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Developmental Differences in the Responsiveness of Gill Na^+,K^+-ATPase to Cortisol in Salmonids

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The ability of cortisol to increase gill Na^+,K^+-ATPase activity was examined in several salmonid species during development. Coho salmon (*Oncorhynchus kisutch*) parr were unresponsive to cortisol *in vitro* (10 μg/ml for 2 days) in November. Responsiveness was significant from January to March, peaking in January just prior to seasonal increases in gill Na^+,K^+-ATPase activity. Gill tissue became unresponsive to *in vitro* cortisol in April when *in vivo* gill Na^+,K^+-ATPase activity peaked. The ability of cortisol to stimulate gill, Na^+,K^+-ATPase activity in postemergent fry (2–3 months after hatching) was examined in chum (*O. keta*), chinook (*O. tschawytscha*), coho, and Atlantic salmon (*Salmo salar*). Initial levels of gill Na^+,K^+-ATPase activity were elevated in chum salmon, which normally migrate as fry. Cortisol (10 μg/ml for 4 days *in vitro*) increased gill Na^+,K^+-ATPase activity in chum salmon fry (48% above initial levels), had a limited but significant effect in chinook salmon fry, and had no effect in coho and Atlantic salmon fry. In an *in vivo* experiment, Atlantic salmon previously exposed to simulated natural photoperiod (SNP) and continuous light (L24) received four cortisol injections of 2 $\mu g \cdot g^{-1}$ every third day. SNP fish responded with increased gill Na^+,K^+-ATPase activity (+66%), whereas L24 fish were not affected. Atlantic salmon presmolts with initially low levels of gill Na^+,K^+-ATPase activity responded to cortisol *in vitro*, whereas smolts with initially high levels of gill Na^+,K^+-ATPase activity were unresponsive. Triiodothyronine (0.01–10 μg/ml), prolactin (0.1–10 μg/ml), growth hormone (0.1–10 μg/ml), insulin (0.01–10 μg/ml), and bovine insulin-like growth factor I (0.01–1 μg/ml) did not affect gill Na^+,K^+-ATPase activity *in vitro*, individually or with cortisol (1–10 μg/ml). Thus, changes in responsiveness to cortisol occur during salmonid development, vary among species, and may be important in the heterochrony that characterizes the parr–smolt transformation.

Salmonid species of the genera *Oncorhynchus, Salmo,* and *Salvelinus* show varying degrees of salinity tolerance at different stages in their life cycle (Parry, 1960; Weisbart, 1968; McCormick and Saunders, 1987; Hoar, 1988). A number of species in these genera are known to undergo a complex developmental event which is adaptive for downstream migration, entry into seawater, and rapid growth in the ocean environment (see McCormick and Saunders, 1987; Hoar, 1988). The timing of the behavioral and physiological changes that occur during this transformation differs among salmonid species, normally coinciding with the differing times of downstream migration (Rounsefell, 1958; McCormick and Saunders, 1987; Hoar, 1988). Whereas genetic differences probably account for many of the differences among species, environmental factors (particularly photoperiod

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and temperature) have been implicated in controlling the timing of transformation within a species.

An increase in salinity tolerance is one of the fundamental changes that occurs during the parr–smolt transformation. Physiological changes in a variety of osmoregulatory organs are coincident with and probably causal to increased salinity tolerance (McCormick and Saunders, 1987). One of the most studied of these is gill Na^+,K^+-ATPase, which increases two- to fivefold during the parr–smolt transformation and is strongly correlated with increased salinity tolerance (Zaugg and McLain, 1970; McCormick *et al.*, 1987). As in other teleosts in seawater, gill Na^+,K^+-ATPase is present in high concentration in the chloride cell, where it generates ionic and electrical gradients which are utilized for ion secretion (Silva *et al.*, 1977).

