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Date \underline{March 16/93}
ABSTRACT

The changes in reproductive parameters and hormones, and blood sodium during the final weeks of sexual maturation in wild coastal salmon, chinook, *Oncorhynchus tshawytscha*, and steelhead, *O. mykiss*, were documented for stocks spawning in two similar river systems on the west coast of Vancouver Island, British Columbia, Canada. Chinook were blood-sampled and sacrificed at intervals during migration from the ocean into brackish Nitinat Lake, through the completion of final maturation in Nitinat River, and at the post-spawning stage. Gonadosomatic index (GSI), hepatosomatic index (HSI), and oocyte germinal vesicle stage and hydration level were measured in sacrificed chinook and steelhead. Stamp River steelhead were serially sampled for blood during the month preceding and the month following final maturation.

Blood samples were analysed by specific radioimmunoassay for gonadotropin (GtH), estradiol-17β (E₂), testosterone (T), 11-ketotestosterone (11-KT), and 17α,20β-dihydroxyprogesterone (17,20-P). Blood sodium was measured by flame photometry.

GSI and hydration level of oocytes increased in females of both species between the earliest stages of maturation and ovulation. Patterns of hormone concentration were similar in chinook and steelhead, but the peaks in concentration of the steroids at each stage were much lower in steelhead. Concentrations of E₂ and T declined from initial levels to ovulation in females, while GtH and 17,20-P increased. In chinook males, T concentration peaked and declined before spermiation, while 11-KT was highest at spermiation, and declined thereafter. The concentration of 11-KT was higher than that of T in steelhead males at spermiation. Little change in GtH and 17,20-P concentration occurred in steelhead males, but these hormones increased in relation to onset of spermiation in chinook.
Abstract

Fertility was compared between chinook and chum salmon, *O. keta*, maturing in brackish and fresh water. Fertility was low in both brackish and freshwater chinook females at the beginning of the chinook migration, when salinity was high in Nitinat Lake. Blood sodium was higher in brackish water females, but there was no correlation between egg mortality and blood sodium at ovulation. Later in the season, as salinity in net pens decreased, chinook fertility increased. At the beginning of the chum migration season, when salinity was lowest, chinook fertility was equal to that of chum maturing at the same location.

The relationship between plasma reproductive hormone concentration and the ability to osmoregulate in sea water was compared in post-spawning steelhead and coho salmon, *O. kisutch*, transferred from fresh water to brackish holding facilities. All hormones declined to low levels in steelhead while still in fresh water, within 3 weeks of spawning, but persisted at spawning levels for at least 6 weeks in coho. Steelhead blood sodium was stable in the range indicating osmoregulatory adaptation as salinity was increased to full sea water shortly after transfer. Coho blood sodium was higher in 2/3 sea water than that of steelhead in full sea water. High levels of reproductive hormones may interfere with the ability of salmonids to osmoregulate in sea water.
TABLE OF CONTENTS

Abstract ........................................................................................................... ii

Table of Contents .......................................................................................... iv

List of Figures .................................................................................................. vi

Acknowledgements ......................................................................................... ix

CHAPTER 1 ....................................................................................................... 1
  GENERAL INTRODUCTION ........................................................................... 1

CHAPTER 2 ....................................................................................................... 9
  ANALYTICAL METHODS COMMON TO MOST EXPERIMENTS ................. 9
    A. RADIOIMMUNOASSAY PROCEDURES ................................................... 9
    B. BLOOD SODIUM MEASUREMENT ....................................................... 13
    C. STATISTICS ......................................................................................... 14

CHAPTER 3 ..................................................................................................... 15
  NORMAL MATURATION OF CHINOOK AND STEELHEAD .................... 15
    A. INTRODUCTION .................................................................................. 15
    B. MATERIALS AND METHODS .............................................................. 16
      A. Sampling of maturing chinook .......................................................... 16
      B. Sampling of steelhead ..................................................................... 21
    C. RESULTS ................................................................................................ 22
      A. Chinook ............................................................................................ 22
        I. Migration and spawning of chinook .............................................. 22
        II. Water quality parameters ............................................................ 23
        III. Changes in reproductive parameters ........................................ 28
        IV. Changes in reproductive hormones ........................................... 36
      B. Steelhead .......................................................................................... 50
        I. Migration and spawning of steelhead ........................................... 50
        II. Changes in reproductive parameters .......................................... 51
        III. Changes in reproductive hormones ........................................... 56
    D. DISCUSSION ......................................................................................... 66
      A. females ............................................................................................. 66
      B. males ................................................................................................. 70

CHAPTER 4 ..................................................................................................... 73
  MATURATION OF CHINOOK AND CHUM IN BRACKISH WATER ............ 73
    A. INTRODUCTION .................................................................................. 73
    B. MATERIALS AND METHODS .............................................................. 74
    C. RESULTS ................................................................................................ 77
      I. Reproductive parameters ................................................................. 77
      II. Reproductive hormones ................................................................. 79
      III. Water quality .................................................................................. 86
Table of Contents

D. DISCUSSION ......................................................... 88

CHAPTER 5 ......................................................... 92
SW TRANSFER OF POST-SPAWNING SALMON AND STEELHEAD ........... 92
A. INTRODUCTION ................................................. 92
B. MATERIALS AND METHODS ..................................... 93
C. RESULTS ......................................................... 94
   I. General observations ......................................... 94
   II. Changes in reproductive hormones ......................... 96
   III. Blood sodium ............................................. 100
D. DISCUSSION ..................................................... 101

CHAPTER 6 ......................................................... 105
SUMMARY AND CONCLUSIONS ..................................... 105
A. NORMAL MATURATION OF CHINOOK AND STEELHEAD .......... 105
   1. Females .................................................... 105
   2. Males ...................................................... 106
B. MATURATION OF CHINOOK AND CHUM IN BRACKISH WATER .... 107
C. SEAWATER TRANSFER OF SALMON AND STEELHEAD .......... 109

REFERENCES ...................................................... 111
LIST OF FIGURES

Figure 1. Nitinat system study area, with inset showing proximity of Nitinat and Stamp systems on the west coast of Vancouver Island, British Columbia. ...................... 18

Figure 2. Stamp/Somass system showing the location of Robertson Creek Hatchery. .... 19

Figure 3. Temperature profiles of Nitinat Lake, Aug. 19 to Sept. 5, 1989, at chinook sampling sites 1-3. ................................................................. 25

Figure 4. Salinity profiles of Nitinat Lake, Aug. 19 to Sept. 5, 1989, at chinook sampling sites 1-3. ................................................................. 26

Figure 5. Dissolved oxygen profiles of Nitinat Lake, Aug. 19 to Sept. 5, 1989, at chinook sampling sites 1-3. ....................................................... 27

Figure 6. Water content of oocytes and eggs, and gonadosomatic index of chinook females migrating from the ocean to spawning grounds and undergoing sexual maturation. . 31

Figure 7. Plasma sodium of chinook salmon migrating from the ocean to spawning grounds and undergoing sexual maturation. ............................. 32

Figure 8. Gonadosomatic index and hepatosomatic index in male chinook migrating to spawning grounds and undergoing sexual maturation. ............. 35

Figure 9. Gonadotropin and Estradiol in female chinook migrating to spawning grounds and undergoing sexual maturation. ............................... 37

Figure 10. Blood sodium, Gonadotropin, Testosterone, and 17α,20β-dihydroxyprogesterone in ovulating female chinook at Robertson Creek Hatchery. ............... 38

Figure 11. Plasma testosterone and 17α,20β-dihydroxyprogesterone in female chinook migrating to spawning grounds and undergoing sexual maturation. ........ 41

Figure 12. Plasma gonadotropin in male chinook migrating to spawning grounds and undergoing sexual maturation. .......................................... 43

Figure 13. Blood sodium, Gonadotropin, Testosterone, 11-ketotestosterone, and 17α,20β-dihydroxyprogesterone in spermatizing male chinook at Robertson Creek Hatchery. . 44

Figure 14. Plasma 11-Ketotestosterone and testosterone in male chinook migrating to spawning grounds and undergoing sexual maturation. .................... 47

Figure 15. Plasma 17α,20β-dihydroxyprogesterone in male chinook migrating to spawning grounds and undergoing sexual maturation. .................... 49
List of Figures

Figure 16. Gonadosomatic index, hepatosomatic index and oocyte water content in maturing Stamp River steelhead females at Robertson Creek Hatchery. .......................... 53

Figure 17. Gonadosomatic index and hepatosomatic index in maturing Stamp River steelhead males at Robertson Creek Hatchery. .............................................. 55

Figure 18. Plasma gonadotropin in serially sampled steelhead females undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. ..................... 58

Figure 19. Plasma estradiol-17β in serially sampled steelhead females undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. ....................... 59

Figure 20. Plasma testosterone in serially sampled steelhead females undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. ....................... 60

Figure 21. Plasma 17α,20β-dihydroxyprogesterone in serially sampled steelhead females undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. .... 61

Figure 22. Plasma testosterone in serially sampled steelhead males undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. ............................. 63

Figure 23. Plasma 11-ketotestosterone in serially sampled steelhead males undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. ............... 64

Figure 24. Plasma 17α,20β-dihydroxyprogesterone in serially sampled steelhead males undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. .... 65

Figure 25. Mortalities to the eyed stage among chinook salmon eggs taken from females in brackish (BW) or fresh (FW) water, October 8-26, 1988, and fertilized with milt from FW or BW males. .................................................. 81

Figure 26. Water content of oocytes and eggs, and gonadosomatic index in chinook salmon females at Nitinat Lake and Hatchery, 1988 and 1989. ......................... 82

Figure 27. Blood and ovarian fluid sodium in chinook salmon females at Nitinat Lake and Hatchery, 1988 and 1989. ................................................................. 83

Figure 28. Plasma hormone levels in chinook salmon females at Nitinat Lake and Hatchery, 1988 and 1989. ................................................................. 84

Figure 29. Plasma hormone and sodium levels, and egg mortality rates in chum salmon females at Nitinat Lake and hatchery, Oct. 28, 1988. .................................. 85

Figure 30. Water quality parameters at Nitinat Lake net pen site, October 18, 1988. .... 87

Figure 31. Plasma hormone levels in spawning (March 10, FW) and spent (March 31, FW; April 9, BW) steelhead. ................................................................. 97
Figure 32. Plasma hormone levels in spawning (Dec 13 and 27, FW) and post-spawning (Jan 19-Feb 22, BW) coho males, winter 1988. 98
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This thesis would not have been initiated, nor completed, were it not for the example of my mother, Isabelle Smith, inspiring me to dream and persist.
Juvenile anadromous Pacific salmonids undergo a smolting process which permits transition from a hypoosmotic to a hyperosmotic medium. After feeding 1 to 6 years in the ocean, where growth and gametogenesis are largely completed, adults return to coastal waters in response to cues related to daylength and temperature (Lam, 1983). From inshore areas they migrate up rivers and streams, reverting to obligatory hyperosmoregulation, and upon completing sexual maturation, deposit their stenohaline eggs in freshwater (Potts and Rudy, 1969).

The degree to which final maturation is completed in seawater (SW) or brackish water (BW; salinity 5-25 ppt) before re-entry into freshwater (FW) varies between species and stocks (Healey, 1991). All salmonid species are capable of completing their entire life cycles in FW (Hoar, 1976). The anadromous forms may proceed upstream directly on arrival at the river mouth, several months in advance of final maturation and spawning, or school for varying periods, in nearby bays and estuaries, in some cases entering FW in fully mature condition.

In some cases, fish may be forced to remain in BW beyond the point of final maturation. For example, if autumn rains are delayed following a dry summer, water levels in coastal rivers may be low, resulting in either dry riverbeds or reduced holding volume accompanied by high temperature and low oxygen. In combination with seal and bear predation these factors may drive fish out of the river or prevent initial entry. In addition, salmon hatchery broodstock programs may, out of convenience or necessity, cause fish to be
held in BW through final maturation. For example, capture of broodstock in estuaries with a
seine net may be more reliable and economical than pursuing them after they have entered
and dispersed in the river. Estuarine-captured broodstock may be ripened (held to maturity) in SW or BW netpens if resources for holding in FW are not available.

Investigators have had mixed success with BW or marine ripening of salmonid broodstock. Fertilization rate of sperm from SW-ripened coho, *Oncorhynchus kisutch*, was equal to that of FW-ripened fish, but fertilization rates of SW-ripened eggs were lower (Clarke et al., 1977). Wertheimer and Martin (1981), held FW-captured coho in BW, and found no negative effect on adults, fertilization rate or survival to eyed stage in embryos. In a subsequent study (Wertheimer, 1984) there were significant losses of pink, *O. gorbuscha*, and coho adults, as well as lower fertility in survivors, under a similar regime. Reduction in fertilization rate was found in chum, *O. keta*, (Stoess and Fagerlund, 1982; Lam et al., 1982) and in coho, by Sower and Schreck (1982), in fish held to maturity in BW or SW. These studies also examined blood osmolality and sodium during maturation. Fertilization rate was high if eggs were taken from individuals with blood sodium levels no higher at ovulation than those of SW-adapted salmon before maturation. Fertilization rates in individuals with blood sodium levels during final maturation which were higher than those of SW adapted fish, were much lower.

Both Lam et al., (1982) and Sower and Schreck (1982) concluded that final maturation in SW is possible in chum and coho. If contact with FW is not permitted soon after ovulation/spermiation, however, viability of gametes declines more rapidly in SW held fish than in FW held fish. Personal observations of fertility rates of chum and chinook salmon, *O. tshawytscha*, from a holding site at brackish Nitinat Lake, near the west coast of Vancouver Island, indicate there may be species differences among salmon in the ability to
produce viable gametes in BW. Fertilization rates for chum eggs consistently averaged 97%, whereas those for chinook averaged 20% with a range between individuals of 0 - 80%. The reason for this difference in fertility of chinook and chum at the same site is not known. Based on the studies cited above, however, I hypothesize that a difference in maturation physiology exists such that hypoosmoregulation is compromised in chinook, but not chum maturing at the BW net pen site. Dysfunction in osmoregulation in fish held in BW may lead to elevated blood sodium, which is correlated with infertility of eggs.

It has been suggested that ovulated salmon have exhausted their energy reserves and cannot meet the higher energy requirements of hypoosmoregulation. Under these conditions fish gradually lose ground in the effort to retain water and keep sodium and chloride out of the blood and body cavity (Lam *et al.*, 1982; Sower and Schreck, 1982). Given the salmon's ability to convert much of its muscle tissue to energy (Ando *et al.*, 1986), fish which have fully utilized their energy reserves should have reduced muscle mass. However, based on observations of hundreds of chinook and chum carcasses during artificial spawning activities at Nitinat hatchery and the net pen site, there is no gross difference in the musculature of ripe chinook or chum killed in BW and those killed in FW. Therefore the energy shortage argument does not seem entirely satisfactory. An alternative possibility is that the hormones controlling reproduction interfere in some way with the ability to osmoregulate in brackish water.

