STATE OF SEXUAL MATURITY OF ATLANTIC SALMON (Salmo salar L.) CAUGHT AT SEA, IN COMMERCIAL FISHERIES OFF NEWFOUNDLAND

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STATE OF SEXUAL MATURITY OF ATLANTIC SALMON (*Salmo salar* L.) CAUGHT AT SEA, IN COMMERCIAL FISHERIES OFF NEWFOUNDLAND.

BY

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ABSTRACT

The state of maturity of male and female Atlantic salmon harvested at sea during 1990 and 1991 at three sites in Newfoundland was investigated. Salmon landed at St. Barbe Bay, Conche and Twillingate were sampled throughout the commercial fishing season (June - August). The methods used to determine the state of maturity in male and female salmon were histological examination of gonadal development and radioimmunoassay of plasma steroid hormone levels. The gonad stage development, gonadosomatic index (GSI), hepatosomatic index (HSI), condition factor (CF), testosterone (T), 11-ketotestosterone (11-KT), vitellogenin (Vg) and 17β-estradiol (E2) levels measured in commercially caught salmon were compared with those of cage-reared and wild salmon of known background to verify the maturity classifications. Stages of gonad development, the levels of T, E2, 11-KT, Vg, and GSI, HSI and CF measured in male and female salmon of known state of maturity were used to develop discriminant functions for predicting the state of maturity in commercially caught salmon. Salmon sampled in St. Barbe Bay in the Gulf of St. Lawrence were 100% spawners-of-the-year, whereas, samples from Conche and Twillingate on the northeast coast of Newfoundland consisted of salmon that would not have returned to their native rivers to spawn. Differences in biological characteristics of salmon from the commercial fishery locations tend to support the maritime origin of the non-maturing component of commercial landings in

Newfoundland. Male and female Atlantic salmon that are not spawners-of-the-year can be recognized by discriminant analysis techniques on the basis of the stage of gonad development, plasma hormone levels, and GSI.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

Atlantic salmon (Salmo salar L.) may spend one (sea-age-1), two (sea-age-2) or more years at sea before returning to their natal streams to spawn. On their return spawning migration, both sea-age-1 and sea-age-2 salmon have traditionally contributed to commercial salmon fisheries harvests in eastern Canadian waters. It has been widely speculated, however, that not all of the commercially caught, sea-age-1 salmon are returning spawners-of-the-year but are instead, non-maturing fish which would have remained at sea for an additional year before returning to spawn as sea-age-2 salmon. It is important to know if this is the case because sea-age-1 salmon that are not spawners-of-the-year would generally attain a larger size and have a higher fecundity if they could be permitted to complete the marine phase of their life history and return to spawn at sea-age-2.

Also, from the point of view of resource management, the recognition of salmon that are not spawners-of-the-year in commercial landings would explain some of the annual variation in the number of large, sea-age-2 salmon returning to spawn in eastern Canadian rivers. One of the problems in forecasting the relative abundance of sea-age-2 salmon (from commercial catches of the sea-age-1 cohort in the previous year) has been in not knowing the proportion that are caught in commercial fisheries

before they reach sexual maturity (Chadwick, 1985). If commercial harvests* could be restricted to spawners-of-the-year, then the potential value of the Atlantic salmon resource would be increased.

The results of several marine tagging and recapture studies conducted by the Department of Fisheries and Oceans, Canada in the past two decades, have been used to support the view that coastal commercial salmon fisheries in Newfoundland in particular, exploit non-maturing salmon. It has been speculated (Turner, 1975a; Turner, 1975b; Peppar, 1982; Peppar, 1983; and Pippy, 1982) that maritime origin (tagged as smolts) adult salmon (sea-age-1, -2, -3) caught off Newfoundland in late May to late July would have missed, had they not been caught, the peak migration period (July) for salmon ascending maritime rivers to spawn. It has been suggested that these fish are not spawners-of-the-year but instead would have remained at sea for another year before ascending their natal rivers to spawn. Reddin and Lear (1990) pointed out that several sea-age-1 salmon, tagged off northeastern Newfoundland, were recaptured the year after tagging, suggesting that they did not spawn in the year tagged but remained at sea and would return to spawn the following year. However, no information on the state of maturity of the recaptured fish was presented. It is also possible, given a swimming speed of about 32 km per day (Pippy, 1982), that salmon

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* **Footnote:** The Newfoundland commercial salmon fishery has been closed since 1992, but the fishery remains open in Labrador and off west Greenland.

present off northeastern Newfoundland in late July could swim the approximately 1,600 km distance in time to return as part of the fall-run to maritime rivers. However, this possibility is usually given only secondary consideration in the literature.

In a previous study based on physiological indicators of relative maturity, Idler et. al (1981) concluded that 86-91% of the female salmon captured in the commercial fishery off northeastern Newfoundland were spawners-of-the-year. Female salmon with a plasma vitellogenin (Vg) level higher than 396 ug/ml were considered to be maturing fish. However, this value was determined from six and 19 females of known maturity in two separate years, and may not completely represent the variability in levels of Vg in maturing Atlantic salmon at sea. Furthermore, the Vg methodology of the time (Idler et al. 1979), was not completely quantitative for plasma Vg (D. Idler, OSC, Memorial University of Newfoundland, personal communication).

The present study was based upon an improved quantitative method for measuring plasma vitellogenin levels. So et al. (1985) succeeded in isolating vitellogenin from landlocked Atlantic salmon plasma and developed a homologous radioimmunoassay to quantify circulating vitellogenin. This new technique was adopted in this study to measure plasma vitellogenin in commercially caught female salmon and to evaluate the seasonal profile of vitellogenin in Atlantic salmon of known background. Several steroid hormones have also been associated with sexual maturation in male and female Atlantic salmon that may be useful to evaluate the state

of maturity of individual fish. The steroid hormone, 11-ketotestosterone (11-KT), was first identified in the plasma of sockeye salmon (Oncorhynchus nerka) by Idler et al. (1960) and was quantified as the major androgen in the plasma of male Atlantic salmon during sexual maturation (Idler et al. 1970). Testosterone (T) was also found in the plasma of sexually maturing male Atlantic salmon (Idler et al. 1970). Extremely high levels of testosterone in sockeye salmon during their upstream migration decreased significantly after spawning (Truscott et al. 1986). The steroid, estradiol (E2), was measured in progressively higher concentrations in the plasma of female brown trout (Salmo trutta) during seasonal maturation (Crim and Idler, 1978).

The objectives of this study are: 1. to develop physiological and histological criteria to discriminate between spawners-of-the-year and non-spawners; 2. to use these criteria to identify spawners-of-the-year and non-maturing salmon in commercial salmon fishery landings.

2. MATERIALS AND METHODS

2.1 Study Areas

Blood and gonad samples were collected from 297 commercially caught virgin sea-age-1 male and female Atlantic salmon of unknown state of sexual maturity. Their state of maturity as determined on the basis of reference profiles of gonad development and steroid hormone levels established for 433 maturing and non-maturing salmon sampled at four reference sites. The salmon sampled from the reference sites included virgin reared and wild fish (Table 1).

Commercial landings were sampled at St. Barbe Bay, Conche and Twillingate, Newfoundland (Fig. 1). St. Barbe Bay, in the Gulf of St. Lawrence, was chosen because of a low incidence of recaptures in the area of salmon tagged as smolts in maritime river systems (Turner, 1975b; Pippy, 1982; Peppar, 1983) which suggests that commercial catches in St. Barbe Bay are predominantly of local origin. Long-term studies in the St. Barbe Bay area indicate that spawning adult salmon in local rivers are about 99% sea-age-1 fish. Therefore, commercially caught sea-age-1 salmon in this area are likely to be spawners-of-the-year. Conche and Twillingate, located on the northeast coast of Newfoundland, were chosen because of the relatively high incidence of recaptures of sea-age-1 salmon, during July and August which were tagged as smolts in rivers of eastern New Brunswick (Saunders, 1969; Turner, 1975b).

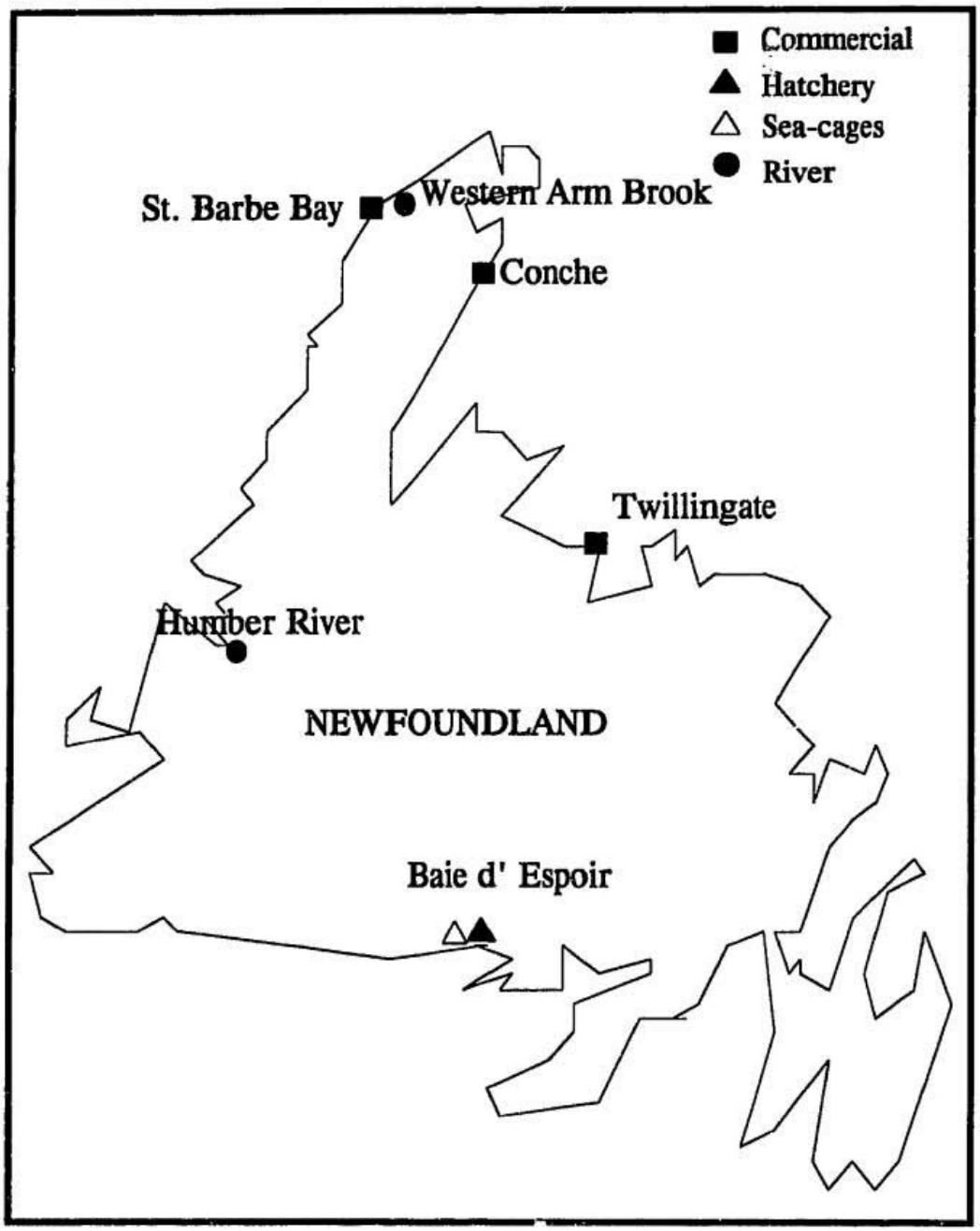


Figure 1. Sampling locations in Newfoundland.

Table 1. Summary of sampling locations, dates, number and sex composition of Atlantic salmon sampled in 1990 and 1991.

Location	Sampling Date	Total Sample Size	Total number of virgin fish at Sea–age 1yr	Number Virgin Males	Number Virgin Females	Percent Virgin Females
Commercial Fishery Sites:						
St. Barbe Bay	Jun. 26–Jul. 14, 1990	37	32	9	23	71.9
St. Barbe Bay	Jun. 28–Jul. 25, 1991	12	4	1	3	75.0
Conche	Jun. 13–Aug. 3, 1990	161	91	57	34	37.4
Conche	Jul. 9–Aug.4, 1991	52	49	32	17	34.7
Twillingate	Jun. 24–Jul. 4, 1991	35	35	13	22	62.9
	Sub–total	297	211	112	99	.
Reference Sites:						
Saint John River Reared Stock						
Hatchery	May 12, 1990	52	0	23	29	55.8
Sea–Cages	Oct. 15, 1990	51	0	21	30	58.8
Sea–Cages	May 4, 1991	49	0	20	29	59.2
Sea–Cages	Jul. 30, 1991	50	0	23	27	54.0
Sea–Cages	Nov. 9, 1991	53	0	27	26	49.1
	Sub–total	255	0	114	141	55.37
Wild Stocks						
Humber River	Jul. 18–Aug. 26. 1991	16	16	5	11	68.8
Western Arm Brook	Aug. 9.&Oct. 18. 1990	12	12	3	9	75.0
Labrador Sea	Oct. 6–Oct. 14. 1991	150	145	34	111	76.6
	Sub–total	178	173	42	131	.

The landings of several fishermen were sampled during the commercial fishing season at St. Barbe Bay and Conche in 1990 and 1991, and during the 1991 season at Twillingate. The season opened on June 5 and closed on October 15 in 1990 and 1991 at all three locations. However, no landings were made before mid-June and few were made after the end of July at either St. Barbe Bay or Conche. The majority of landings from these two locations were in month of July, whereas, those from Twillingate were in late June and early July.

Commercial salmon landings are usually culled into **small** (<2.7 kg) and **large** (≥ 2.7 kg). Small salmon were generally less than 63.0 cm fork length and large salmon were greater than or equal to 63.0 cm fork length. A minimum of 6 male and 6 female small salmon were sampled per week, during the fishery in 1990, and a minimum of 11 males and 11 females were sampled per week, in 1991. Large salmon were fewer in number, therefore, these were sampled opportunistically, throughout the fishing season.

Reference samples of reared salmon were collected at Baie d'Espoir, Newfoundland (Fig. 1) in 1990 and 1991 from hatchery-reared smolts and cage-reared post-smolts which originated from the Saint John River, New Brunswick stock. About 50 male and female salmon were sampled on five occasions (Table 1). The first samples were collected on May 12, 1990 when the cohort (18 month old smolts) was transferred from the hatchery in St. Alban's, Baie d'Espoir into sea-cages located at Roti Bay, approximately 10 km south. Subsequent samples were collected from the

sea-cages on 1. October 15, 1990, prior to winter freeze-up; 2. May 4, 1991, immediately after spring break-up; 3. July 30, 1991, corresponding to the period of sampling in the commercial fishery; and 4. November 9, 1991, during the harvest of maturing adults. The 6.0 m depth sea-cage enclosures were located in an area with a bottom depth of 40 m. The summer water temperature in Roti Bay ranged from 13.0 to 15.0 C and the salinity was 20 parts per thousand at a depth of 2-10 m. The caged fish were captured by sweeping the enclosure with a small mesh seine. Fish likely to be holding near the bottom of the cage were enticed towards the surface prior to sampling by agitating the cage with a long stick. This ensured a random sample, especially during the latter sampling periods, when sexually mature fish tended to occupy the bottom of the cage as they stopped feeding.

Reference samples of wild salmon of a known state of sexual maturity were sampled from three reference sites: 1. the Labrador Sea off the southwest coast of Greenland, 2. Western Arm Brook which flows into St. Barbe Bay, and 3. the Humber River (Fig. 1). The Labrador Sea samples were obtained in October, 1991 from a research cruise. Western Arm Brook samples were collected in August and October, 1990 from a counting fence located at the mouth of the river; and the Humber River samples were collected in July and August in 1991 from a trapnet, located in the estuary of the river. The Western Arm Brook and Humber River salmon were caught during their upstream migration, therefore, were considered to be maturing spawners-of-the-year. On-the-other-hand, the Labrador Sea salmon, caught

in October of 1991, well away from their North American spawning grounds, were not going to spawn in the year of capture.

2.2 Histology

One cubic centimetre of tissue was removed from the middle of the left gonad of all the fish sampled. The tissue was fixed in Bouin's fixative for 24 hours and rinsed in 30% and 50% ethanol for 1 hour each, prior to storage in 70% ethanol. All gonad samples collected in a given day were placed in individual containers and fixed as a batch. The volume of the fixative was at least twice the total tissue volume.

Ovarian and testis tissue (1.0 cm x 0.5 cm x 0.5 cm) from the original sample was dehydrated in successive rinses of 85%, 95% and 100% ethanol and then cleared in two changes of toluene (Appendix 1). The fluids were agitated with magnetic stirrers to reduce the dehydration and clearing times. Cleared tissue blocks were infiltrated with paraffin wax at approximately 60 C and embedded in paraffin for sectioning.

Paraffin-embedded ovarian tissue, especially from adult fish was very friable and required an additional infiltration step for stability during sectioning. The double infiltration technique described by Humason (1979) was used which adds celloidin dissolved in methyl benzoate before the paraffin infiltration (Appendix 1).

At least five good sections were selected from those cut from each tissue block. Ovarian tissue was cut at a thickness of 10-12 um. Testicular tissue was sectioned at 8 um. Sections were mounted on glass slides with albumin, stained with Harris' haematoxylin and eosin (Humason, 1979) (Appendix 2).

Histological specimens were examined under a Leitz (DIALUX-20) compound microscope equipped with a moveable stage. Each slide contained serial sections from the same gonad. The section to be examined was chosen at random using the random number function of an HP 11C calculator.

2.2.1 Oogenesis

Female salmon were classified into ovary development stages on the basis of the most advanced modal oocyte development stage present. The most advanced modal oocyte stage was considered to be representative of the level of ovarian development in individual fish and was determined from the frequency distribution of oogonia and oocytes corresponding to 12 stages of oogenesis. These stages were based on criteria described for Atlantic salmon by Murza and Khristoforov (1993), Chadwick et al. (1986), Sutterlin and MacLean (1984) and Idler et al. (1981) and for adult chum (Oncorhynchus keta) and sockeye salmon by Ishida et al. (1961). For example, an individual fish with oocyte modes at stages 3 and 6 would be classified into stage 6, if the number of oocytes at stage 6 was greater than at stage 3.

The frequency distribution of oocytes was determined by counting all the cells in a randomly chosen histological section of ovary. Oocytes were classified and counted in a series of transects. Oogonia and nucleated oocytes at stages 2-6 were counted at a magnification of 250X and oocytes at stages 7-11 were counted at 25X. Because oil globules often covered the nuclei in stages 7-11, these counts included non-nucleated oocytes.

Female salmon from Western Arm Brook in 1990 that were sampled on their upstream migration were not sacrificed, but were assigned to ovary stage 11 on the basis of the frequency distribution of oocytes recorded in one dead specimen.

The histological protocols used for ovaries with stage 1-11 oocytes (Appendices 1-2) were not suitable for those with stage 12 oocytes. Therefore, stage 12 oocytes were not counted.

The criteria for the 12 stages of oocyte development used to classify the ovaries of Atlantic salmon are described below:

Stage 1. Oogonia - oogonia are round to oval in shape with a small centrally located nucleus; usually in groups or "nests" of 5-40 cells. 5-6 μm in size.

Stage 2. Primary oocyte - the nucleus occupies most of the oocyte and contains one large nucleolus. 10-20 μm in size (Fig. 2).

Stage 3. Early perinucleolus - nucleoli are small, numerous and located around the periphery of the nucleus. Intensely staining clouds of mitochondria ("Balbiani bodies") are concentrated around or adjacent to the nucleus. 20-100 μm in size (Fig. 2).

Stage 4. Mid-perinucleolus - "Balbiani bodies" are fragmented, no longer concentrated near the nucleus, but migrating toward the periphery of the cell. Intensity of cytoplasmic staining fades. 100-200 μm in size (Fig. 3).

Stage 5. Late perinucleolus - "Balbiani bodies" are concentrated in a ring at the periphery of the oocyte, and usually fade from view as they disperse into the cytoplasm; marks the end of the pre-vitellogenic phase of development. 250-400 μm in size (Fig. 4).

Stage 6. Yolk vesicle - clear yolk vesicles appear in the cytoplasm, initially around the periphery and then towards the nucleus. The nucleus has a smooth outline at the beginning of the stage but becomes indented in the latter part of the stage. The "Balbiani bodies" are dispersed. The follicular epithelium or granulosa layer thickens and a thin layer of zona radiata is visible (Fig. 5).

Stage 7. Oil globule - oil globules appear first near the nucleus and then throughout the cytoplasm, often obliterating the nucleus. Oocytes increase dramatically in size from the previous stage. The zona radiata is clearly visible and radial striations are recognizable (Fig. 6).

Stage 8. Primary yolk - yolk globules are concentrated in the outer part of the cytoplasm. Globules stain violet to black (Fig. 7).

Stage 9. Secondary yolk - yolk globules are yellow in colour, larger and more numerous, and occupy a greater part of the cytoplasm than the previous stage. Yolk vesicles are pushed toward the periphery and oil globules disappear (Fig. 8).

Stage 10. Tertiary yolk - yolk globules are small around the nucleus and larger at the periphery. Yolk vesicles form a very thin layer at the periphery of the oocyte (Fig. 9).

Stage 11. Migratory nucleus - nucleus migrates towards one pole; yolk globules are smaller ahead of the migrating nucleus; many yolk globules become fused to form larger yolk mass (Fig. 10-11).

Stage 12 Ripe gonad - oocytes can be expelled easily with gentle pressure to the abdomen.

Legends to Figures 2-11. Stages of ovary development observed in female Atlantic salmon.

Plate-I

Figure 2. Ovary stage 3 (16X) of hatchery-reared female salmon smolt sampled on May 12, 1990 (Sample No. SJ1F29) showing oocytes at stages 2-4.

Figure 3. Ovary stage 4 (16X) of cage-reared female salmon sampled on October 15, 1990 (Sample No. SJ2F28) showing oocytes at stages 3-5.

Figure 4. Ovary stage 5 (16X) of cage-reared female salmon sampled on May 4, 1991 (Sample No. SJ3F50) showing oocytes at stages 5 & 6.

Figure 5. Ovary stage 6 (16X) of cage-reared female salmon sampled on May 4, 1991 (Sample No. SJ3F42) showing oocytes at stage 6.

Plate-II

Figure 6. Ovary stage 7 (16X) of cage-reared female salmon sampled on November 9, 1991 (Sample No. SJ5F9) showing oocyte at stage 7.

Figure 7. Ovary stage 8 (16X) of cage-reared female salmon sampled on November 9, 1991 (Sample No. SJ5F46) showing oocyte at stage 8.

Figure 8. Ovary stage 9 (16X) of wild female salmon sampled at Conche on August 4, 1991 (Sample No. C91F54) showing oocyte at stage 9.

Figure 9. Ovary stage 10 (16X) of wild female salmon sampled at St. Barbe Bay on July 14, 1990 (Sample No. SB90F249) showing oocyte at stage 10.

Plate-III

Figure 10. Ovary stage 11 (16X) of wild female salmon sampled at Conche on July 15, 1991 (Sample No. C91F27) showing oocyte at stage 11.

Figure 11. Ovary stage 11 (16X) of wild female salmon sampled at St. Barbe Bay on July 2, 1990 (Sample No. SB90F215) showing oocyte at stage 11.

