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Plasma prolactin and cortisol concentrations during salinity challenges of coho salmon (*Oncorhynchus kisutch*) at smolt and post-smolt stages*

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ABSTRACT

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Circulating prolactin (PRL) and/or cortisol were measured in relation to hydromineral balance (internal osmotic pressure or plasma Na^+ and K^+) in smolt or post-smolt coho salmon (*Oncorhynchus kisutch*) to evaluate effects of salinity challenges. Freshwater (FW) challenge of seawater (SW)-adapted fish resulted in a peak of circulating PRL concentration 3 days after transfer, irrespective of the developmental stage or the date of transfer. Plasma PRL decreased thereafter, but remained higher at 14–15 days following FW entry than in the SW controls. Circulating cortisol levels were unchanged but a slight reduction of plasma Na^+ and osmolality occurred. Seawater challenge of FW-adapted fish elicited a rapid decline in circulating PRL concentrations to levels found in SW-acclimated fish; levels remained low thereafter. Plasma PRL showed a negative relationship with plasma cortisol and with internal osmotic pressures or natremia. Conversely, high circulating cortisol concentrations were associated with poor hypoosmoregulatory ability, probably reflecting a stress reaction.

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INTRODUCTION

The influence of prolactin (PRL) in the control of hydromineral balance in freshwater (FW) teleosts is well documented: it decreases membrane permeability and has a sodium-retaining action on osmoregulatory surfaces (Nicoll, 1981; Loretz and Bern, 1982; Hirano, 1986). However, its role in salmonid osmoregulatory physiology is less clear. Gonnet et al. (1988) have found no effect of osmotic pressure on PRL release by trout pituitary in vitro. Prolactin also appears not to be mandatory for FW survival of salmonids, since only minor changes occur in plasma Na^+ after hypophysectomy of several species (Donaldson and McBride, 1967; Komourdjian, 1984; Nishioka et al., 1987; Richman et al., 1987). Recent studies have revealed differences in the response of PRL during seawater (SW) adaptation among smolting species (*Salmo salar*: Prunet and Boeuf, 1985; *Oncorhynchus kisutch*: Young et al., 1989) and non-smolting strains of rainbow trout (*Oncorhynchus mykiss*: Prunet et al., 1985).

During smoltification of some anadromous salmonids, pituitary–interrenal activity and plasma cortisol levels increase presumably in preparation for SW entry or other metabolic demand (Fontaine and Hatey, 1954; Oliveireau, 1962, 1975; McLeay, 1975; Specker and Schreck, 1982; Barton et al., 1985; Young, 1986; Sheridan, 1987). Cortisol is well known as a stress-related hormone (Schreck, 1981) and as having a mineralocorticoid function (Maetz, 1969), important for maintaining SW homeostasis. The involvement of cortisol in the development of hypoosmoregulatory mechanisms preparatory to SW entry has been the subject of several investigations. Some recent studies examining developmental changes in circulating cortisol (Young, 1986; Young et al., 1989) have suggested its participation in the development of hypoosmoregulatory ability in coho salmon (*O. kisutch*). In vivo studies suggest that cortisol may increase gill Na^+ , K^+ -ATPase activity in the same species (Richman and Zaugg, 1987; Björnsson et al., 1989), and McCormick and Bern (1989) have demonstrated that cortisol enhances coho salmon gill Na^+ , K^+ -ATPase activity in vitro.

We investigated the effects of salinity on circulating levels of PRL, in a smolting species, the coho salmon, and how they might be correlated to cortisol concentrations and osmotic parameters. Because of the importance of developmental stage to the ability of salmon to adjust to rapid salinity changes, we evaluated the fish during and following smoltification. This allowed us to also consider the role of these hormones relative to osmoregulatory ability.

MATERIALS AND METHODS

Animals and experimental designs

1985 experiments. Coho salmon were obtained from the California Department of Fish and Game's Trinity River Hatchery in early January 1985. They were maintained in Berkeley, CA, under natural photoperiod in circular 180-

l tanks supplied with filtered recirculating chilled (14°C) dechlorinated tap water at a density of 5–12 g/l and were fed twice daily with Oregon Moist Pellet (2% body weight/day); one-third of the tank water was replaced daily.

