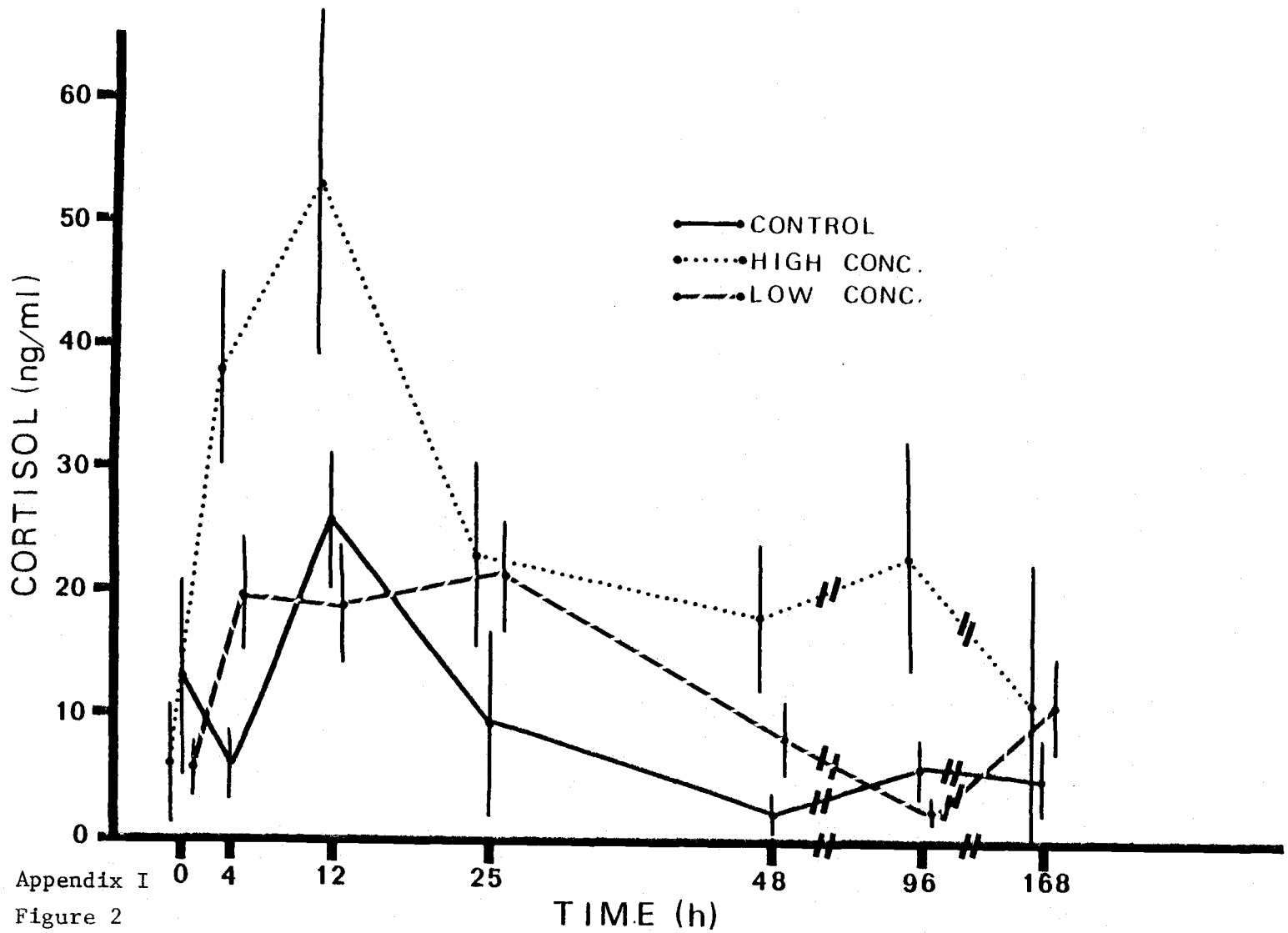
stressed (handled and crowded) fish after 10 h (Fig. 1). Plasma cortisol levels returned to basal value within 192 h.

Physiological responses - coho salmon. To assess the effects of suspended solids on yearling coho salmon (FL = 12.1 cm), we exposed fish to high (2.0-2.5 g/l), low (0.3-0.5 g/l), and control (0.0 g/l) concentrations of suspended topsoil. The fish were offered normal food rations of Oregon Moist Pellet diet during the exposure period. Two replicate tanks maintained for each treatment group. Beginning at 1100, 3 April 1979, blood samples were obtained from six fish in each tank after 0, 4, 12, 24, 48, 96, and 168 h exposure to treatment conditions; the blood plasma was then analyzed for cortisol and sodium content. For comparative purposes we also subjected fish to severe stress (handling and continuous confinement) for 48 h.