PLATE-I

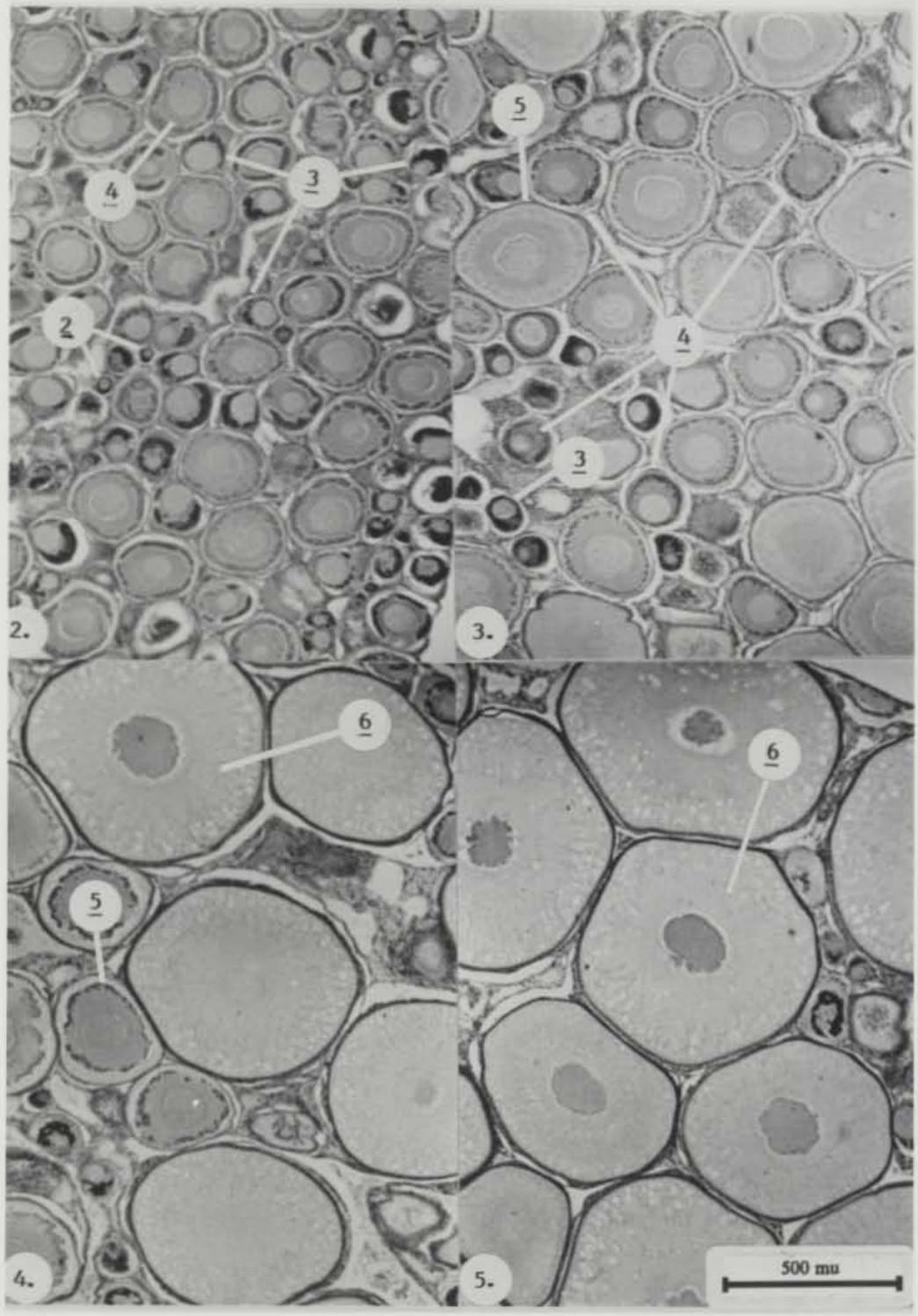


PLATE-II

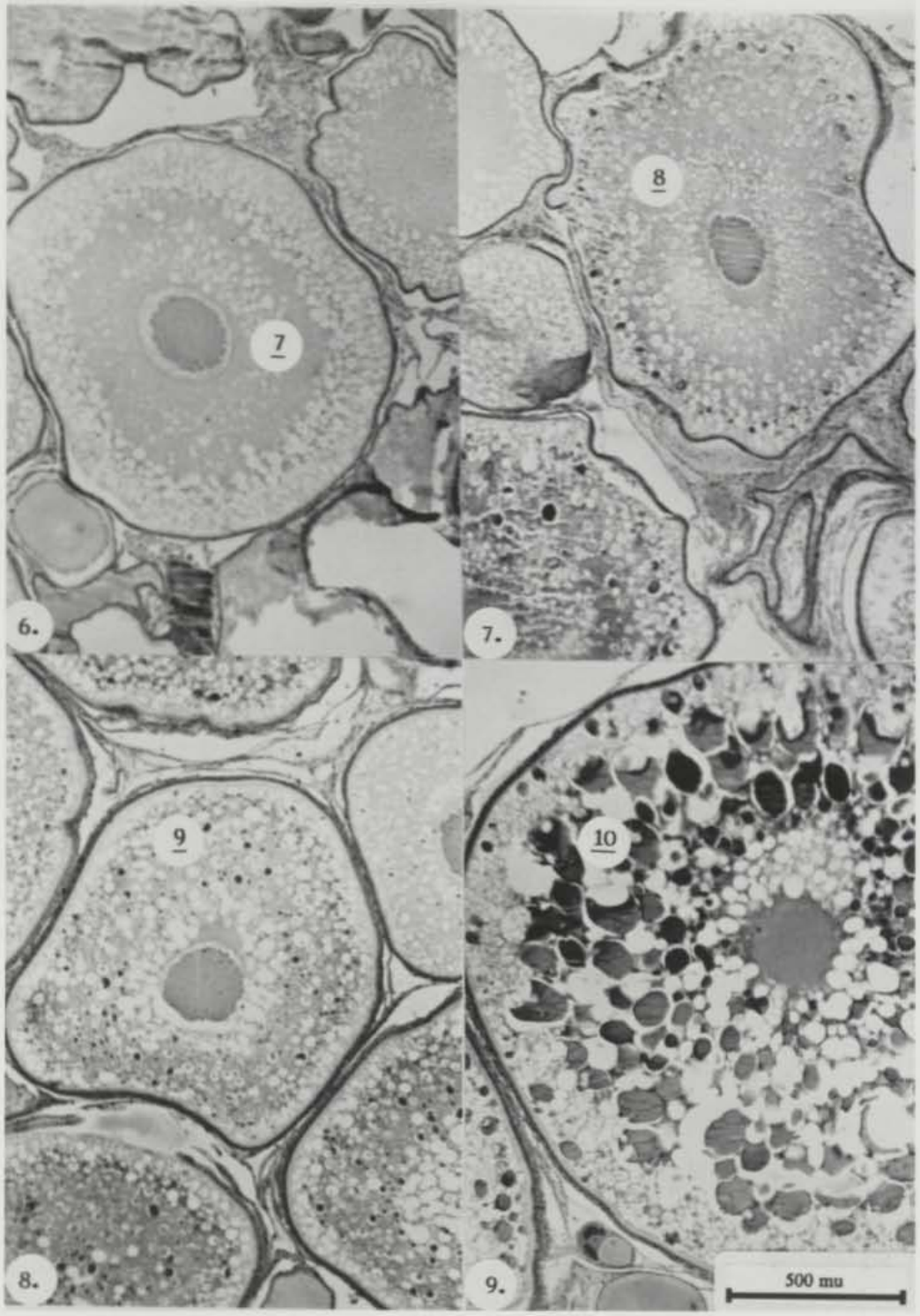
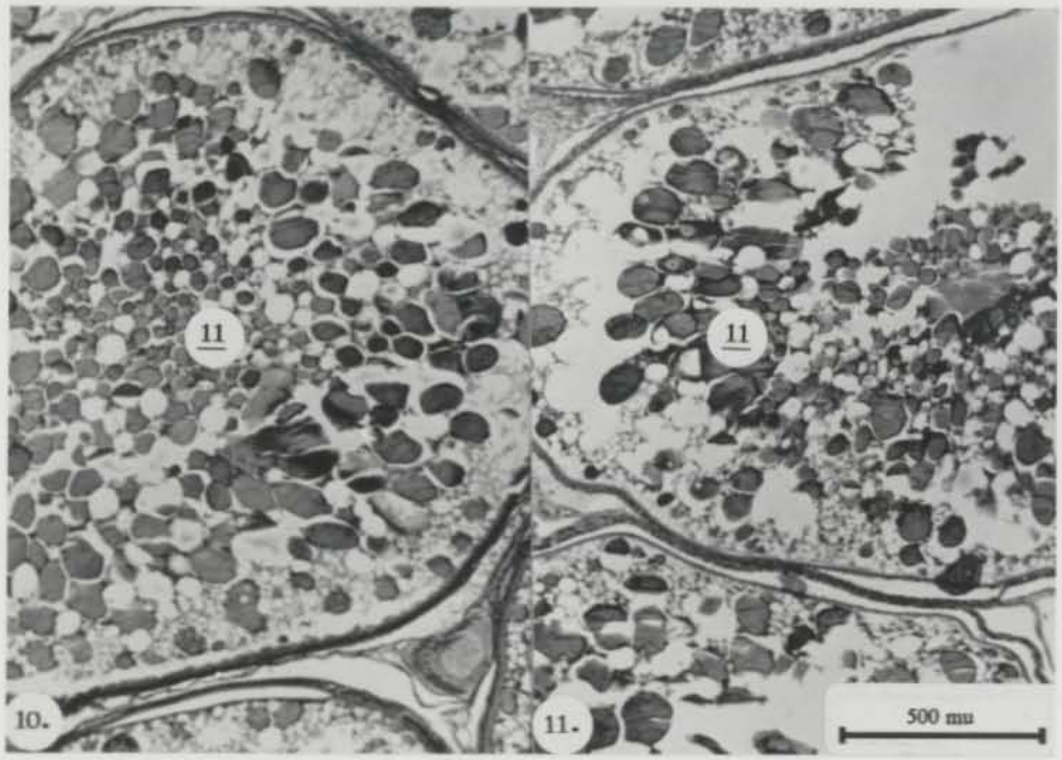


PLATE-III



2.2.2 Spermatogenesis

Testes at stages 1-5 were examined at 1000x magnification. In contrast to female oocytes, the germ cells of salmon testes are too small and too numerous to count. Therefore, male salmon were classified into testis development stages on the basis of the most advanced germ cell stage.

Testis tissue was not obtained from upstream migrating male salmon sampled from Western Arm Brook in 1990, but it was evident from the milt expelled during the blood sampling procedure that these fish were at stage 5.

The most advanced germ cell stage present was determined using criteria described by Hiroi and Yamamoto (1968), and Murza and Khristoforov (1993). These criteria are summarized below:

Stage 1. Primary germ cell - spermatogonia are the largest cells in the testis. Nucleus 7-13 μm cytoplasm 10-20 μm (Fig. 12).

Stage 2. Spermatogonia - some spermatogonia are smaller than in the previous stage and stain darker. Nucleus 5-7 μm cytoplasm 7-14 μm (Fig. 13).

Stage 3. Spermatocyte - germ cells are grouped in distinct cysts; the cytoplasm in individual cells is almost indistinguishable from adjacent cells. Nucleus 3-4 μm (Fig. 14).

Stage 4. Spermatid - cysts are much larger than in the previous stage; germ cells are round in shape and much more numerous. Nucleus 2-2.5 μm (Fig. 15).

Stage 5. Spermatozoa - large cysts with spermatids predominate. The head of mature sperm is round or elliptical in shape. Nucleus 1-1.5 μm (Fig. 16-17).

Stage 6. Ripe gonad - cysts rupture releasing spermatozoa which are expelled easily by gentle pressure to the abdomen. Testes at this stage were not examined histologically.

Legends for Figures 12-17 . Stages of testicular development observed in male Atlantic salmon.

Plate-IV

Figure 12. Testis stage 1 (100X) of cage-reared male salmon sampled on May 4, 1991 (Sample No. SJ3M30) showing primary spermatogonia (1sg).

Figure 13. Testis stage 2 (100X) of cage-reared male salmon sampled on May 4, 1991 (Sample No. SJ3M33) showing primary (1sg) and secondary spermatogonia (2sg).

Figure 14. Testis stage 3 (100X) of cage-reared male salmon sampled on July 15, 1991 (Sample No. C91M11) showing secondary spermatogonia (2sg) and spermatocytes (sc).

Figure 15. Testis stage 4 (100X) of cage-reared male salmon sampled on July 17, 1991 (Sample No. C91M38) showing secondary spermatogonia (2sg), spermatocytes (sc), and spermatids (st).

Plate-V

Figure 16. Testis stage 5 (100X) of cage-reared male salmon sampled on July 30, 1991 (Sample No. SJ4M49) showing secondary spermatogonia (2sg) and large cysts of spermatids (st), and spermatozoa (sz).

Figure 17. Testis stage 5 (100X) of wild male salmon sampled from the estuary of the Humber River on August 26, 1991 (Sample No. H91M14) showing large cysts of spermatids (st) and spermatozoa (sz).

PLATE-IV

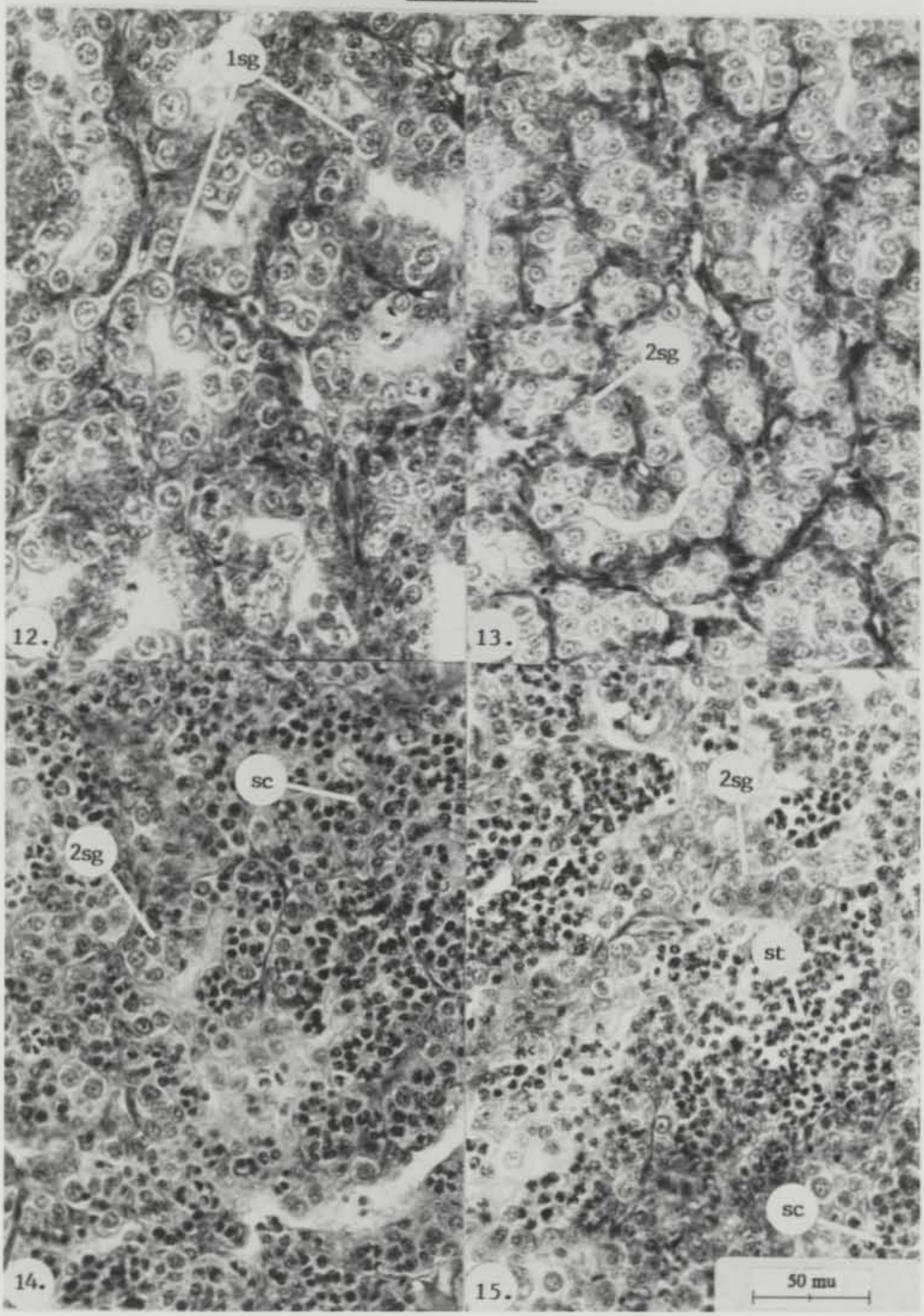
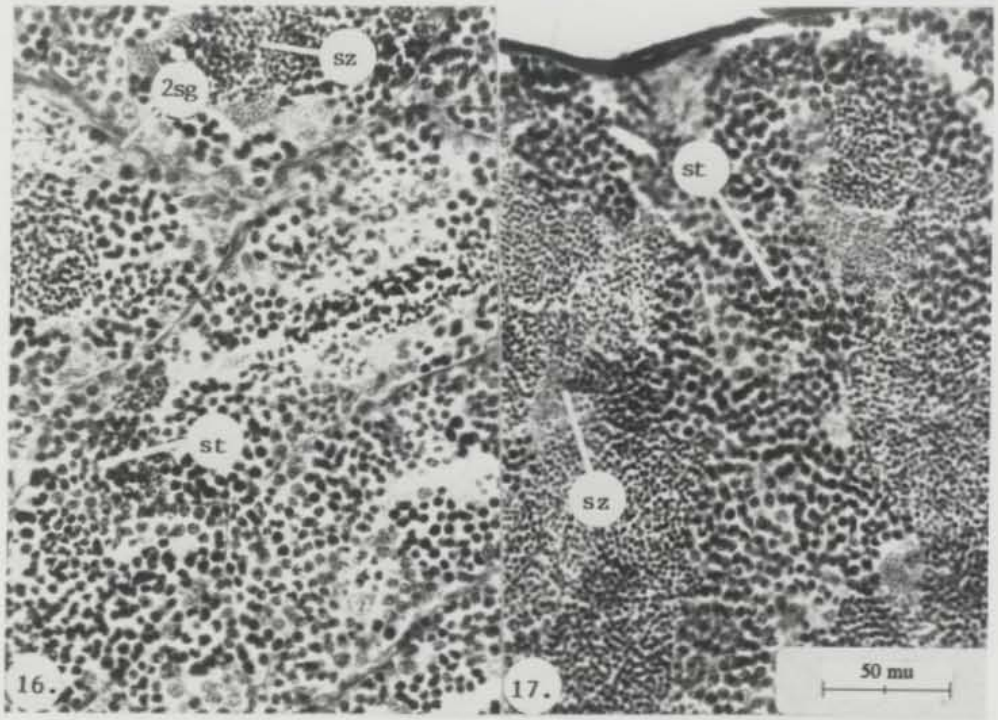


PLATE-V



2.3 Radioimmunoassay

Approximately 1.5 ml of blood was extracted from the caudal vein using a heparinized syringe. The blood was kept on ice, in the syringe, for 1-3 hours before being transferred to 1.5 ml Eppendorf tubes. Tubes were centrifuged at 10,000 rpm for two to four minutes then the plasma layer was removed using a clean Pasteur pipet and transferred to a clean 1.5 ml tube. Plasma samples were frozen and stored at -20.0 C until processed.

All salmon were dead at the time of blood sampling. Salmon from sea-cages were sacrificed quickly with several quick blows to the head immediately before sampling. They were usually dead after the first blow. In commercial landings, the post-mortem period before sampling varied considerably. Salmon that were alive when removed from the commercial gillnets were killed soon after being taken into the boat, and by the time they were landed at the wharf the majority had been dead for about one hour according to fishermen. Some of the blood samples collected from commercial landings were clotted when centrifuged and the extracted plasma was contaminated with ruptured red blood cells. This was found to be the case when post-mortem periods in exceeded two hours based on trials conducted on blood samples collected from sea-cages. Hence, shorter post-mortem periods are recommended. Contaminated samples were discarded and not used in the analysis.

Blood plasma levels of testosterone (T), estradiol-17B (E2), 11-ketotestosterone (11-KT) and vitellogenin (Vg) were measured in duplicate by radioimmunoassay (RIA) as described by Methven et al. (1992). The procedures used for RIAs of 11-KT, T, and E2 are outlined in Appendices 3-6.

2.4 Biological Characteristics

Salmon were sexed and measured for fork length (FLT), to the nearest 0.1 cm; whole weight (WWT) to the nearest 200 gm; gonad weight (GOWT) and liver weight (LWT) to the nearest 0.01 gm. Scale samples for age determination were collected from wild salmon. Scales were removed from an area above the lateral line and just posterior to the dorsal fin. The river-age, sea-age and evidence of previous spawning in wild salmon were determined by means of scale reading according to established criteria (Anon., 1984).

Only virgin male and female salmon that had a sea-age of one year were used in analyses of the state of maturity.

Gonadosomatic index (GSI = GOWT / WWT x 100); hepatosomatic index (HSI = LWT / WWT x 100); and condition factor (CF = FLT^3 / WWT / 100) were calculated for each fish sampled.

2.5 Discriminant Analysis

Discriminant analysis is a multivariate statistical technique used to identify the relationships among distinct groups of observations on the basis of measured parameters. This technique was used to identify the state of maturity in male and female Atlantic salmon on the basis of levels of T, 11-KT, E2, Vg, GSI, HSI, CF and gonad development stage. Maturing spawners-of-the-year were sampled from the Humber River in July-August 1991 and Western Arm Brook in August-October 1990. Non-maturing salmon were sampled from the Saint John River cage-reared stock in May and October 1990 and from the Labrador Sea in October 1991. Those parameters accounting for a significant proportion of the variation between maturing and non-maturing groups were selected for use in the analysis. The selection was done in a stepwise manner using the SAS 'STEPDISC' procedure for personal computers (SAS Institute Inc. 1985). The discriminant analysis and resulting discriminant functions were computed for male and female salmon using the SAS 'DISCRIM' procedure for personal computers (Appendix 7).

According to Titus et al. (1984), a problem often encountered in the application of discriminant analysis, is unequal group sample sizes. They contend that unequal sample sizes may lead to very high percent correct classification, but the improvement over random correct classification may be slight. For such situations they recommend that a chance-corrected procedure be used in interpreting the percentage of agreement between the actual and predicted group memberships. They

describe a statistic, kappa, developed by Cohen (1960) which was found to be useful in interpreting the classification results of discriminant analyses with equal or unequal sample sizes. Kappa expresses the average proportion of individuals that were correctly classified, after removing the effect of correct classifications due to chance. Because the sample sizes for the maturing and non-maturing salmon groups used in the discriminant analysis were unequal, the kappa statistic was used to evaluate the effectiveness of the discriminant function. The equations used in calculation of the kappa statistic are shown in Appendix 8.

The discriminant functions derived for male and female salmon were used to classify the salmon sampled from the Saint John River cage-reared stock (May, July and November 1991) and the commercial fishery into maturing and non-maturing groups. The assumption of a multivariate normal distribution within each maturity group was assumed to have been met.

The accuracy of the discriminant functions in predicting state of maturity was tested by randomly selecting 50% of the observations on salmon of known maturity to derive a discriminant function from which to predict the group membership of the remaining 50% of the observations and vice versa. The percentage of agreement between the actual and predicted state of maturity was evaluated using Cohen's kappa.

3. RESULTS

3.1 Reference Sites

Salmon at each site were predominantly female, but the percentage of females in the Saint John River cage-reared stock was the lowest of the four reference sites (Table 1).

Preliminary RIA results indicated that 11-KT levels in virgin females and Vg and E2 levels in virgin males were either very low or not detectable. The average 11-KT level in females was less than 0.50 ng/ml and the E2 level in males was less than 0.50 ng/ml. Subsequently, only RIAs for levels of Vg and E2 in females and 11-KT in males were carried out.

The RIA and histological parameter values measured in male and female salmon from the four reference sites are given in Appendices 9-12.

3.1.1 Females

The Saint John River hatchery-reared smolts in May 1990 had oocytes at stages 2-5, but more than 50% were at stage 4 (Fig. 18a). In subsequent samples from sea-cages (October 1990 and May 1991) the percentage of oocytes at stage 4 decreased, and the percentage at stages 5-6 increased (Fig. 18b-c). In July 1991 (Fig. 18d), the distribution had changed little from that in the May 1991 samples. However, by November 1991 (Fig. 18e) stage 7 and 8 oocytes were present, as well as some stage 12 oocytes in sexually mature fish.

The ovaries in 96.2% of the hatchery-reared smolts sampled in May 1990, were classified into stage 4 (Fig. 19a). In October 1990, the majority were still at stage 4 (86.7%) (Fig. 19b), but had advanced to stage 5 (58.6%) in May 1991 (Fig. 19c), and were at stage 6 (37%) in July 1991 (Fig. 19d). Stage 6 recorded in July 1991 was the most advanced (Fig. 19d). In November 1991, the majority of immature fish were at stage 6 but 7.4% (2/27) had mature ovaries (stage 12) (Fig. 19e). These fish were spawners-of-the-year.

Plasma levels of E2 and Vg, GSI, HSI and CF were significantly correlated with ovary stages in Saint John River female salmon sampled on five occasions in 1990 and 1991 (Table 2). The highest R^2 values were for Vg and GSI suggesting these parameters were the best indicators of ovary development. Levels of Vg and GSI were highest at stage 12 measured in November 1991 (Fig. 20c-d), but T and E2 levels were highest at stages 7 and 8 which were the next lowest stages recorded

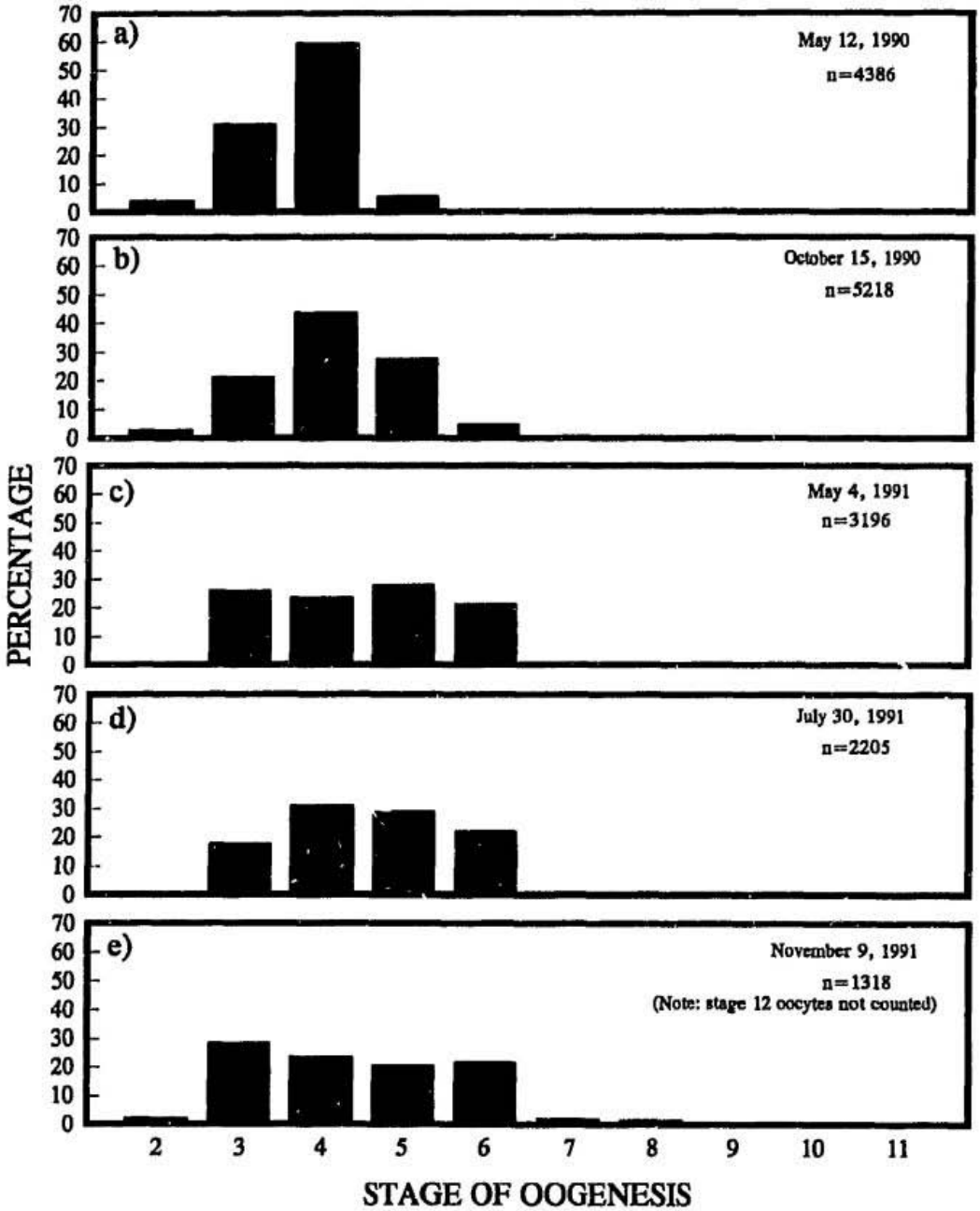


Figure 18. Saint John River: stages of oocyte development in cage-reared female Atlantic salmon on five sampling dates in 1990 and 1991. The values of n represent the number of oocytes counted.