By mid-March, fish in FW (averaging 30 g) appeared silvery with dark fin margins and were classified on this basis of external appearance as “early” smolts. A group of fish was transferred directly to 31‰ artificial seawater (Marine Environment, Incorporated) maintained at 14°C , and was held there until mid-June for FW challenge on the long-term SW-acclimated fish (post-smolts of approximately 50 g). Seawater challenges were performed by the direct transfer of fish into a tank containing 31‰ seawater at 14°C in mid-March (smolts) and mid-June (post-smolts). FW controls were transferred to an identical tank containing FW. The FW challenge was similarly performed.

1987 experiments. Juvenile coho salmon of either sex were obtained from Oregon Aqua-foods, Incorporated. These fish were smolts in their first year of life at the times of testing, based on their ability to survive and grow in SW.

Seawater challenges were undertaken on fish reared at Oregon Aqua-food’s Springfield hatchery, OR, and acclimated to FW. They were transferred to the Hatfield Marine Science Center (MSC), Oregon State University (Newport, OR), and maintained in FW at 18°C for 18 days before the beginning of the experiment (1 July). Fish weighed approximately 20 g. Freshwater challenges were performed on fish reared in flowing natural seawater ponds at Oregon Aqua-food hatchery (Newport, OR) for 24–30 days. They were transferred to the MSC and maintained in 30‰ SW at 15°C for 10 days before the beginning of the experiment. Fish averaged 20 g in mid-June and 39 g during mid-July experiments.

Fish were not disturbed except for feeding twice daily with Oregon Moist Pellet diet (2% body weight/day) until 2 days before the experiments. They were maintained in flow-through circular tanks (380 l) at a density of 5–10 g/l throughout the experiments. Freshwater and SW challenges were performed by switching the FW tap on and the SW tap off or vice versa. In this way, a rapid and progressive change of the salinity of the water occurred, and the water of the whole tank was totally replaced within 2 h; because this was accomplished without moving the fish, handling stress was eliminated as a variable.

Blood collection analysis

Fish were rapidly killed by immersion in a solution of ethyl-*m*-aminobenzoate methane sulfonate (MS222). Blood was collected from the dorsal aorta into heparinized capillary tubes, after severing the caudal peduncle. Plasma

was separated and stored at -20°C for later analysis (prolactin, cortisol, osmolality, Na^{+} and K^{+} concentrations).

Plasma PRL concentration was measured with a homologous radioimmunoassay (RIA) for salmon PRL using highly purified chinook salmon prolactin (Prunet and Houdebine, 1984) according to the method described by Prunet et al. (1985). Plasma cortisol concentration was assayed with a RIA as described by Foster and Dunn (1974) and modified by Redding et al. (1984). Total plasma Na^{+} and K^{+} concentrations were measured with a NOVA 1 sodium-potassium analyser (Nova Biomedical, Newton, MA). Blood osmolality was measured with a Wescor 5100 C vapor pressure osmometer. There was insufficient plasma in some samples to allow for all analyses.

Statistical analysis

Osmolality data were subjected to Student's unpaired *t*-test. Other data followed statistical analysis described by Winer (1971) and Afifi and Azen (1979). They were subjected to one-way analysis of variance (ANOVA) followed by the Fisher's Protected Least Significant Difference (PLSD) multiple comparison test, after testing homogeneity of variance. Plasma prolactin and cortisol data were transformed into their natural logarithms or square roots to increase homogeneity of variance.

RESULTS

Effects of FW challenge

1985 experiments. Transfer of SW-acclimated fish into FW in mid-June caused a 9–10-fold increase in PRL in the plasma of post-smolts (Fig. 1), which reached its maximum after 3 days. Fourteen days after FW contact, plasma PRL concentrations remained high and comparable to levels found in FW-adapted animals. Internal osmotic pressure measured 1 day after the challenge was reduced by approximately 35 mOsm/kg (Fig. 1: insert).

1987 experiments. Although the absolute values of circulating PRL were lower in 1987 after FW entry, plasma PRL followed a similar pattern to that seen in 1985, irrespective of the date of FW exposure: mid-June (Fig. 2) or mid-July (Fig. 3A). Plasma Na^{+} levels decreased slightly but significantly (Fig. 3C) and remained stable at a lower level than SW controls during the course of the experiment. Circulating cortisol (Fig. 3B) or plasma K^{+} (Fig. 3D) did not change significantly.