No mortality occurred in fish exposed to suspended topsoil during treatment or for 10 d thereafter. Mean plasma cortisol concentrations in these fish were similar between replicate groups within a treatment; thus, these results were pooled. The difference between plasma cortisol concentations in fish exposed to high levels of suspended topsoil and in control fish was highly significant ($P < 0.01$) during the experiment (Fig. 2); values in the exposed fish tended to be higher, significantly ($P < 0.05$) at 4 h. But, cortisol levels in fish exposed to low levels of suspended solids were not significantly different from those in control fish. Fish exposed to high concentrations of suspended solids regulated their plasma cortisol to basal levels within 24 h. The maximum concentration of

Appendix I

Figure 2. Plasma cortisol concentration (mean ± SE) in juvenile coho salmon exposed to high (2.0-2.5 g/l), low (0.3-0.5 g/l), and control (0.0 g/l) concentrations of suspended topsoil. n = 12.



Appendix I
Figure 2

cortisol for fish exposed to high suspended solids was approximately 30% of that shown by severely stressed fish (Figs. 2 and 3).

Cortisol concentrations in severely stressed fish rose rapidly within 30 min and continued to increase for 10 h (Fig. 3). By 24 h, cortisol levels had returned to basal levels. Two out of six fish were moribund in the 10 h sample, and two out of six were dead in the 24 h sample.

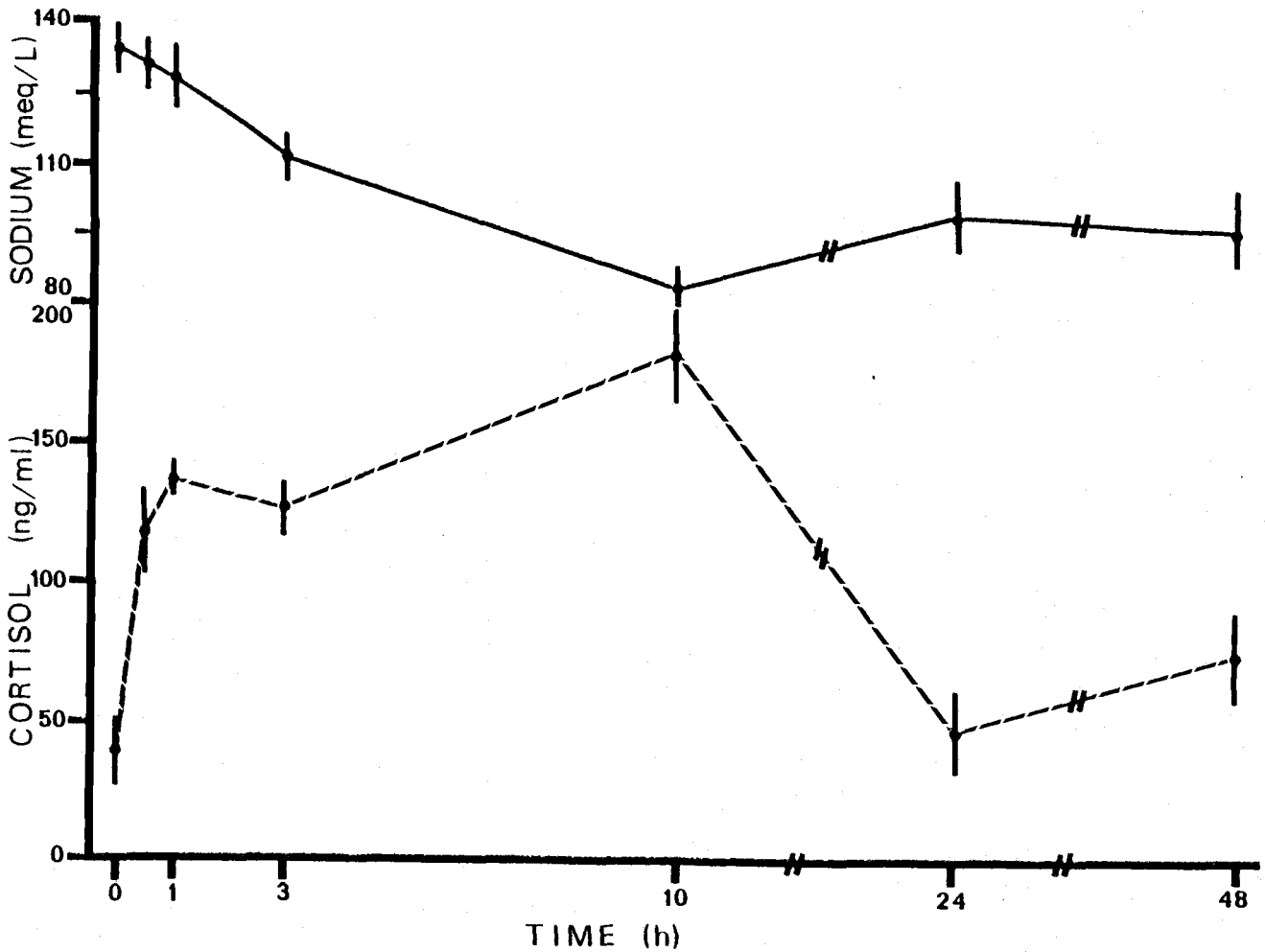
Mean plasma sodium concentrations were similar to juvenile coho salmon exposed to different levels of suspended topsoil. Plasma sodium in severely stressed fish decreased steadily for 10 h (Fig. 3). At 24 h and 48 h, sodium levels tended to compensate toward basal levels, but they were still greatly depressed.