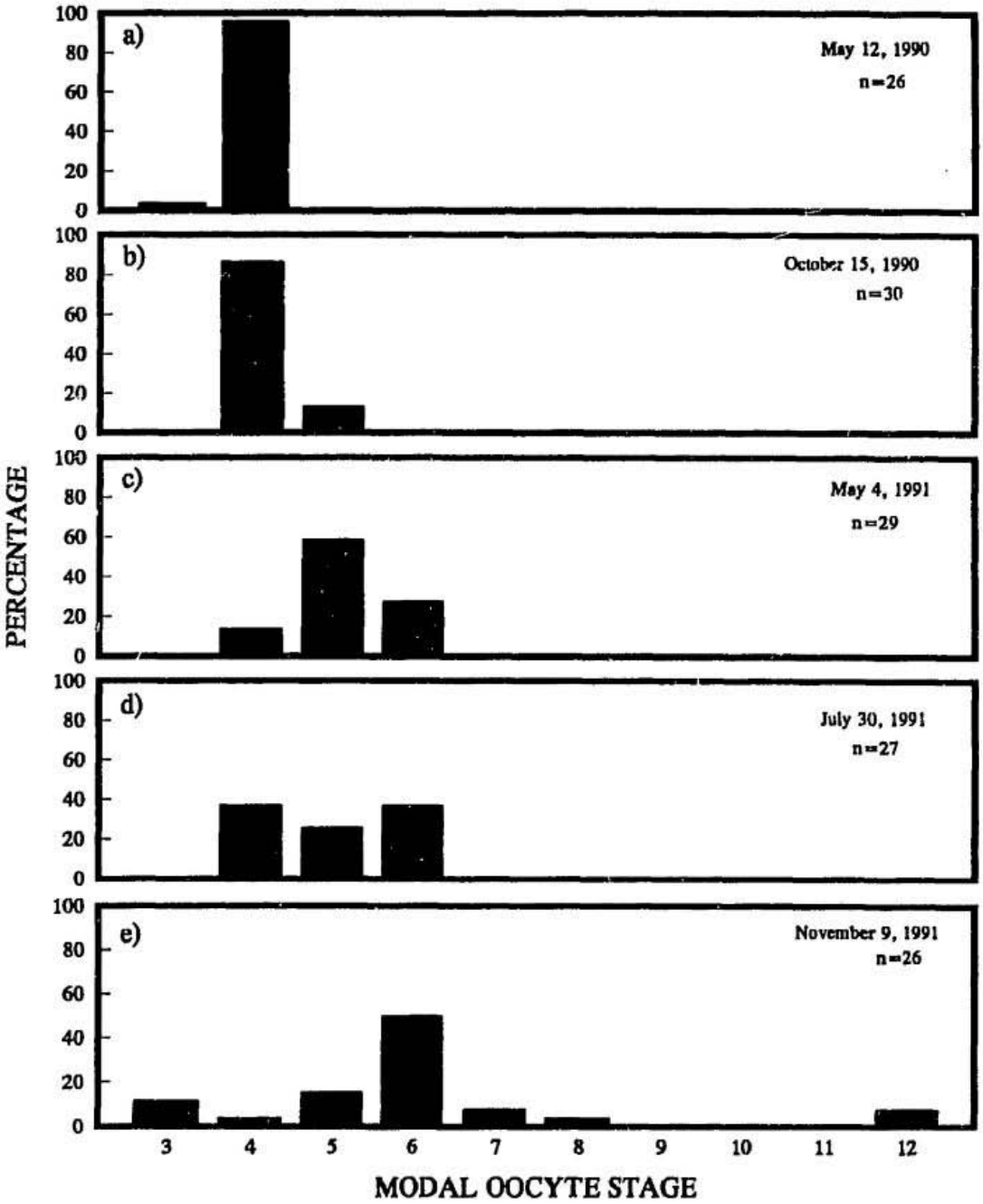


Figure 19. Saint John River: modal oocyte stages in cage-reared female Atlantic salmon on five sampling dates in 1990 and 1991. The values of n represent the number of fish sampled.

Table 2. Relationship between levels of T, E2, Vg, GSI, HSI, and CF and ovary stage in cage-reared Saint John River female Atlantic salmon. '**'= significant relationship at the .05 level and 'ns'=non-significant relationship.

Ovary stage versus:	**R^2**	**p**	
T	.03	.7539	ns
E2	.22	.0001	**
Vg	.69	.0001	**
GSI	.98	.0001	**
HSI	.15	.0076	**
CF	.29	.0001	**

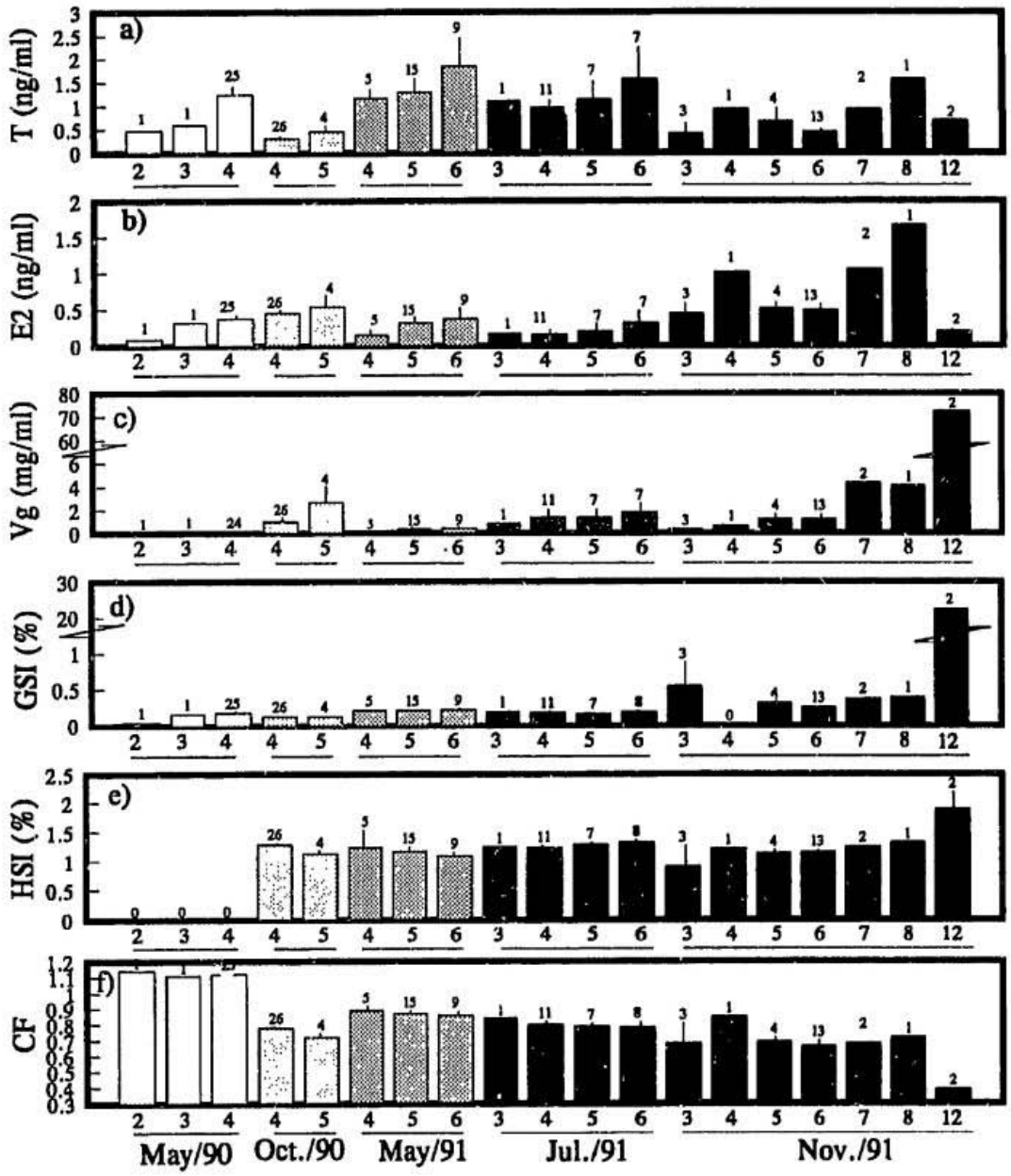


Figure 20. Saint John River: mean levels of T, E2, Vg, GSI, HSI and CF in cage-reared female Atlantic salmon at each modal oocyte stage on five sampling dates in 1990 and 1991. Vertical lines are standard errors and the numbers above lines represent the number of fish sampled.

(Fig. 20a-b). However, plasma levels of these parameters may have continued to increase at stages 9-11 before decreasing at stage 12. There was little change in HSI with increased ovary development except for a sharp increase at stage 12 (Fig. 20e). In contrast to the other parameters, condition factor (CF) decreased with ovary development and dropped sharply at ovary stage 12 (Fig. 20f).

In May 1990, 17.2% (5/29) of the Saint John River female smolts had higher than average (1.2 ng/ml) levels of T. In October 1990, 20.0% (6/30) had much higher than the average (1.24 mg/ml) levels of Vg (Appendix 9a-b), but GSI in these fish was relatively low and their ovaries were at stages 4 or 5, indicating that they were not spawners-of-the-year.

In May 1991, the levels of T, E2, Vg and GSI were still relatively low in all Saint John River females sampled, but 27.6% (8/29) had advanced to ovary stage 6 (Appendix 9c; Fig. 20a-d).

In July 1991, stage 6 was still the most advanced ovary stage present in 27% (7/26) of the Saint John River females sampled (Appendix 9d). However, 14.8% (4/27) of the females had higher than average levels of T (1.19 ng/ml), E2 (0.21 ng/ml) and/or Vg (1.48 mg/ml) (Appendix 9d), suggesting that these fish were potential spawners-of-the-year and that these parameters may be indicators of sexual maturity at this time of year. The minimum levels of T, E2 and Vg recorded in potential spawners were 2.16 ng/ml, 0.61 ng/ml and 1.81 mg/ml, respectively (Appendix 9d). Their ovaries were at stages 4-6 (Appendix 9d).

In November 1991, 7.4% of the females sampled were spawners-of-the-year as evidenced by ovaries at stage 12. This was about one half the percentage that was anticipated based on samples in July 1991. Compared to other females in November, the mature fish had the highest levels of Vg, GSI and HSI, and either the lowest or among the lowest levels of T, E2 and CF recorded (Appendix 9e). The levels of T and E2 recorded in November, were higher in females at stage 7 and 8 than in spawners-of-the-year, but it is very unlikely that these fish were sexually mature because their CF was relatively high, indicating that they had not stopped feeding, and their GSI levels were only 0.35-0.39% compared to 19-25% in fish at stage 12 (Appendix 9e).

The maturing wild female salmon sampled from the Humber River in July-August 1991 had a bi-modal (stages 4 and 7) oocyte frequency distribution (Fig. 21b). The mode at stage 7 was probably representative of spawn in the fall of 1991, while the mode at stage 4 was representative of oocytes that would have continued to develop. These fish were classified into ovary stage 7 or higher (Fig. 22b-c).

The single maturing female sampled from Western Arm Brook (August 1990) had oocytes at stage 11 (Fig. 21c).

Non-maturing wild female salmon sampled from the Labrador Sea in October 1991, had oocytes at stages 3-7 (Fig. 21a). Those with oocytes at stages 3-5 had been at sea for less than one year (sea-age-0), whereas, fish that had stages 3-7 had spent one year at sea (sea-age-1). The majority of the sea-age-0 females were classified into

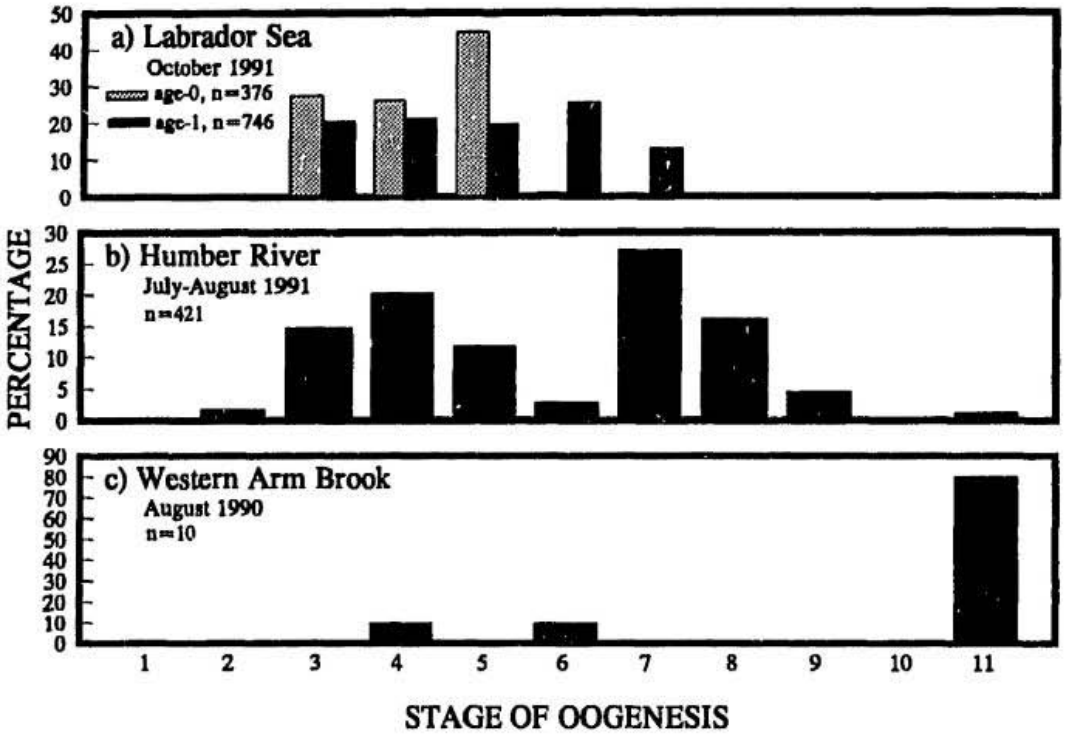


Figure 21. Stages of oocyte development in wild female Atlantic salmon from the Labrador Sea, Humber River and Western Arm Brook. The values of n represent the number of oocytes counted.

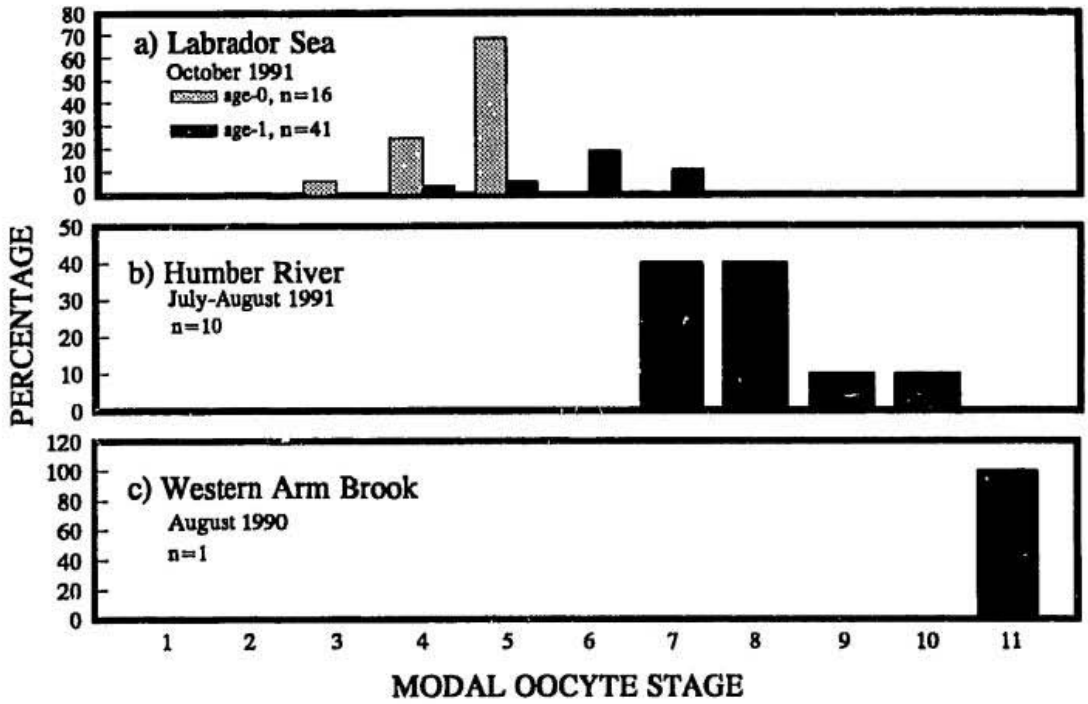


Figure 22. Modal oocyte stages in wild female Atlantic salmon from the Labrador Sea, Humber River and Western Arm Brook. The values of n represent the number of fish sampled.

ovary stage 5 and the majority of the sea-age-1 fish were stage 6 (Fig. 22a).

The plasma levels of T, E2, Vg, and GSI were higher in Humber River females at ovary stage 7 than in Labrador Sea females at the same stage (Fig. 23; Appendix 10b), indicating that the Humber River fish were potential spawners-of-the-year. In Western Arm Brook females sampled in October 1990 these levels were even higher (Fig. 23; Appendix 10c). The lowest values of T, E2, Vg, and GSI recorded in Humber River and Western Arm Brook spawners-of-the-year were 1.40 ng/ml, 0.81 ng/ml, 0.75 mg/ml, and 0.24%, respectively (Appendix 10b-c). The values for T, E2, and GSI were similar to those recorded in Saint John River females that were predicted to be spawners-of-the-year in July 1991. However, the value for Vg was about one half the minimum value in the Saint John River females.

3.1.2 Males

Saint John River (hatchery and cage-reared) male salmon with the exception of precocious smolts in May 1990, did not mature beyond spermatogenic stage 2 until May 1991 (Fig. 24a-c). In the next three months, male gonads developed relatively quickly. In July 1991, 56.5% (13/23) of the males sampled had reached stage 3 or more (Fig. 24d), and by November 1991, 61.5% (16/26) were sexually mature at stage 6 (Fig. 24e). This suggests that the males at stage 3 or higher in the July 1991 sample were potential spawners-of-the-year. Immature males (38.5%; 10/26) in November 1991 were at stage 2 (Fig. 24e).

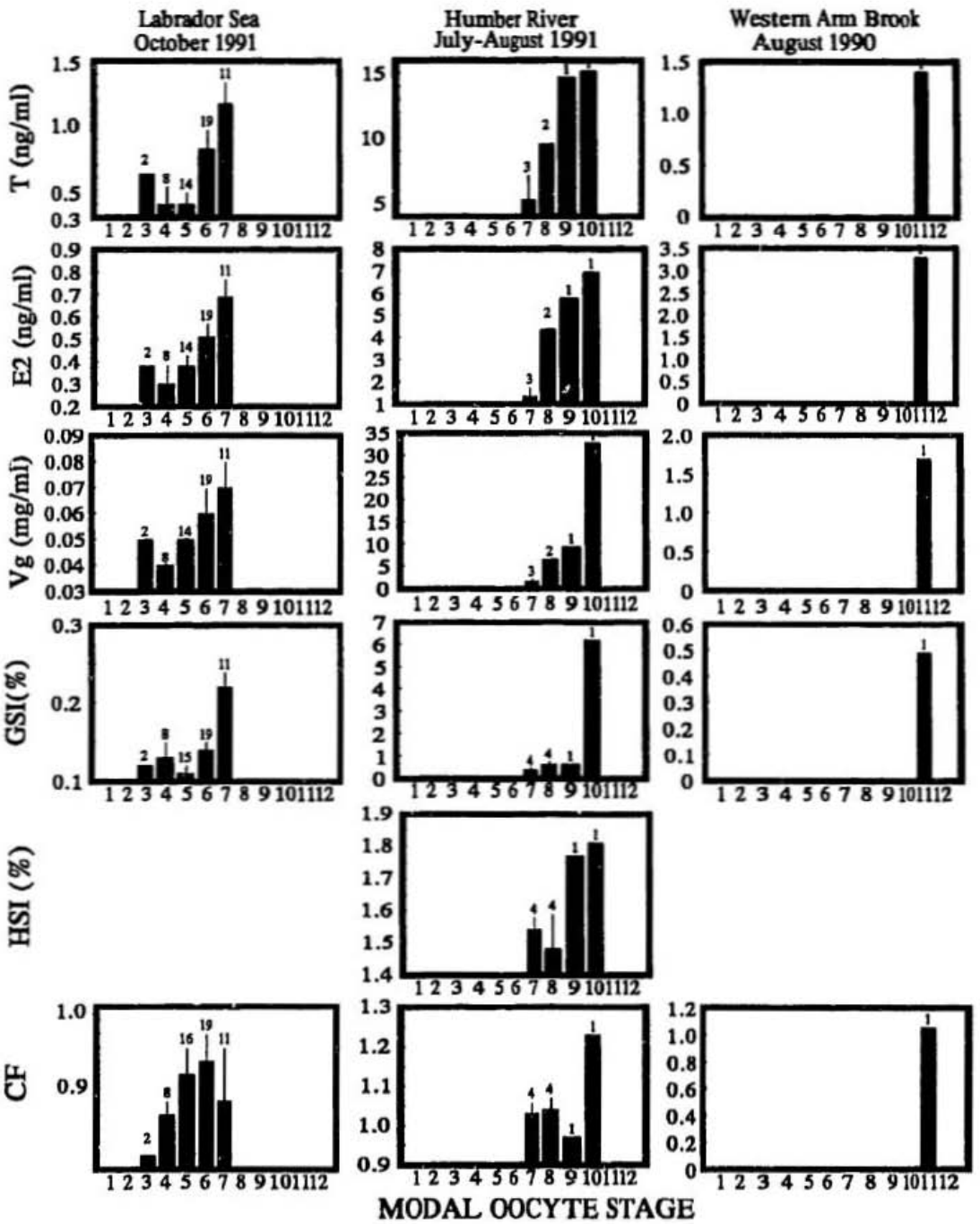


Figure 23. Mean levels of T, E2, Vg, GSI, HSI and CF in wild female Atlantic salmon at each modal oocyte stage from the Labrador Sea, Humber River, and Western Arm Brook. Vertical lines are standard errors and numbers above lines represent the number of fish sampled.

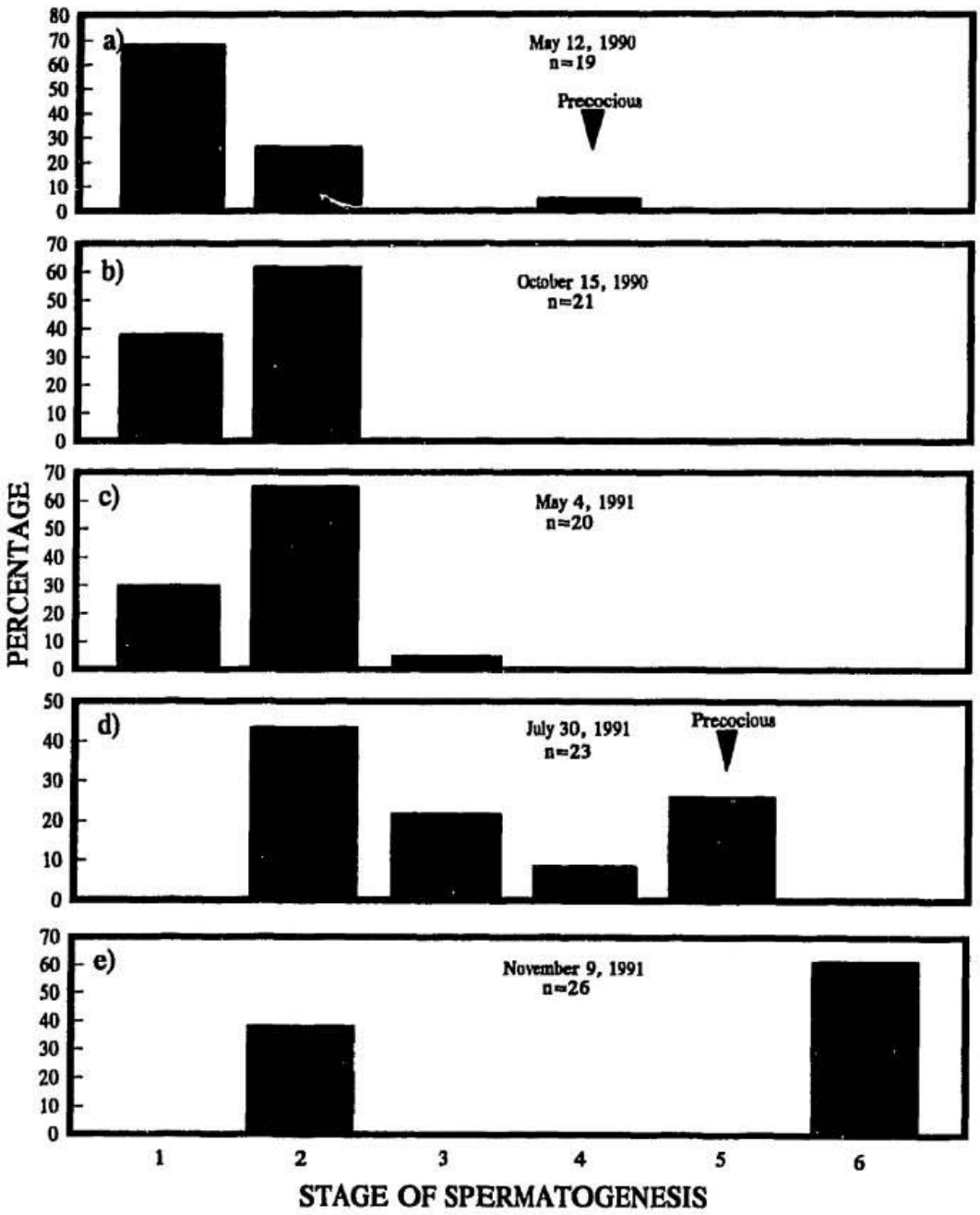


Figure 24. Saint John River: stages of spermatogenesis in cage-reared male Atlantic salmon on five sampling dates in 1990 and 1991. The values of n represent the number of fish sampled.