Specker (1982) suggested that cortisol may be important in regulating several aspects of the parr–smolt transformation. Cortisol has been shown to stimulate gill Na^+,K^+-ATPase activity in a variety of teleosts, including salmonids (Richman and Zaugg, 1987; Björnsson *et al.*, 1987; Madsen, 1990). However, other hormones, including growth hormone (Miwa and Inui, 1985; Richman and Zaugg, 1987; Björnsson *et al.*, 1987), prolactin (Pickford *et al.*, 1970), and thyroid hormones (Miwa and Inui, 1985; Dange, 1986) have been shown to affect gill Na^+,K^+-ATPase activity. McCormick and Bern (1989) have recently shown that cortisol can stimulate gill Na^+,K^+-ATPase activity of coho salmon *in vitro*. The current study was undertaken to determine whether changes in responsiveness to cortisol occur during the parr–smolt transformation, and whether this responsiveness differs among salmonid species. We also determined whether hormones other than cortisol might have direct effects on gill Na^+,K^+-ATPase activity.

MATERIALS AND METHODS

Experiment 1—Coho salmon development. Coho salmon (*Oncorhynchus kisutch*) obtained from Iron Gate Hatchery (California Department of Fish and Game) were reared in 1.5-m diameter, 800-liter capacity outdoor tanks in the Life Sciences Building courtyard at the University of California at Berkeley. A recirculating, biofiltration unit supplied continuous flow, and 50% of the water was replaced every other day. Water temperatures were maintained between 10 and 13° throughout the year. Fish were fed Oregon Moist pellet (Moore–Clarke, LaConner, WA) *ad libitum* twice daily. Fish were starved for 20–24 hr prior to sampling which occurred between 1000 and 1200 hr PST. Eight fish were randomly sampled and killed by a blow to the head. To partially exsanguinate the gills, blood was collected from the caudal vessels, and the first gill arch was removed and placed in preincuabtion medium (described below) on ice. Primary gill filaments were severed just above the septum (approximately one-half their total length of 0.6 to 1.0 cm) so that they could be separated one from another. Filaments were retrieved with suction using a positive displacement pipet, and two to three individual filaments were placed in 0.5 ml preincubation medium in sterile 24-well culture plates (see organ culture method below).

Experiment 2—Postemergent fry. Chum salmon (*O. keta*) were obtained from Minter Creek Hatchery (Washington Department of Fisheries); coho salmon (*O. kisutch*) and fall chinook salmon (*O. tschawytscha*) were obtained from stocks that return to the School of Fisheries, University of Washington, Seattle; Atlantic salmon (*S. salar*) were obtained from a captive stock (originally Penobscot stock) maintained at the Northwest Fisheries Center, National Marine Fisheries Service, Seattle, Washington. Fish were reared in 50-liter, flow-through larval rearing troughs (43 × 64 × 315 cm) supplied with 8.5° fresh water and fed *ad libitum* twice daily. On March 28, six individuals of each species were selected at random and killed by a blow to the head. Ranges of length (snout to tail fork, in cm) and weight (g) were: chum, 4.1–4.3, 0.4–0.5; chinook, 6.0–6.3, 2.4–2.7; coho, 4.9–5.4, 1.2–1.6; Atlantic, 3.1–3.4, 0.2–0.4. The first three pairs of gill arches were removed; because of the small size and difficulty of handling the gill tissue, one half of the gill arch (rather than individual primary filaments) was used for culture. Gill tissue was cultured for 4 days, after which the gill filaments were cut away from the supporting bone and stored as described below.

Experiment 3—Atlantic salmon, in vivo cortisol. Atlantic salmon alevins of Saint John River stock were transported to St. Andrews Biological Station, N.B., Canada, just after hatching in April and reared in 1-m^2,

400-liter capacity tanks supplied with fresh water at 12 liter min^{-1}. Fish were fed with automatic feeders at a rate adjusted for changes in body weight and water temperature. Overhead lighting to all tanks was by standard fluorescent bulbs (40 W) which provided light intensities at the water surface of 430–540 lx.

All fish were initially exposed to simulated natural photoperiod which provided a seasonal change in daylength. On October 14, fish were divided into two groups; one group of fish was exposed to continuous light (24 hr/day; L24), and one group remained on simulated natural photoperiod (SNP). Continuous light at this stage of development has been shown to prevent the normal springtime increases in gill Na^+,K^+-ATPase activity of Atlantic salmon that occur during the parr–smolt transformation (McCormick *et al.* 1987, 1989). Water temperature fluctuated seasonally (6°–18°) during early rearing (April through December) and was then kept relatively constant (4°–6°) during treatment. Fish were fed during the daylight hours of the SNP group.