Fig. 1. Changes in plasma prolactin levels during FW challenge of coho salmon post-smolt on 10 June 1985: SW controls (—□—); SW→FW (—◆—). Insert: changes of internal osmolality measured 1 day after FW entry. Statistical comparisons: significantly different from controls at the same time point with $**P < 0.001$ (ANOVA test) and with (2) $P < 0.01$ (Student's unpaired *t*-test). Each point on the Fig. or value in the insert represents the mean $\pm$ s.e.m.; $n = 9-10$.

Fig. 2. Changes in plasma prolactin levels during FW challenge of coho salmon smolt on 15 June 1987: SW controls (—□—); SW→FW (—◆—). Statistical comparisons: significantly different from controls at the same time point with $**P < 0.001$ and $*P < 0.05$ (ANOVA test). The number of fish studied is indicated near each point, which represents the mean $\pm$ s.e.m.

Effects of SW challenge

1985 experiments. SW entry caused a decrease in plasma PRL, apparent within 24 h after the transfer; this response was similar between smolts (mid-March: Fig. 4) and post-smolts (mid-June: Fig. 5). Levels remained low during the remainder of the experiment (14 days). Values of circulating PRL in FW-adapted controls were much higher in post-smolts (Fig. 5) than in smolts (Fig. 4). Increased salinity resulted in an increase of plasma osmolality 1 day

Fig. 3. Changes in plasma prolactin (A), cortisol (B), Na^+ (C) and K^+ (D) concentrations during FW challenge of coho salmon smolt on 14 July 1987: SW controls (—□—); SW→FW (—◆—). Statistical comparisons: significantly different from controls at the same time point with ** $P < 0.001$ and * $P < 0.05$ (ANOVA test). The number of fish studied is indicated near each point, which represents the mean $\pm$ s.e.m.

after the transfer (compared to FW controls) of 33 mOsm/kg in smolts (Fig. 4: insert); however, a much greater increase (88 mOsm/kg) occurred in post-smolts (Fig. 5: insert), which appeared to have lost hypoosmoregulatory abil-

Fig. 4. Changes in plasma prolactin levels during SW challenge of coho salmon smolt on 12 March 1985: FW controls (—□—); FW->SW (—◆—). Insert: changes of internal osmolality measured 1 day after SW entry. Statistical comparisons: significantly different from controls at the same time point with $**P < 0.001$ and $*P < 0.05$ (ANOVA test) and with (1) $P < 0.001$ (Student's unpaired *t*-test). Each point on the Fig. or value in the insert represents the mean $\pm$ s.e.m.; $n = 7-10$.

Fig. 5. Changes in plasma prolactin levels during SW challenge of coho salmon post-smolt on 10 June 1985: FW controls (—□—); FW->SW (—◆—). Insert: changes of internal osmolality measured 1 day after SW entry. Statistical comparisons: significantly different from controls at the same time point with $**P < 0.001$ (ANOVA test) and with (1) $P < 0.001$ (Student's unpaired *t*-test). Each point on the Fig. or value in the insert represents the mean $\pm$ s.e.m.; $n = 9-10$.

Fig. 6. Changes in plasma prolactin (A), cortisol (B), Na⁺ (C) and K⁺ (D) concentrations during SW challenge of coho salmon smolt on 1 July 1987: FW controls (—□—); FW→SW (—◆—). Statistical comparisons: significantly different from controls at the same time point with ***P* < 0.001 (ANOVA test). The number of fish studied is indicated near each point, which represents the mean ± s.e.m.

ity by this time.

1987 experiments. SW challenge of fish in early July elicited a decline of plasma PRL which quickly stabilized at low values characteristic of SW-adapted animals (Fig. 6A), as seen in the 1985 experiments. Hypoosmoregulatory ability, assessed by plasma Na^+ measurements after the transfer, was poor (Fig. 6C). Plasma cortisol peaked within 1 day of SW exposure, was still high 15 days after transfer, and was positively correlated with plasma Na^+ . Plasma K^+ was apparently unaffected by entry into SW (Fig. 6D).