Plasma levels of T, 11-KT, GSI and CF measured in cage-reared male salmon sampled between May 1990 and November 1991 (Appendix 11a-e) were all correlated with testis stage (Table 3). The highest R^2 values were for T, 11-KT and GSI indicating that these parameters were the best indicators of testis development. Levels of T and 11-KT were relatively low in males at stages 3 and 4, but increased sharply at stage 5 and peaked at stage 6 (Fig. 25a-b), along with GSI (Fig. 25c). All cage-reared males at stage 5 in July had higher than average T and 11-KT levels and GSI, but only a few of those at stages 3 and 4 had higher than average levels for these parameters (Appendix 11d).

In contrast to females, plasma T levels in cage-reared male salmon were correlated with gonad stage but HSI was not (Tables 2-3; Fig. 25).

In November 1991, 61.5% (16/26) of the Saint John River males salmon sampled were spawners-of-the-year. These fish all had testes at stage 6 and levels of T, 11-KT, and GSI that were 5-10 times higher than in stage 2 males which was the next lowest stage recorded. Levels of T ranged from 6.36 to 15.80 ng/ml, 11-KT levels ranged from 15.49 to 63.56 ng/ml, and GSI ranged from 3.0% to 7.5% in spawners-of-the-year in November (Appendix 11e). The highest levels of T, 11-KT, and GSI in stage 2 males were 1.15 ng/ml, 2.05 ng/ml, and 0.11%, respectively.

Table 3. Relationship between levels of T, 11-KT, GSI, HSI and CF and testis stage in cage-reared Saint John River Atlantic salmon. '**'=significance relationship at the .05 level and 'ns'=non-significant relationship.

Testis stage versus:	**R^2**	**p**	
T	.69	.0001	**
11-KT	.64	.0001	**
GSI	.72	.0001	**
HSI	.10	.0657	ns
CF	.13	.0047	**

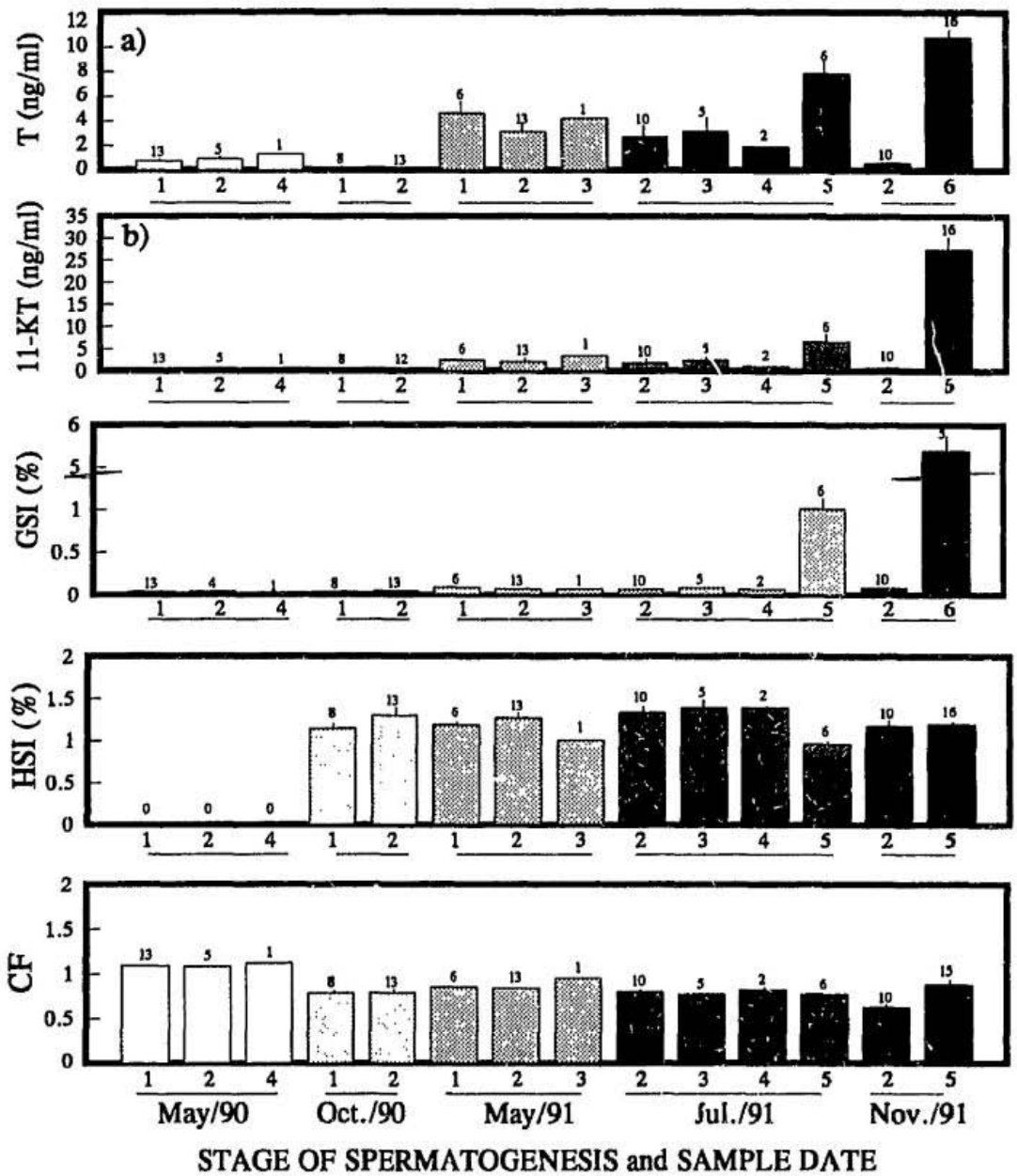


Figure 25. Saint John River: mean levels of T, 11KT, GSI, HSI, and CF in cage-reared male Atlantic salmon at each stage of spermatogenesis on five sampling dates in 1990 and 1991. Vertical lines are standard errors and numbers above lines represent the number of fish sampled.

The percentage of Saint John River males that were sexually mature in November 1991 could have been predicted from the October 1990 sample (61.9%), based on the ratio between the numbers with stage 2 and stage 1 testes (13/21) present in the sea-cages. However, no prediction could have been made based on plasma hormone levels or GSI as mean T, 11-KT and GSI levels were similar for stage 1 and 2 males (Fig. 25a-c; Appendix 11b). In May 1991, the prediction of spawners-of-the-year would have been the same. The levels of T, 11-KT and GSI were similar to those in October 1990 and 66.7% (14/21) of male salmon were at stage 2 (Appendix 11c).

In July 1991, 56.5% (13/23) of the male salmon had developed to testis stage 3 or higher and the levels of T, 11-KT and GSI in these fish were higher than in those at stage 2 (Fig. 25a-c; Appendix 11d), suggesting that they were potential spawners-of-the-year. It is possible that some of the stage 2 males in July 1991 were also spawners-of-the-year, because the percentage of the July 1991 samples that were at stages 3-5 did not fully account for the percentage of male spawners (stage 6) recorded in the November 1991 samples (Fig. 24d-e).

The Humber River male spawners-of-the-year sampled in July-August 1991 were at testis stages 3 and 5 (Fig. 26) and had mean levels of T, 11-KT and GSI which were within the ranges recorded for cage-reared spawners-of-the-year in November (Fig. 27). The large variability in hormone levels observed in Humber River males at the same testis stage precludes the establishment of minimum steroid

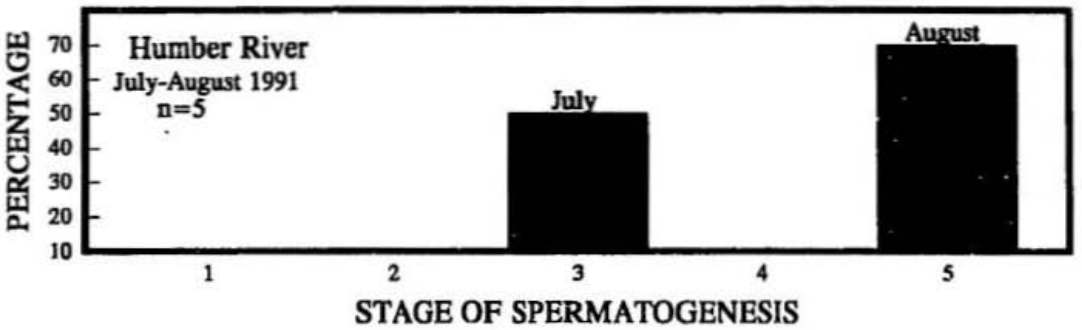


Figure 26. Stages of spermatogenesis in wild male Atlantic salmon from the Humber River. The value of n represents the number of fish sampled.

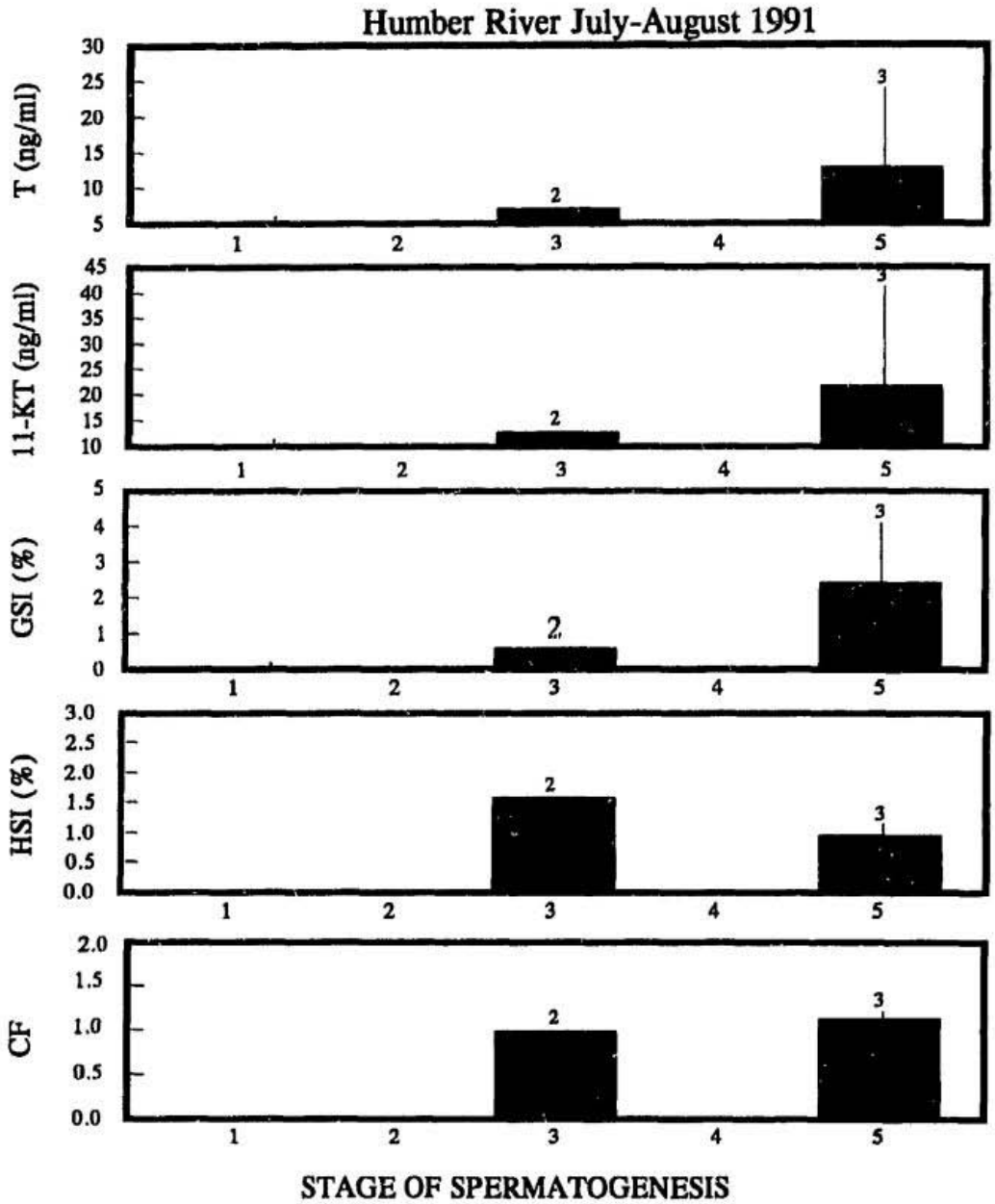


Figure 27. Mean levels of T, 11KT, GSI, HSI and CF in wild male Atlantic salmon at each stage of spermatogenesis from the Humber River. Vertical lines are standard errors and numbers above lines represent the number of fish sampled.

levels to identify spawners-of-the-year in July. For example, The lowest 11-KT level recorded in a maturing male salmon (stage 5) from the Humber River was 0.17 ng/ml and the highest was 60.72 ng/ml (Appendix 12b). Some of the Saint John River male salmon at stage 2, in November, also had 11-KT levels within this range. No gonads were collected from male salmon sampled at Western Arm Brook and those collected from the Labrador Sea sample were unsuitable for histology.

3.2 Commercial Fishery

The presence of ice on the fishing grounds at St. Barbe Bay and Conche in 1991 resulted in lower landings than in 1990, and landings occurred later in the season than in 1990 (Fig. 28).

The RIA and histological parameter values measured in male and female salmon from commercial landings are given in Appendices 13 and 14.

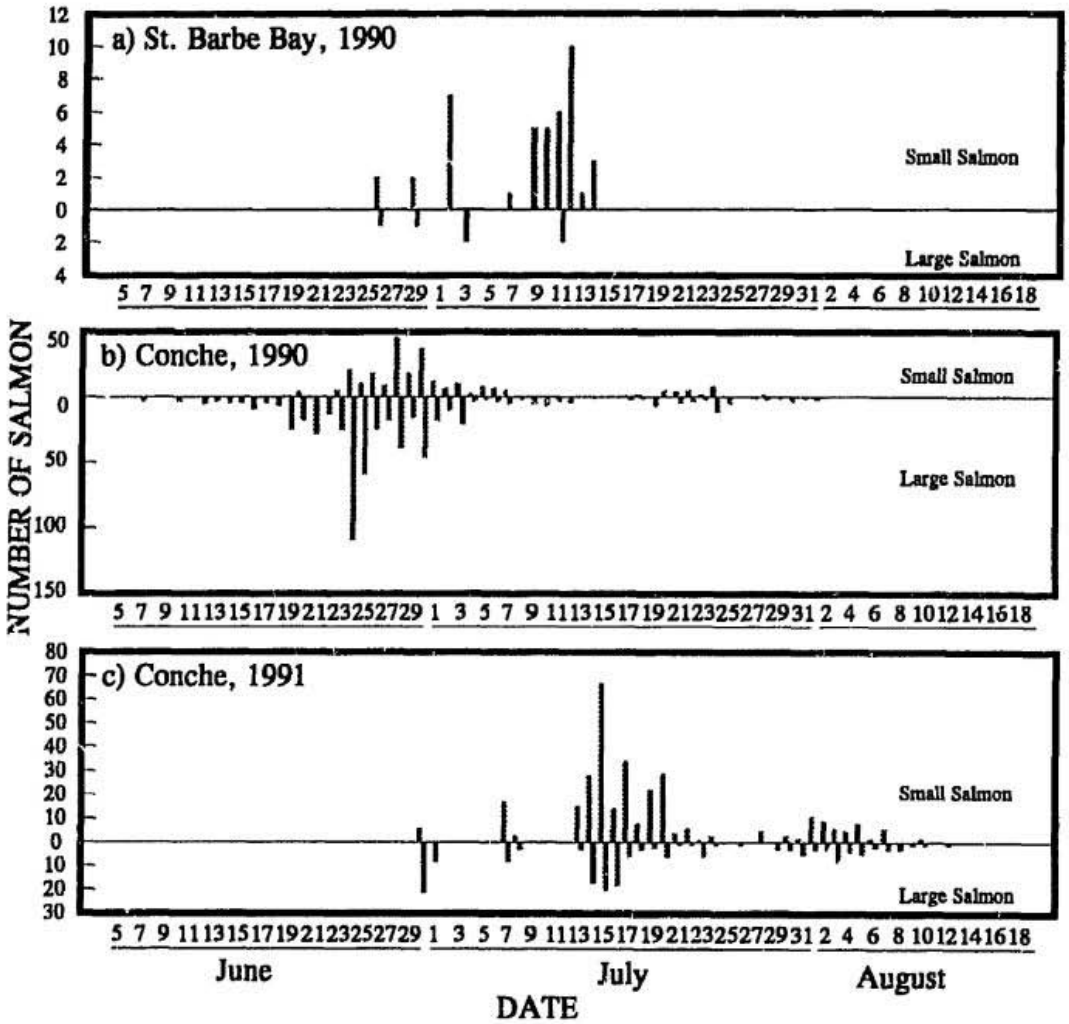


Figure 28. Dates of commercial salmon fishery landings by selected fishermen at St. Barbe Bay and Conche, Newfoundland in 1990 and 1991.

3.2.1 Biological Characteristics

Small salmon were comprised of sea-ages 1 to 2 and large salmon were sea-ages 2-3.

The sex composition of virgin sea-age-1 salmon sampled at Conche in 1990 and 1991 was approximately 36% female; about half the value (73%) recorded from St. Barbe Bay samples (Table 1). The percentage of virgin sea-age-1 females sampled from St. Barbe Bay in 1990 and 1991 was similar to that recorded at Western Arm Brook which flows into the bay (Table 1). This river was probably the destination of many of the fish caught in the St. Barbe Bay fishery.

The average river-age of virgin sea-age-1 salmon from St. Barbe Bay was about 10% higher than at Conche or Twillingate (Table 4).

3.2.2 Females

The percentage frequency distribution of oocytes counted at each development stage was bi-modal in females from St. Barbe Bay, Conche, and Twillingate (Fig. 29a-c). Modes occurred at stages 3-4 and 8-9 in females from all three locations. However, the advanced second mode did not overlap in all locations. Oocytes were most advanced at St. Barbe Bay (stage 10, 20%) compared to Conche (less than 10%) and Twillingate (0%).

Table 4. Number of years spent in freshwater by wild virgin sea–age–1 Atlantic salmon sampled in 1990 and 1991.

Sampling Location	Sample Size	Percentage at each Freshwater–age					Mean Freshwater–age (Years)
		1 yr	2 yrs	3 yrs	4 yrs	5 yrs	
Commercial Fishery Sites:							
St. Barbe Bay 1990	32	0.0	0.0	54.8	32.3	12.9	3.58
St. Barbe Bay 1991	4	0.0	0.0	25.0	75.0	0.0	3.75
Conche 1990	91	0.0	3.5	67.8	28.7	0.0	3.25
Conche 1991	49	0.0	0.0	57.1	38.8	4.1	3.47
Twillingate 1991	35	0.0	14.7	55.9	26.5	2.9	3.18
Reference Sites:							
Humber River July–August 1991	16	0.0	6.7	93.3	0.0	0.0	2.93
Western Arm Brook Aug. & Oct. 1990	12	0.0	0.0	66.7	33.3	0.0	3.33
Labrador Sea October 1991	145	12.0	35.2	39.4	10.6	2.1	2.58

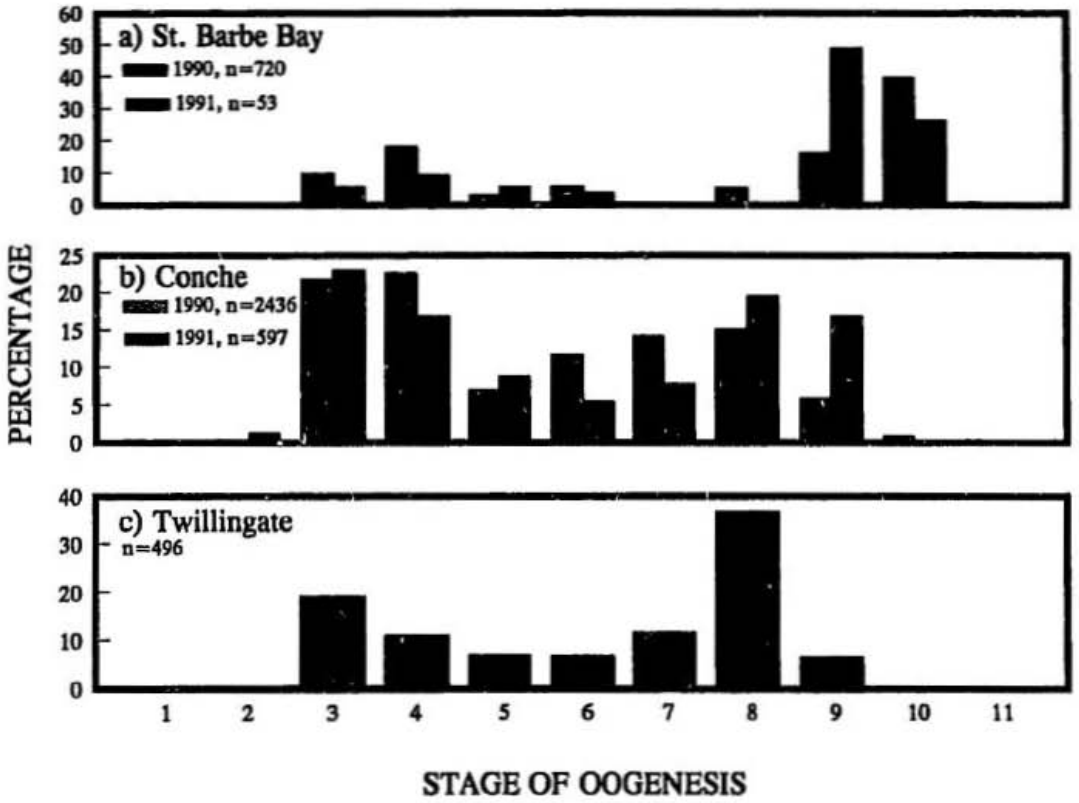


Figure 29. Oocyte stages in wild female Atlantic salmon from commercial landings at St. Barbe Bay, Conche and Twillingate. The values of n represent the number of oocytes counted.

St. Barbe Bay females were classified into ovary stages 8-10 compared to stages 4-10 at Conche, and stages 6-10 at Twillingate 1991 (Fig. 30a-c). The ovaries of females from landings at St. Barbe Bay were more advanced than those at Conche or Twillingate.

The levels of T, E2, Vg and GSI in St. Barbe Bay and Conche females (blood samples from Twillingate were not suitable for RIA) tended to be higher corresponding to more advanced ovary development (Fig. 31a-h). Levels of these parameters increased after stage 8 at St. Barbe Bay (Fig. 31a-d) and after stage 7 at Conche (Fig. 31e-h). However, the T level in one stage 4 female at Conche in 1990 was greater than T levels in some individuals at higher stages (Appendix 13a) and greater than the means at stages 6-10 (Fig. 31e). Plasma levels of T and E2 in St. Barbe Bay females in 1991 (Fig. 31a-b) and Conche females in 1990 (Fig. 31e-f) decreased at stage 10. The levels of T, E2 and GSI were much lower in 1991 than in 1990 at St. Barbe and Conche (Fig. 31e-f & h).

The ovaries of all St. Barbe Bay female salmon exceeded the lowest stage recorded in wild spawners-of-the-year (stage 7) from the Humber River in July and August of 1991. However, a portion of those at Conche and Twillingate were below stage 7. These included females that had not developed past stage 6 and had relatively low levels of Vg (0.09-0.24 mg/ml) compared to the minimum value (0.75 mg/ml) recorded from Humber River females in July 1991.

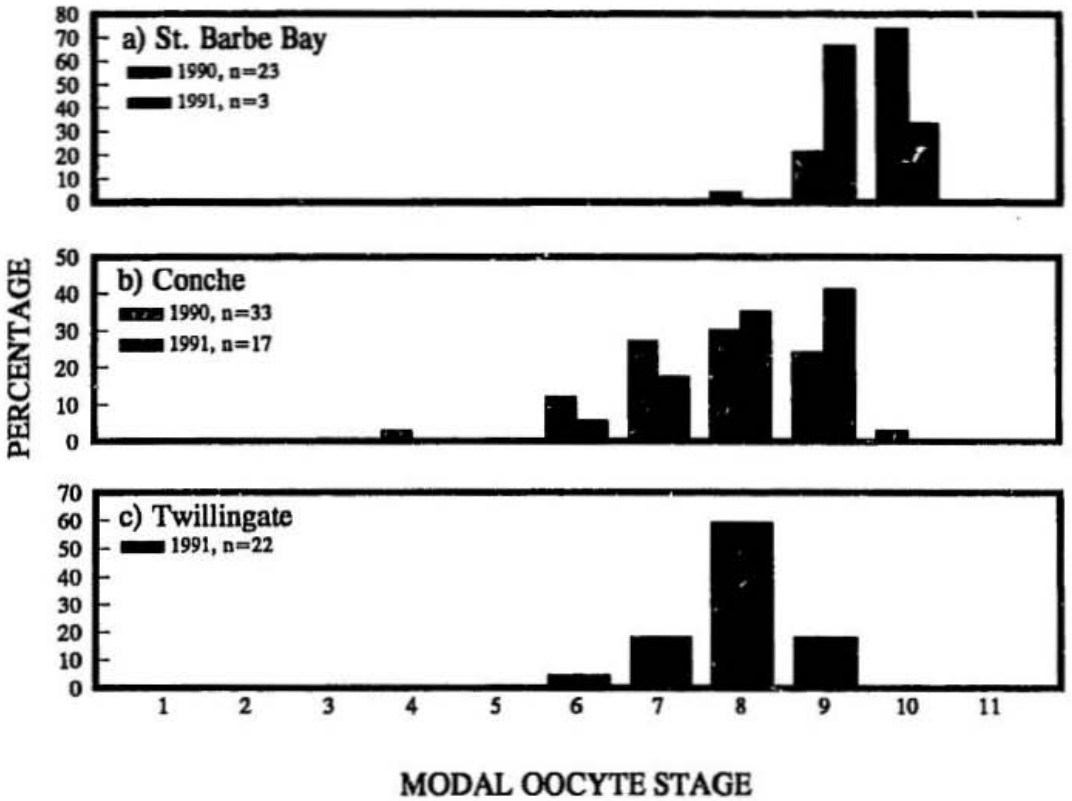


Figure 30. Modal oocyte stages in wild female Atlantic salmon from commercial landings at St. Barbe Bay, Conche and Twillingate. The values of n represent the number of fish sampled.