On April 18 (Day 0), 16 fish from each of the photoperiod treatments were divided into two groups: saline- and cortisol-injected (2 $\mu g \cdot g^{-1}$). Injections were 2 $\mu l \cdot g^{-1}$ body wt administered intraperitoneally on Days 0, 3, 7, and 10. All fish were placed in the same tank, and groups identified by injecting different fins with Alcian blue. On Day 14, fish were killed by a blow to the head, blood was collected from the caudal vessels, and the first gill arch was removed and frozen in SEI buffer at −80°. Na^+,K^+-ATPase activity was measured as described in McCormick *et al.* (1989) and reported as μmol ADP $\cdot$ mg $protein^{-1} \cdot hr^{-1}$.

Experiment 4—Atlantic salmon, presmolt and smolt. Atlantic salmon of mixed, captive stock were raised at the Sea Farm Washington hatchery near Rochester, Washington. Twenty fish were brought to the National Marine Fisheries Service Laboratory in Seattle, Washington and held without food for several days in conditions described previously. On March 31, 6 large fish (16.4–19.5 cm, 54.9–90.6 g) and 6 small fish (8.7–12.1 cm, 7.1–22.7 g) were killed by a blow to the head, the first gill arch was removed, and the primary filaments were isolated and subjected to organ culture as described previously.

Experiment 5—Hypophysectomized and intact coho salmon. Coho salmon obtained as parr from Iron Gate Hatchery (California Department of Fish and Game) were reared at the University of California at Berkeley as described above. Smolt and postsmolt 1-year-old salmon (June to December) were used. Fish were hypophysectomized by the orbital method (Nishioka, 1980), maintained in 5 ppt seawater for several days, and then returned to fresh water for at least 10 days. Hypophysectomized coho salmon do not normally feed, and no food was offered during this period. Intact seawater fish were acclimated to 30 ppt seawater for at least 2 weeks and were fed throughout the experiment.

Organ culture and measurement of gill Na^+,K^+-ATPase activity. *In vitro* methods were modified from that reported by McCormick and Bern (1989). Initial (Day 0) samples were obtained immediately after dissection, placed in 200 μl ice-cold SEI buffer (0.3 *M* sucrose, 0.02 M Na_2 ethylene diamine tetraacetic acid, and 0.05 *M* imidazole, pH 7.3), frozen immediately on dry ice and stored for up to 20 days at −80°. Except where noted, 2–3 primary filaments were maintained in separate incubation wells for each combination of individual fish and dose. Following preincubation at 14° with gentle shaking for 0.5–1 hr, the preincubation medium was removed and replaced with incubation medium containing hormone or hormone vehicle. Gill filaments were incubated at 14° under 95% O_2: 5% CO_2 with gentle shaking for 2–4 days. After culture, gill filaments were removed from culture wells with suction and placed in 200 μl ice-cold SEI buffer and frozen as described above.

The preincubation medium was Minimal Essential Medium (MEM; GIBCO) with Hanks' salts, 25 m*M* Hepes buffer and 4 mg/ml bovine serum albumin (Sigma RIA grade); 250 U/ml penicillin G and 250 μg/ml streptomycin sulfate were added immediately prior to use, and the medium was adjusted to pH 7.55 with NaOH (final osmolarity 298 mOsm). The incubation medium was MEM with Earle's salts, 4 mg/ml bovine serum albumin, 292 μg/ml L-glutamine, 50 U/ml penicillin G, 50 μg/ml streptomycin sulfate, 50 μg/ml gentamicin sulfate, and 5 μg/ml bovine insulin adjusted to be at pH 7.6 when gassed with 95% O_2:5% CO_2. Stock solutions of hormones were prepared in the following concentrations and sterile solvents: cortisol (Sigma H-4001), 10 mg/ml in ethanol; bovine insulin (Sigma I-5500), 10 mg/ml in 0.01 N HCl, native chum salmon growth hormone (gift from H. Kawauchi, Kitasato University) and ovine growth hormone (NIADDK-oGH-19, AFP-7649C), 0.25 mg/ml in 0.01 N NaOH, 0.8% NaCl; native chum salmon prolactin (gift from H. Kawauchi, Kitasato University) 1 mg/ml in 0.01 N NaOH, 0.8% NaCl; tiiodothyronine (sodium salt, Sigma T 2752), 10 mg/ml in 0.1 N NaOH, 0.8% NaCl; recombinant bovine insulin-like growth factor I (Monsanto Corp., St. Louis MO), 0.5 mg/ml in MEM. All solutions, including control and intermediate doses, contained the same amount of hormone solvent as was used for the maximum dose. Experiments with insulin-like growth factor I were conducted in the absence of insulin. The preincubation was designed to remove any endogenous hormones. There was no detectable cortisol in the control incubation medium (less than 0.2 $ng \cdot ml^{-1}$ as measured by radioimmunoassay; Young, 1988).