DISCUSSION

We found that in a smolting species, the coho salmon, plasma PRL concentrations were affected by changes in external salinity. Developmental stage had relatively little influence on the magnitude or duration of changes in circulating PRL.

Although prolactin is known to play an important role in FW adaptation of many euryhaline teleosts, little information is available on changes in plasma PRL in smolting species during FW challenge of SW-acclimated fishes (Hasegawa et al., 1987). In our approach, the effects of a rapid exposure to FW (within 2 h) were observed at different developmental stages (smolt and post-smolt) and duplicated in different years. Our results show that plasma PRL peaks after FW entry with the same pattern and dynamics irrespective of developmental stage (Figs. 1, 2 and 3A). These data match those found in similar studies performed on non-smolting strains of rainbow trout (*O. mykiss*: Prunet et al., 1985) and on a non-salmonid species (*Sarotherodon mossambicus*: Nicoll et al., 1981), and support the numerous studies suggesting the involvement of PRL in maintaining homeostasis in euryhaline (non-salmonid) teleosts in FW (Hirano and Mayer-Gostan, 1978; Clarke and Bern, 1980; Loretz and Bern, 1982; Hirano et al., 1987). In this context, a lowered plasma osmotic pressure has often been related to enhanced prolactin release (Prunet et al., 1985), reflected here in our observations of simultaneous changes of plasma PRL with osmotic pressure or plasma $[\text{Na}^+]$ (Fig. 1 and insert; Fig. 3A,C). Absolute values of plasma PRL in FW differed among the various sampling times and years. These variations could reflect hormone pulsatility, although no diel rhythm for PRL has been observed in Atlantic salmon (Prunet and Boeuf, 1989), or changes with season and developmental stage (Young et al., 1989), or differences between stock or rearing conditions.

Seawater adaptability of various salmonids has been extensively studied during recent years to examine the development of hypoosmoregulatory ability of fish during smoltification, or to test the effects of various hormonal treatments. Some differences are apparent between smolting species which do not show significant changes in plasma PRL concentrations after SW entry

(*S. salar*: Prunet and Boeuf, 1985; *O. keta*: Hasegawa et al., 1987), and a non-smolting strain of rainbow trout (*O. mykiss*) that exhibits a fall in plasma PRL after a similar challenge (Prunet et al., 1985). However, a recent study on *O. kisutch* smolts showed that circulating PRL decreases after progressive exposure (within 18 h) to full-strength SW (Young et al., 1989). Our objectives in studying SW adaptation was to obtain more information on how plasma PRL would respond to a rapidly (within 2 h) increasing external salinity (osmotic stress) in coho salmon. Our results show that the same pattern of decline in PRL was observed after SW exposure, irrespective of the season. This decrease in plasma PRL has been suggested to represent adaptation or preadaptation to SW life (Prunet et al., 1989). This observation is in agreement with data obtained in other studies on salmonids (Prunet et al., 1985; Young et al., 1989). It is noteworthy that plasma PRL levels, after SW entry or after long-term acclimation to SW were always very low and comparable among the different studies. This supports the idea that PRL plays a more important role in FW than in SW.

Prolactin's actions are thought to be synergistic with or antagonistic to the effects of steroid hormones depending on particular situations (Nicoll et al., 1980; Foskett et al., 1983; Hirano, 1986). Recently, Young et al., (1989) observed that the elevation of plasma PRL levels during the coho salmon parr-smolt transformation occurred when plasma cortisol approached basal resting levels and vice versa. This relationship was also seen in the present study (Fig. 6A,B; Fig. 3A,B). Moreover, we have indirect evidence that high concentrations of injected cortisol cause a depression in plasma PRL (J.M. Redding et al., unpublished data, 1987).

Seawater exposure in our experiments caused an increase in plasma cortisol levels (Fig. 6B), as found previously in chinook and coho salmon (Strange and Schreck, 1980; Redding et al., 1984; Young, 1985; Young et al., 1989) but not in Atlantic salmon (Langdon et al., 1984; Nichols and Weisbart, 1985; Langhorne and Simpton, 1986). This supports the notion of genus differences (*Oncorhynchus* and *Salmo*), as already discussed for plasma PRL concentrations (Prunet et al., 1989; Young et al., 1989), for levels of gill Na⁺, K⁺-ATPase activity (Langhorne and Simpton, 1986; Björnsson et al., 1989; Prunet et al., 1989), and for cortisol effects on this enzyme's activity (Langdon et al., 1984; Richman and Zaugg, 1987; Björnsson et al., 1987; McCormick et al., 1987; McCormick and Bern, 1989). These data suggest that *S. salar* smolts may develop a greater hypoosmoregulatory ability while in FW compared to *O. kisutch* smolts (Langhorne and Simpton, 1986).