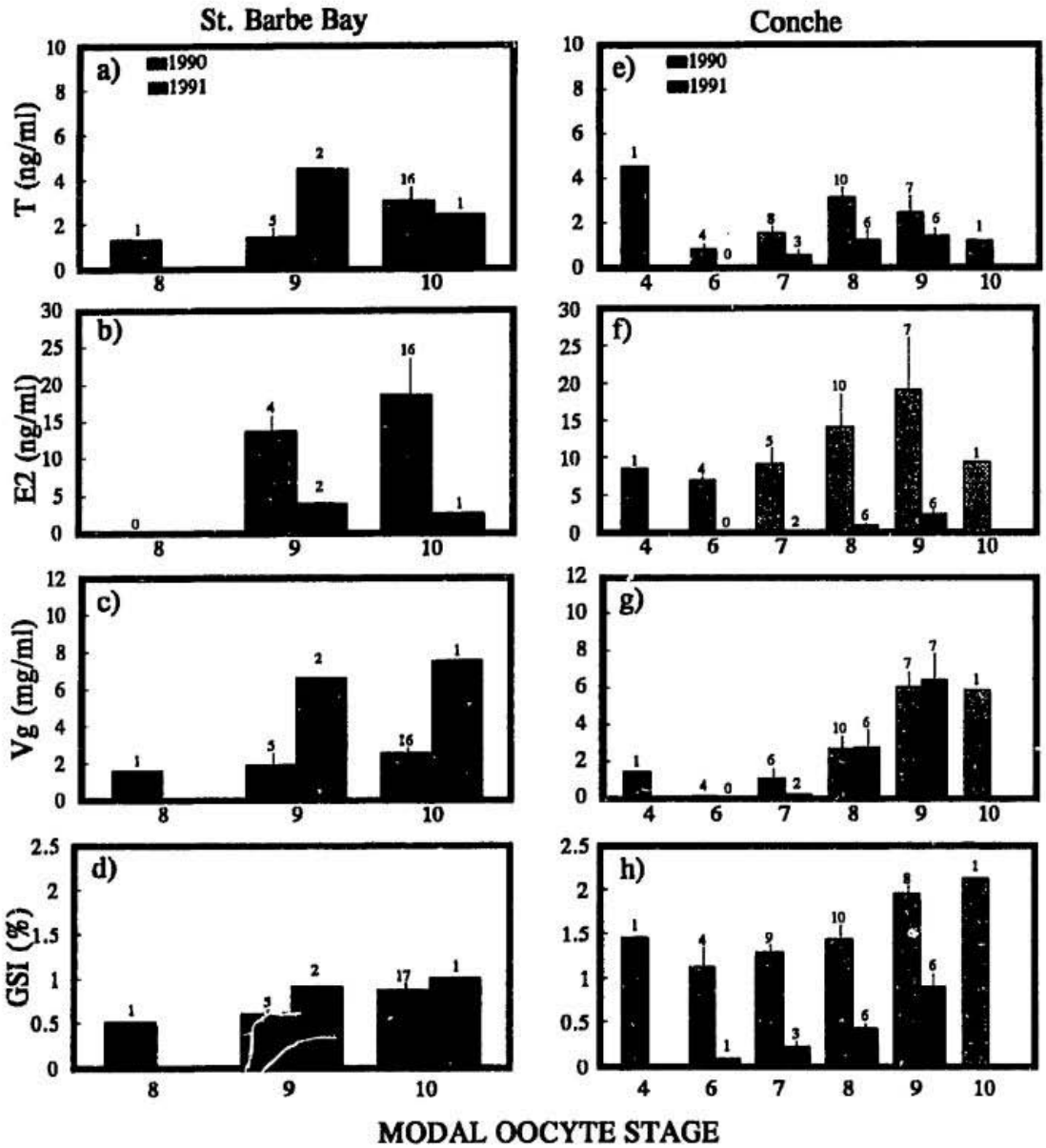


Figure 31. Mean levels of T, E2, VG and GSI in wild female Atlantic salmon at each modal oocyte stage from commercial landings at St. Barbe Bay and Conche. Vertical lines are standard errors and numbers above lines represent the number of fish sampled.

3.2.3 Males

Testis development in all male salmon sampled at St. Barbe Bay, was at stage 3 or higher, but at Conche and Twillingate males were at stage 2 or higher (Fig. 32a-c).

Plasma T and 11-KT levels in male salmon from St. Barbe Bay and Conche were similar at the same testis stage, and tended to decrease between stages 3 and 4 at both locations (Fig. 33a & b-e; Appendix 14). In contrast, GSI levels increased between stages 3 and 4 (Fig. 33a-f). The mean values of T, 11-KT, and GSI recorded at Conche in 1990 were higher than in 1991 (Fig. 33d-f), as with T, E2 and GSI in females (Fig. 31e-f & h).

By comparison with testis stages in males judged to be spawners-of-the-year from the Saint John River in July 1991 and the Humber River in July-August 1991, male salmon in commercial landings in June, July, and August that had reached testis stage 3 and higher were spawners-of-the-year. On this basis all males sampled at St. Barbe Bay were spawners-of-the-year, but stage 2 males at Conche and Twillingate were probably non-maturing fish.

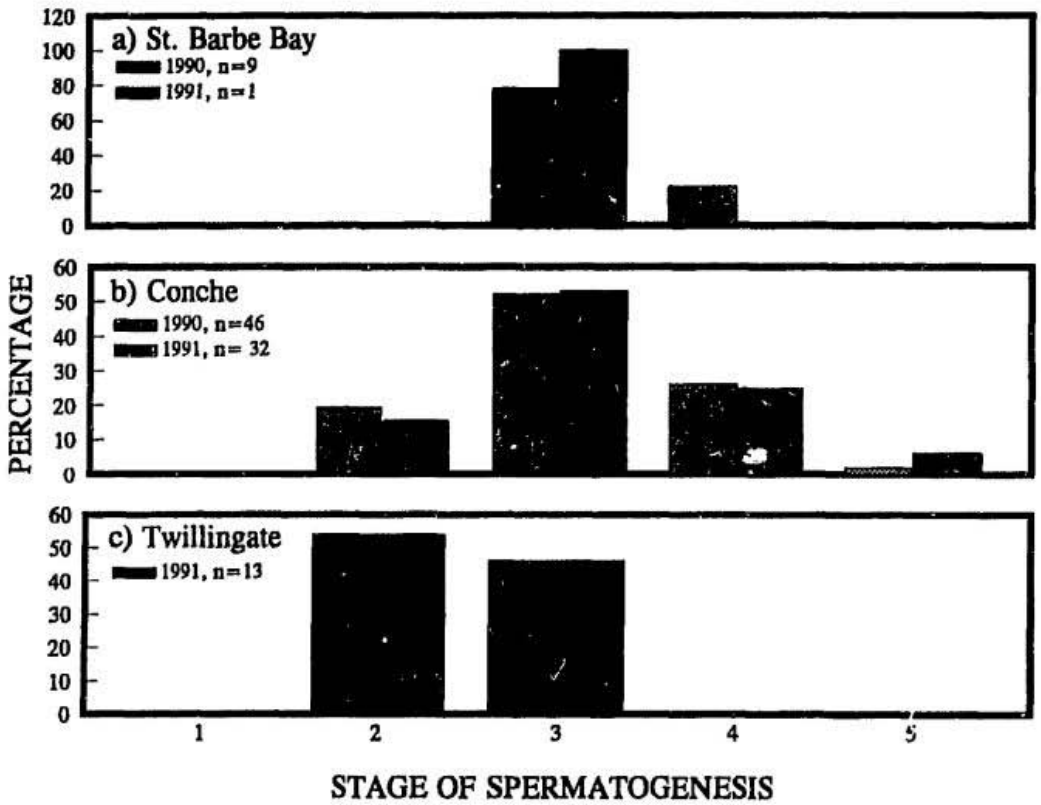


Figure 32. Stages of spermatogenesis in wild male Atlantic salmon from commercial landings at St. Barbe Bay, Conche and Twillingate. The values of n represent the number of fish sampled.

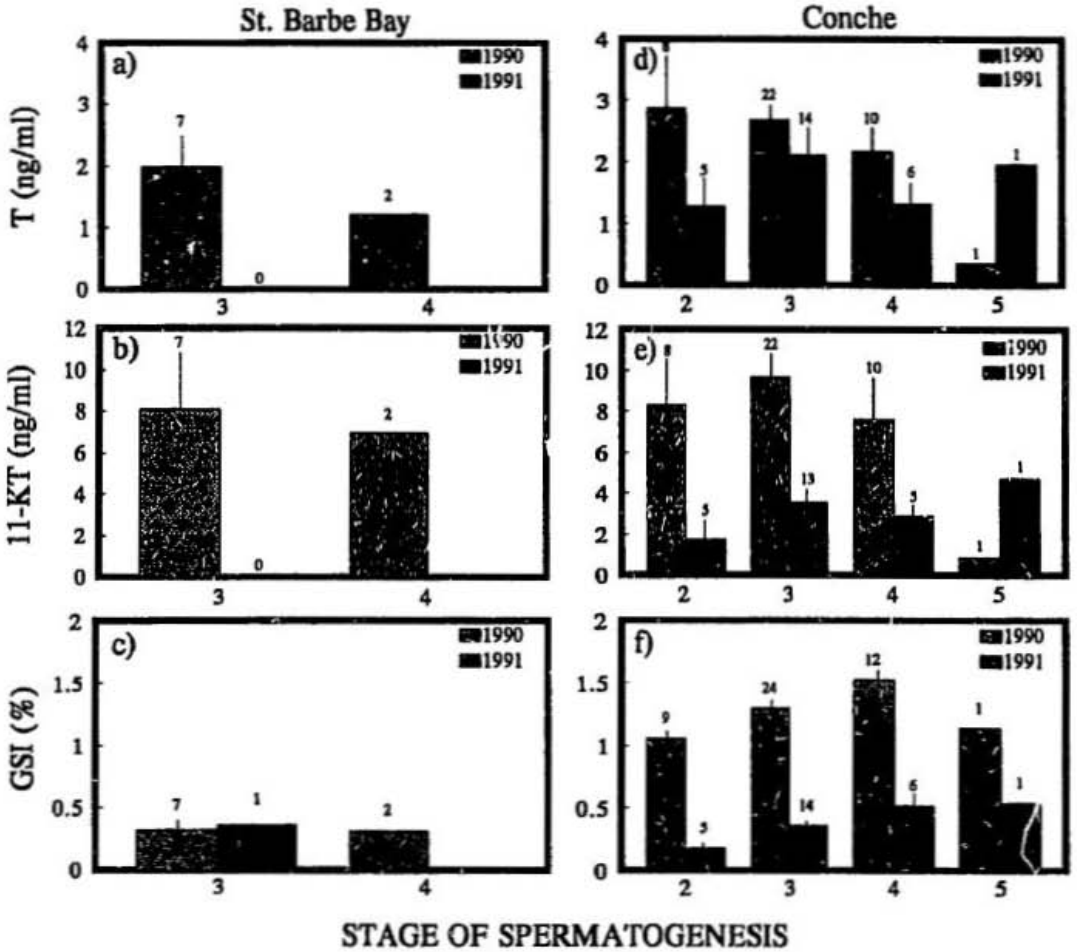


Figure 33. Mean levels of T, 11KT and GSI in wild male Atlantic salmon at each stage of spermatogenesis from commercial landings at St. Barbe Bay and Conche. Vertical lines are standard errors and numbers above lines represent the number of fish sampled.

3.3 Discriminant Analysis

The variation between maturing and non-maturing fish, shown in Table 5, is based on parameters measured in the Saint John River (May and October 1990), Labrador Sea, Humber River, and Western Arm Brook stocks. The parameters selected for discriminant analysis were the levels of E2 and T, ovary stage, and levels of GSI for females; the testis stage, and levels of 11-KT and GSI for males. Because fish sampled from Western Arm Brook were not sacrificed, GSI could not be calculated for these fish, and it was excluded from the discriminant analysis.

The discriminant analysis of the state of sexual maturity of salmon from the four reference sites, indicated that 99.2% of the females and 97.9% of the males could be correctly classified into maturing and non-maturing groups (Table 6) based on the selected parameters. The total estimated rate of correct classification into the maturing group was 93.8% for females and 100.0% for males; and the total estimated rate of correct classification into the non-maturing group was 100.0% for females and 97.4% for males (Table 6). For males and females combined, 95.8% of maturing and 99.3% of non-maturing salmon were correctly classified (Table 7). Cohen's kappa statistic of 0.963 (95% CI=0.927 to 1.000) for females and 0.928 (95% CI=0.857 to 0.999) for males (Table 7) indicates that these classification results were 96.3% and 92.8%, respectively, better than chance alone.

Table 5. Stepwise selection of parameters for discriminant analysis of state of maturity in male and female Atlantic salmon sampled from the Saint John River cage–reared stock (May and October 1990), Labrador Sea (October 1991), Humber River (July–August 1991), and Western Arm Brook (August–October 1990). Parameter values are given in Appendices 10–13.

Parameter	Order of Selection	Partial R**2	F Statistic	Prob > F
Males				
TESTIS STAGE	1	0.6134	65.038	0.0001
11KT	2	0.1398	6.503	0.0147
GSI	3	0.0720	3.028	0.0897
Females				
E2	1	0.6477	209.578	0.0001
T	2	0.0778	9.539	0.0025
OVARY STAGE	3	0.0706	8.506	0.0043
GSI	4	0.0626	7.406	0.0075

Table 6. Results of discriminant analysis of the state of maturity in male and female Atlantic salmon sampled from the Saint John River cage–reared stock (May and October 1990), Labrador Sea (October 1991), Humber River (July–August 1991), and Western Arm Brook (August – October 1990). Parameter values used in the analysis are given in Appendices 10–13.

	PREDICTED MATURITY (based on measured parameters):				
OBSERVED MATURITY:	Number Maturing	Number Non–Maturing	Total	Number Correctly Predicted	Proportion Correctly Predicted
Males:					
Maturing	8	0	8	8	1.000
Non–Maturing	1	38	39	38	0.974
Sub–total	9	38	47	46	0.979
Females:					
Maturing	15	1	16	15	0.938
Non–Maturing	0	110	110	110	1.000
Sub–total	15	111	126	125	0.992
Males and Females combined:					
Maturing	23	1	24	23	0.958
Non–Maturing	1	148	149	148	0.993
Sub–total	24	149	173	171	0.988

Table 7. Calculation of Cohen's kappa for results of discriminant analysis of the state of maturity in male and female Atlantic salmon sampled from the Saint John River cage–reared stock (May and October 1990), Labrador Sea (October 1991), Humber River (July–August 1991), and Western Arm Brook (August–October 1990). Test1 and Test2 refer to subsets of observations on males and females. Test1 used the first 50% of the observations to predict the state of maturity in the second 50% and vice versa for Test 2.

Classified Group	Sample Size Maturing	Non–Maturing	Pc	Po	Kappa	Significance of standard error of Kappa	95% Confidence Interval
Males	8	39	0.703	0.979	0.928	p<.001	0.857 to 0.999
Test 1	5	14	0.562	0.895	0.759	p<.01	0.599 to 0.920
Test 2	3	25	0.781	0.964	0.837	p<.01	0.677 to 0.997
Females	16	110	0.784	0.992	0.963	p<.001	0.927 to 1.000
Test 1	10	54	0.736	1.000	1.000	p<.001	1.000 to 1.000
Test 2	6	56	0.825	1.000	1.000	p<.001	1.000 to 1.000

The kappa statistics for the predicted state of maturity in tested subsets of parameter values for males and females (Table 7) indicate that the discriminant functions have a good agreement with actual maturity, and can be used to predict the state of sexual maturity in salmon.

Analysis of the frequency distribution of the canonical variate scores indicates that the discriminant functions result in only a slight overlap of the female maturing and non-maturing groups (Fig. 34a) and in complete separation of male maturing and non-maturing groups (Fig. 34b).

Application of the discriminant functions to observations on Saint John River cage-reared salmon in May and July 1991, predicted that about 60% of the males and 8-10% of the females were spawners-of-the-year (Table 8). The predicted percentages were the same as those that actually reached sexual maturity in November 1991 (Table 8).

The same discriminant functions applied to virgin sea-age-1 in commercial landings, predicted that 100% of St. Barbe Bay males and females, 95.1% and 79.2% of Conche males, and 100.0% and 64.3% of Conche females were spawners-of-the-year in 1990 and 1991 (Table 8).

The total number of maturing salmon predicted at Conche in 1990 were 58.2% (39/67) male and 67.9% (19/28) male in 1991 (Table 8).

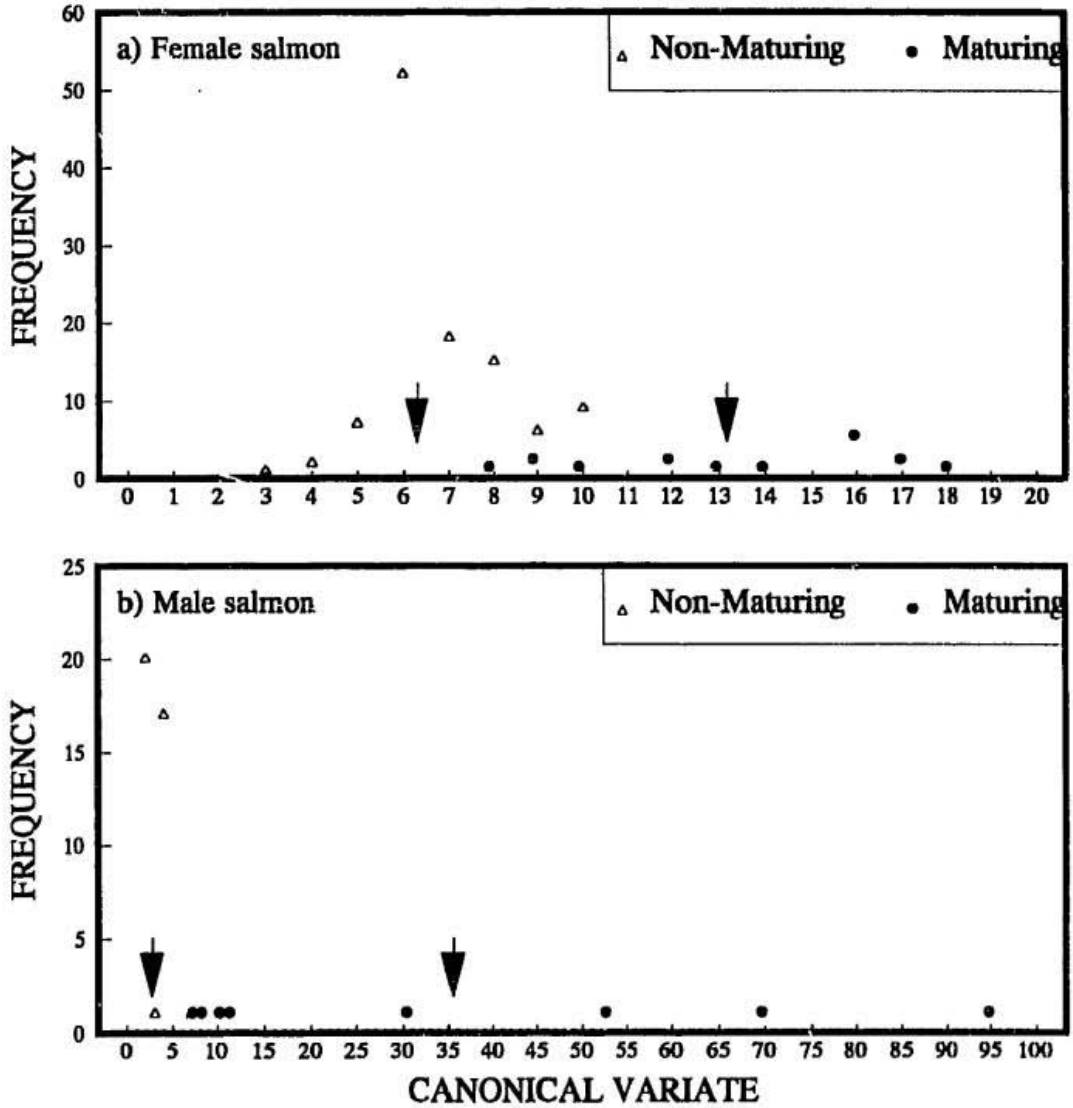


Figure 34. Canonical variate scores for male and female Atlantic salmon used in the discriminant analysis of state of maturity. Arrows indicate the mean values.

Table 8. Results of discriminant analysis of the state of maturity of male and female Atlantic salmon sampled from the Saint John River cage–reared stock (May and July 1991), and commercial landings at St. Barbe Bay and Conche in 1990 and 1991. Numbers in parentheses are percentages.

	PREDICTED MATURITY (based on measured parameters):								
	Males			Females			Total		
Sampling Location and Date	Number Maturing	Number Non–maturing	N	Number Maturing	Number Non–maturing	N	Number Maturing	Number Non–maturing	N
Saint John River, May 1991	12 (60.0)	8 (40.0)	20	3 (10.3)	26 (89.7)	29	15 (30.6)	34 (69.4)	49
Saint John River, July 1991	14 (60.9)	9 (39.1)	23	2 (7.7)	24 (92.3)	26	16 (32.7)	33 (67.4)	49
St. Barbe Bay, 1990	9 (100.0)	0 (0.0)	9	20 (100.0)	0 (0.0)	20	29 (100.0)	0 (0.0)	29
St. Barbe Bay, 1991	0 (0.0)	0 (0.0)	0	3 (100.0)	0 (0.0)	3	3 (100.0)	0 (0.0)	3
Conche, 1990	39 (95.1)	2 (4.9)	41	28 (100.0)	0 (0.0)	28	67 (97.1)	2 (2.9)	69
Conche, 1991	19 (79.2)	5 (20.8)	24	9 (64.3)	5 (35.7)	14	28 (73.7)	10 (26.3)	38

DISCUSSION

The Saint John River reared Atlantic salmon sampled at Baie d'Espoir, Newfoundland were found to be a valid reference for developmental criteria to establish the state of maturity of wild salmon caught in the commercial fishery. Saint John River hatchery-reared female smolts sampled in May 1990 indicated no evidence of acceleration relative to wild smolts. The stage of ovary development recorded in these fish was similar to that in wild smolts of the same parental sea-age recorded by Chadwick et al. (1986). The majority of the parental sea-age-2 Saint John River smolts sampled had ovaries at stage 4, which was the same stage recorded by Chadwick et al. (1986) in wild parental sea-age-2 smolts from Newfoundland and New Brunswick rivers. This finding agrees with an earlier study by Chadwick et al. (1987) which suggested that wild and hatchery-reared smolts from the same wild stock had similar states of ovarian development.

The Saint John River cage-reared post-smolts also showed a similar rate of sexual maturation to that of wild fish. Marshall and Penney (1983) found that hatchery-reared female salmon released as smolts into the Saint John River, New Brunswick, typically returned to the river to spawn after spending two years at sea (80%) and Chadwick et al. (1986) reported that the majority of wild female smolts at Western Arm Brook, Newfoundland returned to spawn after spending only one year at sea. Saint John River cage-reared females sampled at Baie d'Espoir in May 1991,

one year after their transfer into sea-cages (May 1990), would reach sexual maturity in the same length of time (one year) as the wild smolts leaving Western Arm Brook. If the rate of gonad development in Atlantic salmon is correlated with the rate of somatic growth, then after one year growing in sea-cages the Saint John River females would be expected to exhibit more advanced ovaries than smolts from Western Arm Brook, but it was the same in both groups. The ovaries of the parental sea-age-2 fish had progressed to stage 6 after their first year in sea-cages (May 1990-May 1991), which was the same as described in wild smolts from Western Arm Brook, Newfoundland by Chadwick et al. (1986). Hence, the rate of sexual maturation in salmon appears to be similar for reared and wild fish. Additional evidence of this comes from the comparison of oocyte development in sea-age-0 Labrador Sea wild females sampled in October 1991 and Saint John River cage-reared females sampled in October 1990. The most advanced oocyte stage observed (stage 6) was attained in both reference sites. The onset of sexual maturity in female Atlantic salmon, therefore, appears to be triggered by factors other than accelerated somatic growth, such as would occur under hatchery conditions. Murza and Khristoforov (1993) concluded, on the basis of studies of sexual maturation in Atlantic salmon and sea trout (Salvelinus fontinalis), that feeding period duration does not affect the dynamics of oogenesis and Wallace et al. (1987) found that even in vitro, oocytes could maintain sustained growth and differentiation if provided with appropriate nutrients. The data from the present study tend to support these findings.