Gill Na^+,K^+-ATPase activity was measured by the method of McCormick and Bern (1989). Five to ten

minutes prior to assay, tissue was rapidly thawed, removed from SEI buffer, and homogenized in 85 μl SEI buffer with 0.1% Na deoxycholate. The homogenate was centrifuged at 5000*g* for 30 sec, and the supernatant was removed and assayed for Na^+,K^+-ATPase activity and protein content. Twenty microliters of gill homogenate were placed in a final volume of 1 ml assay mixture containing 50 m*M* imidazole, 1 U/ml LDH, 2.5 U/ml PK, 2 m*M* phosphoenolpyruvate (PEP), 0.05 m*M* NADH, 0.5 m*M* ATP, 0.4 m*M* KCN, 45 m*M* NaCl, 2.5 m*M* $MgCl_2$, and 10 m*M* KCl (pH 7.5). A duplicate cuvette run simultaneously contained 0.5 m*M* ouabain. The assay mixture was quickly mixed and the change in absorbance (340 nm) recorded in a Perkin–Elmer recording spectrophotometer. Temperature was maintained at 25° with a water-jacketed cuvette holder.

The rate of NADH oxidation (equimolar to ATP hydrolysis and ADP production) was calculated from the linear rate between 3 and 9 min after initiation of the reaction. Protein content of the homogenate was measured by the method of Larson *et al.* (1986) using bovine serum albumin as standard. Na^+,K^+-ATPase was calculated as the difference in ouabain-sensitive and -insensitive ATP hydrolysis and expressed as μmols ADP · mg protein^{-1} · hr^{-1}.

Statistics. Three or more groups were compared by the nonparametric Kruskall–Wallis test ($P < 0.05$). When there was a significant difference among the groups, pairwise comparisons with control and treatment groups were made by the Mann–Whitney *U* test ($P < 0.05$). Statistical analyses were performed by CRISP (CRUNCH, Berkeley, CA).

RESULTS

Coho salmon development. Initial (*in vivo*) levels of gill Na^+,K^+-ATPase activity from intact fish were constant from November to January, increased from February to peak levels in May, and declined in July (Fig. 1). *In vitro* responsiveness to 10 μg/ml cortisol (expressed relative to initial gill Na^+,K^+-ATPase activities *in vivo*) was positive from November to March, but was statistically significant only in January (Fig. 1). When values were expressed relative to control levels *in vitro* after 2 days in culture, 10 μg/ml cortisol had no significant effect in November, but significantly stimulated gill Na^+,K^+-ATPase activity from January to March (Table 1). In May, when *in vivo* levels of gill Na^+,K^+-ATPase activity were highest, there was no significant

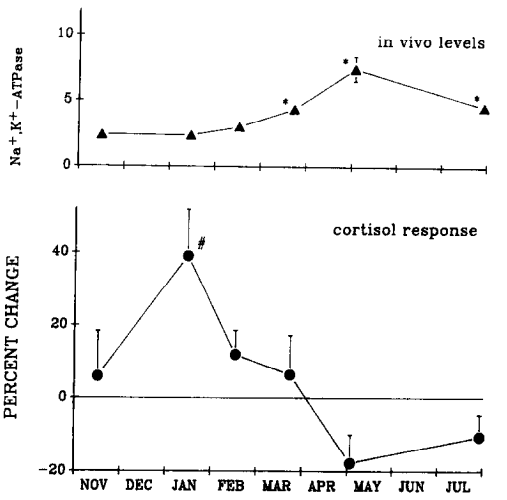

FIG. 1. Temporal changes in initial levels *in vivo* (upper) and responsiveness of gill Na^+,K^+-ATPase activity to 10 μg/ml cortisol *in vitro* for 2 days (lower), in juvenile coho salmon. In the upper figure, * indicates a significant difference from the levels found in the first sampling period (November). Responsiveness is expressed as percent change from initial levels; # significant difference from initial levels. Sample size was eight for each sampling date. Values are mean ± SE.

effect of 10 μg/ml cortisol. In July, when *in vivo* levels had declined from the those in May, responsiveness was again significant (Table 1).