Gill Histology. Gill tissue was sampled from yearling steelhead trout and coho salmon that had been exposed to high, low, and control levels of suspended topsoil for 2 d or 78 d; the tissue was examined after histological preparation. There was no obvious difference in the gross appearance of gill tissue between control and treatment fish.

Physiological performance at elevated ambient temperature. To determine if elevated ambient temperature amplifies the stress associated with exposure to high levels of suspended topsoil, we gradually acclimated yearling steelhead trout (FL = 12.1 cm, Marion Forks Hatchery) to 20°C over a period of 7 d beginning 11 June 1980. On 18 June, fish were exposed chronically to 3-4 g/l suspended topsoil for 48 h. Control fish were also acclimated to 20°C but were not exposed to suspended solids. Both treatment and control groups were

Appendix I

Figure 3. Plasma cortisol and sodium concentrations (mean ± SE) in juvenile coho salmon subjected to handling and continuous crowding in a small livecage; n = 6, except at 24 h when n = 4.



Appendix I
Figure 3

replicated. Six fish from each replicate group were sampled at 0, 3, 9, 24, and 48 h after onset of treatment conditions. Blood samples were analyzed for cortisol, sodium, and hematocrit.

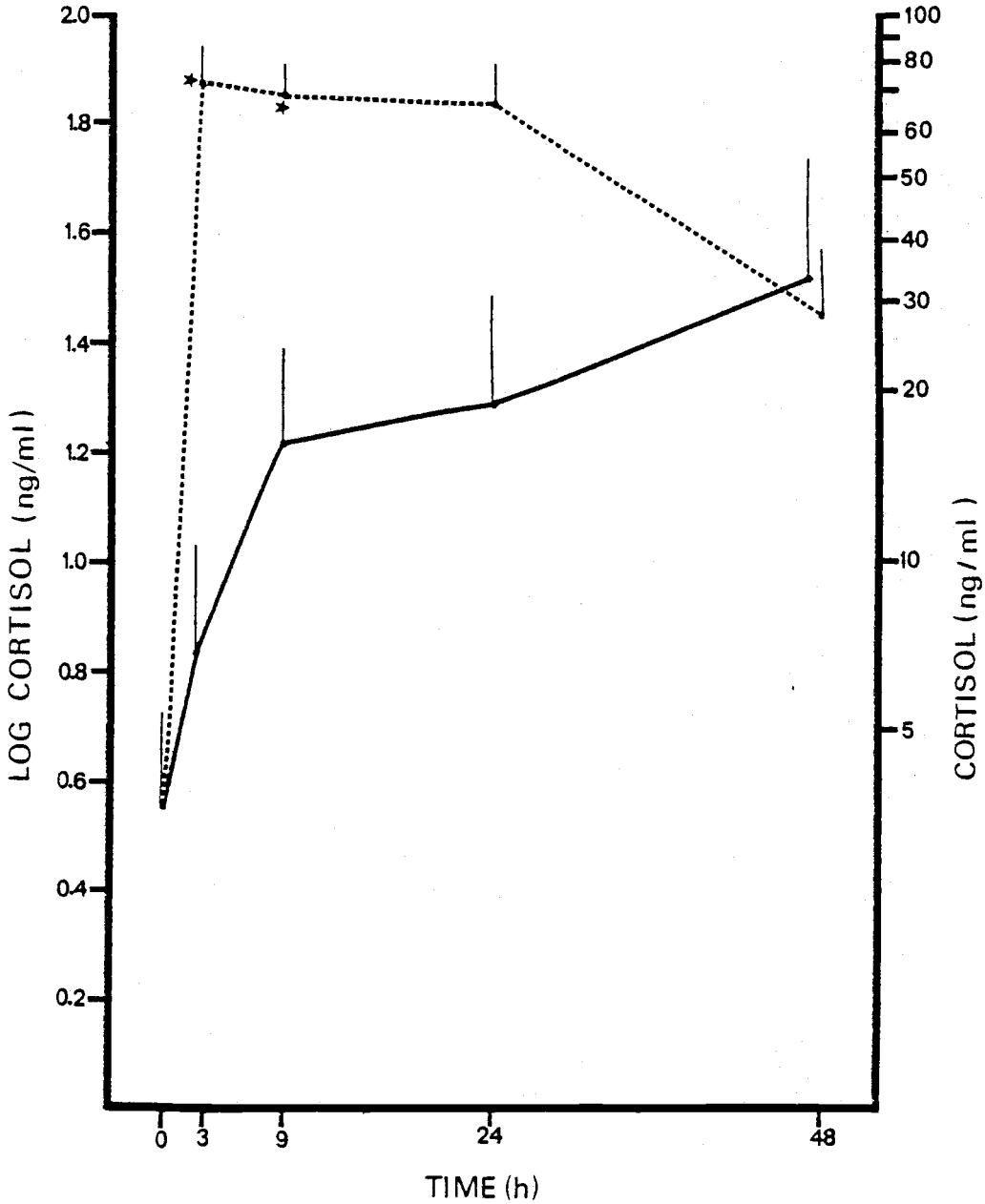
No mortality occurred during the acclimation period or during the 48 h treatment period. Replicate samples were not significantly different from each other; thus, data from these samples was pooled. Exposure to 3-4 g/l suspended topsoil caused a significant ($P < 0.05$) elevation of plasma cortisol compared to unexposed control fish at 3 and 9 h after onset of treatment (Fig. 4). At 24 h, treated fish still tended ($P < 0.10$) to have higher cortisol levels than control fish but there was no difference at 48 h. Unexpectedly, there was a significant and progressive elevation of cortisol in control fish during the 48 h test. Control fish held in 12°C water at approximately the same time and under identical conditions showed no significant change in cortisol levels.