The percentage of Saint John River cage-reared female salmon that reached sexual maturity in November 1991 (7.4%) was only one half the value that had been predicted based on plasma hormone levels from samples of the cohort in the previous July (14.8%). Therefore, the minimum plasma levels of T, E2, and Vg recorded in predicted spawners in July 1991 may have been lower than the minimum values in the portion of these fish that actually reached sexual maturity the following November. The minimum Vg value of 1.81 mg/ml recorded in predicted spawners in July 1991 was much higher than that (0.396 mg/ml) reported by Idler et al. (1981) as the minimum found in female spawners-of-the-year captured at sea in June. This may have been the result of the one month later sampling date in July 1991. However, if the 0.396 mg/ml minimum Vg value for spawners-of-the-year had been applied to the July 1991 Saint John River samples, 73% would have exceeded this level and been predicted to be spawners-of-the-year, as compared to the actual maturity rate of 7.4%. Hence, the value of 1.81 mg/ml was more appropriate for the prediction of spawners-of-the-year in July. However, the actual minimum Vg value for spawners-of-the-year in July may be higher, as suggested by the comparison of predicted and actual maturity rates.

It may not be possible to establish minimum values for single parameters to predict the state of maturity of salmon because of annual variation in the maturity rate of fish preparing to spawn. This was suggested by the lower mean values of T, 11-KT and GSI in males and T, E2 and GSI in females from commercial landings at Conche

in 1991 compared to 1990, in spite of the later sampling date in 1991. There was also wide variation in plasma hormone levels of fish at the same gonad stage, suggesting that if physiological and histological criteria are to be used to predict the state of maturity of salmon then a number of parameters will need to be considered.

The wider distribution of testis and ovary stages and the lower overall mean plasma hormone levels in samples from Conche and Twillingate compared to St. Barbe Bay, supports the conclusion that non-maturing salmon were present in commercial landings at the former two locations. The wider distribution of gonad stages at Conche than at St. Barbe Bay may be related to the larger sample size there, but this would not explain the presence of testis stages 2-3 at Twillingate compared to stages 3-4 at St. Barbe Bay as sample sizes were similar at both locations. The lower mean plasma hormone levels and GSI at each gonad stage in males and females from Conche in 1991 compared to 1990 may represent inter-annual variation in the rate of sexual maturation.

It is not known what environmental influences may have affected gonadal development or hormone levels in the commercial landings between 1990 and 1991. However, photoperiod and temperature have been reported to influence the rate of oogenesis and spermiation in temperate species such as salmonids (Lam, 1983). Low sea surface temperatures which may occurred at Conche in 1991 compared to 1990, due to severe inshore ice conditions, may have resulted in the lower mean plasma hormone and GSI levels. Lower temperatures could also have affected the distribution

and run-timing of salmon (Reddin and Shearer, 1987) in the Conche area resulting in the lower landings in 1991.

Later sampling dates, however, did not account for the more advanced gonad development recorded in male and female salmon at St. Barbe Bay compared to Conche and Twillingate. Ovary and testis stages observed in samples from the three commercial fishery locations were not correlated with the date of sampling except for male salmon at Conche in 1990 ($P < .05$; $R^2 = .29$), indicating that the most sexually mature fish were not simply those caught later in the season.

Discriminant analyses indicated that all virgin sea-age-1 male and female salmon sampled from commercial landings at St. Barbe Bay in 1990 and 1991 were potential spawners-of-the-year but only 97.1% at Conche in 1990 and 73.7% in 1991, were spawners-of-the-year. The remaining 2.9% at Conche in 1990 and 26.3% in 1991 were non-maturing fish.

The lower percentage, determined from discriminant analysis, of female spawners-of-the-year relative to males in commercial landings at Conche in 1991 is suggestive that some of these fish originated in rivers of the maritime provinces of Canada. Sea-age-1 salmon returning to these rivers are predominantly male (Marshall and Penney, 1983), whereas, the majority of sea-age-1 salmon returning to most Newfoundland rivers are predominantly female (Chadwick et al., 1986).

The higher average river-age of sea-age-1 salmon at St. Barbe Bay than at Conche and Twillingate is further evidence that maritime origin salmon were present in commercial landings at the latter two locations. Atlantic salmon from many maritime rivers spend 2 to 3 years in the river before migrating to sea for the first time (Scott and Scott, 1988) compared to 2 to 4 years for Newfoundland rivers (Porter, 1975).

Whether or not maritime origin fish were present in the spawners-of-the-year and non-maturing sea-age-1 salmon identified in commercial landings is more difficult to assess because the sample size was small. However, the predominance of male sea-age-1 spawners-of-the-year at Conche indicates the maritime origin of some fish. If non-maturing sea-age-1 salmon of maritime origin were present they would be predominantly female.

The results of this study indicate that either gonad development or plasma steroid hormone profiles can be used under certain circumstances and/or in combination as a discriminant function to identify the state of maturity of male and female salmon in commercial landings. The discriminant functions derived for males and females distinguished between spawners-of-the-year and non-maturing fish with a high level of reliability and can now be applied to observations on single fish or larger sample sizes. It had not been possible to identify the proportion of commercial landings that were of maritime origin on the basis of the physiological parameters measured. This could now be done using discriminant

analysis provided gonad development and plasma hormone profiles are available for non-maturing maritime and Newfoundland origin salmon.

The closure of the Newfoundland commercial salmon fishery in 1992 should have reduced any interception of non-maturing maritime origin sea-age-1 salmon, and consequently the numbers of sea-age-2 fish returning to maritime rivers in 1993 should have increased as a result, but this was not the case. In 1993, the returns of sea-age-2 Atlantic salmon to New Brunswick and Nova Scotia rivers that flow into the Gulf of St. Lawrence were in fact lower than in 1992 (Anon. 1994). This indicates that either the effect of the interception on stock abundance is relatively low or that the interception was extremely variable as suggested by the wide range of occurrence of non-maturing females in commercial landings at Conche between 1990 and 1991.

The proportion of non-maturing male and female salmon landed at Conche, varied substantially between 1990 and 1991. A sampling program would, therefore, need to be conducted every year in order to evaluate the interception of non-maturing salmon and to predict the impact on river returns of sea-age-2 salmon in the following year. Such a program would require a low cost and 'low-tech' method of determining the state of sexual maturity. This study shows that histological sections of ovaries from females just about to spawn were eosinophilic (pink) whereas those from non-maturing females are basophilic (blue). This observation could become the basis of a test for histological identification of potential spawners-of-the-year.

The oocyte cytoplasm contains organelles, i.e. mitochondria, RER, SER, etc., and other components/inclusions, i.e. yolk, glycogen, lipid, and ribosomes. Several organelles and components are amplified and stockpiled during early stages of oogenesis, especially mRNA (ribosomes) and mitochondria (Balbiani bodies).

The large amount of stockpiled MRNA and mitochondrial DNA (Balbiani bodies) is probably responsible for the basophilic staining of small oocytes in salmon ovary stages 3-6. Bouin fluid damages or dissolves mitochondria, but not their DNA (Carleton & Drury 1957).

Salmon oocytes accumulate yolk (Vg) in later stages of oogenesis (7-12). Bouin fixative dissolves the lipid components of yolk and leaves behind the protein basis, which is demonstrated as a finely granular eosinophil mass in haematoxylin and eosin staining (Galigher & Kozloff 1971). This Bouin-induced proteinaceous yolk residue predominates in the cytoplasmic staining of oocytes in salmon ovary stages 7-11. The transition from prevalence of nucleic acids to yolk platelets occurs in salmon ovary stage 7 and this is reflected in the variable cytoplasmic staining.

REFERENCES

Anonymous. 1984. Atlantic salmon Scale Reading. ICES Report of the Atlantic salmon Scale Reading Workshop, Aberdeen, Scotland, 23-28 April, 1984.

Anonymous. 1994. Report on the status of Atlantic salmon stocks in eastern Canada in 1993. DFO Atlantic Fisheries Stock Status Report 94/2, 118 p.

Carleton, H.M. and R.A.B. Drury. 1957. Histopathological Technique. 3 rd ed. Oxford University Press. Toronto.

Chadwick, E.M.P. 1985. Fundamental research problems in the management of Atlantic salmon, Salmo salar L., in Atlantic Canada. J. Fish. Biol. **27** (Supplement A), 9-25.

Chadwick, E.M.P., R.G. Randall, and C. Léger. 1986. Ovarian development of Atlantic salmon (Salmo salar) smolts and age at first maturity, p. 15-23. *In* D.J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. publ. Fish. Aquat. Sci. 89.

Chadwick, E.M.P., R.R. Claytor, C.E. Léger, and R.L. Saunders. 1987. Inverse correlation between ovarian development of Atlantic salmon (Salmo salar) smolts and sea age. Can. J. Fish. Aquat. Sci. 44:1320-1325.

Cohen, J. 1960. A coefficient of agreement for nominal scales. Educ. Psychol. Measur., 20:37-46.

Crim, L.W. and D.R. Idler. 1978. Plasma gonadotrophin, estradiol and vitellogenin and gonad phosvitin in relation to the seasonal reproductive cycles of female brown trout. Ann. Biol. Anim. Bioch. Biophys., 18(4), 1001-1005.

Galigher, A.E. and E.N. Kozloff. 1971. Essentials of Practical Microtechnique. 2nd ed. Lea & Febiger. Philadelphia.

Hiroi, O. and K. Yamamoto. 1968. Studies on the maturation of salmonid fishes-II. Changes in the testis of the masu salmon, (Oncorhynchus masou), during anadromous migration. Bulletin Faculty of Fisheries, Hokkaido University. Vol. 20, 1968: 252-264.

Humason, G.L.. 1979. Animal tissue techniques, [fourth ed.]. W.H. Freeman and Company. San Francisco.

Idler, D.R., I.I. Bitners and P.J. Schmidt. 1960. Isolation and identification of 11-ketotestosterone in salmon plasma. Can. J. Biochem. Physiol. Vol. 38: 1053-1057 p.

Idler, D.R., D.A. Horne and G.B. Sangalang. 1970. Identification and quantification of the major androgens in testicular and peripheral plasma of Atlantic salmon (Salmo salar) during sexual maturation. Gen. Comp. Endocrinol. 16, 257-267.

Idler, D.R., S.J. Hwang and L.W. Crim. 1979. Quantification of vitellogenin in Atlantic salmon (Salmo salar) plasma by radioimmunoassay. J. Fish. Res. Board Can. 36:574-578.

Idler, D.R., S.J. Hwang, L.W. Crim, and D. Reddin. 1981. Determination of sexual maturation stages of Atlantic salmon (Salmo salar) captured at sea. Can. J. Fish. Aquat. Sci. 38: 405-413.

Ishida, R., K. Takagi and S. Arita. 1961. Criteria for the differentiation of mature and immature forms of chum and sockeye salmon in northern seas. Bulletin 5, Int. North Pac. Fish. Comm., August, 1961. p. 27-47.

Lam, T.J. 1983. Environmental influences on gonadal activity in fish p. 65-101. In W.S. Hoar, D.J. Randall, and E.M. Donaldson [ed.] Fish Physiology, Vol IX: p. 469. Academic Press.

Marshall, T.L. and G.H. Penney. 1983. Spawning and river escapement requirements for Atlantic salmon of the Saint John River, New Brunswick. CAFSAC Research Document 83/66.

Methven, D.A., L.W. Crim, B. Norberg, J.A. Brown, G.P. Goff, and I. Huse. 1992. Seasonal reproduction and plasma levels of sex steroids and vitellogenin in Atlantic halibut (Hippoglossus hippoglossus). Can. J. Fish. Aquat. Sci. 49: 754-759.

Murza, I.G. and O.L. Khristoforov. 1993. Estimation of degree of maturity of gonads and prediction of age of attainment of sexual maturity in Atlantic salmon and sea trout (Salmo trutta L.) (methodological instructions). Can. Translat. Fish. Aquat. Sci. No. 5599, 106 pp.

Peppar, J.L. 1982. Atlantic salmon smolt investigations, Restigouche River system, New Brunswick. Can. MS Rep. Fish. Aquat. Sci. No. 1641. vii + 15 p.

Peppar, J.L. 1983. Adult Atlantic salmon (Salmo salar L.) investigations, Restigouche River system, New Brunswick, 1972-80. Can. MS Rep. Fish. Aquat. Sci. No. 1695. viii + 33 p.

Pippy, J. [Chairman] 1982. Report of the working group on the Interception of Mainland Salmon in Newfoundland. Can. MS Rep. Fish. Aquat. Sci. 1654: x + 196 p.

Porter, T.R. 1975. Biology of Atlantic salmon in Newfoundland and Labrador. Department of Environment, Fisheries and Marine Service Information Report Series No. NEW/N-75-2: 11 p.

Reddin, D.G., and W.H. Lear. 1990. Summary of marine tagging studies of Atlantic salmon (Salmo salar L.) in the Northwest Atlantic area. Can. Tech. Rep. Fish. Aquat. Sci. 1737: iv + 115 p.

Reddin, D.G., and W.M. Shearer. 1987. Sea-surface temperature and distribution of Atlantic salmon in the Northwest Atlantic Ocean. American Fisheries Society Symposium, 1:262-275.

SAS Institute Inc. 1985. SAS/STAT Guide for Personal Computers, Version 6 Edition. Cary, NC: SAS Institute Inc., 378 pp.

Saunders, Richard L. 1969. Contributions of salmon from the Northwest Miramichi River, New Brunswick, to various fisheries. J. Fish. Res. Bd. Canada 26: 269-278.

Scott, W.B., and M.G. Scott. 1988. Atlantic Fishes of Canada. Can. Bull. Fish. Aquat. Sci. 219: 731 p.

So, Y.P., D.R. Idler and S.J. Hwang. 1985. Plasma vitellogenin in landlocked Atlantic salmon (Salmo salar Ouananiche): isolation, homologous radioimmunoassay and immunological cross-reactivity with vitellogenin from other teleosts. Comp. Biochem. Physiol. Vol. 81B, No. 1, pp. 63-71.

Sutterlin, A.M., and D. MacLean. 1984. Age at first maturity and the early expression of oocyte recruitment processes in two forms of Atlantic salmon (Salmo salar) and their hybrids. Can. J. Fish. Aquat. Sci. 41: 1139-1149.

Titus, K., J.A. Mosher and B.K. Williams. 1984. Chance-corrected classification for use in discriminant analysis:ecological applications. The American Midland Naturalist Vol. 111 No. 1, 1-7 p.

Truscott, B., D.R. Idler, Y.P. So, and J.M. Walsh. 1986. Maturational steroids and gonadotrophin in upstream migratory sockeye salmon. Gen. Comp. Endocrinol. 62, 99-110.

Turner, G.E. 1975a. Exploitation of Miramichi Atlantic salmon based on smolts tagged in 1968, 1969 and 1970. Resource Development Branch, Maritimes Region, Technical Report Series No. MAR/T-75-3. v + 11 p.

Turner, G.E. 1975b. Migration route and timing of Miramichi River salmon (Salmo salar L.) as indicated from recaptures of tagged smolts and adults. Resource Development Branch, Maritimes Region, Technical Report Series No. MAR/T-75-7. v + 11 p.

Wallace, R.A., K. Selman, M.S. Greeley, Jr., P.C. Begovac, Y-W. P. Lin, R. McPherson, and T.R. Petrino. 1987. Current Status of Oocyte Growth. Proceedings of the third annual symposium on reproductive physiology of fish. D.R. Idler, L.W. Crim and J.M. Walsh [ed.] pp. 167-177.

Appendix 1. Protocols used for dehydrating and double embedding of gonad tissue from male and female Atlantic salmon *.

Males

85% ethanol	30 min.
95% ethanol	30 min.
100% ethanol I	15 min.
100% ethanol II	30 min.
Toluene I	15 min.
Toluene II	15 min.
Wax I (Paraplast +; 60.0 C)	2 1/2 - 3 hrs.
Wax II (Paraplast +; 58.0 C)	overnight

Females

85% ethanol	30 min.
95% ethanol	30 min.
100% ethanol	30 min.
Drain	

Double Embedding:

Methyl benzoate + 2% celloidin I	75 min.
Methyl benzoate + 2% celloidin II	75 min.
Drain	
Toluene I	15 min.
Toluene II	30 min.
Wax I (Paraplast +; 60.0 C)	2 1/2 - 3 hrs.
Wax II (Paraplast +; 58.0 C)	overnight.

Note1: The alcohols, methyl benzoate and toluenes were agitated with magnetic stirrers.

Note2: Paraplast + (melting point 56.0 - 58.0 C) was used for Wax I and Wax II.

* Protocols according to S.J. Walsh, Department of Fisheries and Oceans, St. John's, Newfoundland.

Appendix 2. Protocols used for staining of gonad tissue from male and female Atlantic salmon *.

Xylene	3 min.
Xylol (50:50 xylene:ethanol)	2 min.
100% ethanol	3 min.
95% ethanol	2 min.
85% ethanol	2 min.
70% ethanol	2 min.
50% ethanol	2 min.
Distilled water I	3 min.
Harris's haematoxylin**	1 min.
Rinse in running tap water	5 min.
Distilled water II	1 min.
Aqueous eosin Y	2 min.
Distilled water I (vigorous rinse; 3x)	
Distilled water II (vigorous rinse; 3x)	
85% ethanol (vigorous rinse; 3x)	
95% ethanol	5 min.
100% ethanol	5 min.
Xylol	3 min.
Xylene I	3 min.
Xylene II	3 min.
Permount mounting medium on slide.	
Place cover slip on slide and lay flat to dry.	

* Protocols according to S.J. Walsh, Department of Fisheries and Oceans, St. John's, Newfoundland.

** A fresh solution of Harris's haematoxylin was made up about one week before commencement of the staining procedure.

Appendix 3. Protocols used for extraction of steroid hormones from plasma of male and female Atlantic salmon *.

PREPARATION OF PLASMA SAMPLES

1. Untreated or suspected low level samples: 200 μl plasma required. Treated or suspected high level samples: 100 μl plasma required.

2. Add 10 μl of isotope (label) for the recovery estimate. Vortex 1 min. and incubate 0.5-1.5 hrs at room temperature.

3. Add 1.5 mls of ether, vortex 1 min. Freeze the aqueous layer in a dry-ice/EtOH bath. Pour off the unfrozen ether layer, which contains the steroid, into a clean tube.

4. Repeat step 3.

5. Dry down the ether under N_2 and add 1 ml of distilled EtOH to the resultant solute. Vortex 30 sec. Allow the samples to sit at room temperature for at least 1 hour or overnight at 4 C (refrigerator) before doing the recovery estimates.

6. Add 100 μl of each sample to scintillation vials and 10 ml liquid Scintran Brand scintillation cocktail supplied by BDH Chemicals Ltd. Shake.

7. To count tubes - add 10 ml scintillation cocktail + 10 μl recovery isotope.

8. To background tubes - add 10 ml cocktail.

9. Count 100 μl samples, count tubes and background tubes for 20 min. in gamma counter.

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* Protocols according to C. Short and L. Crim, MSRL, MUN.

Appendix 4. Protocols used for preparation of the standard curves for RIAs of steroid hormones in plasma of male and female Atlantic salmon *.

PREPARATION OF STANDARD CURVE

The standard curve is prepared in ethanol (EtOH). The final volumes are dried down under N_2 in the RIA tube and brought back to 100 μl with assay buffer.

Stock solution: 1mg/10ml EtOH

Prepare: 100X = 100 μl stock brought to 10 ml EtOH
10,000X = 100 μl (100X) brought to 10 ml EtOH

The dilutions are made in a 10 ml Class A volumetric flask with a disposable micropipet to measure the 100 μl aliquots.
Subsequent 2X dilutions of the 10,000X are prepared as follows: (first a volume of the 10,000X is poured into tube #1 to allow access to the pipet)

- Add 0.5 ml EtOH to each tube.

- Pipet exactly 0.5 ml of the Ag solution into each successive tube from the previous one.

- Vortex, and change pipet tip each time.

Final dilutions:

Tube #:

1	2	3	4	5	6	7	8	9	10	11

Concentration (pg/100ul):

1000	500	250	125	62.5	31.25	15.6	7.8	3.9	1.95	0.98

---

* Protocols according to C. Short and L. Crim, MSRL, MUN.

Appendix 5. Protocols used for RIAs of 11-ketotestosterone in plasma of male and female Atlantic salmon *.

11-KETOTESTOSTERONE

Important Note: The assay buffer, label and antibody must remain cold for this assay. Label and antibody should be made up ahead of time and stored in refrigerator prior to preparing the standards.

Assay Buffer

$NaH_2PO_4.H_2O$ (0.028M)	3.87 g
Na_2HPO_4 (0.061M)	8.66 g
NaCl (0.154M)	9.00 g
Distilled H_2O	1000 ml

pH 7.0
After adjusting the Ph, add 1.0 g Gelatin/litre (0.1%) and heat to dissolve.

Label

For recovery: 1000 cpm/10μl
For assay : 10,000 cpm/100μl

The isotope is stored at -20 C. An appropriate volume is dried down under N_2 and brought up to volume with assay buffer. (Check a 10 μl aliquot of the stock to determine how much is needed for the entire assay).

Antibody

The antibody is stored at -20 C in 50 μl aliquots which are already diluted 100X in EtOH and must be dried down before adding buffer. Use an antibody to buffer dilution of 1:25,000 (43%).

1:25,000/100 = 1:250 (100X dilution)
Therefore, 100 μl Ab : 25,000 μl Buffer

Preparation of Samples (See Appendix 3).

Standard Curve (See Appendix 4).

APPENDIX 5 Continued

Assay Protocol

Tube #	ID	Std.#	Vol.	Buffer	AB	Isotope	Charcoal
1,2	CT	-	-	800µl	-	100µl	-
3,4	0 AB	-	-	200µl	-	"	600µl
5,6	0 Hor.	-	-	100µl	100µl	"	"
7,8	0.98	11	100µl	"	"	"	"
9,10	1.95	10	"	"	"	"	"
11,12	3.9	9	"	"	"	"	"
13,14	7.8	8	"	"	"	"	"
15,16	15.6	7	"	"	"	"	"
17,18	31.2	6	"	"	"	"	"
19,20	62.5	5	"	"	"	"	"
21,22	125.0	4	"	"	"	"	"
23,24	250.0	3	"	"	"	"	"
25,26	500.0	2	"	"	"	"	"
27,28	750.0	1	75µl	"	"	"	"
29,30	1000.0	1	100µl	"	"	"	"
31,32up	sample						

- Add standards and samples (100µl) to RIA tubes (in EtOH) and dry down under N_2.

- Add assay buffer, vortex. Once assay buffer has been added to tubes, the tubes should be kept in ice/water bath.

- Add 100µl isotope (10,000cpm/100µl)

- Add antibody (at appropriate dilution)

- Incubate overnight at 4 C.

APPENDIX 5 Continued

-Make up charcoal separation solution:

Dextran-coated Charcoal

Kodak Charcoal RIA Grade (0.25% w/v)	1.25 g
Dextran T-70 (0.25% w/v)	0.125 g
Assay Buffer	500 mls

- Transfer tubes to ice bath and add 600 µl charcoal suspension (@ 4 C)

- Incubate in ice/water bath for 60 min. Vortex.

- Centrifuge at 3400 rpm for 25 min.

- Dump into scintillation vials and add 10 ml scintillation cocktail. Vortex.

- Count standards and samples for 2 min. each in scintillation counter.

---

* Protocols according to C. Short and L. Crim, MSRL, MUN.

Appendix 6. Protocols used for RIAs of testosterone and estradiol in plasma of male and female Atlantic salmon *.

TESTOSTERONE & ESTRADIOL

Assay Buffer

50 nM Phosphate, 100 mM Chloride, 0.1% Azide. 0.1% Gelatin

Na_2HPO_4	6.30 g/l
KH_2PO_4	0.75 g/l
NaCl	5.86 g/l
NaN_3	1.00 g/l
DH_2O	1000 ml

Ph 7.4
After adjusting the Ph, add 1.0 g gelatin/litre and heat to dissolve.

Label

The isotope is stored at 4 C. Make the appropriate assay buffer such that there is approximately 10,000 cpm/100μl. **NOTE:** This isotope is stored in buffer, not ethanol and does not need to be dried down.

The recovery estimates will be done using the old tritiated isotope. Using the dilution of 1000cpm/10μl, the old procedures still apply.

Antibody

The antibody is stored at 4 C. Determine appropriate working dilution. The dilution is made up is assay buffer.

Preparation of Samples (See Appendix 3).

Standard Curve (See Appendix 4).

Separation

Add 1.0 ml of separating reagent to all tubes except 1&2.

APPENDIX 6 Continued

Assay Protocol

Tube #	ID	Std.#	Vol.	Buffer	AB	Isotope	Charcoal
1,2	CT	-	-	-	-	100µl	-
3,4	0 AB	-	-	300µl	-	"	1.0 ml
5,6	0 Hor.	-	-	200µl	100µl	"	"
7,8	0.98	11	100µl	"	"	"	"
9,10	1.95	10	"	"	"	"	"
11,12	3.9	9	"	"	"	"	"
13,14	7.8	8	"	"	"	"	"
15,16	15.6	7	"	"	"	"	"
17,18	31.2	6	"	"	"	"	"
19,20	62.5	5	"	"	"	"	"
21,22	125.0	4	"	"	"	"	"
23,24	250.0	3	"	"	"	"	"
25,26	500.0	2	"	"	"	"	"
27,28	750.0	1	75µl	"	"	"	"
29,30	1000.0	1	100µl	"	"	"	"
31,32up	sample		100µl				

NOTE: all reagents were at room temperature.
- Add standards/samples to duplicate RIA tubes (in EtOH), dry down under N_2.

- Add 200µl assay buffer, vortex.

- Add 100µl antibody and 100µl isotope (working dilution), Vortex, let stand 1 hr. at room temperature (take out separating reagent, shake bottle, allow to come to room temperature).

APPENDIX 6 Continued

- Add 1.0 ml of separating reagent (all except CT's), vortex and let sit at room temperature for 25 min.

- Centrifuge at 3000 rpm for 15 min.

- Aspirate supernatant carefully. Count 1.0 min. in gamma counter (may be left overnight before counting).

---

* Protocols according to C. Short and L. Crim, MSRL, MUN.