Postemergent fry. Initial levels of gill Na^+,K^+-ATPase activity in chum salmon were more than twofold greater than in the other three species ($P < 0.05$). There was no significant effect of *in vitro* cortisol (0.1–10 μg/ml) for 4 days on gill Na^+,K^+-ATPase activity of postemergent fry of coho or Atlantic salmon ($P < 0.05$; Fig. 2). In chinook salmon, 10 μg/ml cortisol significantly increased gill Na^+,K^+-ATPase activity relative to Day 4 control levels (35% increase), but not from initial levels. In chum salmon, 10 μg/ml cortisol resulted in a significant increase in gill Na^+,K^+-ATPase activity relative to Day 4 control levels (95% increase) and initial levels (48% increase).

Atlantic salmon, in vivo. Gill Na^+,K^+-ATPase activity in control (vehicle-

TABLE 1
EFFECT OF *in Vitro* CORTISOL (2 DAYS) ON Na^+,K^+-ATPase ACTIVITY IN GILL TISSUE OF COHO SALMON

	Nov. 15	Jan. 13	Feb. 14	Mar. 22	May 1	July 25
Body length (cm)	11.9 ± 0.1	12.3 ± 0.2	12.7 ± 0.1	13.2 ± 0.2	14.9 ± 0.4	17.2 ± 0.4
Na^+,K^+-ATPase activity (μmol ADP · mg protein^{-1} · hr^{-1})						
Control	2.3 ± 0.1	2.6 ± 0.1	2.9 ± 0.1	3.6 ± 0.3	5.4 ± 0.5	3.5 ± 0.4
10 μg · ml^{-2}	2.4 ± 0.2	3.2 ± 0.2	3.3 ± 0.2	4.4 ± 0.3	5.8 ± 0.6	4.3 ± 0.5
% Change	7 ± 9	23* ± 7	15* ± 5	23* ± 16	11 ± 8	22* ± 7

Note. Sample size was eight fish for each sampling date. Initial values of gill Na^+,K^+-ATPase activity are contained in Fig. 1. Values are mean ± SE.
* Significantly different from control levels ($P < 0.05$, Mann–Whitney U test).

injected) Atlantic salmon was 4.4 ± 0.4 μmol ADP · mg protein^{-1} · hr^{-1} (mean ± SE) in fish under simulated natural photoperiod (SNP) and 1.8 ± 0.3 μmol ADP · mg protein^{-1} · hr^{-1} in fish reared under continuous light (L24) ($P < 0.05$). Four cortisol injections (2 μg · g^{-1}) over a 10-day period resulted in a 66% increase in gill Na^+,K^+-ATPase activity in the SNP group ($P < 0.05$), whereas there was no significant effect of cortisol on gill Na^+,K^+-ATPase activity of the L24 group (Fig. 3).

Atlantic salmon, presmolt and smolt. Presmolt and smolt of Atlantic salmon were distinguished by differences in size, and high initial levels of gill Na^+,K^+-ATPase activity, which were more than twofold higher in smolts in late March (Table 2). In presmolts, *in vitro* cortisol treatment resulted in a dose-dependent increase in gill Na^+,K^+-ATPase activity; 10 μg/ml cortisol resulted in a 53% increase over Day 2 control levels and an 8% increase over initial levels; the former was statistically significant ($P < 0.05$). Smolts, with initially high levels of gill Na^+,K^+-ATPase activity, did not respond to *in vitro* cortisol treatment.