Regardless of the duration of any holding period between arrival in inshore waters and SW/FW transition there are a number of reproductive processes, controlled by the hypothalamo-pituitary-gonadal axis, which take place before viable gametes are produced (see Fostier *et al.* 1983; Wallace and Selman, 1981). Some of these, such as vitellogenesis, are initiated while fish are still feeding in the marine environment, well before FW entry
(Wallace and Selman, 1981). Others, such as ovulation (Nagahama, 1990) and spermiation (Liley et al., 1986; Scott and Sumpter, 1989), are more abrupt, and occur just days or hours before gametes are fully formed and ready for release. All are regulated by a sequence of interdependent hormonal actions which defines the precise timing of events.

Seasonal changes in reproductive hormones and their correlation with developmental state of the gonads have been studied in rainbow trout, *O. mykiss*, (Baynes and Scott, 1985; Liley et al., 1986a; b; Scott et al., 1983), brown trout, *Salmo trutta*, (Crim and Idler, 1978), sockeye salmon, *O. nerka*, (Schmidt and Idler, 1962; Truscott et al., 1986), as well as pink, *O. gorbuscha*, (Dye et al., 1986), coho, (Sower and Schreck, 1982; Fitzpatrick et al., 1986), chum, (Ueda et al., 1984a; Hirano et al., 1990), masou, *O. masou* (Sufi et al., 1982; Yamauchi, 1983) and amago salmon, *O. rhodurus*, (Ueda et al., 1985; Kagawa et al., 1983).

The most important hormones have been identified as a pituitary glycoprotein, gonadotropin (GtH), and several steroids including an estrogen, estradiol-17β (*E₂*), the androgens testosterone (*T*) and 11-ketotestosteron (11-KT), and the progestin 17α, 20β-dihydroxyprogestosterone (17,20-P). Although the mechanisms of action of these hormones are not fully understood, they are considered to be the critical links in the chain of maturation, in mediating gametogenesis and final maturation.

Gonadotropin is a dimeric pituitary glycoprotein believed to direct or initiate most processes in the course of gonadal development and maturation. Two GtH's with distinct beta subunit amino acid sequences and differing carbohydrate content are recognized in salmonids (Idler and Ng, 1983; Suzuki et al., 1988a;b; Kawauchi et al., 1989; Swanson et al., 1991). Designated GtH I and II, they are somewhat homologous to mammalian FSH and LH, respectively, in their beta subunit amino acid sequences (Itoh et al., 1988). They are also referred to as vitellogenic and maturational GtH, respectively.
Vitellogenic GtH (vGtH) is thought to be important in early gonadal development, when highest plasma levels are recorded (Suzuki et al., 1988c). In immature females it promotes thecal cell secretion of T, aromatization of T to E₂ by granulosa cells (Dickhoff and Swanson, 1989) and uptake of the yolk precursor molecule, vitellogenin, by oocytes (Tyler et al., 1991). In immature males testicular androgenesis is promoted by endogenous vGtH (Swanson et al., 1989). As fish approach final maturation vGtH decreases in the blood and maturational GtH (mGtH) begins to rise (Suzuki et al., 1988c). Accordingly, the actions and measurement of mGtH are of exclusive concern in this study and all subsequent uses of the term "GtH" refer to the maturational form.

Incubations of GtH in vitro with whole follicles (Scott et al., 1982) and separated thecal and granulosa layers (Kagawa et al., 1982; Kanamori et al., 1988) reveal stage- and dose-dependent responses in their metabolism of E₂, T, 17-hydroxyprogesterone, and 17,20-P. Similar incubations of testicular tissue indicate stimulus of T, 11-KT (Schulz, 1986a) and 17,20-P (Ueda et al., 1984b) secretion in rainbow trout, and direct control of sodium ion transport by sperm duct epithelium in brook trout, Salvelinus fontinalis, spermiation (Marshall et al., 1989).

Among the sex steroids, the first indication of the onset of final maturation in females is a decline in plasma E₂, signalling the end of vitellogenesis (Scott et al., 1983). Receptors for E₂ are located in the liver, and during the oocyte growth stage E₂ is bound at cytosolic and nuclear sites (Maitre et al., 1985; Pottinger, 1986; Pottinger and Pickering, 1990) stimulating vitellogenin manufacture and release into blood. A decline in E₂ is seen at the end of oocyte growth, followed rapidly by a drop in circulating vitellogenin (Wallace et al., 1987). The factor which initiates the decline in E₂ is not known.
Testosterone is present in the blood of salmonids during most phases of gametogenesis in either sex (Scott and Sumpter, 1983; Baynes and Scott, 1985; Sumpter et al., 1984). Specific binding has been demonstrated in testis Sertoli cells (Schulz and Blum, 1988; Foucher and le Gac, 1989) and pituitary of trout (Peute et al., 1989). The action of T in spermatogenesis has not been determined, though its multiple binding sites in trout testes suggest its importance (Schulz and Blum, 1988). In the ovary, where it may function purely as a substrate for aromatization (Kanamori et al., 1988), no receptor-like binding has yet been demonstrated. Nevertheless, T rises (often sharply, in some reports) at the completion of gametogenesis (Sumpter et al., 1984; Truscott et al., 1986; Fitzpatrick et al., 1986), presaging the next phase of steroidogenesis which brings on final maturation.

11-KT is the major male-specific androgen produced by the salmonid testis (Idler et al., 1971). Testicular production of 11-KT is responsive to exogenous GtH in vivo, more so than that of T, especially around the time of spermiation (Ueda et al., 1985). In vitro studies show that sensitivity of 11-KT synthetic pathways to GtH is highest, and level of response greatest, in testicular tissue at the end of spermatogenesis (Schulz and Blum, 1990). Miura et al. (1991) demonstrated that 11-KT is the only androgen capable of stimulating all phases of spermatogenesis in vitro in fragments of eel testis. As is the case for T, plasma 11-KT is not strongly associated with any particular germ cell stage, but the consistently higher levels seen around final maturation make it a useful indicator of normal reproductive function (Scott and Sumpter, 1989).

First identified in significant amounts in spawning sockeye salmon by Idler et al (1960), 17,20-P was shown by Jalabert (1976) to be the most potent hormonal stimulator of in vitro maturation (germinal vesicle breakdown or GVBD) in rainbow trout folliculated or denuded eggs. Similar findings have been reported for oocytes of amago salmon and rainbow
trout (Nagahama et al., 1983). Jalabert et al. (1978) demonstrated the effectiveness of 17,20-P as an in vivo inducer of final oocyte maturation in coho. 17,20-P was also shown to be a potent stimulus of spermiation in testes of these same species (Ueda et al., 1985). In males, the beginning of spermiation has a better correlation with the first appearance and rise of 17,20-P in plasma than it does with either T or 11-KT (Schulz and Blum, 1990). Sangalang and Freeman (1988) demonstrated 17,20-P to be the major steroid produced by spermiated testes and mature, pre-ovulatory ovaries of Atlantic salmon.

Osmoregulation is known to be under hormonal control in salmonids. Growth hormone (Bolton et al., 1986; 1987) and cortisol (Redding et al., 1984; Bjornsson et al., 1987) are involved in SW adaptation of salmon smolts. The action of prolactin is required in FW adaptation of salmon previously adapted to SW (Hirano et al., 1985; Hasegawa et al., 1986). Although this thesis is not concerned directly with the osmoregulatory hormones, there is the possibility that reproductive hormones may influence the process of osmoregulation. Sea water adaptability is impaired by exogenous androgens in salmon smolts (Lundqvist et al., 1989). In maturing adults held in SW blood sodium is elevated as plasma sex steroids increase despite levels of cortisol, growth hormone, and prolactin similar to those of SW adapted fish (Hirano et al., 1990). State of osmoregulatory adaptation will be assessed on the basis of blood and ovarian fluid sodium concentration.

In order to assess the physiological response of fish to an environmental disturbance or constraint, it is necessary to have a clear picture of the physiological profile under "normal" undisturbed conditions. In Chapter 3 of this study I examined changes in endocrine status, reproductive parameters, and osmoregulation in chinook salmon, from their first appearance in inshore waters to the completion of spawning. This is the first examination of reproductive physiology in coastal chinook salmon. Endocrine profiles and
reproductive parameters were also studied in the FW phase of adult steelhead trout, a species entering FW considerably in advance of final maturation and capable of survival and reentry of SW after reproduction. Reproduction in resident rainbow trout has been well documented (Scott et al., 1983), but how well those findings apply to steelhead is speculative at present. In terms of their reproductive physiology, chinook and steelhead, arguably the most endangered of the Pacific salmon in British Columbia, are the least studied. As successful spawning of the remaining populations becomes of greater concern our knowledge of the basis of this function will require greater detail, of the kind provided in this chapter.

In Chapter 4, the BW maturation of chinook was examined, with emphasis on viability of eggs matured in Nitinat Lake and Nitinat River. One aspect of osmoregulation, the ability to maintain blood sodium levels within a certain range while maturing in BW was also examined. This may help to identify abnormalities in blood sodium or hormone concentration, which correlate with reduced fertility. Some critical comparisons were also made with chum salmon under the conditions of estuarine capture and artificial spawning, as this species is known to spawn normally under slightly saline conditions.

Chapter 5 compares the post-spawning response to SW transfer of adult coho, which normally die after spawning, and steelhead, which are capable of recovery and SW re-entry after spawning. Coho (jacks and adult males), of smaller size than chinook, were used in this study for ease of handling. There are also indications from scale circuli patterns that precociously maturing male coho (jacks) may return to the ocean and resume feeding. Reproductive hormones and blood sodium were measured to determine whether SW adaptability returns in the presence of high levels of these hormones after reduction of gonadal mass through spawning.
A. RADIOIMMUNOASSAY PROCEDURES

Plasma sex steroid levels were measured using radioimmunoassays described and validated by van Der Kraak et al. (1984) for testosterone (T), 17β-estradiol (estrogen or E2) and 17α-hydroxy-20β-dihydroxyprogesterone (17,20-P), and by Dye et al. (1986) for 11-ketotestosterone (11-KT). In preparation for assay, plasma samples were diluted 20-fold in steroid assay buffer and incubated at 70°C for 1 hour in covered 12 x 75 mm borosilicate culture tubes. After cooling to room temperature for 1/2 hour the tubes were centrifuged at 1,335 g for 15 min at 4°C then decanted into plastic tubes and stored at -20°C. The denaturation of steroid binding proteins, advocated by Scott et al. (1982), eliminates the need for plasma steroid extraction prior to assay.

The assay buffer was a 0.05 M phosphate buffer comprised of 5.75 g/l dibasic sodium phosphate and 1.315 g/l monobasic sodium phosphate plus 1.0 g/l gelatin, in distilled water. After heating to 37°C for 1 hour to dissolve the gelatin and adjusting pH to 7.6, 65 mg/l sodium azide was added to prevent bacterial growth.

Standards of 11-KT (lot 2697, Syndel Laboratories Ltd., Vancouver, B.C.), testosterone (T-1500, Sigma), 17 beta-estradiol (E-8875, Sigma) and 17,20-P (Q-1850, Steraloids Inc., Wilton, NH), dissolved in ethanol at 1 mg/ml initially, were serially diluted at 10-fold intervals to 10 ng/ml in the assay buffer. The working standards were prepared by further serial-dilution at 2-fold intervals from 5,000 to 39 pg/ml. Tritiated steroids were
obtained from Amersham Canada Ltd. (Oakville, Ontario) for 11-KT (TRK.676), testosterone (TRK.402) and 17 - estradiol (TRK.322). Tritiated 17,20-P was made at the West Vancouver Laboratory by Helen Dye from labelled 17α-hydroxyprogesterone, using the method of Scott et al (1982).

Antibody to 11-KT was a gift from Dr. T. G. Owen (Helix Biotech Ltd., Richmond, B.C.), and was used at a dilution of 1:3500 or 1:4000. Remnants from each batch were frozen and added to the next batch made up from 1:100 stock. Antibodies to testosterone (61-315) and estrogen (61-305, ICN Immunobiologics) were prepared as stock solutions by adding 5 ml assay buffer to 1 vial of lyophilized antibody (1:5). Aliquots were removed and diluted to 1:21 (E2) or 1:50 (T) as needed for the assays while unused portions were stored frozen at the stock dilution. Antibody to 17,20-P, a gift from Dr. A. P. Scott (MAFF Fisheries Laboratory, Lowestoft, England), was stored at 1:100 and used at working dilutions of between 1:4,000 and 1:16,000.

In the radioimmunoassays of 11-KT and 17,20-P duplicate 12 x 75 mm borosilicate tubes contained 100 ul of standard or denatured plasma, 50 ul of antibody and 50 ul of tritiated steroid (2,000 cpm). For testosterone and estrogen the tubes contained 200 ul of standard or denatured plasma, 200 ul of radiolabelled steroid (5,000 cpm) and 200 ul of antibody. Activity of the tritiated hormone (total counts) was monitored in each assay by including duplicate tubes wherein standard, antibody and charcoal (see below) were replaced by buffer. Non-specific binding and reference controls had standard and antibody in the former case, and standard only in the latter case, replaced with assay buffer. After vortexing, all tubes were incubated overnight at room temperature.

The reaction was stopped the following day by immersion in ice-water for 15 minutes. Ice-cold dextran-coated activated charcoal (0.5 g/l dextran T-70 [Pharmacia (Canada) Ltd.,]
Dorval, Quebec) and 5.0 g/l activated charcoal [C-5260, Sigma] in assay buffer) at 1 ml (11-KT and 17,20-P) or 200 ul (testosterone and estrogen) was added (except in total counts tubes) to bind any unbound steroid. After vortexing and a further 10 min stand in ice-water, tubes were centrifuged at 1,335 g for 10 min at 4° C and decanted into glass scintillation vials. Each vial received 10 ml of Scinti Verse II (So-X-12, Fisher) or Ecolite (ICN) and was vigorously shaken and counted on a LKB Wallac 1214 RackBeta liquid scintillation counter (Wallac Oy, Turku, Finland) for 3 min each.

Plasma GtH was measured by slight modification of the method of Peter et al. (1984). Antibody and hormone for standards and labelling were received from Dr. J. P. Sumpter (Brunel University, Uxbridge, England). Phosphate buffer stock for the iodination [0.5 M, 0.84 g potassium dihydrogen phosphate (KH₂PO₄, BDH) and 6.3 g dibasic sodium phosphate (Na₂HPO₄, S-9763, Sigma) in 100 ml double distilled deionized water (DDI), pH 7.5] was prepared fresh each time. Column buffer was a 0.08 M barbital buffer [5.0 g sodium barbital (B 22-500, Fisher), 3.25 g sodium acetate (BDH), 0.1 g thimerosal (T-5125, Sigma) and 34.2 ml 0.1 N hydrogen chloride made up to 1 l in DDI, pH 8.6]. Assay buffer was made by adding Bovine serum albumin (BSA, Sigma) to 0.08 M barbital buffer at the rate of 5 g/l.