Appendix 7. SAS program used for discriminant analysis and classification of the state of maturity in male and female Atlantic salmon.

```
*FILE:FEDISC.SAS;
*MARCH 7, 1994;
OPTIONS PAGESIZE=58 LINESIZE=132 NOCENTER;
LIBNAME LIB 'c:\SALMAT\ssd';
*************************************************************************
DISCRIMINATION AND CLASSIFICATION OF THE STATE OF MATURITY OF MALE
AND FEMALE SALMON BASED ON PLASMA HORMONE LEVELS, Vg, GSI, CF AND
STAGE OF GONADAL DEVELOPMENT.
*************************************************************************;
*CODES USED TO REFER TO SAMPLING LOCATIONS AND DATES*;
   *SJ1 = SAINT JOHN RIVER MAY 1990;
   *SJ2= SAINT JOHN RIVER OCT. 1990;
   *SJ3= SAINT JOHN RIVER MAY 1991;
   *SJ4= SAINT JOHN RIVER JULY 1991;
   *SJ5= SAINT JOHN RIVER NOV. 1991;
   *LS91=LABRADOR SEA OCT. 1991;
   *H91= HUMBER RIVER JULY 1991;
   *W90= WESTERN ARM BROOK 1991;
   *SB90=ST. BARBE BAY, JULY 1990;
   *SB91=ST. BARBE BAY, JULY 1991;
   *C90= CONCHE, JULY 1990;
   *C91= CONCHE JULY 1991;
   *TW91=TWILLINGATE 1991;
*************************************************************************
SELECTION OF THE CALIBRATION DATASET
*************************************************************************;
DATA MAT; SET LIB.VIRGSUM;
*NOTE: ONLY VIRGIN SEA-AGE-ONE FISH IN DATASET VIRSUM;
    if loc='SJ1' OR loc='SJ2' OR LOC='H91'
            OR LOC='W90' OR LOC='LS91';
WWT=WWT*1000;
*****SELECTION OF FEMALE SALMON*****;
  TITLE'MATURITY IDENTIFICATION OF FEMALE ATLANTIC SALMON';
  TITLE2'MATURITY DATA FROM SJ1-SJ2, H91, W90, LS91';
    IF SEX=2;
     IF LOC='W90' THEN DO;
      STAGE=11;
     END;
*MATURING GROUPS;
IF LOC='H91' THEN MATCLASS= 'MATURING    ';
IF LOC='W90' THEN MATCLASS= 'MATURING    ';
*NON-MATURING GROUPS;
IF LOC='LS91' THEN MATCLASS='NON-MATURING';
IF LOC='SJ1' THEN MATCLASS= 'NON-MATURING';
IF LOC='SJ2' THEN MATCLASS= 'NON-MATURING';
```

APPENDIX 7 Continued

```
KEEP LOC T KT E Vg GOWT WWT GSI CF SA STAGE MATCLASS;
PROC PRINT; RUN;
*********************************************************************
SELECTION OF QUANTITATIVE VARIABLES FOR THE DISCRIMINANT FUNCTION
*********************************************************************;
 PROC STEPDISC data=mat;
  CLASS MATCLASS;
 *VARIABLES TO BE TESTED - FEMALE;
   VAR T E Vg GSI CF STAGE;
   *NOTE1: VARIABLE KT NOT MEASURED IN FEMALES;
   *NOTE2: VARIABLE HSI MISSING IN MOST OBSERVATIONS, NOT INCL.;
RUN;
*********************************************************************
DISCRIMINATION
*********************************************************************;
PROC DISCRIM data=mat POOL=TEST OUTSTAT=LIB.FEDISC2;
title2'DISCRIMINANT FUNCTION BASED ON SJ1-SJ2 H91, W90, LS91';
 CLASS MATCLASS;
 VAR E T STAGE;
RUN;
*********************************************************************
CALCULATION OF CANONICAL COEFFICIENTS OF SELECTED VARIABLES
*********************************************************************;
 PROC CANDISC DATA=MAT;
  CLASS MATCLASS;
 *SELECTED VARIABLES - FEMALE;
    VAR E T STAGE;
RUN;
*********************************************************************
*********************************************************************
TEST ACCURACY OF CLASSIFICATION TECHNIQUES
BY RANDOM SELECTION OF ONE HALF OF THE OBSERVATIONS IN THE INPUT
DATASET FOR THE DISCRIMINANT FUNCTION AND THE OTHER HALF TO BE
CLASSIFIED. THE TWO RESULTANT DATASETS ARE TESTED AGAINST EACH OTHER.
*********************************************************************;
*****CREATE TWO DATASETS FROM FIRST AND SECOND HALF OF ORIGINAL*****;
DATA MAT2; SET MAT; RETAIN SEED1 1613219081;
   RANNUM=RANUNI (SEED1);
   KEEP LOC T KT E Vg GSI CF STAGE MATCLASS RANNUM;
 PROC SORT DATA=MAT2; BY RANNUM;
RUN;
*191 OBS IN FEMALE DATASET;
DATA TEST1; SET MAT2 (FIRSTOBS=1 OBS=95);
DATA TEST2; set MAT2 (FIRSTOBS=96 OBS=191);
 PROC SORT DATA=TEST1; BY LOC;
 PROC SORT DATA=TEST2; BY LOC;
*****DISCRIMINANT FUNCTION BASED ON FIRST HALF-TEST1*****;
 PROC DISCRIM DATA=TEST1 POOL=TEST OUTSTAT=TESTDIS1 NOPRINT;
```

APPENDIX 7 Continued

```
    CLASS MATCLASS;
     VAR E T STAGE;
*****DISCRIMINANT FUNCTION BASED ON SECOND HALF-TEST2*****;
 PROC DISCRIM DATA=TEST2 POOL=TEST OUTSTAT=TESTDIS2 NOPRINT;
    CLASS MATCLASS;
    VAR E T STAGE;
RUN;
*************************************************************************
*****TEST OF CLASSIFICATION TECHNIQUE*****
*************************************************************************;
*****TEST FIRST HALF WITH SECOND HALF*****;
 PROC DISCRIM DATA=TESTDIS2 TESTDATA=TEST1 TESTLIST
        TESTOUT=TEST1OUT; TESTID LOC;
    CLASS MATCLASS;
    TESTCLASS MATCLASS;
    VAR E T STAGE;
  TITLE2'MATURITY DATA FROM SJ1-SJ2, H91, W90, LS91';
  TITLE3' CLASSIFICATION OF TEST DATASET TEST1-FIRST HALF';
******TEST SECOND HALF WITH FIRST HALF*****;
 PROC DISCRIM DATA=TESTDIS1 TESTDATA=TEST2 TESTLIST
        TESTOUT=TEST2OUT; TESTID LOC;
    CLASS MATCLASS;
    TESTCLASS MATCLASS;
    VAR E T STAGE;
  TITLE3' CLASSIFICATION OF TEST DATASET TEST2-SECOND HALF';
RUN;
*************************************************************************
*************************************************************************
CLASSIFICATION OF UNKNOWNS
*************************************************************************;
DATA UNKNOWNS; SET LIB.VIRGSUM;
 IF LOC='SJ3' OR LOC='SJ4' OR LOC='SJ5' OR
   LOC='SB90' OR LOC='SB91' OR
   LOC='C90' OR LOC='C91' OR
   LOC='TW91';
 IF SEX=2;
 MATCLASS='UNKNOWN     ';
 KEEP LOC STAGE T KT E Vg GSI CF MATCLASS;
RUN;
PROC DISCRIM DATA=LIB.FEDISC2 TESTDATA=UNKNOWNS TESTLIST
        TESTOUT=UNKOUT; TESTID LOC;
 CLASS MATCLASS;
 TESTCLASS MATCLASS;
 VAR E T STAGE;
 TITLE2'CLASSIFICATION OF FEMALE SALMON OF UNKNOWN MATURITY';
RUN;
```

Appendix 8. Calculation of Cohen's kappa statistic, statistical significance, and 95% confidence interval for observed and predicted state of maturity in male and female Atlantic salmon *.

Test data classification matrix:

OBSERVED MATURITY:	PREDICTED MATURITY:			
	Maturing	Non-Maturing	Row Total	Row Prop.
Maturing	8	0	8	0.17
Non-Maturing	1	39	40	0.83
Col. Total	9	39	48	
Col. Prop.	0.19	0.81		
Row X Col.	0.03	0.68		

Calculation of kappa:

Po = sum of the observed proportion of agreement

$$Po = \frac{\text{sum matrix diagonal elements}}{\text{sum of all elements}} = \frac{47}{48} = 0.979$$

Pc = sum of the chance expected proportion of agreement

a. row proportions = $\frac{\text{row total}}{\text{sum of all elements}}$

b. column proportions = $\frac{\text{column total}}{\text{sum of all elements}}$

c. Pc = sum of (row proportion X column proportion)

Pc = 0.708

$$\text{Kappa} = \frac{Po-Pc}{1-Pc} = 0.929$$

Calculation of 95 % CI (2 x Sk) - using appropriate standard error of K:

$$\text{Standard Error } K = Sk = \text{SQRT}\ \frac{Po(1-Po)}{N(1-Pc)^2} = \text{SQRT } 0.005 = 0.071$$

95% CI = K +/- 2 x Sk = 0.858 to 0.999

Test of significance (Z):

Approximate standard error of K (SEk) (under Ho: K = 0)

$$Sek = \text{SQRT}\ \frac{Pc}{N(1-Pc)} = \text{SQRT } 0.051 = 0.225$$

$$Z = \frac{K-0}{Sek} = \frac{0.929}{0.225} = 4.128$$

---

* Summary of calculations adapted from Titus et al., 1984.

Appendix 9. Parameter values measured in blood and ovary samples from female Atlantic salmon sampled from the Saint John River cage–reared stock. The values of 'Stain' refer to the colour of stained ovary sections – 'A'= Acidophilic (pink); 'B'=Basophilic (blue); and 'AB'=intermediate (mauve). 'CF' refers to Condition Factor.

a) Sampling Date: May 12, 1990

Sample No.	T ng/ml	E2 ng/ml	Vg mg/ml	GSI %	HSI %	CF	Ovary Stage	Stain
3	1.44	0.07	0.10	0.20	.	1.13	4	B
5	2.20	0.10	0.12	0.18	.	1.02	4	B
7	1.54	0.24	0.11	0.16	.	1.06	.	.
8	2.58	0.37	0.12	0.17	.	1.14	4	B
9	2.46	0.41	0.15	0.18	.	1.10	4	B
11	3.13	0.39	0.13	0.18	.	1.16	4	B
12	3.68	0.61	0.15	0.23	.	1.11	4	B
13	0.36	0.48	0.10	0.22	.	1.01	4	B
14	0.02	0.35	0.13	0.20	.	1.05	4	B
16	0.48	0.09	0.06	0.05	.	1.14	.	B
18	0.97	0.25	0.13	0.17	.	1.11	4	B
26	0.73	0.22	0.06	0.17	.	1.05	4	B
28	0.77		0.05	0.17	.	1.14	4	B
29	0.60	0.32	0.16	0.16	.	1.11	3	B
30	0.74	0.30	0.48	0.16	.	1.11	4	B
31	0.64	0.13	0.10	0.17	.	1.09	4	B
33	0.27	0.18		0.10	.	1.22	4	B
35	0.84	0.38	0.11	0.13	.	1.17	4	B
36	1.03	0.75	0.15	0.19	.	1.12	4	B
37	0.87	0.31	0.10	0.25	.	1.16	4	B
38	1.20	0.84	0.07	0.19	.	1.11	4	B
42	1.74	0.54	0.11	0.19	.	1.12	4	B
43	1.16	0.53	0.07	0.02	.	1.09	.	.
44	0.70	0.48	0.91	0.14	.	1.07	4	B
46	0.95	0.27	0.06	0.25	.	1.21	4	B
47	0.97	0.50	0.11	0.20	.	1.21	4	B
49	0.91	0.33	0.07	0.17	.	1.03	4	B
50	0.96	0.52	0.22	0.12	.	1.24	4	B
51	0.92	0.38	0.15	0.15	.	1.05	4	B
MEAN	1.20	0.37	0.15	0.17	.	1.12		
STD	0.86	0.19	0.17	0.05	.	0.06		
N	29	28	28	29	.	29		

b) Sampling Date: October 15, 1990

Sample No.	T ng/ml	11KT ng/ml	E2 ng/ml	Vg mg/ml	GSI %	HSI %	CF	Ovary Stage	Stain
2	0.49		0.87	0.05	0.12	1.07	0.79	4	B
3	0.27		0.47	0.01	0.11	0.98	0.76	4	B
4	0.19		0.38	0.11	0.10	1.06	0.73	4	B
5	0.15		0.21	0.06	0.22	1.86	0.88	4	B
6	0.23		0.53	0.15	0.13	1.43	0.73	4	B
7	0.14		0.36	0.18	0.12	1.92	0.95	4	B
8	0.18		0.75	0.17	0.12	1.21	0.79	4	B
11	0.42		0.73	0.01	0.27	1.53	0.90	4	B
12	0.33		0.40	0.10	0.14	1.03	0.79	4	B
17	0.36		0.45	0.17	0.10	1.13	0.72	4	B
21	0.11		0.38	3.29	0.10	1.36	0.77	4	B
22	0.31		0.56	5.14	0.12	1.08	0.74	5	B
23	0.20		0.54	5.06	0.11	1.36	0.72	4	B
25	0.50	0.00	0.43	6.35	0.12	1.27	0.77	4	B
28	0.33		0.65	8.97	0.13	1.24	0.73	4	B
29	0.52		0.84	5.13	0.15	1.12	0.77	5	B
30	0.29		0.45	0.15	0.12	1.31	0.69	4	B
33	0.53		0.56	0.16	0.11	1.12	0.80	4	B
34	0.45		0.42	0.17	0.13	1.29	0.87	4	B
35	0.70		0.73	0.17	0.11	1.28	0.65	5	B
36	0.54		0.49	0.18	0.13	1.23	0.79	4	B
37	0.52		0.69	0.16	0.13	1.31	0.76	4	B
39	0.49		0.34	0.10	0.09	1.23	0.66	4	B
40	0.30		0.40	0.17	0.12	1.32	0.76	4	B
44	0.16		0.14	0.16	0.13	1.03	0.80	4	B
46	0.21		0.18	0.19	0.14	1.44	0.76	4	B
48	0.34		0.35	0.13	0.11	1.05	0.77	4	B
49	0.31		0.37	0.15	0.15	1.23	0.77	4	B
50	0.10		0.37	0.19	0.11	1.45	0.79	4	B
51	0.29		0.27	0.16	0.13	1.08	0.73	5	B
MEAN	0.33	.	0.47	1.24	0.13	1.27	0.77		
STD	0.15	.	0.19	2.38	0.04	0.22	0.06		
N	30	1	30	30	30	30	30		

c) Sampling Date: May 4, 1991

Sample No.	T ng/ml	E2 ng/ml	Vg mg/ml	GSI %	HSI %	CF	Ovary Stage	Stain
1	0.24	0.04	0.16	0.17	0.91	0.83	4	B
2	0.41	0.05	0.20	0.21	0.89	0.86	6	B
4	0.51	0.09	0.09	0.19	0.82	0.96	6	B
5	0.86	0.13	0.09	0.23	1.06	0.93	5	B
7	1.14	0.02	0.04	0.20	2.42	0.81	5	B
9	0.51	0.17	0.11	0.18	1.02	0.88	5	B
10	1.02	0.15	0.06	0.20	2.01	0.93	5	B
13	3.96	1.15	0.18	0.25	1.35	0.81	5	B
16	0.88	0.14	2.95	0.20	0.89	0.98	5	B
17	0.93	0.09	0.09	0.22	1.68	0.87	5	B
19	3.27	0.91	0.07	0.26	0.92	0.84	5	B
20	1.02	0.11	0.02	0.20	0.94	0.89	4	B
21	0.54	0.08	0.43	0.20	0.90	0.89	5	B
27	0.68	0.09	0.86	0.23	1.47	0.87	6	B
28	1.91	0.39	0.09	0.22	0.98	0.87	4	B
29	0.64	0.27	0.82	0.23	1.19	0.83	5	B
31	4.62	1.03	0.90	0.27	1.21	0.77	6	B
32	0.04	0.32	0.03	0.20	1.25	0.95	5	B
33	1.08	0.31	0.08	0.18	0.96	0.83	6	B
35	0.79	0.15	0.09	0.17	1.00	0.82	5	B
37	3.02	0.61	0.08	0.25	1.08	1.02	6	B
38	0.89	0.23	0.06	0.21	0.93	0.82	5	B
40	0.79	0.11	0.07	0.20	1.03	0.90	4	B
42	4.89	1.07	1.27	0.26	1.28	0.75	6	B
44	0.68	0.07	0.05	0.20	0.92	0.84	5	B
45	0.97	0.14	0.05	0.22	0.87	0.98	5	B
46	1.13	0.15	0.05	0.18	1.23	0.85	6	B
47	2.14	0.35	0.08	0.22	1.31	0.83	5	B
50	2.17	0.54	0.32	0.25	1.27	0.77	5	B
MEAN	1.44	0.31	0.32	0.21	1.17	0.87		
STD	1.30	0.33	0.60	0.03	0.36	0.07		
N	29	29	29	29	29	29		

d) Sampling Date: July 30, 1991

Sample No.	T ng/ml	E2 ng/ml	Vg mg/ml	GSI %	HSI %	CF	Ovary Stage	Stain
1	0.38	0.06	0.82	0.17	1.17	0.82	4	B
2	0.50	0.09	0.54	0.26	1.15	0.98	6	B
3	0.82	0.07	0.22	0.19	1.21	0.76	5	B
5	0.54	0.08	0.14	0.18	1.24	0.82	5	B
7	0.76	0.19	0.76	0.16	1.18	0.77	4	B
8	0.50	0.06	1.14	0.15	1.29	0.76	6	B
9	3.63	0.81	1.81	0.25	1.48	0.79	6	B
11	0.83	0.10	0.78	0.16	1.45	0.80	4	B
12	0.54	0.02	0.10	0.19	1.34	0.83	6	B
16	1.44	0.10	0.37	0.13	1.24	0.84	4	B
17	1.13	0.17	0.53	0.14	1.12	0.79	4	B
23	1.06	0.07	1.42	0.25	1.43	0.68	6	B
24	0.48	0.05	1.73	0.21	1.23	0.82	6	B
25	3.31	0.77	5.46	0.16	1.52	0.69	5	B
26	0.51	0.00	1.05	0.16	1.16	0.79	5	B
27	0.78	0.15	1.61	0.23	1.11	0.80	5	B
28	0.51	0.19	1.42	0.16	1.33	0.75	5	B
30	1.39	0.11	1.04	0.13	1.19	0.88	5	B
34	0.86	0.12	0.18	0.17	1.20	0.78	4	B
35			1.78	0.23	1.47	0.83	6	B
38	4.79	1.11	6.11	0.17	1.23	0.51	6	B
39	0.92	0.19	0.35	0.32	1.40	0.82	5	B
42	0.72	0.11	0.11	0.17	1.11	0.94	6	B
43	0.73	0.12	0.53	0.19	1.10	0.85	4	B
46	1.11	0.17	0.89	0.19	1.26	0.84	4	B
48	2.16	0.01	7.72	0.18	1.44	0.71	4	B
50	0.36	0.07		0.17	1.48	0.79	6	B
MEAN	1.19	0.21	1.48	0.18	1.28	0.79		
STD	1.10	0.28	1.93	0.03	0.13	0.09		
N	26	26	26	27	27	27		

e) Sampling Date: November 9, 1991

Sample No.	T ng/ml	11KT ng/ml	E2 ng/ml	Vg mg/ml	GSI %	HSI %	CF	Ovary Stage	Stain	Comment
2	0.56		0.56	2.50	0.17	1.19	0.75	5	B	
4	0.66		0.72	9.5	0.22	1.49	0.79	6	B	
8	0.50		0.69	2.64	0.35	1.26	0.61	7	AB	
9	1.35		1.43	5.94	0.39	1.27	0.75	7	B	
11	1.31		0.70	0.61	0.36	1.00	0.68	5	B	
14	0.73		0.59	0.94	0.28	1.33	0.45	6	B	
18	0.13		0.06	0.12	0.21	0.93	0.89	6	B	
19	0.34		0.36	0.42	0.28	1.21	0.73	5	B	
20	0.36		0.49	0.24	0.27	1.13	0.77	6	B	
22	0.54		0.50	0.28	0.23	1.28	0.42	6	B	
24	0.49		0.33	0.46	0.26	1.12	0.72	6	B	
25	0.83		0.65	0.39	0.19	1.47	0.43	3	B	
26	0.28		0.68	0.22	0.26	1.14	0.70	6	B	
27	0.27		0.41	5.96	0.28	1.20	0.70	6	B	
28	0.08		0.66	1.85	0.24	1.09	0.45	6	B	
29	0.57	0.04	0.21	26.17	24.74	1.60	0.39	12	.	Ripe gonad
31	0.55		0.06	0.10	0.34	1.14	0.74	.	.	
32	0.94		1.02	0.70		1.23	0.85	4	B	
34	0.32	0.13	0.45	1.46	0.23	1.21	0.61	5	B	
44	0.67	21.30	0.31	0.10	0.22	1.33	0.68	6	B	
46	1.57		1.67	4.05	0.39	1.33	0.72	8	A	
47	0.68		0.74	0.89	0.26	1.08	0.72	6	B	
48	0.83		0.91	4.68	0.32	1.16	0.67	6	B	
49	0.05		0.13	0.11	0.22	0.96	0.66	6	B	
50	0.43		0.38	115.75	19.17	2.19	0.39	12	.	Ripe gonad
52	0.29		0.54	0.30	1.21	0.22	0.77	3	A	
53	0.11		0.19	0.36	0.21	1.04	0.65	3	A	
MEAN	0.39	7.16	0.56	6.57	1.98	1.21	0.66			
STD	0.40	12.25	0.38	22.40	5.94	0.31	0.15			
N	27	3	27	27	26	27	27			

Appendix 10. Parameter values measured in blood and ovary samples from wild female Atlantic salmon at three reference sites. The values of 'Stain' refer to the colour of stained ovary sections – 'A'=Acidophilic (pink); 'B'=Basophilic (blue); and 'AB'=intermediate (mauve). 'CF' refers to Condition Factor.

a) Sampling Location: Labrador Sea, October 1991

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Ovary Stage	Stain
Sea-Age-0												
07-Oct					38.8	0.06		1.02	1	0		
07-Oct	0.21		0.07	0.02	38.2	0.12		0.90	3	0	4	B
07-Oct	0.24		0.15	0.03	38.8	0.09		0.90	3	0	4	B
07-Oct	0.29		0.30	0.06	37.2	0.07		0.90	2	0	5	B
07-Oct	0.87		0.37	0.04	36.6	0.15		0.86	3	0		
08-Oct					41.5	0.11		0.95	2	0		
09-Oct					34.8	0.08		0.92	3	0		
09-Oct					40.0	0.06		0.97	2	0		
09-Oct					36.4	0.08		0.89		0		
09-Oct					36.0	0.10		0.90	3	0		
09-Oct					34.9	0.07		0.90	2	0		
09-Oct					38.4	0.06		0.94	1	0		
09-Oct					38.3	0.17		1.06	4	0		
09-Oct					37.8	0.08		0.93		0		
09-Oct					40.0	0.09		0.94	2	0		
09-Oct	0.33		0.50	0.27	38.2	0.09		0.96	3	0		B
09-Oct					35.4	0.09		0.92	2	0		
09-Oct					39.9	0.00		0.99	2	0	5	B
09-Oct					34.1	0.06		0.94	2	0		
09-Oct					36.2	0.05		0.99	4	0	5	B
09-Oct	0.16		0.18	0.05	37.7	0.12		0.89	4	0	5	B
09-Oct	0.08		0.21	0.04	34.7	0.07		0.91	2	0	4	B
09-Oct					36.8	0.10		1.04	4	0		
10-Oct	0.15		0.22	0.05	38.8	0.05		0.82	2	0	3	B
10-Oct					37.4	0.06		0.99	2	0		
10-Oct					38.6	0.08		1.09	2	0		
10-Oct					38.6	0.06		0.93	2	0		
10-Oct					32.3	0.08		0.94	2	0		
10-Oct	0.00		0.17	0.06	38.3	0.04		1.04	1	0	5	B
10-Oct					45.6	0.17		1.86	5	0		
10-Oct					35.1	0.08		1.11	2	0		
11-Oct	0.22		0.24	0.04	40.1	0.09		1.01	2	0	5	B
11-Oct									2	0		
11-Oct					34.5	0.14		0.87	4	0		
11-Oct					35.7	0.00		1.11	3	0		
11-Oct					39.6	0.06		1.00	2	0		
11-Oct					36.3	0.09		0.89	3	0		
11-Oct					36.4	0.06		0.63	3	0		
12-Oct					38.1	0.10		0.97	2	0		
12-Oct	0.03		0.34	0.04	32.2	0.18		0.90	3	0	5	B
12-Oct					37.5	0.12		0.98	4	0		
12-Oct					33.7	0.12		1.01	3	0		
13-Oct	0.57		0.49	0.07	35.8	0.05		0.90	3	0	5	B
13-Oct	0.36		0.48	0.05	34.8	0.09		0.96	2	0	5	B
13-Oct	1.00		0.64	0.04	33.2	0.19		0.91	4	0		B
13-Oct	0.30		0.49	0.05	32.9	0.06		0.89	2	0	5	B
13-Oct	0.82		0.75	0.07	32.5	0.15		0.93	3	0	5	B
13-Oct	0.39		0.38	0.04	33.7	0.10		0.93	3	0	4	B
13-Oct	0.13		0.26	0.04	30.3	0.12		0.84	4	0		
MEAN	0.34	.	0.35	0.06	36.72	0.09	.	0.96	2.64	0	.	.
STD	0.29	.	0.18	0.06	2.80	0.04	.	0.15	0.92	0	.	.
N	18	0	18	18	48	48	0	48	47	49	16	18
Sea-Age-1												
06-Oct	0.43		0.14	0.04	66.2	0.16		0.91	1	1	6	B
06-Oct					60.0	0.13		0.82	3	1	5	B
06-Oct	1.06		0.83	0.05	61.5	0.17		0.94	3	1	4	B