Recent studies on coho salmon suggested that cortisol was involved in the development of hypoosmoregulatory mechanisms (Young, 1986) and its elevation in FW smolts was related to the development of good hypoosmoregulatory ability prior to migration to the ocean (Young et al., 1989). Other studies indicate that cortisol acts directly on the gill of coho salmon to stim-

ulate Na^+ , K^+ -ATPase activity (McCormick and Bern, 1989). The internal osmotic pressure or plasma Na^+ after SW challenge as observed in smolts in 1985 (Fig. 4: insert) is suggestive of a good hypoosmoregulatory ability compared to the post-smolts of the same year (Fig. 5: insert) or to the smolts of 1987 (Fig. 6C). Thus, when cortisol was followed after SW exposure in our 1987 experiments, its extended elevation observed in smolts cannot be related to good hypoosmoregulatory capacity. This suggests that at this developmental stage, cortisol is simply responding as a general stress factor, induced by the novel water (Strange et al., 1977; Schreck, 1981), the transfer to SW of fish of limited hypoosmoregulatory ability or the too rapid elevation of external salinity. This is in agreement with the previous observations that the elevation of cortisol during salinity challenges of smolts displaying optimal hypoosmoregulatory ability are only relatively transitory. Additionally, in fully SW-acclimated fish, the resting levels of circulating cortisol are similar between FW and SW (Figs. 6B,3B: see controls).

In conclusion, the changes in salinity caused changes in circulating prolactin which were inversely correlated with internal osmotic pressure or natriemia, irrespective of developmental stage. Plasma PRL also showed a negative relationship with plasma cortisol. Conversely, prolonged elevation of circulating cortisol (stress factor?) after SW exposure was associated with poor hypoosmoregulatory ability. It is evident from this study, that the roles and interactions of prolactin and cortisol in determining good adaptability to SW or FW in salmonids require further clarification.