Iodination of salmon GtH followed the procedure of Peter et al. (1984) for carp GtH, with modification. The shipping vial containing 10 ul (1 mCi) Na¹²⁵I (Amersham) was used as the reaction vessel. The solution was first buffered with 70 ul 0.5 M phosphate buffer before the addition of 5 ug of sGtH (41.6 ul of a 120 ug/ml solution in phosphate buffer). Mixing of this volume was achieved by drawing up once and expelling from a pipette tip within the reaction vessel. Following this, 40 ul of 0.5 M phosphate buffer and 25 ul chloramine T [10 mg chloramine T (Calbiochem) in 10 ml 0.05 M phosphate buffer] were added to the vessel. After gentle shaking of the recapped vessel for 90 s the reaction was
stopped with the addition of 100 ul sodium metabisulphate [24 mg sodium metabisulphate (Fisher, S-244) in 10 ml 0.05 M phosphate buffer] and 200 ul potassium iodide [100 mg potassium iodide (Fisher P-411C) in 10 ml 0.05 M phosphate buffer].

The contents of the vessel were transferred to a 1.1x20 cm Sephadex G-50 column primed with 2 ml 5% BSA in barbital buffer (1 g BSA in 20 ml barbital buffer). Two hundred ul potassium iodide, used to flush the vessel, were transferred to the column as well. When this volume (approximately 690 ul) had percolated into the bed, the column was flushed through with column buffer during the collection of 16 fractions of 1 ml (20 drops) each. Activity was counted in 10 ul aliquots from each fraction in a gamma counter (Picker, Pace-1) to identify the first peak (usually appearing in fractions 6-10), corresponding to labelled sGtH. Low activity fractions and the second peak containing inorganic $^{125}$I were discarded. Degradation of the tracer was slowed by adding 200 ul of 5% BSA in column buffer to the stock solution. Tracer stock was viable for about 1 month when stored at 4° C.

The RIA for GtH was conducted at 4° C, encompassing 4 days on a 24-hour cycle, or 2 days on a 12-hour cycle. Duplicate 12 x 75 borosilicate glass culture tubes contained either 50 ul of standard (0.9-240 ng/ml) or plasma, 200 ul GtH antibody (1:10,000-1:14,000) in 1:100 normal rabbit serum [NRS, (Calbiochem, 869019)] in assay buffer, and approximately 5,000 cpm/200 ul tracer. Assay buffer replaced standards in maximum binding and non-specific binding controls, and NRS replaced anti-GtH in non-specific binding controls. The rack holding assay tubes was vigorously shaken and incubated at 4° C. At 24 (or 12) hours, tubes were again shaken and incubated at 4° C for a further 24 (12) hours, followed by addition of 200 ul goat antibody to rabbit gamma-globulin (GARGG) (Calbiochem 539844, Terochem, prepared by the addition of 20 ml assay buffer to 1 vial of lyophilized GARGG). After a final incubation of similar duration, the tubes were shaken prior to centrifugation at 3000 rpm for
20 min at 4° C. In the 12-hour version of the assay, tubes were held at room temperature 1 hour after each shake, except in the last instance. Centrifuged tubes were immediately aspirated and counted in a LKB Wallac 1272 Clinigamma counter.

In calculating plasma steroid or GtH concentration, non-specific binding counts were first subtracted from counts for each standard or plasma tube. Counts were then expressed as percentage of the reference counts (between 0 and 100 %). Steroid or protein concentration was determined by reference to the standard curve of percent binding versus log concentration. Samples which bound less than 20% (off the linear part of the standard curve) were diluted in assay buffer and reassayed. Those which bound more than 80 % were interpreted as having undetectable levels of hormone.

B. BLOOD SODIUM MEASUREMENT

Plasma sodium and potassium were determined by the flame photometric technique of Blackburn and Clarke (1987). Briefly, 5 ul of plasma was diluted in 1 ml lithium diluent and aspirated into a Turner (Case Instrument) clinical flame photometer. Up to 20 point-standards at 160 mMol/l sodium : 8 mMol/l potassium (160/8) and 140/5 were interspersed in an average run of 250 plasmas. Additionally standard curves consisting of 0 - 250 in steps of 50 mMol/l were run at the beginning, middle and end of each run of plasmas. Use of this method removes the necessity of running duplicate samples for each plasma.

C. STATISTICS

All data are expressed as mean ± standard error. Tukey HSD test was used to determine differences between several means (P < 0.05). Analysis of serial sampling data was by repeated measures ANOVA and Tukey HSD test. Arcsin and log\(^{10}\) transformations
were used to obtain homogeneity of variance. Differences between two means were determined by t-test. Statistical analyses were performed using Systat version 5.0.
CHAPTER 3

NORMAL MATURATION OF CHINOOK AND STEELHEAD

A. INTRODUCTION

The first aim of this chapter is to describe changes in reproductive condition and endocrines in chinook from the time of their first appearance in inshore marine waters, to completion of spawning. Identification of irregularity in physiological parameters in salmon maturing in BW requires knowledge of changes in those parameters during normal SW/FW transition and final maturation. Reproductive parameters and hormones were measured in chinook males and females in SW just at the end of the feeding and growth phase and the beginning of final maturation, through the SW/FW transition period to the post-spawning stage. One aspect of osmoregulatory function, blood sodium, was also analysed. For this purpose chinook from two similar river systems on the west coast of Vancouver Island were examined. The majority of samples were obtained from Nitinat River chinook, while post-spawning (spent) samples were obtained from Stamp River chinook.

A similar description of reproductive parameters and endocrinology during final maturation was made in (Stamp River) steelhead, a species which often spends several months in FW before spawning. Although steelhead could not be sampled during the SW/FW transition period comparison of final maturation in the two species may be instructive. Adaptation to FW and final maturation occur more or less concurrently in Nitinat River chinook, but are separated by several months in Stamp River late summer steelhead. It was anticipated that any involvement of reproductive hormones in FW
adaptation or conversely, influences that osmoregulatory processes exert on reproductive physiology, would be manifested as differences in hormone profiles around the time of final maturation in the two species. In addition I expected to find differences in reproductive hormone profiles which help to explain the difference in post-spawning survival potential between chinook and steelhead.

Reproductive condition was evaluated on the basis of gonadosomatic index (GSI), hepatosomatic index (HSI), external signs of gender, and skin pigmentation. In females, oocyte germinal vesicle position and degree of hydration (or water content) were recorded. Changes in reproductive hormone levels were related to gonadal development and migratory stage.

B. MATERIALS AND METHODS

A. Sampling of maturing chinook

a. Nitinat chinook

Chinook salmon (4-6 yr, 5-20 kg) in Nitinat Lake were captured by gillnet during the period Aug 19 to Sept 10, 1989. First appearance of fish in the lake was determined by sonar scanning and test fishing. Fishing was carried out between dusk and dawn at three locations (Fig. 1) in the lake: Site 1: outlet (seaward) end, Aug. 19 (4 females, 5 males); Site 2: mid way along the length of the 20 km lake, Aug. 29 (4 females, 5 males); and Site 3: inlet end, Sept. 5 (5 females, 5 males). A 600 m x 12 m, 12 cm mesh monofilament gillnet was set across the lake for one hour between midnight and dawn. As fish were landed over the
stern, external signs of maturation were recorded and blood was obtained by caudal puncture in 10 ml heparinized vacutainer tubes fitted with 18 gauge 3.5 cm hypodermic needles.

Blood samples were kept on ice 1 hr before spinning in an IEC portable benchtop centrifuge.

Plasma samples and fish were held on ice for up to 2 hr until the vessel returned to shore, at which time plasmas were frozen at -20° C, and fish were sampled for body weight, gonads and livers. Gonads and livers were weighed to obtain GSI (gonad weight x 100/ body weight) and HSI (liver weight x 100/ body weight). In the case of females the oocyte germinal vesicle position was recorded. A sample of 50 oocytes from each female was weighed to the nearest 0.1 g. Oocytes were reweighed after 24 h drying at 80° C followed by 1 h in a desiccator at room temperature. This was also done with chinook eggs donated by a salmon sportfishing guide from 8 females angled in Barkley Sound 25 km northwest of Nitinat Lake's outlet, Aug. 5-15, 1989.

Three methods were employed in the daytime capture of fish in the freshwater phase. Seine and gill nets were used in the middle reaches of the river to obtain maturing fish in transit (5 females, 5 males), while crowding and dipnetting was the method used for mature fish in the hatchery raceways (8 females, 11 males). Blood samples as well as gonad, liver and body weights were obtained in the same manner as in the Nitinat Lake sampling program.

Temperature, salinity and oxygen were measured at the surface and at depths of 1, 3, 6, and 10 meters in Nitinat Lake during the sampling period.
Figure 1. Nitinat system study area, with inset showing proximity of Nitinat and Stamp systems on the west coast of Vancouver Island, British Columbia. Also shown are Nitinat Lake chinook sampling sites 1-3, the "Lakehead" area, and adult chinook netpen maturation site, in relation to Nitinat Hatchery.
Figure 2. Stamp/Somass system showing the location of Robertson Creek Hatchery.
b. Stamp River chinook

As sampling of Nitinat chinook was carried out during the hours of both daylight and darkness 24-hour blood sampling program was conducted at Robertson Creek Hatchery (RCH) (Fig. 2) to determine the effect of sampling time on endocrine and blood sodium levels. Stamp River chinook have a migration pattern similar to that of Nitinat River chinook. On Oct. 25, 1990, seven unovulated females (5-13 kg) were selected on the basis of ovarian firmness as determined by palpation. Seven males were selected on the basis of a general healthy appearance, as all males examined at this stage were spermiating and expressed milt when handled. Fish were removed to covered, flow-through aluminum containers of 200 l capacity, and allowed to acclimatize for 2 hours before being anaesthetized (0.4 ml 2-phenoxyethanol/l) and sampled.

Samples of blood were taken from all 14 fish at 1530 h Oct. 24 and 1330 h Oct. 25, 1990. For sampling, water flow was interrupted in sequence at each unit containing one fish. Three-fourths of the water was drained away and an emulsified 50:50 2-phenoxyethanol: water mixture was added through a gap in the lid to give a final concentration of 0.5 ml/l. After three minutes lids were removed, narcotized fish were rolled on their sides in the water and 5 ml of blood was removed from the caudal vasculature. Lids were replaced and water flow was reestablished at 20 litres per minute within 5 min of the initial interruption. At 2000, 0040, 0530, and 0920 h 5 fish of each sex were sampled, the remaining two of each sex served as controls for handling stress. After the final blood sample, fish were killed with a blow to the head and sampled for germinal vesicle stage in oocytes, as well as GSI and HSI.

Spent chinook were captured by gillnet in Glover Creek adjacent to the RCH site in Nov. 1989. Specimens sacrificed on Nov. 5 and 13 (4 females, 6 males) were sampled
following the procedure described above for Nitinat chinook. In addition 5 males and 5 females were bled and transferred to individual holding tanks on Nov. 8. On Nov. 11 dead fish were removed from the tanks and sampled for GSI and HSI while live fish were anaesthetized and bled. On Nov. 13 the same procedure was followed and the remaining 4 live fish were sacrificed and sampled for GSI and HSI.

B. Sampling of steelhead

Stamp River steelhead (2.63 ± 0.65 kg) were collected from surplus broodstock in holding ponds at RCH in December/January 1987-88. These fish were maintained in a 2 x 30 x 2.5 m deep covered concrete raceway. To control fungus the raceway (and fish) were flushed for 1 h each week with malachite green at 1 ppm until the approach of final maturation in the hatchery brood fish, in the spring of 1988. On sampling days fish (6 females, 5 males) were crowded to one end of the raceway, dipnetted individually from the raceway into a 400 l aluminum tub with 2-phenoxyethanol at 0.5 ml/l, bled, palpated and swabbed with malachite (5 ppt). Seven ml blood were collected weekly or biweekly between February 5 and March 10, 1988, and the approximate dates of ovulation or spermiation were recorded. These fish were not sampled for GSI and HSI on the final sample date as they were held beyond final maturation and had lost many eggs on the floor of the raceway. Other fish of the same stock and original holding pond were sacrificed on several occasions during the serial-sampling program, to provide GSI and HSI data. Steelhead maturing in 1988, were sacrificed on Feb. 1, 12 and 19 and on March 3. For fish maturing in 1989, from which examples of earlier stages of maturation were sought, samples were obtained on Dec. 8 and 27/88 and Jan. 15/89.
Blood plasma samples from all chinook and steelhead were analyzed for GtH, T, and 17, 20-P by specific RIA described in Chapter 2. Female plasma samples were analyzed for E₂ and male plasma samples for 11-KT. Representative plasma samples from females were analyzed for 11-KT to confirm undetectable levels, and likewise for E₂ in males.

C. RESULTS

A. Chinook

I. Migration and spawning of chinook

a. Nitinat chinook

In 1989 chinook first appeared in Nitinat Lake on Aug. 19 and were captured at Site 1 at the outlet end of the lake on that date. These first fish were silvery, resembling midsummer or feeding fish in coloration, and the sexes could not be externally differentiated. Fish were less silvery when captured in the middle reach of the lake (Site 2) on Aug. 29. Chinook appeared at the inlet end of the lake (Site 3) on Sept. 5. Skin colour was coppery, and females were distinguishable from males on the basis of abdominal width. Their presence at the mouth of Nitinat River, 2 km away, was confirmed 2 days later. Catch size on the 3 sampling dates in the lake were 9, 13 and 89, respectively, with only 4 females in each of the first 2 catches.

The number of fish at the mouth of the river and adjacent bay increased to Sept. 26. During this period darker fish with brown, red, and yellow flanks were seen in the lower tidal pools of the river, but few ventured above the tidal influence before Oct. 2. After Oct. 2
fish in full spawning colours, devoid of silvery scales, were seen from Nitinat Hatchery to the mouth of the river. The large schools at the head of the lake began to diminish after that date. Spawning began on Oct. 8 and continued until Nov. 4. Most spawning occurred in the river 2 km on either side of the hatchery.

b. Stamp River chinook

Chinook adults entered the Somass River enroute to the Stamp River around Sept. 7 in 1989. Peak migration through the fish ladder at Stamp Falls occurred between Oct. 5 and 15. Peak spawning activity occurred between Oct. 18 and 30 in Glover Creek. Spent chinook were observed in Glover Creek between Oct. 23 and Nov. 8.

II. Water quality parameters

a. Temperature (Fig. 3)

Nitinat Lake was stratified with respect to temperature at all sampling sites. Surface temperature ranged from 19’ C at Site 1 to 22’ C at the head of the lake and decreased, most rapidly between 1 and 3 m, to a uniform 13’ C at 10 m depth over the length of the lake. Surface temperature remained relatively constant at the head of the lake from mid-summer until Sept. 27 when a slow decline began in response to the change of season. Summer temperatures in the river (12’-17’ C) persisted until this time, then fell to 9’ C at the beginning and 7’ C at the end of the peak spawning period.