There were no significant differences between plasma sodium levels in control and treatment fish (Fig. 5). Blood hematocrit level was higher in the treatment group at 3 h (Fig. 5).

Osmoregulatory performance in 75% seawater. To assess the effect of exposure to suspended solids on osmoregulatory performance in seawater, we used a seawater challenge test that is commonly used to evaluate the effects of stress (Wedemeyer and McLeay 1981). The fish did not survive a transfer from freshwater to full-strength synthetic seawater (Instant Ocean®); therefore, we used 75% seawater. We chose 3 d as the duration of the challenge test because in a preliminary

Appendix I

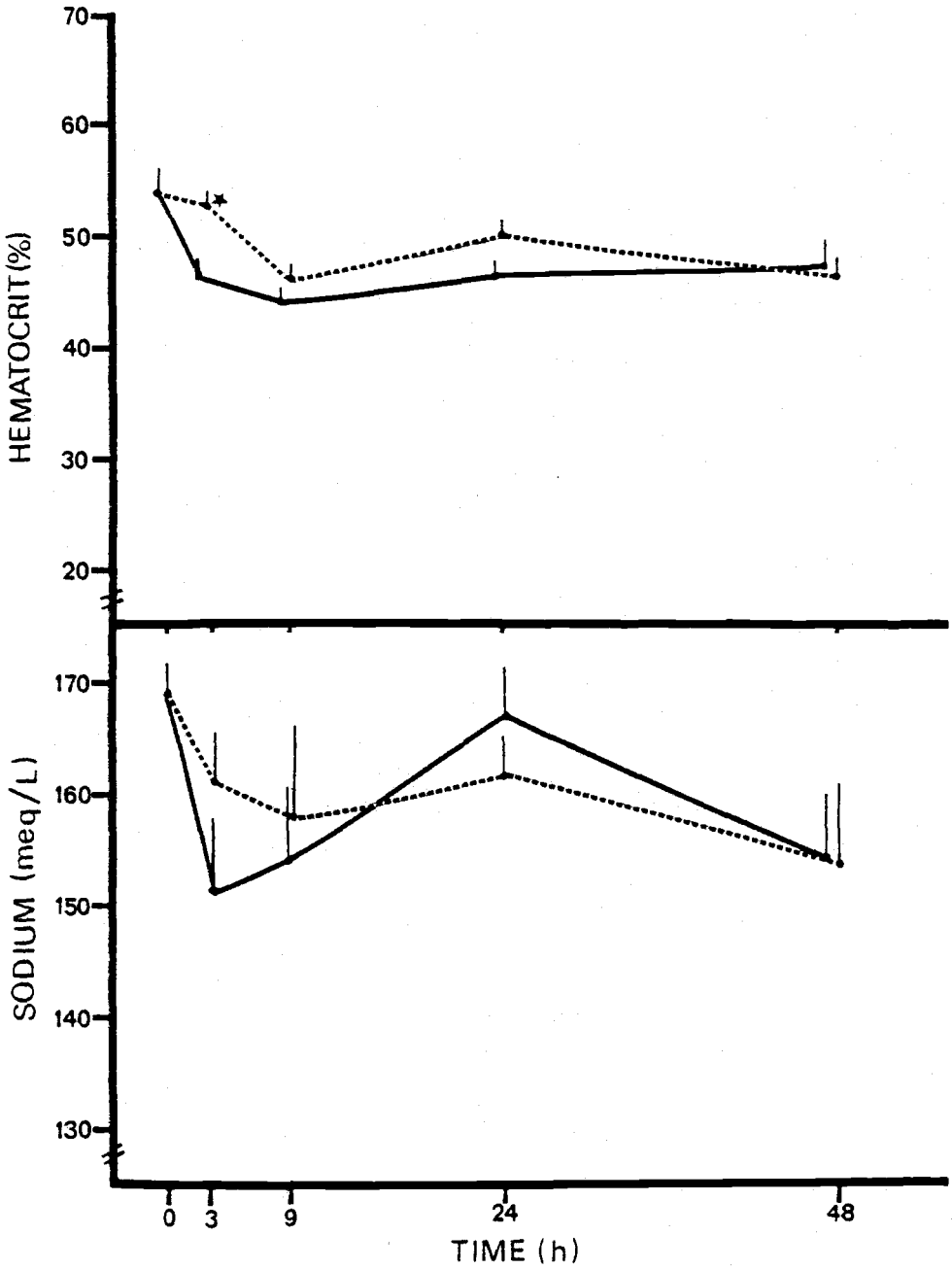
Figure 4. Plasma cortisol concentration (mean + SE) in yearling steelhead trout acclimated to 20°C and explosed to high (2-4 g/L; dotted line) and control (0.0 g/L; solid line) concentrations of suspended topsoil. Stars indicate significant differences ($P < 0.05$) between treatment and control groups.