Hypophysectomized and intact coho salmon. The possible *in vitro* effects of other hormones were tested on gill tissue from intact and hypophysectomized 1-year-old coho salmon (Table 3). Triiodothyronine (0.01–10 μg/ml), prolactin (0.1–10 μg/

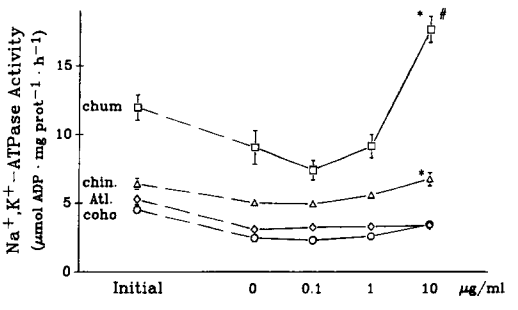

FIG. 2. Initial levels (*in vivo*) and changes in gill Na^+,K^+-ATPase activity in response to cortisol (4 days *in vitro*) in postemergent chum, chinook, coho, and Atlantic salmon. # indicates a significant change from initial levels, * significant change from control (no cortisol) levels at Day 4. Sample size was 6 for control and cortisol concentration, values are mean ± SE.

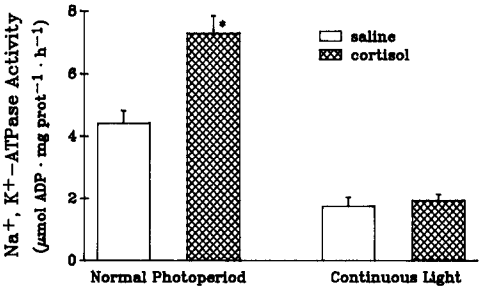

FIG. 3. Effect of 4 cortisol injections (2 μg · g^{-1} body wt) over a 10-day period on gill Na^+,K^+-ATPase activity in Atlantic salmon exposed to simulated natural photoperiod or continuous light. * indicates a significant increase over control (saline-injected) levels. Gill Na^+,K^+-ATPase activity of saline-injected) controls in simulated natural photoperiod and continuous light groups were significantly different ($P < 0.05$). Sample size was seven to eight fish for each group, values are mean ± SE.

TABLE 2
EFFECT OF *in Vitro* CORTISOL EXPOSURE FOR 2 DAYS ON GILL Na^+,K^+-ATPase ACTIVITY IN LARGE (>16 cm) AND SMALL (≤12 cm) JUVENILE ATLANTIC SALMON

		Cortisol for 2 days		
	Initial	Control	1.0 μg/ml	10 μg/ml
Large fish				
Activity	12.8 ± 1.2	10.4 ± 1.2	11.2 ± 1.2	10.3 ± 1.6
Percentage change	—	0.0	10 ± 6	4 ± 17
Small fish				
Activity	4.9 ± 0.6	3.6 ± 0.5	4.2 ± 0.4	5.3 ± 0.9
Percentage change	—	0.0	23 ± 11	53* ± 27

Note. Experiment was conducted in late March. Results are expressed as activity (μmol ADP · mg protein^{-1} · hr^{-1}) and as percentage change from control levels (%), mean ± SE.
* Significantly different from control levels ($P < 0.05$, Kruskall–Wallis test).

ml), growth hormone (0.1–10 μg/ml), insulin (0.01–10 μg/ml), and insulin-like growth factor I (0.01–1 μg/ml) did not affect gill Na^+,K^+-ATPase activity *in vitro*, individually or when combined with cortisol (1–10 μg/ml). A combination of growth hormone (1 μg/ml) and insulin-like growth factor I (1 μg/ml) tested in gill tissue from hypophysectomized coho salmon was also without effect (data not shown).

DISCUSSION

Taken together, the results of these experiments indicate that the stimulatory effect of cortisol on gill Na^+,K^+-ATPase activity is most apparent when the fish are near their normal time of seaward migration. The period of maximal sensitivity of the gill to cortisol is seasonal in coho and Atlantic salmon (and perhaps other salmonid species), probably mediated through changes in day length. It seems likely that the influence of development and photoperiod are dependent on genetically determined features of the life cycle for individual salmonid species.

Coho salmon fry (2 months after hatching, in March) and parr (10 months after hatching, in November) were unresponsive to cortisol *in vitro* (Figs. 1 and 2; Table 1). Just prior to and during the springtime increases in gill Na^+,K^+-ATPase activity, gill tissue of coho salmon responded significantly to cortisol *in vitro* (Table 1); this responsiveness is more dramatic and transient if expressed relative to the initial levels of gill Na^+,K^+-ATPase activity (Fig. 1). At the peak of the parr-smolt transformation when initial levels of gill Na^+,K^+-ATPase activity are highest, gill tissue is