Water temperatures in Stamp River fall from September highs of around 14.5’ C to 7’ in November, and 2’ in December. Temperature rises to 4’ in February and 7’ in March.
b. Salinity (Fig. 4)

A salinity gradient was recorded at all lake sampling sites. The highest surface salinity was found at Site 1 (28 ppt) where an increase to 30 ppt between 1 and 3 m occurred. At Sites 2 and 3 surface salinity was slightly lower (25 and 21 ppt, respectively), but likewise increased to 30 ppt in the first 3 m of depth.

c. Oxygen (Fig. 5)

Dissolved oxygen in the surface layer at Site 1 ranged from 10 to 12 ppm during the sampling period. A slow decrease to 8 ppm occurred between 1 and 3 m, below which no change was seen. From Site 2 to the head of the lake surface oxygen levels were lower than those at Site 1, and were depleted to lower levels at depth. Oxygen level fell to 5 ppm at about the 4 m mark. Chinook cannot survive at oxygen levels less than 5 ppm, and throughout most of the length of the lake the movements of adult salmon are restricted to the top 4 m of water.

d. hydrogen sulphide

Under normal conditions there is a stable layer of highly toxic hydrogen sulphide in the anoxic zone from 4 m depth to the bottom of the lake. While the concentration of H₂S was not measured directly during the course of this study its presence was confirmed in both 1988 and 1989 when inversions occurred at various times of the year, bringing the characteristic smell to the surface. Mortalities of chum adults were observed in the fall of 1988 and 1989, following the peak chinook migration period.
Figure 3. Temperature profiles of Nitinat Lake, Aug. 19 to Sept. 5, 1989, at chinook sampling sites 1-3.
Figure 4. Salinity profiles of Nitinat Lake, Aug. 19 to Sept. 5, 1989, at chinook sampling sites 1-3.
Figure 5. Dissolved oxygen profiles of Nitinat Lake, Aug. 19 to Sept. 5, 1989, at chinook sampling sites 1-3.
III. Changes in reproductive parameters

A. Females

a. Gonadosomatic index

*Nitinat chinook*

A steady rise in GSI (Fig. 6) took place in females from the first sample within the confines of Nitinat Lake (12%) up to the penultimate sample in mid-river, prespawning fish (20%). No change was in evidence between the mid-river, unovulated fish and ovulated fish at the hatchery. Gonad weight averaged over all stages of maturation was 2.00 ± 0.53 kg.

*Stamp chinook*

Gonadosomatic index in Stamp River serially sampled females was similar (22 ± 1.7%) to that of ripe Nitinat females. Mean gonad weight was 2.12 ± 0.52 kg. In spent females GSI fell to 1.79 ± 0.76%.

b. Germinal vesicle stage

*Nitinat chinook*

Oocytes from females in the first 3 samples (Aug. 19 and 29, Sept. 5) taken in Nitinat Lake were in the central (premigrating) germinal vesicle (CGV) stage. In 75% of mid-river fish (Oct. 10) oocytes were in migrating germinal vesicle (MGV) stage, and 25% had oocytes in the peripheral germinal vesicle (PGV) stage. In ripe fish at the hatchery (Oct. 14) 90% were at PGV or germinal vesicle breakdown (GVBD).
Stamp chinook

Among the serially sampled fish 3 of 7 females were ovulated, or partially so, and their oocytes were in the GVBD stage. Of the unovulated fish 2 were in GVBD and 2 were in PGV stage.

c. Water content of oocytes

Oocytes of females angled in the ocean west of Barkley Sound in August had the lowest hydration level at 50.2% (Fig. 6). Water content of oocytes from BW females was obtained from the Sept. 5 Nitinat Lake sample (52%). Upon ovulation at Nitinat Hatchery 57% of the egg mass was comprised of water.

d. Hepatosomatic index

Nitinat chinook

There was a slight though not significant increase in HSI between Aug. 19 and Sept. 21 in Nitinat Lake females. Thereafter a slight decrease in the mid-river sample occurred, followed by a further decrease to 1.53% at ovulation.

Stamp chinook

Hepatosomatic index of Stamp females at ovulation was significantly lower (1.00%, P < 0.003) than in Nitinat females at ovulation. There was a significant increase to 1.66% in spent fish after oviposition.
e. Blood sodium (Fig. 7)

Nitinat chinook

The highest blood sodium concentrations (205 mM/l) in females were encountered in samples taken at the lower end of Nitinat Lake (SW). Significant decreases occurred in the 2 subsequent lake fish samples. Blood sodium in FW female groups was lower than in most BW groups except the Sept. 5 lake sample, and followed a decreasing trend from mid-river through pre-ovulatory and ovulated fish. The lowest blood sodium (128 mM/l) levels of any FW fish were recorded from ovulated females at Nitinat hatchery.

Stamp chinook (Fig. 10)

Plasma sodium was slightly higher in ovulated Stamp females than in Nitinat females. In spent females blood sodium was higher than in ovulated females at Nitinat or RCH.
Figure 6. Water content (Water) of oocytes and eggs, and gonadosomatic index (GSI) of chinook females migrating from the ocean to spawning grounds and undergoing sexual maturation. SW = seawater (west coast of Vancouver Island); S1-S3 = Sites 1-3 (Nitinat Lake); MR = mid-river; Mature = ovulated at Nitinat Hatchery; Spent = spent fish at Robertson Creek Hatchery. Each value represents the mean + SEM. * indicates significant difference from previous sample.
Figure 7. Plasma sodium of chinook salmon migrating from the ocean to spawning grounds and undergoing sexual maturation. Sites 1-3: Nitinat Lake; Mid R = mid-river; Mature = ovulated or spermiated at Nitinat Hatchery; Spent = spent fish at Robertson Creek Hatchery. Each value represents the mean + SEM. * indicates significant difference from previous sample.
B. Males

a. Gonadosomatic index (Fig. 8)

*Nitinat chinook*

Gonadosomatic index in male chinook increased between Aug. 19 (Site 1) and Sept. 5 (Site 3). In FW mid-river males before spermiation GSI was higher than in ripe males at Nitinat Hatchery.

*Stamp chinook*

In males that were spent or holding with spent females in Glover Creek GSI varied widely (3.5-10.0%) averaging 6.00%, as compared with 6.34 ± 0.88% in Stamp River males used in the 24-hour serial sampling study on newly mature fish.

b. Hepatosomatic index (Fig. 8)

*Nitinat chinook*

No changes were seen in HSI among males captured in Nitinat Lake through to the mid-river sample. A highly significant increase (to 1.87%, P < 0.01) in HSI took place between mid-river fish and ripe fish at the hatchery.

*Stamp chinook*

In recently spermiated males, sacrificed after serial sampling, HSI was significantly lower (1.06%) than in spermiated Nitinat males. Hepatosomatic index in spent males was higher than in the recently spermiated males.
c. Blood sodium

*Nitinat chinook*

Changes in blood sodium (Fig. 7) of male chinook showed the same trend as in females from Nitinat Lake through to the mid-river stage. Wider variation in male blood sodium level was seen in all lake samples however. A slight rise was seen in mid-river males. No changes took place between the mid-river stage and spermiated males at the hatchery.

*Stamp chinook (Fig. 13)*

Mean blood sodium in Stamp males was similar to that in Nitinat males. Blood sodium was significantly lower in spent males.
Figure 8. Gonadosomatic index (GSI) and hepatosomatic index (HSI) in male chinook migrating to spawning grounds and undergoing sexual maturation. Sites 1-3: Nitinat Lake; Mid R = mid-river; Mature = spermiated fish at Nitinat Hatchery; Spent = spent fish at Robertson Creek Hatchery. Each value represents the mean ± SEM. * indicates significant difference from previous HSI sample. GSI values which are similar as determined by Tukey HSD (P > 0.05) are identified by the same superscript.
IV. Changes in reproductive hormones

A. Females

a. Gonadotropin (GtH)

*Nitinat chinook*

Plasma levels of GtH varied only slightly around 7.5 ng/ml in fish captured in Nitinat Lake (*Fig. 9*), with a trend toward increasing values from the mouth to the head of the lake. The rise in GtH values between lake females and those in mid-river fish was not significant. There was a significant increase to a mean of 15.6 ng/ml in ovulating females sampled at the hatchery.

*Stamp chinook*

The distribution of oocytes among germinal vesicle and ovulatory stages between females was mirrored in the profiles of some plasma hormones. While there are indications of significant differences in some reproductive hormone levels between females, in light of the small sample sizes within groups analysis of these data was not conducted. As well, there was scant evidence of diurnal cycling of plasma hormone and blood sodium concentrations in individuals, and the 24-hour profiles have not been reported here. However, hormone and sodium values for 3 ovulated and 7 spermiated Stamp River fish at the beginning of serial sampling were compared to those for mature Nitinat fish to assess the validity of taking samples of spent fish from Glover Creek at RCH.
Figure 9. Gonadotropin (GtH) and Estradiol (E2) in female chinook migrating to spawning grounds and undergoing sexual maturation. Sites 1-3: Nitinat Lake; Mid R = mid-river; Mature = ovulated fish at Nitinat Hatchery; Spent = spent fish at Robertson Creek Hatchery. Each value represents the mean + SEM. * indicates significant difference from previous sample.
Figure 10. Blood sodium (Na), Gonadotropin (GtH), Testosterone (T), and 17α,20β-dihydroxyprogesterone (17,20-P) in ovulating female chinook at Robertson Creek Hatchery.
Plasma GtH (*Fig. 10*) level in ovulated Stamp chinook was similar to that in ovulated Nitinat females. Gonadotropin was significantly elevated, relative to recently ovulated females in sacrificed spent females and in serially sampled females, and remained at this level or increased in serial samples.

**b. Estradiol-17β (E2)**

*Nitinat chinook*

Highest plasma E2 (79 ± 16.2 ng/ml) was measured in females caught on Aug. 19 at the outlet of Nitinat Lake (Site 1). In the two subsequent lake captures (Sites 2 and 3) E2 was at or below the limit of detection (4 ng/ml) (*Fig. 9*). E2 was higher in the mid-river females than in Site 2 and 3 females, and was depleted from the plasma of ovulated females at the hatchery.

*Stamp chinook*

No E2 was detectable in the plasmas of peri-ovulatory and spent chinook at RCH.

**c. Testosterone (T)**

*Nitinat chinook*

Plasma T (*Fig. 11*) was detected in all females sampled in the lake (31 to 242 ng/ml), and variation was extreme at all sites. Coefficient of variation was lowest in the Site 1 sample (13%) and highest in the Site 2 and 3 samples (41 and 55%, respectively). Mid-river females had the highest T levels while at ovulation, levels had returned to those seen in lake fish.
Stamp chinook (Fig. 10)

Extreme variability in plasma T level was seen in ovulating fish at RCH as well (214 ± 98 ng/ml). Plasma T was at its lowest in spent fish, and did not change in the serial samples.

d. 17α,20β-dihydroxyprogesterone (17,20-P)

Nitinat chinook

Lake female 17,20-P levels (Fig. 11) were a few ng/ml above the detection limit of 4 ng/ml and no trend toward an increase in lake fish was in evidence. No change was seen in mid-river fish, but the concentration was greatly elevated in ovulated fish at the hatchery (1018 ± 373 ng/ml).
Figure 11. Plasma testosterone (T) and 17α,20β-dihydroxyprogesterone (17,20-P) in female chinook migrating to spawning grounds and undergoing sexual maturation. Sites 1-3: Nitinat Lake; Mid R = mid-river; Mature = ovulated fish at Nitinat Hatchery; Spent = spent fish at Robertson Creek Hatchery. Each value represents the mean + SEM. * indicates significant difference from previous sample.
**Stamp chinook**

Plasma 17,20-P level was as high (*Fig. 10*) in ovulating Stamp females as in mature Nitinat females. There was a significant drop in 17,20-P in both the sacrificed and serially sampled groups of spent fish. There was a slight decline indicated in the two spent females which survived through subsequent samples.

**B. Males**

*a. Gonadotropin*

**Nitinat chinook (Fig. 12)**

GtH in males at Site 1 in the lake was 6.9 ng/ml, while the hormone was not detected in the mid-river males. In spermiated males GtH increased (14.6 ng/ml) significantly over the highest level recorded in the lake.

**Stamp chinook (Fig. 13)**

Recently mature males at RCH showed somewhat lower levels of GtH overall than Nitinat males. Gonadotropin reached its highest plasma level in spent males sacrificed at RCH (*n*=6, 29.1 ng/ml), but was lower than this in spent males which were serially sampled.
Figure 12. Plasma GtH in male chinook migrating to spawning grounds and undergoing sexual maturation. Sites 1-3: lower, mid and upper Nitinat Lake; Mid R = mid-river; Mature = spermiated fish at Nitinat Hatchery; Spent = spent fish at Robertson Creek Hatchery. Each value represents the mean + SEM. * indicates significant difference from previous sample.
Figure 13. Blood sodium (Na), Gonadotropin (GtH), Testosterone (T), 11-ketotestosterone (11-KT), and 17α,20β-dihydroxyprogesterone (17,20-P) in spermiating male chinook at Robertson Creek Hatchery.
b. **11-Ketotestosterone (11-KT)**

**Nitinat chinook**

The level of plasma 11-KT (*Fig. 14*) was significantly lower in the first two lake samples than in the last lake sample at Site 3. Concentrations in mid-river males were similar to those of Site 3 males, but increased significantly in spermiated fish sampled at the hatchery.

**Stamp chinook**

11-KT level was slightly lower in spermiating Stamp males (*Fig. 13*) than in Nitinat males at the same stage, but was significantly lower than either of these groups in spent fish.

c. **Testosterone**

**Nitinat chinook**

Testosterone (*Fig. 14*) level in males was high in the first sample, at Site 1 (130 ng/ml) and declined significantly in the subsequent lake samples. There was a significant increase to a peak in plasma T in the mid-river sample followed by a marked decline in spermiated males sampled at the hatchery.

**Stamp chinook**

Spermiating males at RCH had plasma T levels (*Fig. 13*) similar to those in males at Nitinat hatchery. Plasma T in spent sacrificed males at RCH was significantly lower than in spermiated males. Starting values in the serially sampled group of spent males were similar to this and did not change in subsequent samples.
d. 17α,20β-dihydroxyprogesterone

Nitinat chinook (Fig. 15)

Plasma 17,20-P in males was at or slightly above the detection limit of the assay (4 ng/ml) in lake samples from Sites 1-3, showing little change over the 3-week period. Similar levels were seen in mid-river fish, but a steep increase occurred in spermiated fish at the hatchery.
Figure 14. Plasma 11-KT and T in male chinook migrating to spawning grounds and undergoing sexual maturation. Sites 1-3: lower, mid and upper Nitinat Lake; Mid R = mid-river; Mature = spermiated at Nitinat Hatchery; Spent = spent fish at Robertson Creek Hatchery. Each value represents the mean ± SEM. * indicates significant difference from previous sample.