Appendix I
Figure 4

Appendix I

Figure 5. Plasma sodium and blood hematrocrit levels (mean + SE) in yearling steelhead trout acclimated to 20°C and exposed to high (2-4 g/l; dotted line) and control (0.0 g/l; solid line) concentrations of suspended topsoil. Star indicates significant differences ($P < 0.05$) between treatment and control groups.



Appendix I
Figure 5

test, plasma sodium concentrations in control fish increased initially and then decreased after 3 d in 75% SW. If exposure to suspended solids reduced osmoregulatory capacity, plasma sodium levels in treated fish should have remained elevated after 3 d relative to those in controls.

Yearling steelhead trout and coho salmon that were exposed to high, low, or control concentrations of suspended topsoil for 2 d or 7-8 d (see design and results for physiological responses) were transferred to 75% seawater. After 3 d, plasma samples were taken and analyzed for sodium level. There were no significant differences in sodium level between treatment and control fish in either steelhead trout or coho salmon.

Tolerance to disease challenge. To determine if exposure to suspended solids alters a fish's tolerance to a pathogenic organism, yearling steelhead trout (FL = 9.6 cm) were treated for 2 d with 2.5 g/l suspended topsoil and then exposed to the water-borne pathogen, Vibrio anguillarum. The dose and duration of the inoculation were predetermined to induce approximately 50% mortality in untreated fish. Control groups consisted of (1) untreated fish exposed to Vibrio, (2) treated fish not exposed to Vibrio, and (3) untreated fish not exposed to Vibrio. Mortality was monitored twice daily for 11 d. Postmortem bacteriological analysis was used to confirm the infection by Vibrio; survivors were also tested. Percent mortality and mean day to death were calculated for each group. Mortalities which could not be attributed to Vibrio were not included in the calculations.

Exposure to suspended topsoil caused a significantly ($P < 0.05$) higher mortality rate in fish before and after inoculation with *Vibrio* (Table 1). Replicate groups had similar mortality rates. Exposure to suspended topsoil caused some fish to die before the inoculation with *Vibrio*.

DISCUSSION

Exposure to relatively high concentrations of suspended topsoil did not seem to be severely stressful to yearling salmonid fishes; but it did induce some physiological changes that are characteristic of sublethal stress. Our findings generally corroborate those of Noggle (1978) who reported a slight increase in blood glucose levels in coho salmon after they were exposed to very high concentrations of suspended glacial deposits. Sherk et al. (1975) also found sublethal hematological effects in several estuarine species after exposure to suspensions of fuller's earth; but other species were unaffected.

Plasma cortisol level has been used as general indicator of stress in many species of fish (Schreck and Lorz 1978; Donaldson 1981). High concentrations of suspended topsoil, such as might occur in aquatic systems adjacent to logging or road construction activities (Brown and Krygier 1971), caused a temporary elevation of plasma cortisol levels in both yearling steelhead trout and coho salmon. This suggests that exposure to suspended topsoil was stressful to the fish initially; but acclimation seemed to occur within several days. The magnitude of the cortisol repsonse was moderate compared with that of fish that were

Appendix I

Table 1. Mortality after challenge with *Vibrio anguillarum* in yearling steelhead trout exposed to 2.5 g/l suspended topsoil for 2 d. The two columns for *Vibrio* challenged groups represent results for replicate experiments.

	Suspended Solids			Control		
	Vibrio		Unchallenged	*Vibrio*		Unchallenged
Total number at start	25	25	25	25	25	25
Death before *Vibrio* challenge	6	5	6	2	1	0
Deaths caused by *Vibrio*	14	16	0	10	15	0
Percentage of challenged fish that died from *Vibrio*	74[a]	80[a]	0	43	63	0
Mean day to death	4.5	3.7	0	4.5	4.8	0
Deaths not attributable to *Vibrio*	0	1	4	1	4	1
Survivors after 11 d	5	3	15	12	5	24
Number of survivors with with *Vibrio* infection	1	0	0	0	1	0

[a]Significantly different from controls ($P < 0.05$).

severely stressed by handling and confinement, implying that exposure to suspended solids was not severely stressful.