TABLE 3
SUMMARY OF EFFECT OF VARIOUS HORMONES *in Vitro* ON Na^+,K^+-ATPase ACTIVITY IN GILL TISSUE FROM INTACT AND HYPOPHYSECTOMIZED COHO SALMON

	Intact (FW)	Intact (SW)	Hypophysectomized (FW)
Cortisol	+	+	+
Growth hormone (salmon, bovine)	−/−*	−/−	−/−
Prolactin (salmon, ovine)	−/−		−/−
T3	−/−	−/−	−/−
Insulin (bovine)	−/−		−/−
IGF-I (recombinant bovine)	−/−		−/−

Note. Fish were adapted to fresh water (FW) or 30 ppt seawater (SW). +, increase; −, no effect.
[a] Without cortisol/with cortisol.

again unresponsive to cortisol. This refractoriness to cortisol when gill Na^+,K^+-ATPase activity is initially high was also seen in large Atlantic salmon smolts (Table 2). When peak levels of gill Na^+,K^+-ATPase activity subsequently declined in late July, gill tissue of coho salmon once again responded to cortisol *in vitro* (Table 1). The seasonal differences in the ability of cortisol to increase gill Na^+,K^+-ATPase activity *in vitro* indicate that changes in responsiveness to cortisol may be an important determinant of the timing of the seasonal increases in gill Na^+,K^+-ATPase activity that occur in several anadromous salmonids.

As in the present study, McCormick and Bern (1989) found that presmolt coho salmon with initially low levels of gill Na^+,K^+-ATPase activity responded to 10 μg/ml cortisol by increasing gill Na^+,K^+-ATPase activity to levels significantly greater than initial levels. It was proposed that the increase was the result of *de novo* synthesis of Na^+,K^+-ATPase. When cortisol prevents or partially prevents *in vitro* decreases in gill Na^+,K^+-ATPase activity, cortisol may be exerting its effect either by increasing *de novo* synthesis of the enzyme, or protecting the enzyme from degradation or both. The response to cortisol was found to be highly specific (e.g., the enzyme was not activated by cortisone) and resulted in increased ouabain binding sites, indicating an increase in the number of sodium pumps.

It seems likely that the seasonal changes in responsiveness to cortisol are in part photoperiod-dependent. Previous studies have found that photoperiod changes can alter the timing of many aspects of the parr–smolt transformation, including changes in gill Na^+,K^+-ATPase activity (see McCormick and Saunders, 1987; and Hoar, 1988 for review). In the present study, *in vivo* cortisol treatment was found to be effective in increasing gill Na^+,K^+-ATPase activity in Atlantic salmon held under simulated natural photoperiod, but not in those exposed to continuous light (Fig. 3). The latter treatment has been shown to prevent the normal springtime increases in salinity tolerance and gill Na^+,K^+-ATPase activity of Atlantic salmon (McCormick *et al.*, 1987, 1989). Further studies will be needed to determine whether continuous light also abolishes *in vitro* responsiveness to cortisol, and whether responsiveness to cortisol can be altered by changing the timing or magnitude of increased daylength.

Among the salmonids there is considerable variation in time of residence in fresh water, the timing of downstream migration, and the timing of the parr-smolt transformation (Rounsefell, 1958; Hoar, 1988). For instance, pink and chum salmon normally migrate downstream within 2 months after hatching in fresh water, whereas Atlantic salmon may spend up to 5 years in fresh water before entering seawater. The differences in the timing of downstream migration and the parr–smolt transformation among salmonids are, by definition, a heterochrony. McCormick and Saunders (1987) suggested that the endocrine system, in coordination with environmental and developmental change, might be responsible for this heterochrony.

It is not yet clear whether all species of migratory salmonids undergo the same physiological changes during the parr–smolt transformation. Certainly those which undergo seasonal migrations such as coho salmon, Atlantic salmon, and steelhead trout undergo a number of similar physiological changes (McCormick and Saunders, 1987; Hoar, 1988). Facultatively anadromous species such as brook trout (*Salvelinus fontinalis*) apparently do not undergo any physiological change prior to entry into estuaries, where adaptation to seawater is gradual (McCormick *et al.*, 1985). Our knowledge of the physiological and biochemical changes in pink salmon (*O. gorbuscha*) and chum salmon, which migrate seaward and have increased salin-

ity tolerance soon after hatching, is even more limited. Sullivan *et al.* (1983) have shown that gill Na^+,K^+-ATPase activity of pink salmon increases 40% between 40 and 70 days after hatching. In the present study we observed high gill Na^+,K^+-ATPase activity in 2-month-old chum salmon, levels which were almost threefold greater than coho and Atlantic salmon of similar size and age.