*Stamp chinook*

In recently spermiated males at RCH (*Fig. 13*), plasma 17,20-P was slightly lower than in Nitinat males of uncertain spermiation date. Plasma 17,20-P concentrations in spent males remained high in both groups of spent fish at RCH.
Figure 15. Plasma 17,20-P in male chinook migrating to spawning grounds and undergoing sexual maturation. Sites 1-3: lower, mid and upper Nitinat Lake; Mid R = mid-river; Mature = spermiated fish at Nitinat Hatchery; Spent = spent fish at Robertson Creek Hatchery. Each value represents the mean + SEM. * indicates significant difference from previous sample.
B. Steelhead

I. Migration and spawning of steelhead

The migrating population of Stamp River steelhead in 1988 and 1989 was estimated to be approximately 2500 in both years. In the lake-fed Stamp/Somass system complete drying of the riverbed in late summer and fall does not occur in the lower reaches as it does in Nitinat River, and determination of the exact timing of the first migrants is not possible. Given the propensity of this late-summer stock to enter FW several months before final maturation it is also difficult to identify a pronounced peak in spawning. In 1989 steelhead were recorded at the Stamp Falls ladder as early as Sept. 20, and numbers increased until counting was discontinued on Nov. 10, but a significant proportion of the run, comprised of both hatchery and wild fish, may remain below the ladder for months.

The fish examined in this study were hatchery stock and the timing of their arrival and maturation (and spawning in Glover Creek) in the 1988 brood year will be taken as representative. Adult steelhead first appeared at RCH on Oct. 4 and continued to arrive until Nov. 5. Fish had lost all silvering in the scales and were brownish dorsally and whitish ventrally. The sexes were distinguishable on the basis of snout shape and the red lateral stripe in males. These fish were held and blood-sampled when adults entered Glover Creek to pair and begin final maturation on Feb. 1, 1988. First spawning of steelhead began in early March in Glover Creek and continued until March 29. Secondary sexual characteristics and spawning coloration were similar to those of adults first entering the hatchery in October. Synchronization of spawning activity was not as pronounced as in chinook; during most observation periods immature, ripe and spent fish could be seen.
holding over the same gravels. The last spent steelhead were observed dropping to the lower reaches of Glover Creek on April 5.

II. Changes in reproductive parameters

A. Females

a. Germinal vesicle stage

Germinal vesicle stage was variable between females in each sample except for March 3 (all GVBD) in 1988 broodstock, and Dec. 8 (all MGV) in 1989 broodstock. Oocytes in the least mature, CGV stage, were not present in these fish. Oocyte stage was a mixture of PGV and GVBD in all samples between Dec. 8 and ovulation in spring. GVBD stage was seen in 25, 33, 40, 80, and 80% of females on Dec. 27, Jan. 15, Feb. 1, Feb. 12, and Feb. 19, respectively. Two germinal vesicle stages in the same ovary (PGV and GVBD) were seen in two females.

b. Gonadosomatic index

Lowest GSI (13.1 ± 1.7%) was seen in females (Weight: 2.43 ± 0.72 kg) of most recent arrival at the hatchery, on Dec. 8, 1988 (Fig. 16). In ovulated fish, representing 20, 10, and 100% of the last three samples respectively, GSI was marginally higher (18.1 ± 1.9%, P = 0.053). Highest GSI occurred in Feb. 1, 1988 females however, where no difference in GSI was seen between PGV and GVBD females.
c. Water content of oocytes

Water content rose slightly in the samples collected from adults maturing in 1988, from 51.3% in maturing oocytes to 55.6% in ovulated eggs, although the difference was not significant (Fig. 16). There was no difference in hydration level between oocytes examined in 1989 broodstock, representing the earliest obtainable, and the earliest oocytes collected from 1988 broodstock.

d. Hepatosomatic index (Fig. 16)

The highest HSI (1.44%) occurred in females in the earlier stages of final maturation, and the lowest (1.07%) occurred at ovulation. Although the difference between these two dates was significant, HSI fluctuated in the intervening samples.
Figure 16. Gonadosomatic index (GSI), hepatosomatic index (HSI) and oocyte water content (Water) in maturing Stamp River steelhead females at Robertson Creek Hatchery. The data are presented in the correct order with respect to time elapsed since arrival at the hatchery, with values for fish sampled early from the 1989 spawners presented ahead of values for fish sampled late from the 1988 spawners. Numbers inside bars represent sample sizes. Each value represents the mean ± SEM. Values which are similar as determined by Tukey HSD (P > 0.05) are identified by the same superscript.
B. Males

a. Gonadosomatic index

In male steelhead (Weight: 2.88 ± 0.62) sacrificed at RCH there was a slight increase in GSI (Fig. 17) on Feb. 1, followed by a significant decline in the next sample. Values were in the 3.5% range in early samples of 1989 spawners. GSI fluctuated (4.1-2.7%) in fish sampled after more prolonged holding in 1988 spawners. Milt production by mature male steelhead was not large when compared to the volumes produced by chinook, but fluid was present in the sperm ducts of most steelhead at the time of gonad removal. Milt volume was not measured in the sacrificed males, but there did not appear to be an increase in the last sample from 1988 spawners.

b. Hepatosomatic index

There were no significant increases in HSI between consecutive samples (Fig. 17). There was a significant increase between the first two samples and the last sample (March 3).
Figure 17. Gonadosomatic index (GSI) and hepatosomatic index (HSI) in maturing Stamp River steelhead males at Robertson Creek Hatchery. The data are presented in the correct order with respect to time elapsed since arrival at the hatchery, with values for fish sampled early from the 1989 spawners presented ahead of values for fish sampled late from the 1988 spawners. Numbers inside bars represent sample sizes. Each value represents the mean ± SEM. HSI values which are similar as determined by Tukey HSD (P > 0.05) are identified by the same superscript. * indicates significant difference from previous GSI value.
III. Changes in reproductive hormones

A. Females

a. Gonadotropin (Fig. 18)

Six female steelhead were serially sampled in this phase of the study, which relates plasma hormone profiles to ovulation date. The study was begun on Feb. 5 1988, and terminated on March 10. Two fish were found to have ovulated on Feb. 10, three more on Feb. 28, and the last one on March 9. Plasma GtH was low or undetectable until a few days before ovulation. During ovulation GtH rose from about 8.5 to 25 ng/ml, and continued to rise in ovulated fish for 2-3 weeks after ovulation.

b. Estradiol-17β (Fig. 19)

A consistent decline in E₂ occurred in all females. In pre-ovulatory fish E₂ ranged between 1.9 and 11.6 ng/ml, reaching a fairly uniform level of 2 ng/ml at ovulation, dropping to below the 1 ng/ml range or to below the limit of detection afterwards.

c. Testosterone (Fig. 20)

The pattern of plasma T concentration was similar to that of E₂ in relation to ovulation, with the difference that starting and final T values were higher. Levels declined between the first sample and ovulation in all fish, and generally declined further after ovulation.
d. 17α,20β-dihydroxyprogesterone (Fig. 21)

Changes in 17,20-P titre were variable between females. In most fish 17,20-P titre was the highest of the steroid hormones studied around the time of ovulation. However, in various fish, levels were high before and declining at ovulation while in others, highest levels were attained after ovulation.

B. Males

a. Gonadotropin

Gonadotropin was not detected in the plasma of any male steelhead.

Five males were sampled in the study, and an attempt was made to determine the time of spermiation among them. Milt was first expressed by 4 males on Feb. 10, and by the fifth on March 4, but quantities varied greatly between fish. One male died between Feb. 26 and March 4.
Figure 18. Plasma gonadotropin (GtH) in serially sampled steelhead females undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. Sample dates are standardized to the day of ovulation for each fish. F1-6 = females 1-6.
Figure 19. Plasma estradiol-17β (E2) in serially sampled steelhead females undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. Sample dates are standardized to the day of ovulation for each fish. F1-6 = females 1-6.
Figure 20. Plasma testosterone (T) in serially sampled steelhead females undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. Sample dates are standardized to the day of ovulation for each fish. F1-6 = females 1-6.
Figure 21. Plasma 17α,20β-dihydroxyprogesterone (17,20-P) in serially sampled steelhead females undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. Sample dates are standardized to the day of ovulation for each fish. F1-6 = females 1-6.
b. Testosterone (Fig. 22)

Plasma T was lower in males than in females at the outset of sampling and declined to the end of the study by an average of 75%.

c. 11-ketotestosterone (Fig. 23)

Throughout the sampling period 11-KT levels in males were equal to, or greater than, T levels. Most fish showed a decline from highest levels of 11-KT approximately 10 days before spermiation to lower levels at spermiation. A further decline followed spermiation in all fish, but the percent change within fish was not as large as for T.

d. 17α,20β-dihydroxyprogesterone (Fig. 24)

The level of 17,20-P in males did not reach the levels seen in females, remaining relatively low (15 ng/ml) at spermiation. A gradual rise beginning 1 week after spermiation was clear in two males which survived to the end of sampling, but in the other two there was no change.
Figure 22. Plasma testosterone (T) in serially sampled steelhead males undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. Sample dates are standardized to the day of first milt expression for each fish. M1-5 = males 1-5.
Figure 23. Plasma 11-ketotestosterone (11-KT) in serially sampled steelhead males undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. Sample dates are standardized to the day of first milt expression for each fish. M1-5 = males 1-5.
Figure 24. Plasma 17α,20β-dihydroxyprogesterone (17,20-P) in serially sampled steelhead males undergoing sexual maturation at Robertson Creek Hatchery, Feb.-March, 1988. Sample dates are standardized to the day of first milt expression for each fish. M1-5 = males 1-5.
D. DISCUSSION

A. females

Reproductive factors in female chinook salmon at the end of their SW residence and transition into the FW environment have been documented in this portion of the study. Additionally, reproductive hormone concentrations in the peri-ovulatory stage of chinook, maturing shortly after FW entry, and steelhead, maturing over a longer period in FW, have been examined in detail.

Both species were found to gain gonadal mass (GSI) between earlier and later stages of oogenesis. Other studies have found that GSI may increase (Ueda et al., 1984a) or undergo little change (McBride et al., 1986) in maturing salmon. Hydration level of oocytes rose consistently as females of both species matured, indicating that water is absorbed during the last stages of maturation. In chinook this constitutes an increase in water demand between first entry of brackish Nitinat Lake and ovulation in the river (approx. 0.07 kg/ovary).

Oocyte germinal vesicle stage was variable between individuals in most samples from both chinook and steelhead. Asynchronous maturation in a spawning population is not unusual in salmonid species which can maintain fertility of gametes for several days after final maturation (see Wallace and Selman, 1981). The two most advanced stages (PGV and GVBD, respectively) were not seen in chinook in the brackish water environment of Nitinat Lake, indicating a degree of synchronization between migratory stage and oocyte maturation. Only in steelhead were oocytes of different maturation stage seen in the same ovary (PGV and GVBD). The fact that these were the two most advanced stages suggests
that oocytes progress more rapidly from PGV to GVBD than they do from one stage to the next in earlier stages.

Blackburn and Clarke (1987) tested osmoregulatory ability in juvenile salmon immersed in SW for 24 h, and found that fish which had completed smoltification maintained blood sodium level below 170 mM/l. Levels above 200 mM/l were observed in one sample of lower Nitinat Lake adult chinook newly arrived from the ocean. Similar high levels recorded by Sower and Schreck (1982) in maturing coho were associated with high adult mortality and low egg survival. The high blood sodium level in Nitinat chinook may have been a normal transient effect related to cessation of feeding and beginning of final maturation, but the possibility of stress or netting injuries contributing to high sodium influx in these fish cannot be discounted. Higher catches in subsequent lake samples, further into the brackish lake, allowed the rejection of fish suffocated by the net. Blood sodium levels in these fish fall within the normal range for adult salmon in SW (Sower and Schreck, 1982; Lam et al., 1982). Blood sodium decreased after transition from Nitinat Lake to FW in female chinook and declined further at ovulation.

Similarities were seen in the patterns of change of reproductive hormones in chinook and steelhead females. Prior to ovulation gonadotropin was at basal levels, rose around the time of ovulation, and reached highest levels in post-ovulation or spent fish of both species. This is a common pattern in salmonids (Scott et al., 1989; Dye et al., 1986) where an increase of mGtH at the end of gametogenesis is believed to initiate production/release of 17,20-P, GVBD, and ovulation (Kawauchi et al., 1989). The continuous rise of GtH after ovulation and spawning may be a consequence of reduced steroid feed-back inhibition of pituitary gonadotrops (Jalabert, 1980; Scott et al., 1983) with the sequential decline of E2, T, and 17,20-P, respectively.
A marked drop in plasma E\textsubscript{2} took place in Nitinat Lake females after first entry of fish from the ocean. Estradiol was low or declining after the first sample from steelhead as well, dropping to basal levels before ovulation. Hepatic production of vitellogenin would be expected to substantially decline within days of E\textsubscript{2} disappearance from the blood (Wallace et al., 1987), and circulating vitellogenin would be rapidly depleted by oocyte uptake (Tyler et al., 1991). A large part of any increase in GSI after the decline in E\textsubscript{2} in both chinook and steelhead may therefore be attributable to water of hydration. Plasma E\textsubscript{2}, associated more with vitellogenesis than final maturation, was higher in mid-river Nitinat chinook than in the last fish sampled in the lake. Between these two sampling dates the commencement of autumn rains caused a rise in river level and an increase in the depth of the FW layer over the length of the lake. The occurrence in the mid-river sample of fish at a stage of maturation, assessed on the basis of plasma E\textsubscript{2} level, which would be expected in fish midway along the lake suggests fish may be induced to move more rapidly into FW by decreasing temperature or increasing river flow.

Between the decline of E\textsubscript{2} and the rise of 17,20-P at ovulation plasma T rose to a peak in mid-river chinook, then declined thereafter. Similar preovulatory peaks of androgen were reported in coho (Sower and Schreck, 1982), chum (Ueda et al., 1984a), and rainbow trout (Sumpter et al., 1984). In serially sampled steelhead T declined from the first sampling date in every female irrespective of ovulation date, and was depleted to nearly undetectable levels in the last sample. The sampling procedure itself, or some environmental influence, may have been acting to affect T, but not other hormone levels. These observations nevertheless suggest that a reduction or cessation of T production occurs in steelhead, as well as in chinook, sometime before ovulation.
A surge in plasma 17,20-P occurred at ovulation in chinook. The plasma concentration was much higher in chinook than in steelhead at ovulation in this study, and about two-fold higher than any previously reported values (Dye et al., 1986; Ueda et al., 1984a; Van Der Kraak et al., 1984; Scott et al., 1983) for this hormone in a salmonid species. The reason for the exceptionally high level in chinook is not clear. Samples from mature fish in this study may have been taken nearer to the event of egg expulsion from the follicle, and release of 17,20-P into the body cavity, than in other studies. If rapid absorption takes place blood levels would accurately reflect the levels in the body cavity. Rapid response in blood 17,20-P level to changes in volume of eggs and ovarian fluid in the body cavity seems to occur in rainbow trout either manually stripped (Springate et al., 1984) or allowed to spawn naturally (Liley and Rouger, 1990). This and the fact that the ovary gradually loses steroidogenic capacity after spawning (Van Der Kraak and Donaldson, 1986) explains the significant decrease in plasma 17,20-P in spent chinook.