Low concentrations of suspended topsoil had no significant effect on cortisol levels in the fish. The concentration of suspended solids in natural, unperturbed streams in the Pacific Northwest is usually below 0.5 g/l (Brown and Krygier 1971); our results suggest that exposure to such levels is not stressful for yearling salmonids. We could detect no consistent effect of suspended topsoil on the histological appearance of gill tissue. This finding is contrary to the general observation that exposure to suspended solids causes damage to the gill epithelia as reported by Herbert and Merkins (1961), Sherk (1975), and Noggle (1978).

The combination of high ambient temperature and exposure to high concentrations of suspended topsoil was not additive or synergistic with respect to the response of plasma cortisol. Again, this finding suggests that exposure to suspended solids was not severely stressful and did not seriously compromise the fish's ability to tolerate elevated ambient temperature. This finding is contrary to the results of Rodgers (1972) who found a higher mortality rate associated with higher ambient temperature in the four-spined stickleback, Apeltes quadracus, after a 24 h exposure to a suspension of silt.

Osmotic imbalance is usually symptomatic of severe stress (see Chapter II). Osmoregulatory performance was not impaired during exposure to suspended topsoil at 12° or 20°C; nor did such exposure compromise the fish's ability to regulate plasma sodium levels after transfer from freshwater to 75% seawater. This observation supports

the conclusion that exposure to suspended solids is not severely stressful to yearling salmonids.

Exposure to suspended topsoil significantly increased the fish's mortality rate after a water-borne challenge of the pathogenic bacterium Vibrio anguillarum. This increased mortality may have been due to a direct facilitation of microbial entry into the fish via abraded epithelia. Alternately, elevation of plasma cortisol level during exposure to suspended topsoil (see Fig. 1) may have suppressed the fish's immune system (Ellis 1981), thereby promoting the bacterial infection. Treatment of yearling coho salmon with cortisol increased the mortality rate after a challenge of Vibrio anguillarum (unpublished results). The incidence of fin-rot in rainbow trout (Salmo gairdneri) was increased after exposure to suspended solids (Herbert and Merkens 1961).

Exposure to suspended topsoil at concentrations up to 3 g/l can cause some physiological changes that are characteristic of sublethal stress. This stress does not seem to be severe; but it may compromise the fish's ability to resist disease.

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Appendix II. MOUNT ST. HELENS ASH CAUSES SUBLETHAL STRESS RESPONSES IN STEELHEAD TROUT[1]

by

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KEYWORDS: Salmo gairdneri, corticosteroids, stress, suspended solids

Abstract

Corticosteroid and hematocrit levels were elevated in juvenile steelhead trout exposed to suspensions of Mount St. Helens ash or comparable suspensions of topsoil or kaolin clay. The physiological responses resulting from exposure to suspended solids are symptomatic of sublethal stress in fishes.

The recent eruption of Mount St. Helens in Washington State resulted in the deposition of volcanic ash on large areas of the Pacific Northwest. Many streams and rivers in the affected areas contained extremely high concentrations of suspended volcanic ash after the eruption. Future periodic increases in the level of suspended volcanic ash may result from new eruptions and from the erosion of existing ash deposits.

Many of the affected streams and rivers support large populations of economically valuable salmonid fishes. Volcanic ash containing silicate glass particles has extremely sharp and angular surface characteristics[3] which may be abrasive to a fish's epithelial tissue, particularly that of the gills. Consequently, fish exposed to significant amounts of suspended volcanic ash could die or suffer deleterious sublethal stress. We define stress as the sum of non-specific and specific physiological responses to any set of stimuli that deviate from homeostatic conditions, significantly reduce an amimal's capacity to perform necessary biological functions, and eventually lead to either adaptation or exhaustion[4,5,6].