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REFERENCES

- Afifi, A. and Azen, S., 1979. Statistical Analysis: a Computer Oriented Approach. Academic Press, New York, NY.
- Barton, B.A., Schreck, C.B., Ewing, R.D., Hemmingsen, A.R. and Patiño, R., 1985. Changes in plasma cortisol during stress and smoltification in coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol., 59: 468–471.
- Björnsson, B.Th., Yamauchi, K., Nishioka, R.S., Deftos, L.J. and Bern, H.A., 1987. Effects of hypophysectomy and subsequent hormonal replacement therapy on hormonal and osmoregulatory status of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol., 68: 421–430.
- Björnsson, B.Th., Young, G., Lin, R.J., Deftos, L.J. and Bern, H.A., 1989. Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma calcium regulation, osmoregulation and calcitonin. Gen. Comp. Endocrinol., 74(3): 346–354.
- Clarke, W.C. and Bern, H.A., 1980. Comparative endocrinology of prolactin. Horm. Proteins Peptides, 8: 105–197.
- Donaldson, E.M. and McBride, J.R., 1967. The effects of hypophysectomy in the rainbow trout *Salmo gairdneri* (Rich) with special reference to the pituitary–interrenal axis. Gen. Comp. Endocrinol., 9: 93–101.
- Fontaine, M. and Hatey, J., 1954. Sur la teneur en 17-hydroxycorticostéroïdes du plasma de saumon (*Salmo salar*). C.R. Acad. Sci. (Paris), Ser. D, 239: 319–321.
- Foskett, J.K., Bern, H.A., Machen, T.E. and Conner, M., 1983. Chloride cells and the hormonal control of teleost fish osmoregulation. J. Exp. Biol., 106: 255–281.
- Foster, L.B. and Dunn, R.T., 1974. A single antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. Clin. Chem., 20: 365–368.
- Gonnet, F., Prunet, P., Tonon, M.C., Dubourg, P., Kah, O. and Vaudry, H., 1988. Effects of osmotic pressure on prolactin release in rainbow trout: in vitro studies. Gen. Comp. Endocrinol., 69: 252–261.
- Hasegawa, S., Hirano, T., Ogasawara, T., Iwata, M., Akiyama, T. and Arai, S., 1987. Osmoregulatory ability of chum salmon, *Oncorhynchus keta*, reared in fresh water for prolonged periods. Fish Physiol. Biochem., 4(2): 101–110.
- Hirano, T., 1986. The spectrum of prolactin action in teleosts. In: C.L. Ralph (Editor), Comparative Endocrinology: Developments and Directions. Alan Liss, New York, NY, pp. 53–74.
- Hirano, T. and Mayer-Gostan, N., 1978. Endocrine control of osmoregulation in fish. In: P.J. Gaillard and H.H. Boer (Editors), Comparative Endocrinology. Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 209–212.
- Hirano, T., Ogasawara, T., Bolton, J.P., Collie, N.L., Hasegawa, S. and Iwata, M., 1967. Osmoregulatory role of prolactin in lower vertebrates. In: R. Kirsch and B. Lahlou (Editors), Comparative Physiology of Environmental Adaptations. S. Karger, Basel, pp. 112–214.
- Komourdjian, M.P., 1984. Monovalent plasma ion regulation in the hypophysectomized Atlantic salmon. Gen. Comp. Endocrinol., 56: 278–282.
- Langdon, J.S., Thorpe, J.E. and Roberts, R.J., 1984. Effects of cortisol and ACTH on gill Na^+/K^+ ATPase, SDH and chloride cells in juvenile Atlantic salmon (*Salmo salar* L.). Gen. Comp. Endocrinol., 61: 203–213.
- Langhorne, P. and Simpton, T.H., 1986. The interrelationship of cortisol, gill (Na^+/K^+)ATPase and homeostasis during the parr–smolt transformation of Atlantic salmon (*Salmo salar* L.). Gen. Comp. Endocrinol., 61: 203–213.
- Loretz, C.A. and Bern, H.A., 1982. Prolactin and osmoregulation in vertebrates. Neuroendocrinology, 35: 292–304.
- Maetz, J., 1969. Observations on the role of the pituitary–interrenal axis in the ion regulation of the eel and other teleosts. Gen. Comp. Endocrinol., Suppl., 2: 299–316.