Plasma 17,20-P concentrations at ovulation were near the highest recorded for each individual or increased for a short time thereafter, in most steelhead females. Levels in the final samples from 5 of the 6 females were lower than at ovulation. Dead eggs in significant numbers were present on the raceway floor after ovulation. Stripping eggs generally acts to lower plasma 17,20-P levels (Liley and Rouger, 1990) and partial loss of eggs in some of these steelhead may be involved in the variability of patterns between females. As fertility checks were not performed the significance of an uninterrupted fall in 17,20-P from the first sample in one female is not known. Lowest levels of 17,20-P at ovulation occurred in steelhead, possibly as a result of lower GtH stimulation in the period preceding ovulation.
B. males

As in females, blood sodium in male chinook captured at the lower end of Nitinat Lake was quite high. The trend in subsequent lake samples was toward lower levels as fish gained access to lower salinity in the lake. Blood sodium declined further as maturation approached and the fish entered FW. A significant decline in blood sodium occurred between newly spermiated and spent males. This may take place during removal of sodium (Marshall et al., 1989) from the sperm duct lumen in the formation of seminal fluid.

Gonadosomatic and hepatosomatic indices were not very useful in the evaluation of maturation in male chinook. As a relatively small percentage of total weight, small changes in gonad or liver weight may be obscured by increase in body weight due to water absorption by somatic tissues (Kirschener, 1991) on the entry of BW and FW. Conversely, a significant decline in body weight may occur as a result of starvation. Analysis of gonad and liver weights in relation to some measure of condition coefficient (body weight in relation to length) may be a more useful way of monitoring change in those parameters.

Gonadotropin is usually found to be lower in males than in females at a given stage of maturation (Dye et al., 1986; Fitzpatrick et al., 1986; Sumpter and Scott, 1989). In this study GtH was about equal between the sexes in Nitinat chinook. Lowest levels of plasma GtH were seen in mid-river Nitinat males, a finding which suggests, as do the E2 values for mid-river females, that chinook in earlier stages of maturation will enter the river as soon as access is available.

In Stamp steelhead males, GtH was undetectable throughout the maturation process, even after spermiation. This does not exclude the possibility of a role for GtH in spermiation. A peak in plasma GtH below the detection limit of the assay used in this study was found in spermiating resident rainbow trout males Sumpter and Scott (1989). The
striking difference in GtH levels between chinook and steelhead males may help to explain
the difference in post-spawning life histories between these species. In chinook, gonadal
androgenesis is driven to a higher level than in steelhead by high levels of GtH. High
androgen level in salmonids induces hyperadrenocorticism and elevated cortisol, which
mobilizes energy reserves (Robertson and Wexler, 1959; Donaldson and Fagerlund, 1969)
and eventually leads to tissue degeneration. Androgens decline in mature and post-
spawning chinook, but not to the low levels seen in mature steelhead. The magnitude of the
prematuration peak, or perhaps the persistence of appreciable post-spawning androgen
levels may not allow the cessation of cortisol production. Without elimination of cortisol,
tissue catabolism in chinook would continue unabated.

Levels of 11-KT increased steadily in males migrating out of Nitinat Lake and up the
river, to peak in mature fish at the hatchery. Testosterone level peaked before that of 11-KT
and was much lower than 11-KT in mature males. In steelhead males 11-KT was higher
than T at spermiation, and both androgens were declining. In sockeye (Truscott et al., 1986)
and pink salmon (Dye et al., 1986) plasma T was lower than 11-KT and fell steadily toward
final maturation. In coho (Fitzpatrick et al., 1986) T level fell while 11-KT rose with the
approach of spawning. Taken together these observations suggest that 11-KT has some
function in late spermatogenesis not shared by T (Schulz and Blum, 1990), but this function
has not been determined (Scott and Sumpter, 1989).

In Nitinat chinook males a 20-fold surge of 17,20-P at the time of final maturation
was similar, albeit of somewhat smaller amplitude, to that in females. Unlike females
however, the rise in 17,20-P in males continued after final maturation. Salmonids continue
to produce milt for days or weeks after initial spermiation. The persistent high levels of
17,20-P during this period was interpreted by Ueda et al. (1985) as evidence for control of
spermiation by this hormone. However, its major effect may be solely on milt volume. Only GtH is able to stimulate \( \text{Na}^+ \) transport from the sperm duct lumen (Marshall et al. 1989), which creates the ionic environment required by maturing sperm.

In general 17,20-P in male steelhead did not approach the levels in chinook, and did not trend toward a notable peak at spermiation. Differences in milt volumes between spermiated steelhead and chinook seem to be correlated with the differences in 17,20-P levels between the two species. Both the low levels of GtH and 17,20-P and low milt volumes in steelhead may be an effect of prolonged confinement in concrete raceways. The absence of redd building activities by females may have deprived males of the stimulus Liley et al. (1986) identified as complementary to 17,20-P and milt production in this species.
A. INTRODUCTION

The experience at Nitinat hatchery has been that fertility of chinook females completing final maturation in net pens in brackish Nitinat Lake is lower than in females maturing in FW. The major objective of this phase of the study was to determine whether fertility of fish maturing in BW differs from that of fish maturing in FW. If such a difference exists can it be related to differences in plasma hormones or other physiological features? For example, Sower and Schreck (1982) found that fertility of SW-held coho was adversely affected in individuals with high blood sodium shortly before, but not necessarily during ovulation. Blood sodium level in female coho maturing normally in FW was between 135 and 150 mM/l, while in SW-maturing fish levels ranged between 175 and 200 mM/l.

Peaks in chum salmon migration and spawning occur 3-4 weeks after those of chinook in the Nitinat system. A substantial proportion (40-70%) of chum eggs incubated at Nitinat hatchery come from females captured and held to final maturation in the lake. Fertilization rate is high in egg lots from these fish maturing at the same net pen location where attempts to obtain comparable fertility in chinook have been largely unsuccessful. Similar high fertility was seen in chum captured and held at another BW location on the west coast of Vancouver Island (Lam et al., 1982). Because fertility of females maturing in BW and FW is equal in chum but not in chinook, the question is raised as to whether physiological differences can be detected in chum and chinook that may relate to the ability to produce viable eggs in BW.
A secondary aim of this chapter was to examine hormone profiles and blood sodium levels in preovulatory chinook delayed at the Nitinat lakehead by low water levels in the river. The question in that situation is whether reproductive hormone profiles in the blood of BW females are different from those normally seen in FW females at a similar stage of maturation. It is also of interest to know if blood sodium level in these fish indicates osmotic imbalance in BW and whether this correlates with hormone profiles.

B. MATERIALS AND METHODS

In October of 1988 maturing chinook females were captured by seine on several occasions in Nitinat Lake and held to final maturation and ovulation in BW net pens near the mouth of Nitinat River. In the first group, females were held in the net pen until several had ovulated, a practice followed with females maturing at the hatchery, and eggs were taken from 8 fish on Oct. 8. Eggs were obtained on the same date from a group of females allowed to mature in FW (n=8). The post-ovulation age of eggs at the time of fertilization varied by as much as 5 days within these groups. Blood and ovarian fluid was not collected from these fish as sampling equipment was not available at the net pen site on this occasion. Both groups of eggs were fertilized with sperm from males maturing in FW.

Eggs were transported with several volumes of air in sealed plastic buckets and fertilized, after temperature acclimatization, within 3 h of collection. In clean, dry 10 l plastic buckets 10 ml sperm was mixed into each batch of eggs (approx. volume 2 l) and allowed to stand for 5 min. Approximately 2 volumes of water were poured into each bucket, gently stirred and allowed to stand for 1 min. The eggs were then rinsed with several volumes of water and poured into upwelling Heath tray incubators (Heath Techna Corp.)
supplied with 8°C well water at 11 l/min. The same fertilization procedures were followed
with subsequent groups of eggs, with modifications noted.

A second group of eggs from fish of relatively recent ovulation, was taken on Oct. 14. From fish captured and/or determined to be unripe on Oct. 10, 6 net pen and 6 hatchery females were randomly selected. Shortage of space at the net pen site did not allow for the holding of male broodstock in great numbers, but 3 were maintained for comparison with FW males. Half the eggs of three Oct. 14 females from each location were fertilized with sperm from net pen males, and the other half with sperm from FW males. Each of these 6 males fertilized eggs from one hatchery and one net pen female. Eggs of the 3 remaining females from each location were fertilized with sperm from FW males, such that each of 6 different males fertilized all the eggs of 1 female. Eggs that had been divided and fertilized with the two types of sperm were incubated in trays divided with plexiglass partitions.

After rains commencing Oct. 12 had brought the river level up, and the numbers of fish holding at the lakehead had decreased, a third group of net pen females, evaluated as new arrivals, was established, on Oct. 15. These fish (n=14) were checked every 2 days and their eggs, ovarian fluid and blood taken when ripe between Oct. 17-26. A third group of eggs ovulated in FW was obtained from females (n=16) arriving ripe at the hatchery during the same period, but blood and ovarian fluid were not obtained from these fish. Eggs from both types of females were fertilized with sperm from FW males. Mortality was recorded for each of the above tests at the eyed egg stage.

Hormone profiles, blood sodium, and other reproductive parameters of the last group of BW females (Oct. 17-26) in 1988 were compared with those from females arriving newly ripe at the hatchery on Oct. 12, 1989. The first rains came earlier in 1989 than in 1988, but it was possible in both years to differentiate, on the basis of coloration and general condition,
between ripe fish which had been delayed in the lake or lower river and those which came in fresh. Fish which had been delayed in the lake were very dark-skinned and those delayed in the lower river were infected with patches of fungus. Varying proportions of the eggs from delayed fish were in the so-called "water-hardened" state; enlarged, hard to the touch, and infertile. Lighter coloured, nonfungused fish were judged to be most similar to the Oct. 17-26, 1988 fish among the FW types encountered in 1989. Eggs from these females (n=8) were fertilized with sperm from FW males.

A comparison of hormone levels, blood sodium, and egg hydration was also made between the 2 above-mentioned groups and females captured at the lakehead on Sept. 21, 1989. Chinook had been holding in this area (see Fig. 1) for several weeks, and 200 were seined and transferred to the net pen site for holding to maturity. Five females were sacrifice-sampled 2 hours after capture. Mortalities of 50% in the main group forced the decision to release the survivors on Sept. 23. Although no ovulated eggs were obtained for fertility tests, it was important to document the physiological condition of fish that had been delayed in BW.

On Nov. 4 of 1988 chum salmon females maturing at the net pen site (n=7) and hatchery (n=7) were sampled. Reproductive hormone and sodium levels were analyzed in the plasmas of these fish. After fertilization of eggs (approx. volume 1 l) from both locations with milt (5 ml) from FW males, mortality was recorded at the eyed stage.

In the interest of simplifying the language of description, eggs from females maturing in BW are referred to as "BW eggs". Similar abbreviations yield the terms "FW eggs", "BW milt" and "FW milt", respectively for gametes matured at a given location. The eggs produced by one female are referred to as a "batch".
C. RESULTS

I. Reproductive parameters

a. Egg fertility

Chinook

Highest mortalities occurred in chinook eggs taken at the lake pens on Oct. 8, 1988 (Fig. 25). Mortality was above 90% in the eggs from 3 females in this group. Losses in eggs taken at the hatchery were highest on Oct. 8 as well, and 48% mortality was the highest at that location.

Mean mortality was lower on Oct. 14 than on Oct. 8 in eggs taken at both locations. Fertility of milt obtained from BW and FW males was compared in this test. High mortality (58-98%) occurred in 2 of the 3 batches of BW eggs divided and fertilized with BW and FW milt, and in the third batch, mortalities were below 8%. Within these batches, mortality was similar whether fertilization was with FW or BW milt. In the 3 undivided batches of BW eggs, fertilized with FW milt from 3 previously unused males, mortality did not exceed 20%.

After fertilizations by the same males used on divided batches of BW eggs the highest mortality in divided batches of FW eggs was 6.2%. In the 3 undivided batches of FW eggs, fertilized with the last 3 FW males, mortality ranged between 15 and 76%.

Lowest mortalities among BW eggs occurred in those obtained between Oct. 17-26 and fertilized with FW milt. Mortality was high in three batches of these eggs (100, 23, and 22%, respectively), but relatively low in the other 11 batches. In the FW females fertilized over the same period mortality ranged between 1.5 and 15.2%. Mortality among FW eggs was lowest in those fertilized on Oct. 12, 1989.
In many groups of eggs from BW and FW and on most dates, mortality varied widely. In the Oct. 8 BW eggs for example, mortality ranged from 2.5 to 100%, but a bimodal distribution was not indicated, rather the range of possible mortality rates between "high" and "low" were represented. Neither Tukey's HSD test nor the Mann-Whitney test identified differences between any groups. There was only a low correlation between blood sodium and mortality.

*Chum (Fig. 29)*

There was no difference in mortality to the eyed stage between chum eggs from lake pen and hatchery ripened females (4 and 5.3%, respectively) fertilized with sperm from FW males.

*b. Gonadosomatic index (Fig. 26)*

*Chinook*

Females sampled at the lakehead in 1989 had significantly lower GSI than both the BW and FW ovulated groups.

c. *Germinal vesicle stage*

Only the fish sampled at the lakehead in 1989 held oocytes in PGV stage; eggs from all ovulated fish were in GVBD.
d. Water content of oocytes

Chinook

Oocytes from unovulated females at the lakehead had the lowest hydration level (Fig. 26). Ovulated eggs from the lake pen fish were significantly higher in water content, and eggs from hatchery females contained more water than lake pen eggs.

e. Blood and ovarian fluid sodium

Chinook

High blood sodium (191 mM/l) was seen in lakehead females (Fig. 27). In lake pen females at ovulation, blood sodium was significantly higher than in hatchery females, and significantly lower than in lakehead females. Ovarian fluid (Fig. 27) sodium was also higher in lake pen fish (158 mM/l) than in hatchery fish (144 mM/l).

Chum (Fig. 29)

Blood and ovarian fluid sodium did not differ between net pen and hatchery chum females (blood: 148 and 147 mM/l, ovarian fluid: 150 and 151 mM/l, respectively).

II. Reproductive hormones

a. Gonadotropin

Chinook

Plasma GtH (Fig. 28) was lowest in the unovulated females caught at the lakehead on Sept. 21, 1989. Between the ovulated fish at the lake pens or hatchery, highest levels were encountered in the lake pen group, though the difference was not significant.
Chum

Plasma GtH (Fig. 29) in chum females ovulating at the lake pens was 15.4 ± 6.3 ng/ml, compared to 23.6 ± 12.9 ng/ml at the hatchery.

b. Testosterone

Chinook

There was no difference in T level (Fig. 28) in the plasmas obtained from females at the 3 locations (range 128-156 ng/ml).

Chum

Testosterone (Fig. 29) in lake pen females was significantly elevated compared to hatchery females.

c. 17,20-P

Chinook

In lakehead females 17,20-P was only a few ng/ml above the detection limit (Fig. 29). In ovulated fish at the lake pens and hatchery the levels were much higher, 450 and 1020 ng/ml respectively, and significantly different (P < .03).