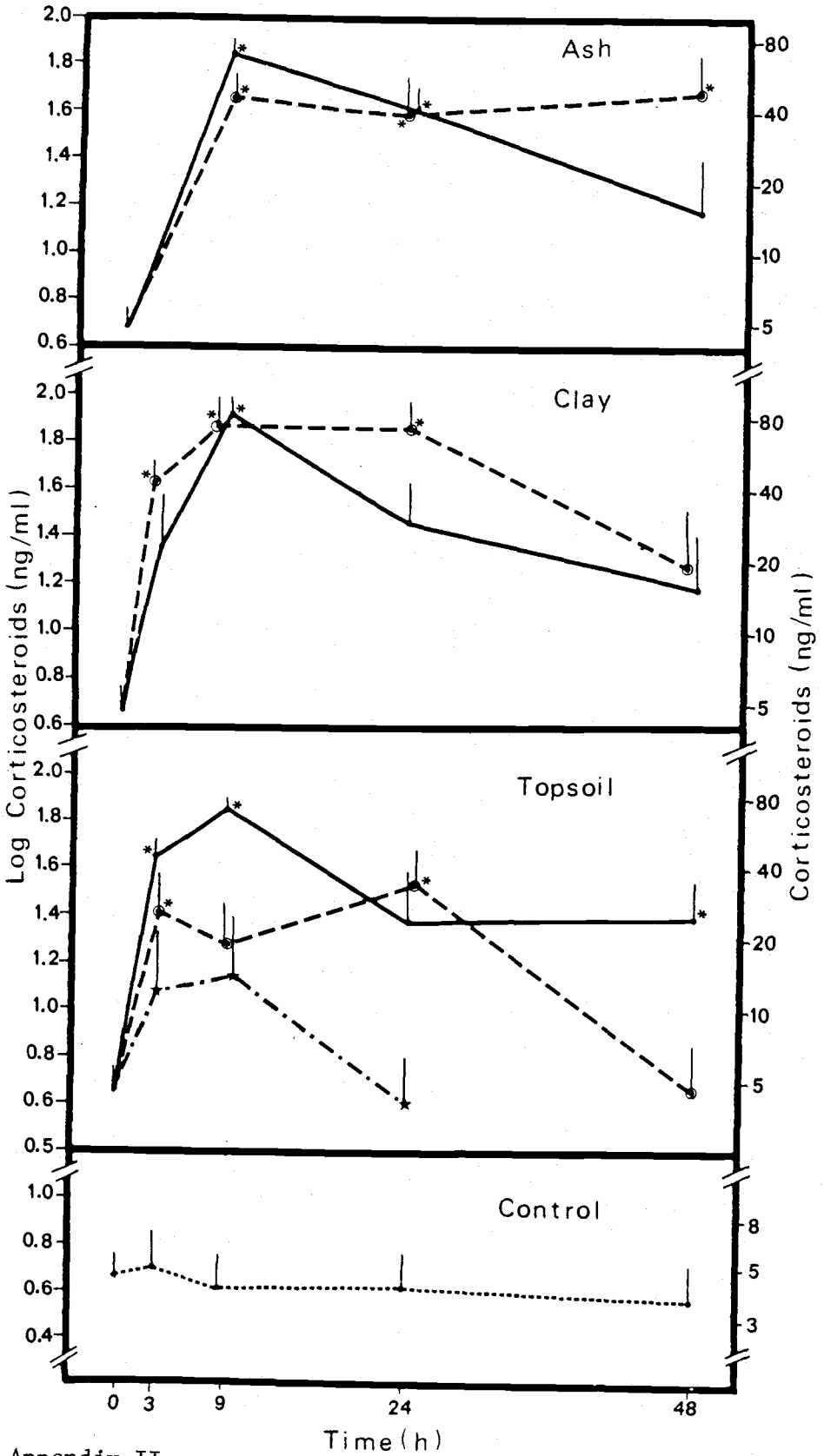
To assess the immediate physiological effects of chronic exposure to suspended volcanic ash[7], we subjected steelhead trout (Salmo gairdneri) to a relatively high concentration (2-3 g/l) and a relatively low concentration (0.5 g/l) of suspended ash[8]. For comparison, we also subjected fish to similar concentrations of suspended topsoil and kaolin clay[9]. Slurries of the appropriate concentrations were pumped into 100-l circular tanks (61 cm diam.) at a rate of 2 l/min for 48 hours. For topsoil, fish were also exposed

to one acute dose of 3 g/l. Control groups received a similar flow of clear well water. Water quality characteristics did not vary significantly between treatment groups[10]. Each tank initially contained 40 juvenile steelhead trout (mean fork length, 11.4 cm) which were acclimated for 7 days. Fish were sampled at 0, 3, 9, 24, and 48 hours after the imposition of treatment conditions[11]. We assayed blood samples for plasma corticosteroid hormones, plasma sodium, and hematocrit[12]. In fish, these parameters vary in relation to stress and correlate in some cases to a reduction in the animals' capacity to perform necessary functions--e.g., growth, osmoregulation, and disease resistance[13,14,15]. We also prepared histological samples of gill tissue[16] from fish in each treatment group to determine if histopathological effects resulted from exposure to suspended solids.

Exposure to suspended volcanic ash, topsoil, and kaolin clay for 48 hours caused no mortality. Significant increases in the concentration of plasma corticosteroids occurred in fish exposed to the three types of suspended solids (Fig. 1). High and low concentrations of suspended solids elicited similar responses in all groups. There was an apparent trend toward acclimation--i.e., a return to basal levels--within 48 hours in most groups. Fish exposed to a single acute dose of suspended topsoil tended to have a slightly higher plasma corticosteroid level 3 and 9 hours after imposition of treatment, but by 24 hours corticosteroid levels were identical to those of control fish. Blood hematocrit levels of fish exposed to the three types of suspended solids were consistently higher than those of control fish at 9 and 24 hours (Table 1). We detected no significant

Appendix II

Figure 1. Plasma corticosteroid concentration (mean + SE) increased in steelhead trout after chronic exposure to high (2-3 g/l, solid lines) and low (0.5 g/l, dashed lines) levels of suspended volcanic ash, kaolin clay, and water at 12° C. Fish experiencing one acute exposure to 3 g/l suspended topsoil tended to have higher corticosteroid levels (dashed dotted line). Asterisks indicate significant differences ($P < 0.05$; Student's t-test) between treatment and control groups.