The purpose of the interspecific comparisons in the present study was to determine whether changes in responsiveness to cortisol could account for the changes in the timing of the parr–smolt transformation among salmonid species. Chum salmon migrate to sea as fry, soon after emergence from the gravel; fall chinook migrate during their first spring; coho and Atlantic salmon migrate during their second spring or later. Only chum salmon fry demonstrated increased gill Na^+,K^+-ATPase activity relative to initial levels when exposed to 10 μg/ml cortisol *in vitro*. Chinook salmon fry had a limited but significant capacity to respond to cortisol, and coho and Atlantic salmon fry had no response. The response of the tested species correlates with their time of seawater migration and with their relative salinity tolerances at the fry stage (chum > chinook > coho; Weisbart, 1968). There was no apparent dose dependence in the response of gill tissue of chum salmon to cortisol *in vitro,* perhaps owing to the high initial levels of gill Na^+,K^+-ATPase activity in chum salmon at this stage. In this regard it would be of considerable interest to examine several developmental stages of the chum salmon to determine if there are temporal changes in responsiveness to cortisol similar to that seen for coho salmon.

It is interesting that chinook salmon gill Na^+,K^+-ATPase activity increased significantly in response to cortisol in comparison with controls *in vitro* (Fig. 2), although the increase was not as marked as that for chum salmon. This particular stock of chinook (fall chinook) is one that migrates seaward in its first spring. The present experiment was done in late March, approximately 6 weeks before the fish migrate. The results using fall chinook compare favorably with the experiments using yearling coho salmon in that the stimulatory effect of cortisol *in vitro* is observed within several months before the fish normally enter seawater. It would be interesting to compare the effects of cortisol on fall chinook (age 0 migrants) and spring chinook (age 1 migrants) to determine whether genetically determined variations in responsiveness to cortisol occur within species.

Cortisol had the capacity to increase Na^+,K^+-ATPase activity (relative to control levels) in gill tissue from presmolt coho salmon in fresh water, postsmolts in fresh water and seawater, and hypophysectomized postsmolts in fresh water (Table 3). None of the other hormones tested (prolactin, growth hormone, triiodothyronine, insulin, and IGF-I) had a direct effect on coho salmon gill Na^+,K^+-ATPase activity, nor did any of these hormones show any additive or synergistic effect with cortisol. Although these negative results do not eliminate the possibility that these (and other) hormones directly affect gill Na^+,K^+-ATPase activity under some circumstances, they do indicate that cortisol has a predominant role in regulating gill Na^+,K^+-ATPase. Indeed, it is possible to explain at least some of the reported *in vivo* endocrine effects on gill Na^+,K^+-ATPase activity by their effects on the interrenal. Young (1988) has shown that growth hormone *in vivo* and *in vitro* stimulates the ability of the coho salmon interrenal to produce cortisol in response to ACTH. Similarly, *in vivo* thyroxine treatment also sensitizes the interrenal to ACTH (Young and Lin, 1988).

The results of the present study provide evidence that changes in responsiveness to cortisol are important in determining the timing of increased gill Na^+,K^+-ATPase activity in smolting salmonids. Other factors, however, are also likely to play an im-

portant role. Circulating cortisol levels increase in coho and Atlantic salmon in spring, as do plasma growth hormone, thyroxine, and insulin (Specker and Schreck, 1982; Thorpe *et al.* 1987; Young *et al.*, 1989; Prunet *et al.*, 1989; Plisetskaya *et al.*, 1988). Young (1989) has shown that the *in vitro* capacity of the coho salmon interrenal to produce cortisol in response to ACTH changes seasonally, with peak sensitivity occurring in April. Since circulating levels of cortisol are regulated by a variety of environmental and endocrine factors (including stress) which can result in large and rapid changes, the relationship between circulating cortisol and increased gill Na^+,K^+-ATPase activity is likely to be a complex one. Further investigations of the possible endocrine or paracrine regulation of gill responsiveness and of gill cortisol receptors should provide insight into other factors controlling the parr–smolt transformation.

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