- McCormick, S.D. and Bern, H.A., 1989. In vitro stimulation of Na^+/K^+ ATPase activity and ouabain binding by cortisol in coho salmon gill. *Am. J. Physiol.*, 256: R707-R715.
- McCormick, S.D., Nishioka, R.S. and Bern, H.A., 1987. In vitro corticoid and growth hormone regulation of Na^+/K^+ ATPase activity in gill tissue of coho salmon. *Am. Zool.*, 27: 74A.
- McLeay, D.J., 1975. Sensitivity of blood cell counts in juvenile coho salmon (*Oncorhynchus kisutch*) to stressors including sublethal concentrations of pulpmill effluent and zinc. *Fish. Res. Board Can.*, 32: 2357-2364.
- Nichols, D.J. and Weisbart, M., 1985. Cortisol dynamics during seawater adaptation of Atlantic salmon, *Salmo salar*. *Am. J. Physiol.*, 248: R651-659.
- Nicoll, C.S., 1981. Role of prolactin in water and ion balance in vertebrates. In: R.B. Jaffe (Editor), *Prolactin*. Elsevier, New York, NY, pp. 127-166.
- Nicoll, C.S., White, B.A. and Leung, F.C., 1980. Evolution of prolactin, its functions, and its receptors. In: R.M. McLeod and U. Scapagnini (Editors), *Central and Peripheral Regulation of Prolactin Functions*. Raven Press, New York, NY, pp. 11-25.
- Nicoll, C.S., Wilson, S.W., Nishioka, R.S. and Bern, H.A., 1981. Blood and pituitary prolactin levels in tilapia (*Sarotherodon mossambicus*; Teleostei) from different salinities as measured by a homologous radioimmunoassay. *Gen. Comp. Endocrinol.*, 44: 365-373.
- Nishioka, R.S., Richman, N.H., Young, G., Prunet, P. and Bern, H.A., 1987. Hypophysectomy of coho salmon (*Oncorhynchus kisutch*) and survival in fresh water and seawater. *Aquaculture*, 65: 343-352.
- Olivereau, M., 1962. Modifications de l'interréale du smolt (*Salmo salar* L.) au cours du passage d'eau douce en eau de mer. *Gen. Comp. Endocrinol.*, 2: 565-573.
- Olivereau, M., 1975. Histophysiologie de l'axe hypophyso-corticosurrénalien chez le saumon de l'atlantique (cycle en eau douce, vie thalassique et reproduction). *Gen. Comp. Endocrinol.*, 27: 9-27.
- Prunet, P. and Boeuf, G., 1985. Plasma prolactin level during transfer of rainbow trout (*Salmo gairdneri*) and Atlantic salmon (*Salmo salar*) from freshwater to sea water. *Aquaculture*, 45: 167-176.
- Prunet, P. and Boeuf, G., 1989. Plasma prolactin levels during smoltification in Atlantic salmon *Salmo salar*. *Aquaculture*, 82: 297-305.
- Prunet, P. and Houdebine, L.M., 1984. Purification and biological characterization of chinook salmon prolactin. *Gen. Comp. Endocrinol.*, 53: 49-57.
- Prunet, P., Boeuf, G. and Houdebine, L.M., 1985. Plasma and pituitary prolactin levels in rainbow trout during adaptation to different salinities. *J. Exp. Biol.*, 235: 187-196.
- Prunet, P., Boeuf, G., Bolton, J.P. and Young, G., 1989. Smoltification and seawater adaptation in Atlantic salmon (*Salmo salar*): plasma prolactin, growth hormone and thyroid hormones. *Gen. Comp. Endocrinol.*, 74, 3: 355-364.
- Redding, J.M., Schreck, C.B., Birks, E.K. and Ewing, R.D., 1984. Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in fresh water and during seawater acclimation in yearling coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.*, 56: 146-155.
- Richman, N.H. and Zaugg, W.S., 1987. Effects of cortisol and growth hormone in osmoregulation in pre- and de-smoltified coho salmon (*Oncorhynchus kisutch*). *Gen. Comp. Endocrinol.*, 65: 189-198.
- Richman, N.H., Nishioka, R.S., Young, G. and Bern, H.A., 1987. Effects of cortisol and growth hormone replacement on osmoregulation in hypophysectomized coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.*, 67: 194-201.
- Schreck, C.B., 1981. Stress and compensation in teleostean fishes: responses to social and physical factors. In: A.D. Pickering (Editor), *Stress in Fish*. Academic Press, London, New York, NY, pp. 295-321.

- Sheridan, M., 1987. Effects of thyroxin, cortisol, growth hormone and prolactin on lipid metabolism of coho salmon. *Gen. Comp. Endocrinol.*, 65: 220–238.
- Specker, J.L. and Schreck, C.B., 1982. Changes in plasma corticosteroids during smoltification of coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.*, 46: 53–58.
- Strange, R.J. and Schreck, C.B., 1980. Seawater and confinement alters survival and cortisol concentration in juvenile chinook salmon. *Ichthyological notes. Copeia*, 2: 351–353.
- Strange, R.J., Schreck, C.B. and Golden, J.T., 1977. Corticoid stress responses to handling and temperatures in salmonids. *Trans. Am. Fish. Soc.*, 106: 213–218.
- Winer, B.J., 1971. *Statistical Principles in Experimental Design*. MacGraw-Hill, New York, NY.
- Young, G., 1985. In vitro studies on interrenal gland activity during smoltification of coho salmon. *Aquaculture*, 45: 391–392.
- Young, G., 1986. Cortisol secretion in vitro by the interrenal of coho salmon (*Oncorhynchus kisutch*) during smoltification: relationship with plasma thyroxine and plasma cortisol. *Gen. Comp. Endocrinol.*, 63: 191–200.
- Young, G., Björnsson, B.Th., Prunet, P., Lin, R.J. and Bern, H.A., 1989. Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma prolactin, growth hormone, thyroid hormones and cortisol. *Gen. Comp. Endocrinol.*, 74(3): 335–345.