Appendix II
Figure 1

Appendix II

Table 1. Percent hematocrit (+S.D.) increases in juvenile trout after chronic treatment with suspended volcanic ash, topsoil, and kaolin clay. Hematrocrit responses for fish treated with high and low concentrations of suspended soilds were statistically similar; thus the values shown represent the pooled mean for both treatment concentrations. Sample size is shown in paretheses. Asterisks indicate significant ($P < 0.05$, Student's t-test) differences between treatment and control groups.

	Time (hours)				
Treatment	0	3	9	24	48
Control	39 + 1.3 (46)	46 + 1.5 (19)	40 + 1.2 (25)	42 + 1.8 (25)	42 + 1.8 (22)
Ash	-	-	44 + 1.5 (20)	46 + 1.4 (18)	44 + 1.2 (22)
Topsoil	-	47 + 1.0 (21)	51 + 1.2* (17)	51 + 2.5* (12)	51 + 1.9* (16)
Clay	-	45 + 1.6 (21)	47 + 1.2* (23)	48 + 1.2* (21)	42 + 1.7 (20)

alteration in the concentration of plasma sodium during exposure to the three types of suspended solids, nor did we observe any consistent histological effects on gill tissue.

Our results indicate that exposure to fairly high concentrations of suspnded volcanic ash, topsoil, or clay for short periods does not induce mortality; however, such exposure causes physiological responses that are commonly associated with sublethal stress in fishes. Exposure to both high and low levels of supended solids produced similar responses for plasma corticosteroids, suggesting a dose-independent effect within the range of concentrations that we used. The general pattern of corticosteroid responses was remarkably similar for the three types of supended solids--a rapid elevation to a maximal level of about 70-80 ng/ml, followed by an apparent trend toward adaptation. In our laboratory, this general pattern is typical for steelhead trout that are exposed chronically to a moderately severe physical disturbance such as netting and confinement in crowded conditions. The truncated appearance of the corticosteroid response for fish exposed to a single high dose of topsoil indicates that continuous exposure to suspended solids is necessary to elicit a significant effect at a concentration of 2-3 g/l.

Another indication of physiological disequilibrium during exposure to suspended solids was the consistent increase in blood hematocrit values compared with control levels at 9 and 24 hours after treatment was initiated. Interpretation of hematocrit response in fishes is difficult because it is influenced by many factors and is highly variable in unperturbed fish. In the present case, higher hematocrit

levels may evidence a compensatory mechanism for impaired respiratory performance. Hypoxia can cause hematocrit levels to increase in fish[17]. If suspended solids reduce the transport efficiency of oxygen across gill epithelia, either by irritating epithelial cells or by some physiochemical process, then elevated hematocrit levels may compensate for the loss of efficiency. We detected no consistent effect on the appearance of gill tissue, nor did the concentration of plasma sodium change significantly; thus, it seems unlikely that suspended ash acts abrasively on gill epithelia or impairs ionic exchange across gill surfaces under our test conditions. The mucous covering of normal gill tissue probably is adequate protection against direct physical injury by suspended ash during short-term exposure.

Mount St. Helens volcanic ash, topsoil, and kaolin clay induce some physiological responses that are characteristic of sublethal stress in fishes. The responses that we observed have been correlated in some cases with reduced performance capacity in fishes. We infer that exposure to volcanic ash and other types of suspended solids may significantly lower a fish's chances for survival, especially during periods when other sublethal deleterious factors, e.g., high water temperature, are imposed simultaneously.

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7. Mount St. Helens volcanic ash was collected in July 1980 from Moses Lake, Washington. Others[1] have described physical and chemical properties of this ash.

8. The concentration of suspended solids in typical coastal streams of the Pacific Northwest seldom exceeds 1.0 g/l, even after a rainstorm in an area with high sediment loading rates (personal communication, Dr. Robert Beschta, Dept. Forest Engineering, Oregon State University).

9. Sandy loam topsoil (44 percent < 75 μm diameter) from an excavation near Corvallis, OR, and kaolin clay (Anglo-American Clays Co., Atlanta, GA) were used.

10. Dissolved oxygen ($\bar{x}$ = 9 mg/l), pH (7.0), conductivity (225 mmhos), and alkalinity (80mg $CaCO_3$/l) were measured.

11. Fish exposed to volcanic ash were not sampled at 3 hours; fish exposed to one acute dose of topsoil were not sampled at 48 hours.

12. Corticosteroids were assayed acording to R.J. Strange and C.B. Schreck, J. Fish. Res. Board Can. 35, 345 (1978); in salmonids this assay yields a value that is at least 80 percent cortisol, the rest

being either cortisol or other corticosteroids. Plasma sodium was measured with a Perkins-Elmer 51-Ca flame photometer.

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16. We excised the most anterior gill arch from each fish, fixed it in Bouin's solution for 24 hours, imbedded it in paraffin, and stained 7-µm sections with eosin and hematoxylin.

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