Chum

In lake pen females 17,20-P levels (Fig. 29) averaged 233 ± 192 ng/ml. High variability was seen in hatchery females as well (178 ± 103 ng/ml), and the difference between the 2 groups was not significant.
Figure 25. Mortalities to the eyed stage among chinook salmon eggs taken from females in brackish (BW) or fresh (FW) water, October 8-26, 1988, and fertilized with milt from FW or BW males, eg., BW/FW = BW female fertilized with FW milt. Values represent mean + SEM.
Figure 26. Water content (Water) of oocytes and eggs, and gonadosomatic index (GSI) in chinook salmon females at Nitinat Lake and Hatchery, 1988 and 1989. Solid bars: unovulated fish holding at lakehead, Sept. 21, 1989; Diagonal hatching: recently ovulated at net pens, Oct.17-26, 1988; Cross hatching: recently ovulated at hatchery, Oct. 12, 1989. Values represent mean + SEM. * indicates significant difference from preceding value.
Figure 28. Plasma hormone levels in chinook salmon females at Nitinat Lake and Hatchery, 1988 and 1989. GtH=gonadotropin, T=testosterone, 17,20-P=17α,20β-dihydroxyprogesterone. Solid bars: unovulated fish holding at lakehead, Sept. 21, 1989; Diagonal hatching: recently ovulated at net pens, Oct.17-26, 1988; Cross hatching: recently ovulated at hatchery, Oct. 12, 1989. Values represent mean + SEM. Hormone levels which were similar as determined by Tukey HSD Test (P > 0.05) are identified by the same superscript.
Figure 29. Plasma hormone (see Fig. 28 for abbreviations) and sodium levels, and egg mortality rates in chum salmon females at Nitinat Lake and hatchery, Oct. 28, 1988. Solid bars: recently ovulated at net pens; Hatched bars: recently ovulated at hatchery. Values represent mean + SEM. * indicates significant difference from preceding value.
III. Water quality

a. Temperature

Surface temperature had decreased to 10° C following the onset of seasonal rains (Oct. 18) at the lake pen site in 1988. The thermocline (13.5° C) was encountered at 1 m (Fig. 30), inverted due to the density difference between FW and BW. Temperature in the first 2 m at the net pen site had fallen to 7° C by the Nov. 4 chum sampling date. On Sept. 21, 1989 the lakehead surface temperature was 16° C, with a thermocline at 1 m.

b. Salinity

The surface water was nearly fresh (2 ppt) at the net pen site in 1988, on Oct. 18, but salinity increased sharply (Fig. 30) within the first metre to 22 ppt and reached 24 ppt at 2.5 m, the depth of the net pen. The FW layer extended to 2 m on Nov. 4. The salinity profile at the lakehead was similar to the profile at Site 3 in 1989.

c. Oxygen

Between 2 and 2.5 m, oxygen decreased from 6.2 to 4.3 ppm at the net pen site (Fig. 30), on Oct. 18. Oxygen level remained above 6 ppm to a depth of 3 m on Nov. 4. At the lakehead in 1989, the anoxic layer was located 3 m below the surface.
Figure 30. Water quality parameters at Nitinat Lake net pen site, October 18, 1988.
D. DISCUSSION

Mortalities were higher, though not significantly so, in chinook eggs taken from females at the net pen site during a period of high salinity and temperature, and low oxygen (Oct. 8 and 14) than in eggs taken after Oct. 17. The incidence of mortality was also higher in eggs from FW females taken earlier in the season than in those taken later. These were fish arriving at the hatchery soon after the first rise in river level permitted access to FW. Their exact ovulation dates are unknown, but it can be assumed with some confidence that they had been in BW only a few days prior to arrival at the hatchery. Plasma hormone and sodium levels seen in the Sept. 21, 1989 lakehead chinook may be representative of their physiological state prior to FW entry. If so, effects of high prematuration blood sodium, similar to those postulated by Sower and Schreck (1982), may have contributed to the high mortality rate in the first eggs taken at the hatchery.

Mortalities from the last group of chinook eggs taken at the net pens were almost as low as in FW chinook eggs taken at the same time, and in chum eggs from the net pens and hatchery one week later. By the time the third sample of chinook eggs was taken, higher flow rate from the river increased the depth of the FW layer, resulting in higher dissolved oxygen and lower temperature and salinity in the top 2 m of water at the net pen site. These same conditions existed when the chum were sampled for fertility. Normally these conditions prevail during the majority of the hatchery's chum spawnings at the net pen site.

Although mean egg mortalities were slightly higher for late October net pen chinook than for hatchery chinook and chum, the higher average for net pen chinook was caused by 3
of the 11 fish examined. One of these, suffering 100% egg mortality, had higher blood and ovarian sodium than the average for the group. The other 2, each suffering 20% mortality, had higher than average blood sodium but average ovarian fluid sodium. A strong correlation between high blood and ovarian fluid sodium and egg mortality was reported by Sower and Schreck (1982) in coho held to maturity in SW, and similarly for chum (Lam et al., 1982; Morisawa et al., 1979) and pink salmon Wertheimer (1984) in SW and BW.

In the current study there was no significant correlation between blood sodium and egg mortality. However, high sodium was seen in the blood of preovulatory chinook at the lakehead before the river level rose. Similar high levels were seen by Sower and Schreck (1982) in preovulatory coho held in SW. Blood sodium was somewhat lower at ovulation, but in some of these fish, low fertility nevertheless developed. A similar process may be at work in chinook in Nitinat Lake. Brackish water and FW eggs used in the Oct. 8 fertility tests came from fish which in the preovulatory stage may have been exposed to the same water conditions as those experienced by the Sept. 21 lakehead fish. High blood sodium developed in these fish, and it may be in this period that the future viability of unovulated oocytes is compromised. It would appear that the process in question does not affect the entire ovary simultaneously, or that it is reversible by the availability of FW, as no batches of eggs taken in FW on Oct. 8 sustained mortalities as high as those seen in BW eggs taken on the same date.

Although the influence of concurrent high temperature and low oxygen may be directly or indirectly involved, it is clear that some chinook females produce a higher proportion of infertile eggs during late summer conditions of high salinity existing at the Nitinat Lake net pen site. Fertility of males does not seem to be as strongly affected.
During the periods of lowest river flow, unacceptably high mortality of chinook adults occurs during net pen holding in BW, as seen in the group of fish from which the Sept. 21 sample was obtained. High mortality occurred in this group in the 2 days following capture. I suggest that this is due to high temperature and low oxygen whose combined effects are to increase the ventilation rate of gills. The increasing volume of water passing the gills might increase the dehydrating and hypernatremic effects of high salinity on the fish, leading to osmotic imbalance and death. Fertility of eggs in the survivors of these conditions would almost certainly be diminished.

In years when the change of season coincides with arrival of chinook at the lakehead, and cooler, well oxygenated water of low salinity becomes available at the net pen site, the practice of estuarine capture and maturation may be a viable option for chinook broodstock. Chum salmon normally arrive at the lakehead later in the season than chinook, when water salinity, temperature, and oxygen levels have moderated somewhat. High survival of adults and eggs is usually obtained when chum broodstock are held in the net pens. It appears this is not due necessarily to a superior hypoosmoregulatory ability of chum as compared to chinook, but rather to an improvement in water conditions between the peaks in chinook and chum migration.

Highest blood sodium was seen in chinook delayed at the lakehead by low river water. Plasma T was as high in lakehead females as in ovulated females, but 17,20-P and GtH were low. In mature females at the net pens, T and GtH were at levels similar to those in females maturing at the hatchery, but 17,20-P levels, although much higher than in lakehead fish, were half those in hatchery females.

The reduction of 17,20-P in fish maturing in BW suggests a degree of suppression of maturation processes under saline conditions, as seen by Sower and Schreck (1982) in coho.
Plasma 17,20-P levels in mature BW fish were many times higher than in fish holding at the lakehead, not surprisingly as lakehead fish were not mature. In order to determine whether delayed entry of FW may have curtailed maturation in lakehead fish, a comparison of maturation timing between groups from the same school held in BW and FW would be required. The question of which reproductive hormones might correlate with reduced ability to osmoregulate in BW is of greater concern in this thesis however, and the observations in lakehead fish do not strongly implicate 17,20-P. Lakehead fish had blood sodium levels higher than the critical level of 170 mM/l defined by Blackburn and Clarke (1987) for smolts adapting to SW. At the same time T levels were as high in the lakehead fish as those in mature fish. Based on the observation that blood sodium rose while prolactin, the freshwater-adapting hormone, remained low in SW-maturing chum Hirano et al. (1990) suggested that: "high levels of maturational (and other) steroids such as 17,20-P are involved in the development of the impaired hypoosmoregulatory ability in Pacific salmon at the time of spawning". The possibility that 17,20-P may interfere with the ability to osmoregulate in SW, cannot be ruled out. The observation that T was high in Nitinat lakehead chinook points to this hormone as having a likelihood of involvement in the failure to hypoosmoregulate as well.
CHAPTER 5

SW TRANSFER OF POST-SPAWNING SALMON AND STEELHEAD

A. INTRODUCTION

As demonstrated in Chapter 4, delayed FW entry of maturing chinook, in which reproductive hormones are high, results in high blood sodium. Fertility of chinook maturing in BW or FW in the early part of the season, following protracted BW residency, is lower than that of chinook maturing later in the season, when access to FW increases. In these situations high levels of reproductive hormones may be interfering with hypoosmoregulation.

After completion of spawning steelhead do not inevitably undergo further decline of condition to the point of death, as is the case in chinook and the other species of Pacific salmon (Healey, 1991). A proportion of post-spawning steelhead populations travels downstream after spawning and readapts to SW (Hart, 1980). This leads to the question of whether there are post-spawning differences in reproductive hormones between steelhead and salmon which affect the ability to adapt to SW. In particular, these contrasting life-histories provide an opportunity to examine responses in sex hormone levels and osmoregulatory adaptation to the challenge of SW transfer. For example, although there are similarities in the profiles of reproductive hormones in the last few weeks of maturation in chinook and steelhead the peaks of concentration for most hormones are considerably lower in steelhead. I suggest this is evidence for the existence of a greater degree of proximate control over reproductive hormones in steelhead than in other salmon. This allows a wider range of responses to environmental conditions, including salinity.
Mayer et al. (1990) could not find differences in the levels of 5 endogenous androgens to correlate with the difference in SW adaptability in mature and immature Atlantic salmon parr. On the other hand, high plasma levels of exogenous sex steroids impaired SW adaptability during smoltification in amago salmon (Miwa and Inui, 1986). Reproductive hormone levels in post-spawning in chinook and mature steelhead are considerably higher than in the Atlantic salmon parr of Mayer et al. (1990). Will the higher reproductive hormone levels of mature salmonids permit the delineation of a more clear-cut relationship between impairment of SW adaptability and reproductive endocrinology? Does SW transfer precipitate a reduction of circulating reproductive hormones in the SW readaptation process of steelhead?

These questions are addressed in the current section which focuses on post-mature steelhead and coho. Spent steelhead and spermiating male coho were transferred to SW facilities and blood-sampled over a period of weeks as salinity was gradually increased. Both mature jack and adult coho were examined in this study to determine whether a difference in endocrine levels and post-spawning survival and SW adaptability exists. Blood was analysed for hormone and sodium levels, and observations were made on behaviour and condition.

B. MATERIALS AND METHODS

On March 31 1988, Glover Creek steelhead were captured by electroshock holding over the spawning gravel in the only available spawning area in the creek, blood-sampled, tagged, and transported by tank truck to holding in 1/3 SW (10 ppt) at Pacific Biological Station (PBS) in Nanaimo, B.C. Salinity was increased to 2/3 SW on April 10 and to full SW (29 ppt) on April 21. Fish were anesthetized and force-fed once (whole or cut herring), and sampled on a weekly or biweekly basis.
Sampling was conducted on mature male coho during the winter of 1988 and spring of 1989 at Big Qualicum and Robertson Creek Hatcheries, and subsequently at PBS. At RCH, spermiating coho jacks were blood-sampled once and discarded on December 27, for comparison with mature adult males. Blood sampling was kept to a minimum in the serially sampled adults and jacks to conserve their health through to SW transfer. At Big Qualicum Hatchery adult male coho were blood-sampled as captured between Dec. 11 and 13 to provide a representative sample for spermiating adults. A group of unsampled spermiating adult males was collected at the same time.

Coho jacks from RCH (n=8) and adults (n=8) from Big Qualicum were transported to 1/3 SW at PBS on Dec. 17 and 18. Salinity was increased to 1/2 SW on Jan. 11, 1989 and to 2/3 SW on Feb. 4. Fish at PBS were anaesthetized (0.4 ml/l 2-phenoxyethanol) and force-fed oxytetracycline treated fish food (Oregon Moist Pellets: Moore-Clarke, LaConner, Wash.) and stripped of milt once weekly. Blood sampling was conducted on a biweekly basis until Feb.

22.

C. RESULTS

I. General observations

A. Activity, feeding, growth, and survival

Steelhead

Steelhead were alert and energetic from the beginning of the study. Force-feeding of steelhead with herring pieces was conducted on April 9, thereafter active feeding took place
when whole herring was offered on non-sampling days. Growth was evident in some fish. Survival over the 2 months of sampling was 60%.

Coho

Jacks were more active than adults, but not as active as steelhead, and relatively easy to catch. Force-feeding was necessary in all feeding sessions. No growth took place in adults or jacks. Over the 2 month sampling period mortality was 100% in jacks and 70% in adults. In dead fish and those killed on Feb. 22, stomachs were full to capacity with undigested food.

B. Milt production and skin colour

Steelhead

Milt was expressed by all males when collected at RCH. Only one fish was producing milt after the first week in 1/3 SW, and this ceased after the third week.

Dorsal spots began to lighten and a silvery sheen characteristic of SW fish returned to the flanks, with fading of the red lateral stripe, around the third week after capture in all but the milt-producing male, which remained darker than the other steelhead.

Coho

Both adults and jacks expressed milt through to the end of the study. All coho remained dark and kept their spawning colours.
II. Changes in reproductive hormones

A. Gonadotropin

Steelhead

All samples, including the first from fish electroshocked in FW, showed nearly undetectable levels of GtH (1.5 ng/ml). Average values from ovulated and spermiated steelhead sampled 3 weeks earlier (March 10) are included here (Fig. 31) to illustrate the magnitude of the decline in hormone levels. GtH was higher in the 2 males which died after SW transfer, and in the spermiating male, but was undetectable after the first 2 SW samples.

Coho

Plasma GtH in FW was similar in coho adult males and jacks (Fig. 32). By the time the first sample in BW was taken, one month after transfer from FW, jack mortalities had reduced their number by 50% to 4 fish. GtH was not detectable in two of these fish (below 3 ng/ml), but was relatively high in the other two. On Feb. 4, plasma GtH could be detected in only one of the three remaining fish, and on Feb. 22 no jacks were left alive.

In adult males, 5 fish survived to the first BW sample, with similar GtH levels within the group (15.4 ng/ml). GtH was detectable at around 6 ng/ml in two of three survivors on Feb. 4, and reached 6.5 and 19 ng/ml in the two surviving to Feb. 22.
Figure 31. Plasma hormone levels in spawning (March 10, FW) and post-spawning (March 31, FW; April 9, BW) steelhead, spring 1988. Solid bars: gonadotropin, diagonal hatching: testosterone, cross hatching: 17,20-P, plain: 11-ketotestosterone. Numbers above groups of bars give sample size. Values represent mean + SEM.
Figure 32. Plasma hormone levels in spawning (Dec 13 and 27, FW) and post-spawning (Jan 19-Feb 22, BW) coho males, winter 1988. Solid bars: gonadotropin, diagonal hatching: testosterone, cross hatching: 17,20-P, plain: 11-ketotestosterone. Numbers above groups of bars give sample size. Values represent mean + SEM.
B. Testosterone

Steelhead

Highest testosterone (20 ng/ml) was measured in one of the males that died before the third week in BW, and in the spermiating male. In all other steelhead, including females, T was undetectable at all sampling times in SW.

Coho

Plasma T in jack coho before transfer to BW averaged 49 ± 12 ng/ml, decreasing significantly in the next sample.

Similar T levels were seen in adult male coho before transfer (64 ± 33 ng/ml), halving in the first month in BW. In the second month, T dropped to below 1/4 of the FW value.

C. 11-Ketotestosterone

Steelhead

No spent fish had measurable levels of plasma 11-KT.

Coho

High levels of 11-KT were recorded in FW jacks. These levels remained unchanged one month later in BW, and were halved in the last survivors on Feb. 4.

Plasma 11-KT level in adult males in FW was similar to that in jacks, and followed a similar course to the Feb. 4 sample. No further decline was seen in the last survivors of this group on Feb. 22.
D. 17α, 20β-Dihydroxyprogesterone

Steelhead

17,20-P was not detectable in the plasmas of male or female steelhead.

Coho

Plasma 17,20-P was high in FW jacks and did not change during the first month in BW. In the Feb. 4 sample levels had declined significantly to 1/2 the FW value.

High levels of 17.20-P were also present in FW adult males. Levels declined after 1 month in BW and rose again in the last sample.

III. Blood sodium

Steelhead

Spent steelhead electroshocked from Glover Creek before transport to PBS had plasma sodium levels of 155 mM/l. Seventeen days later, after 1 week in 10 ppt BW, plasma sodium was still at 155 mM/l. After 1 month (May 28) in full SW plasma sodium was unchanged. There was no difference between males and females.

Coho

In jacks and adult coho in FW, blood sodium was similar to that in steelhead (153 and 154mM/l, respectively). One month later, after 1 week in 20 ppt BW, jacks had levels of blood sodium similar to those in adults (164 and 163, respectively). Two weeks later, still in 20 ppt, blood sodium level in jacks was 160 mM/l, and in the 3 adults 172 mM/l. Two adults surviving another 18 days in 2/3 SW to the final sample had blood sodium levels of 173 mM/l.
All reproductive hormones dropped below the detection limit in spent steelhead. This occurred within 1 week of spawning in Glover Creek. Estimation of spawning time of these fish is based on the observation that floods had pushed all gravid and spent fish out of the creek 10 days before electroshocking began. When the flood subsided, only gravid fish were seen in the first week; in the following week spent fish were in the majority. A decline in GtH starting about 20 days after ovulation in spent female rainbow trout has been reported (Scott et al., 1983). The GtH level remained elevated for a longer period in males, which produce milt for many weeks after the onset of spermiation (Sumpter and Scott 1989). A post-spawning decline in T and 17,20-P to undetectable levels in resident rainbow trout was also more immediate in females than in males, but still required 30 days. The more rapid decline of all sex hormones in male and female steelhead after spawning may be an important difference between resident and anadromous strains of this species. Rapid elimination of reproductive hormones from the body may facilitate the changes in osmoregulatory hormones required before reentry of SW by steelhead.

In mature, hand-stripped male coho reproductive hormones slowly declined over two months in BW. Spermiation was also maintained during this period. It is not clear to what degree continued high testicular production of steroids is required to maintain plasma levels in this range. Idler and Truscott (1963) suggested that persistence of gonadal steroids in the blood of spent sockeye may be due in part to impaired clearance. I suggest that the persistence of GtH in the blood of coho provides sufficient stimulus to the gonads for continued steroid and milt production. Production eventually declines, perhaps as energy reserves are depleted after many weeks.
The continued responsiveness of the gonads to GtH may shed light on other questions regarding general (including osmoregulatory) systems failure as spawning condition is maintained. Changes in the histology of kidneys, liver, interrenals, gills, and stomach of migrating and maturing pink salmon were described as incremental degeneration by McBride et al., (1986). Loss of function and responsiveness to hormonal signals presumably follows a similar slow course in those organs, and eventually reaches the gonads as well. No selective shutting down of systems is in evidence, in fact the potential for complete return of normal function of all organs seems to persist at least until the time of spawning. In sockeye salmon gonadectomized a few weeks before final maturation general health and proper function of all other organs returned to normal (McBride et al., 1963). Similarly in chinook, gonadectomy a few days before final maturation allowed extension of normal life for several months (McLean, 1993 in press).

In contrast, coho jacks and adult males gonadectomized after the onset of spermiation (in a study conducted as part of the current chapter, but which yielded little additional information) did not regain vigour. For example, as in the intact fish there was no resumption of digestive and peristaltic activity in the gastrointestinal tract. Cumulative changes in many systems eventually do define a point in maturation after which recovery is not possible. In the natural situation continued production of GtH in Pacific salmon may suffice to explain the persistence of high levels of sex steroids. The sex steroids may initiate, directly or indirectly a process of degeneration in all tissues, and also interfere with the ability to osmoregulate in SW.

Concurrent with the decline in sex steroid levels, hypoosmoregulatory ability developed almost immediately in steelhead after spawning. Blood sodium did not vary as salinity was increased in the holding tank and was normal in 29 ppt for SW adapted salmon,
within 1 month after transfer from FW. Morisawa et al. (1979) recorded similar levels during the prespawning SW migrations of chum salmon. Salinity in the coho tank was not increased to full SW in the light of evidence that the continuing high levels of sex steroids would not permit survival in high salinity. In 20 ppt BW blood sodium in coho was higher than that in steelhead in full SW. I suggest that the disappearance of reproductive hormones from the plasma of spent steelhead, prior to departure from the spawning area in the creek, constitutes a preadaptation for a return to SW after spawning. This reduction of reproductive hormones may be the event which initiates abandonment of the redd and downstream movement of spent fish. A rapid decline in sex steroids immediately after spawning would also remove any degenerative/catabolic activity which may be stimulated by the levels circulating in spawning fish.

Any correlation between clearance of reproductive hormones and return of hypoosmoregulatory ability does not amount to proof of direct osmoregulatory action, however. Decline of sex steroids may create the permissive environment for the resumption of production of the osmoregulatory hormones. Cortisol (Bjornsson et al., 1987; Redding et al., 1984), growth hormone (Clarke et al., 1977; Richman and Zaugg, 1987), and prolactin (Bolton et al., 1987; Hirano et al., 1987), are known to directly control function of the organs of osmotic/ionic regulation through their effects on membrane permeability and Na⁺/K⁺-ATPase activity. Concurrent measurement of reproductive hormones and prolactin during the SW maturation of farmed Atlantic salmon was performed by Anderson et al. (1991). Prolactin peaked and declined before androgen and E₂ levels rose and no change in osmotic ability occurred. Similar studies of the relationship between reproductive hormones, cortisol, and growth hormone have not been reported. There is also a need for studies of the effects of exogenous sex steroids on hypoosmoregulation, cortisol, and growth hormone in non-
maturing fish in order to elucidate links which may exist between the controlling mechanisms of reproduction and osmoregulation.
A. NORMAL MATURATION OF CHINOOK AND STEELHEAD

1. Females

The changes in reproductive parameters and hormones during the final weeks of sexual maturation in wild coastal salmon; chinook, *Oncorhynchus tshawytscha,* and steelhead, *O. mykiss,* were documented for stocks spawning in two similar river systems on the west coast of Vancouver Island, British Columbia, Canada. The two species are similar in that the bulk of their growth and oogenesis take place in the marine environment while spawning occurs in freshwater after the growth phase. Both species normally make the transition from seawater to freshwater before final maturation. The major difference in life-history is that chinook spawn in the fall and die soon after; steelhead although entering freshwater in fall, spawn in spring and often survive to return to the ocean.

Oocytes of both species sequester vitellogenin from the blood until 2 or 3 weeks before final maturation, and continue to hydrate through to ovulation. There are no differences in GSI, HSI or oocyte water content between the species at ovulation.

Patterns of hormone concentration are similar in chinook and steelhead, but the peaks in concentration of the steroids at each stage are lower in steelhead. A decline in E₂ preceded a rise in maturational GtH, which is believed to initiate final maturation in salmon (Dickhoff and Swanson, 1989), by about two weeks. Presumably vitellogenesis continues in steelhead for several months after FW entry. Plasma T was relatively high in both species at
the beginning of the decline in E₂, and declined towards ovulation. GtH was lower at ovulation in steelhead than in chinook, not reaching the levels seen in ovulating chinook until several days after ovulation.

The somewhat lower level of the maturation inducing hormone, 17,20-P, in steelhead may be due to the lower level of GtH stimulation leading up to ovulation in that species. In chinook females an abrupt rise in plasma 17,20-P, from basal levels to the highest levels, occurred at ovulation.

Blood sodium was elevated in chinook captured in high salinity water near the outlet of Nitinat Lake. At all other times and locations during unimpeded migration blood sodium was in the range of normal values for unstressed salmon (Lam et al., 1982; Sower and Schreck, 1982; Blackburn and Clarke, 1987).

2. Males

Gonadotropin was detectable in the first samples of Nitinat male chinook before FW entry and rose as maturation commenced, peaking in spent fish. In steelhead males GtH was not detectable at any time during final maturation. The difference in GtH production may explain the considerably lower levels of maturational steroids produced in steelhead as compared to chinook.

Testosterone and 11-KT rose with approaching maturation in chinook. Between the mid-river sample of sub-mature fish and the sample of mature fish at Nitinat hatchery T declined and 11-KT rose, such that 11-KT was higher than T at spermiation. Both steroids declined after spermiation in chinook. 11-KT concentration was higher than that of T in steelhead at spermiation as well. These patterns, seen in other salmonid males during
maturation (Dye et al., 1986; Ueda et al., 1984a), indicate that 11-KT is more involved with the later stages of spermatogenesis than is T.

A surge of 17,20-P accompanies spermiation in chinook, while in steelhead 17,20-P level was maintained at quite low levels. It is not clear whether the low levels of 17,20-P in spermiating steelhead males is normal for the species or a consequence of suppression resulting from the holding conditions for these fish. In chinook 17,20-P continued to rise after the onset of initial spermiation, and large volumes of milt were obtained from spent fish; evidence which supports the concept of 17,20-P as the maturational hormone in male as well as female salmon (Ueda et al., 1985; Schulz and Blum, 1990), but is also consistent with the view of Marshall et al. (1989) that 17,20-P is involved with the production of seminal fluid.

B. MATURATION OF CHINOOK AND CHUM IN BRACKISH WATER

In 1988, mortality was higher in eggs taken earlier in the season from both BW and FW chinook than in eggs taken later in the season at the same locations. The first females maturing in FW had recently left BW after extended residence in an environment of high salinity and temperature. The first females maturing at the BW net pen site were exposed to the same conditions for a longer period. Fertility was highly variable in the first females from either location, and the high average mortalities were due to exceptionally high mortality in a few batches of eggs.

Subsequent samples taken from the net pens and hatchery after the beginning of autumn rains had lower mortality rates, but the incidence of high mortality remained
greater in BW eggs. Mortality rates after fertilizations with sperm from males maturing at either location suggest that maturation site does not affect fertility of sperm. A few batches of eggs having exceptionally high mortality again brought the average up for eggs from either location. The occurrence of batches of eggs with near 100% mortality was reported for coho by Sower and Schreck (1982) and for chum by Lam *et al.* (1982) who linked the phenomenon with high blood sodium in females before or during ovulation.

Fall weather had brought about a marked lowering of temperature and salinity and an elevation of dissolved oxygen in the lake by the time eggs were taken from the third group of chinook in 1988. Mortality was slightly higher in these eggs than in eggs taken well into the rainy season at the hatchery in 1989, and considerably lower than in the first 2 groups of 1988. One batch of eggs in the last BW group suffered 100% mortality and the majority of the others had mortalities comparable to those sampled in the hatchery over the same period. Blood sodium in the BW females was significantly higher than in hatchery females. In particular sodium was highest in the female contributing 100% mortality. However, a clear correlation between blood sodium and mortality of eggs, as found by Sower and Schreck (1982), could not be demonstrated in this study. The occurrence of high mortality in the first group of FW eggs, from females exposed to high salinity in the days immediately preceding ovulation, suggests this is a sensitive period of final maturation.

An important finding of this study was the fact that mortality in the majority of the last batches of chinook eggs from the net pens was no higher than in chum eggs taken at the net pens and hatchery one week later in the 1988 season. Toward the end of the chinook migration period in Nitinat Lake improvement of environmental conditions, stemming from lower salinity and temperature, and higher oxygen, are more favourable to net pen maturation. The perceived difference between chum and chinook does not reflect a basic
physiological difference leading to differences in ability to withstand holding in BW. The past differences in egg mortality rate between BW chum and chinook instead reflect the difference in time of arrival at the lakehead. Chum arrive later when conditions are already more favourable, and do not normally experience osmoregulatory difficulties. In years when substantial rains precede or coincide with the arrival of chinook at the lakehead estuarine capture and holding of chinook should be considered as a viable option.

C. SEAWATER TRANSFER OF SALMON AND STEELHEAD

The hypothesis that transfer of steelhead into SW may bring about a rapid decline in reproductive hormones that facilitates SW readaptation could not be tested in this study. Reproductive hormones declined to basal levels immediately after spawning, before fish had abandoned the spawning area in the creek. The rapid post-spawning decline in reproductive hormones sets steelhead apart from coho (and other Pacific salmon) which continue to produce and secrete GtH, 17,20-P and androgens for some weeks after spermiation.

Hand-stripping of milt does not have the effect of lowering sex hormones or GtH, or improving hypoosmoregulatory ability in mature coho. Steelhead resumed feeding, lost their spawning colours, and adapted to SW soon after disappearance of reproductive hormones from the blood and transfer from FW. Coho are not capable of any of these physiological changes, and appear to be "locked" into a program of high steroid production. That the gonads of mature coho continued to respond to GtH stimulation is reason to believe other organs, including those involved in hypoosmoregulation, were operative and capable of responding to hormonal stimulation. Coho were unable to regulate blood sodium however,
and the presence of high levels of sex steroids in the blood at this time suggests interference by the sex steroids in the control mechanisms of osmoregulation.

Other methods of examining the hypothesis of steroid interference in (hypo) osmoregulatory ability may be available in the use of gonadectomized or otherwise sexually dysfunctional fish, where steroid metabolism by the gonads can be eliminated.
REFERENCES


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