(continued next page)

Sea-Age-1 continued

07-Oct	0.13	0.37	0.06	61.7	0.11	0.98	3	1	6	B
07-Oct	1.47	0.90	0.05	66.7	0.22	1.08	2	1	7	B
07-Oct	1.87	0.79	0.03	60.2	0.15	0.97	3	1	6	B
07-Oct				61.3	0.12	0.94	2	1		
07-Oct	1.07	0.30	0.03	58.3	0.13	0.93	3	1	7	B
07-Oct				65.4	0.16	0.94	3	1		
08-Oct	0.94	0.60	0.07	66.6	0.23	0.84	2	1	6	B
08-Oct	1.67	0.45	0.05	62.8	0.16	0.86	3	1	7	B
08-Oct	0.14	0.13	0.04	63.2	0.10	0.96	2	1	4	B
09-Oct				53.5	0.17	0.63	2	1		
09-Oct				62.2	0.21	1.09	4	1		
09-Oct	1.07	0.22	0.04	64.7	0.09	1.00	2	1	6	B
09-Oct				58.0	0.16	0.99	2	1		
09-Oct	0.22	0.40	0.11	61.2	0.35	0.96	3	1	/	AB
09-Oct				61.3	0.19	1.06	3	1		
09-Oct	0.72	0.74	0.09	66.2	0.23	0.81	2	1	7	B
09-Oct	1.25	0.75	0.07	69.1	0.17	0.85	2	1	7	B
09-Oct	1.58	1.18	0.05	57.6	0.17	0.93	2	1	6	B
09-Oct	0.65	0.46	0.09	65.4	0.17	0.84	1	1	7	B
09-Oct	0.40	0.19	0.03	62.2	0.06	0.95	2	1	5	B
09-Oct	0.43	0.24	0.04	58.8	0.11	0.86	3	1	6	B
09-Oct	0.33	0.26	0.03	65.1	0.14	1.10	3	1	5	B
09-Oct				63.0	0.40	0.92	4	1		
10-Oct	0.60	0.09	0.09	65.4	0.18	0.86	3	1		B
10-Oct				61.0	0.10	0.76	3	1		
10-Oct	0.18	0.22	0.07	61.4	0.08	0.97	3	1	6	B
10-Oct	0.31	0.55	0.05	64.9	0.15	0.87	1	1	6	B
10-Oct	0.18	0.17	0.04	56.1	0.14	0.91	3	1	4	B
10-Oct	1.54	1.16	0.05	68.3	0.23	0.87	2	1	7	B
10-Oct				61.3	0.11	0.97	3	1		
10-Oct				65.6	0.23	0.89	1	1		
10-Oct	0.90	0.50	0.03	60.3	0.25	0.95	2	1	4	B
10-Oct	0.43	0.25	0.05	60.7	0.11	0.98	3	1	6	B
11-Oct	2.49	0.78	0.31	61.4	0.15	1.00	3	1	6	B
11-Oct	1.03	0.79	0.04	60.2	0.22	1.02	3	1	6	B
11-Oct	0.50	0.48	0.04	58.5	0.09	0.94	2	1	6	B
11-Oct				62.2	0.05	0.90	1	1		
11-Oct	1.11	0.53	0.05	69.3	0.18	0.95	1	1	3	B
11-Oct	0.25	0.41	0.05	65.5	0.12	1.11	2	1	6	B
11-Oct				60.2	0.09	1.01	3	1		
11-Oct	1.64	0.75	0.07	62.5	0.16	0.86	3	1	7	B
11-Oct	2.98	1.04	0.06	63.4	0.36	1.20	4	1	7	B
11-Oct	0.81	0.93	0.05	66.7	0.11	0.96	3	1	6	B
11-Oct	1.28	0.63	0.04	61.2	0.12	0.99	4	1	6	B
11-Oct				58.4	0.13	0.91	3	1		
11-Oct	1.36	0.61	0.04	64.2	0.23	0.98	3	1	5	B
11-Oct	0.38	0.39	0.04	60.1			3	1	5	B
11-Oct	0.38	0.42	0.07	63.9	0.13	0.98	2	1	5	B
11-Oct	1.16	0.29	0.07	65.4	0.15	0.92	3	1	6	B
11-Oct	0.51	0.59	0.06	60.8	0.21	0.95	4	1	7	B
11-Oct				60.8	0.13	0.89	2	1		
12-Oct				59.4	0.14	0.94	3	1		
12-Oct				62.3	0.11	0.84	2	1		
12-Oct	0.27	0.42	0.05	62.5	0.17	1.15		1	6	B
12-Oct				65.2	0.27	0.99	1	1		
12-Oct				64.7	0.18	0.97	2	1		
12-Oct				61.2	0.17	0.87	3	1		
12-Oct				62.4	0.22	0.87	1	1		
13-Oct	0.39	0.48	0.06	61.8	0.10	0.79	4	1	6	B
13-Oct				64.3	0.14	0.91	2	1		
13-Oct	1.52	0.70	0.05	65.5	0.13	0.99	2	1	3	B
MEAN	0.88	. 0.53	0.06	62.52	0.16	. 0.94	2.51	.	.	.
STD	0.60	. 0.28	0.04	3.09	0.07	. 0.09	0.84	.	.	.
N	42	0 42	42	64	63	0 63	63	64	42	43

Appendix 10 continued

b) Sampling Location: Humber River, July–August 1991

Sample Date	T ng/ml	11–KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Ovary Stage	Stain
18–Jul	3.02	0.01	1.06	0.75	49.0	0.24	1.39	0.90	2	1	7	B
21–Jul	3.67	0.02	0.81	1.88	49.0	0.35	1.49	1.04	3	1	7	B
24–Jul	8.67	0.08	4.75	6.65	50.8	0.70	1.45	1.01	3	1	8	A
25–Jul	9.05	0.12	2.15	2.27	50.4	0.44	1.60	1.03	3	1	7	AB
25–Jul	10.37	0.03	3.95	6.57	49.4	0.66	1.56	1.02	3	1	8	A
25–Jul	3.44	0.02	1.92	1.17	48.3	0.33	1.14	0.99	3	1	.	.
25–Jul	14.65	0.08	5.79	9.46	51.2	0.65	1.72	0.92	3	1	9	A
29–Jul					52.1	0.49	1.46	0.94	3	1	7	A
29–Jul					53.1	0.36	1.10	0.91	3	1	8	A
09–Aug					51.1	0.85	1.61	1.03	3	1	8	A
19–Aug	15.17	0.09	6.96	32.70	52.1	6.19	1.76	1.18	3	1	10	A
MEAN	8.50	0.06	3.42	7.68	50.59	1.02	1.48	1.00	2.9	.	.	.
STD	4.86	0.04	2.28	10.59	1.53	1.72	0.21	0.08	0.3	.	.	.
N	8	8	8	8	11	11	11	11	11	11	11	11

e) Sampling Location: Western Arm Brook, August–October 1990

Sample Date	T ng/ml	11–KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Ovary Stage	Stain
09–Aug	1.40	0.00	3.29	1.70	52.8	0.49		1.05	4	1	11	A
18–Oct	7.81	0.00	3.33	2.17	51.4			0.97	3	1	.	.
18–Oct	4.45	1.44	3.64	1.52	57.2			0.98	3	1	.	.
18–Oct	66.97	1.33	17.40	2.21	62.2			1.00	4	1	.	.
18–Oct	8.96	0.00	4.03	1.75	52.7			1.22	3	1	.	.
18–Oct	48.47	1.79	8.59	0.42	52.7			1.13	3	1	.	.
18–Oct	51.58	1.17	4.70	2.13	55.2			1.12	3	1	.	.
18–Oct	2.50	0.00	2.24	0.31	59.7			1.06	3	1	.	.
18–Oct	5.96	0.00	4.80	1.74	58.4			1.05	4	1	.	.
MEAN	22.01	0.64	5.78	1.55	55.81	0.49	.	1.07	3.3	.	.	.
STD	25.83	0.77	4.71	0.71	3.76	.	.	0.08	0.5	.	.	.
N	9	9	9	9	9	1	0	9	9	9	9	9

Appendix 11. Parameter values measured in blood and testis samples from male Atlantic salmon from the cage–reared Saint John River stock. 'CF' refers to Condition Factor.

a) Sampling Date: May 12, 1990

Sample No.	T ng/ml	11–KT ng/ml	Vg mg/ml	GSI %	HSI %	CF	Testis Stage	Comment
1	0.45	0.26	0.17	0.01	.	1.11	No samp.	
2	1.32	0.00	0.13	0.01	.	1.12	4	Precocious
4	1.52	0.00		0.01	.	1.06	2	
6	1.71	0.00		0.07	.	1.11	1	
10	1.57	0.18		0.04	.	1.08	2	
15	0.68	0.92	0.10	0.06	.	1.01	1	
17	0.58	0.00		0.04	.	1.11	1	
19	1.79	0.70		0.06	.	1.12	1	
20	0.92	0.37		0.05	.	1.11	1	
21	0.87	0.00		0.05	.	1.17	No samp.	
22	0.69	0.00		0.08	.	1.00	No samp.	
23	0.54	0.00	0.02	0.06	.	1.03	1	
24	0.93	1.12	0.05	0.06	.	1.10	2	
25	0.31	0.00		0.08	.	1.11	1	
27	0.32	0.00		0.03	.	1.05	2	
32	1.11	0.00		0.07	.	1.15	No samp.	
34	0.51	0.00		0.03	.	1.07	1	
39	0.69	0.00	0.13	0.01	.	1.06	1	
40	0.64	0.00		0.01	.	1.09	1	
41	0.51	0.00		0.04	.	1.09	1	
45	0.62	0.00		0.01	.	1.07	1	
48	0.60	0.00		0.02	.	1.12	1	
52	0.43	0.00		0.00	.	1.12	2	
MEAN	0.84	0.15	0.10	0.04	0.00	1.09		
STD	0.45	0.32	0.06	0.03	0.00	0.04		
N	23	23	6	23	23	23		

b) Sampling Date: October 15, 1990

Sample No.	T ng/ml	11–KT ng/ml	Vg mg/ml	GSI %	HSI %	CF	Testis Stage
1	0.32	0.00		0.08	2.34	1.11	2
9	0.16	0.00		0.04	1.16	0.78	1
10	0.51	0.00		0.05	1.30	0.71	1
13	0.22	0.00	0.16	0.06	1.20	0.77	2
14	0.16	0.00		0.04	1.29	0.76	1
15	0.12	0.00		0.05	1.31	0.80	1
16	0.17	0.00		0.04	0.91	0.83	1
18	0.20	0.00		0.03	1.13	0.85	1
19	0.21	0.00		0.06	0.95	0.83	1
20	0.40	0.00	0.17	0.04	1.05	0.78	1
24	0.01	0.00		0.06	1.53	0.68	2
26	0.11			0.04	1.37	0.86	2
27	0.07	0.00		0.04	1.11	0.71	2
31	0.12	0.00	0.15	0.04	1.25	0.75	2
32	0.20	0.00		0.04	1.26	0.76	2
38	0.11	0.00		0.05	1.22	0.78	2
41	0.20	0.00		0.06	1.30	0.75	2
42	0.12	0.00	0.21	0.02	1.02	0.76	2
43	0.11	0.00	0.18	0.05	1.06	0.78	2
45	0.12	0.00		0.04	1.12	0.77	2
47	0.00	0.00		0.05	1.00	0.84	2
MEAN	0.17	0.00	0.17	0.05	1.23	0.79	
STD	0.12	0.00	0.02	0.01	0.29	0.09	
N	21	20	5	21	21	21	

c) Sampling Date: May 4, 1991

Sample No.	T ng/ml	11–KT ng/ml	E2 ng/ml	GSI %	HSI %	CF	Testis Stage
3	3.18	2.83	0.07	0.10	1.17	0.84	1
6	3.02	2.26	0.09	0.10	1.28	0.82	2
8	0.63	0.13	0.17	0.04	1.00	0.89	2
11	1.93	0.08	0.11	0.04	1.36	0.88	2
12	0.70	0.32	0.06	0.06	1.06	0.86	1
14	6.20	3.66	0.21	0.07	1.11	0.83	1
15	5.41	3.42	0.16	0.07	1.37	0.77	2
18	1.17	0.34	0.18	0.04	1.00	0.88	2
22	5.51	5.54	0.17	0.10	1.15	0.79	2
23	0.70	0.00	0.12	0.06	1.74	0.91	2
24	4.84	5.32	0.22	0.04	1.22	0.87	2
25	0.63	0.02	0.09	0.06	1.58	0.88	2
30	7.39	1.95	0.13	0.11	1.32	0.83	1
34	4.12	3.81	0.19	0.08	1.18	0.90	1
36	4.25	3.51	0.14	0.07	1.00	0.95	3
39	1.48	0.42	0.04	0.06	1.03	0.80	2
41	6.16	1.50	0.17	0.11	1.26	0.86	1
43	9.08	5.37	0.24	0.12	1.28	0.78	2
48	4.24	3.75	0.06	0.13	1.15	0.79	2
49	1.78	0.08	0.06	0.05	1.26	0.81	2
MEAN	3.62	2.22	0.13	0.08	1.23	0.85	
STD	2.50	1.99	0.06	0.03	0.19	0.05	
N	20	20	20	20	20	20	

d) Sampling Date: July 30, 1991

Sample No.	T ng/ml	11–KT ng/ml	E2 ng/ml	GSI %	HSI %	CF	Testis Stage	Comment
4	4.23	5.34	0.04	0.11	1.60	0.77	3	
6	6.94	5.25	0.05	0.04	1.52	0.77	2	
10	0.82	0.40	0.04	0.04	1.24	0.87	2	
13	5.46	3.53	0.06	0.14	1.72	0.91	2	
14	1.12	0.26	0.06	0.07	1.23	0.77	2	
15	7.06	4.61	0.11	0.11	1.58	0.81	3	
18	1.53	0.95	0.03	0.06	1.38	0.79	4	
19	5.36	5.51	0.05	0.83	1.00	0.76	5	Precocious
20	11.62	8.01	0.04	1.42	0.87	0.81	5	Precocious
21	0.86	0.00	0.00	0.04	1.29	0.89	2	
22	8.93	14.06	0.00	1.20	0.87	0.83	5	Precocious
29	8.07	5.73	0.03	1.13	1.03	0.77	5	Precocious
31	1.26	0.00	0.07	0.05	1.21	0.76	2	
32	0.32	0.00	0.03	0.06	1.46	0.80	3	
33	8.45	7.92	0.11	0.11	0.95	0.79	2	
36	3.25	2.19	0.15	0.08	1.12	0.73	3	
37	0.68	0.00	0.21	0.05	1.78	0.82	2	
40	0.93	0.23	0.10	0.09	1.17	0.74	3	
41	0.71	0.32	0.06	0.06	1.16	0.71	2	
44	5.20	6.07	0.10	0.64	0.99	0.71	5	Precocious
45	2.27	0.92	0.09	0.07	1.40	0.85	4	
47	0.81	0.00	0.09	0.06	1.22	0.75	2	
49	8.08	0.78	0.07	0.87	1.02	0.74	5	Precocious
MEAN	4.09	3.13	0.07	0.32	1.25	0.79		
STD	3.43	3.67	0.05	0.45	0.26	0.05		
N	23	23	23	23	23	23		

e) Sampling Date: November 9, 1991

Sample No.	T ng/ml	11–KT ng/ml	GSI %	HSI %	CF	Testis Stage	Comment
1	6.08	27.70	6.59	1.15	0.83	6	Ripe gonad
3	10.92	23.87	6.65	1.14	0.85	6	Ripe gonad
5	0.79	0.80	0.10	0.96	0.76	2	.
6	10.75	44.61	6.34	1.08	1.05	6	Ripe gonad
7	11.88	18.39	4.92	1.15	0.86	6	Ripe gonad
10	11.52	22.31	3.00	1.01	0.76	6	Ripe gonad
12	0.51	0.82	0.10	1.42	0.65	2	.
13	13.67	63.56	3.77	1.09	0.83	6	Ripe gonad
15	0.57	0.91	0.11	1.17	0.62	2	.
16	11.04	30.70	4.78	1.20	0.47	6	Ripe gonad
17	9.79	15.49	5.97	1.07	0.96	6	Ripe gonad
21	13.11	33.88	7.50	1.11	1.05	6	Ripe gonad
23	0.72	0.98	0.08	1.46	0.66	2	.
30	0.64	0.00	0.06	1.28	0.43	2	.
33	9.58	20.74	3.22	1.16	0.87	6	Ripe gonad
35	1.15	2.05	0.05	1.04	0.70	2	.
36	0.52	0.56	0.09	0.74	0.36	2	.
37	11.72	24.59	3.60	1.38	0.43	6	Ripe gonad
38	0.17	0.40	0.09	1.07	0.66	2	.
39	6.36	19.91		1.54	0.00	6	Ripe gonad
40	11.81	21.27	3.74	1.24	0.83	6	Ripe gonad
41	7.54	22.85	4.21	1.26	1.06	6	Ripe gonad
42	15.80	26.47	4.74	1.20	0.80	6	Ripe gonad
43	0.60	0.85	0.09	1.29	0.71	2	.
45	0.45	0.29	0.10	1.28	0.65	2	.
51	10.34	21.07	5.29	1.26	1.48	6	Ripe gonad
MEAN	6.87	17.12	3.01	1.18	0.74		
STD	5.40	16.09	2.66	0.17	0.28		
N	26	26	25	26	26		

Appendix 12. Parameter values measured in blood and testis samples from wild male Atlantic salmon at three reference sites. 'CF' refers to Condition factor.

a) Sampling Location: Labrador Sea, October 1991

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Testis Stage
Sea-Age-0											
06-Oct	0.55	0.32	0.19		37.7	0.04		0.82	2	0	.
06-Oct	0.14	0.00	0.13		40.4	0.08		1.03	1	0	.
07-Oct					41.4	0.05		0.95	3	0	.
07-Oct	0.62	0.48	0.22		36.8	0.03		0.94	3	0	.
09-Oct					31.7	0.03		0.69	2	0	.
09-Oct	0.16	0.14	0.31		33.2	0.06		0.89	5	0	.
10-Oct					36.0	0.05		1.01	2	0	.
10-Oct	0.41	0.59	0.01		41.2	0.02		0.97	1	0	.
11-Oct	0.30	0.07	0.12		38.0	0.06		0.91	1	0	.
11-Oct					38.3	0.04		0.97	2	0	.
11-Oct					34.5	0.04		0.91	2	0	.
11-Oct	0.47	0.55	0.46		37.4	0.04		0.99	3	0	.
12-Oct					37.8	0.03		0.92	1	0	.
12-Oct					33.8	0.03		1.04	3	0	.
12-Oct					36.8	0.05		1.02	2	0	.
13-Oct	1.53	0.57	0.67		33.5	0.04		0.84	4	0	.
13-Oct	0.38	0.12	0.52		37.0	0.04		0.97	2	0	.
14-Oct					33.2	0.04		1.02	3	0	.
14-Oct					35.8	0.03		0.87	2	0	.
MEAN	0.50	0.32	0.29	.	36.55	0.04	.	0.93	2.32	.	.
STD	0.41	0.24	0.22	.	2.76	0.01	.	0.09	1.06	.	.
N	9	9	9	.	19	19	.	19	19	19	.
Sea-Age-1											
06-Oct					61.6	0.04		1.04	3	1	.
07-Oct					61.8	0.04		0.94	1	1	.
08-Oct	0.64	1.36	0.23		65.0	0.05		1.02	3	1	.
09-Oct	0.03	0.00	0.15		58.8	0.25		0.94	5	1	.
10-Oct	0.35	1.20	0.21		71.5	0.04		0.89	3	1	.
10-Oct					55.1	0.05		1.23	6	1	.
11-Oct					71.5	0.04		0.94	3	1	.
11-Oct					64.8	0.05		1.05	3	1	.
11-Oct	0.84	1.52	0.42		66.2	0.04		1.03	3	1	.
11-Oct	1.67	10.22	0.24		69.7	0.04		1.03	3	1	.
11-Oct	0.34	0.58	0.29		62.5	0.03		1.14	4	1	.
11-Oct	1.75	6.06	0.32		59.5	0.06		0.99	3	1	.
12-Oct					63.2	0.05		1.03	2	1	.
13-Oct					68.0	0.05		1.04	3	1	.
13-Oct					64.1	0.06		1.02	1	1	.
MEAN	0.80	2.99	0.27	.	64.22	0.06	.	1.02	3.07	.	.
STD	0.67	3.75	0.09	.	4.68	0.05	.	0.08	1.28	.	.
N	7	7	7	.	15	15	.	15	15	15	.

b) Sampling Location: Humber River July-August, 1991

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Testis Stage
25-Jul	9.63	17.59	0.27		51.0	0.80	2.06	1.03	3	1	3
25-Jul	4.47	7.73	0.63		55.9	0.43	1.10	0.95	3	1	3
04-Aug	1.04	0.17	0.20		37.3	0.80	1.29	0.97	3	1	5
19-Aug	2.69	4.29	0.31		55.7	0.69	0.69	1.15	3	1	5
26-Aug	34.81	60.72	0.51		51.5	5.80	0.89	1.29		1	5
MEAN	10.53	18.10	0.38	.	50.28	1.70	1.20	1.08	3.0	.	.
STD	13.95	24.68	0.18	.	7.61	2.30	0.53	0.14	.	.	.
N	5	5	5	.	5	5	5	5	4	5	5

c) Sampling Location: Western Arm Brook, August-October 1990

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Testis Stage
18-Oct	35.07	167.10	0.29		50.8			1.31	4	1	.
18-Oct	38.26	122.13	0.91	0.16	57.2			1.25	3	1	.
18-Oct	27.53	237.12	0.33	0.24	57.2			1.17	3	1	.
MEAN	33.62	175.45	0.51	0.20	55.07	.	.	1.24	3.3	.	.
STD	5.51	57.95	0.35	0.06	3.70	.	.	0.07	0.6	.	.
N	3	3	3	2	3	.	.	3	3	3	.

Appendix 13. Parameter values measured in blood and ovary samples from wild female Atlantic salmon at three commercial fishery locations in 1990 and 1991. The values of 'Stain' refer to the colour of stained ovary sections – 'A'=Acidophilic (pink); 'B'=Basophilic (blue); and 'AB'=intermediate (mauve). 'CF' refers to Condition Factor.

a) Sampling Location: Conche, 1990

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Ovary Stage	Stain
15-Jun	3.11		10.43	1.15	54.0	0.27		0.81	3	1	8	AB
19-Jun	1.18		10.73	1.60	55.0	1.90		0.99	3	1	8	A
24-Jun	3.54		16.71	8.06	59.0			0.86	3	1	.	.
24-Jun	6.25		51.92	2.98	58.0	1.41		0.80	3	1	8	A
25-Jun	2.45	0.00			53.0	1.64		0.83	4	1	7	B
25-Jun	0.49	0.99			54.0	1.48		0.81	3	1	7	B
26-Jun	2.65		10.47	4.34	56.5	1.66		0.88	3	1	8	A
26-Jun	3.63		6.40	0.19	54.0	1.81		0.82	3	1	8	AB
28-Jun					59.0	2.09		0.90	4	1	9	A
30-Jun	4.35		13.86	5.54	60.0	2.18		0.84		1	9	A
30-Jun	3.70		8.58	2.65	57.5	1.56		0.79	4	1	8	A
03-Jul	2.84		12.72	3.04	60.0	1.34		0.89	4	1	7	AB
03-Jul	3.14		14.47	3.37	57.0	1.42		0.83	3	1	8	A
05-Jul	1.64		3.44	1.64	62.0	1.09		0.81	3	1	7	B
05-Jul	1.13		10.97	6.84	55.0	1.87		0.88	3	1	9	A
05-Jul	1.22		9.41	5.91	53.5	2.13		0.83	4	1	10	A
05-Jul	4.57		8.52	1.50	56.0	1.46		0.83	4	1	4	B
05-Jul	1.33		7.10	1.57	56.5	1.45		0.85	4	1	7	B
05-Jul	5.74		57.27	5.37	57.5	1.88		0.85	4	1	9	A
05-Jul	2.14		27.92	2.63	55.5	1.63		0.78	3	1	9	AB
09-Jul	1.37		6.53	0.09	66.0	0.97		0.96	3	1	6	B
13-Jul					58.0	1.39		0.86	3	1	7	B
16-Jul	0.67		9.15	5.60	53.5	2.09		0.82	4	1	9	A
20-Jul	1.48		9.47	7.77	55.0	1.74		0.68	4	1	9	A
24-Jul	3.30		9.75	5.93	59.0	1.44		0.78	3	1	8	A
24-Jul	1.39		15.14	0.12	61.5	1.18		0.98		1	7	B
24-Jul	1.31	0.05		0.16	62.5	1.16		0.96	4	1	7	B
24-Jul	2.33		9.56	4.73	62.5	1.13		0.71	4	1	8	AB
25-Jul	0.95		6.77	0.18	65.5	0.88		0.92	3	1	6	B
25-Jul	0.70		8.12	0.17	52.5	1.71		0.92	3	1	6	B
27-Jul	2.19		9.21	0.21	53.5	1.83		0.85	3	1	8	AB
30-Jul	1.06		7.42	0.19	64.0	0.90		0.84	3	1	7	B
30-Jul	0.43		6.89	0.24	65.5	0.94		0.92	3	1	6	B
31-Jul	1.71		4.87	8.79	53.0	2.21		0.95	3	1	9	A
MEAN	2.31	0.35	13.23	3.09	57.79	1.51	.	0.85	3.4	.	.	.
STD	1.49	0.56	12.34	2.76	3.98	0.44	.	0.07	0.5	.	.	.
N	32	3	29	30	34	33	0	34	32	34	34	34

b) Sampling Location: Conche, 1991

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Ovary Stage	Stain
09-Jul	1.03	0.03			57.0	0.29	1.77	0.88	3	1	7	AB
09-Jul	0.66		1.29	3.25	57.5	0.60	1.19	1.09	5	1	8	A
15-Jul	0.69		1.34	4.59	65.0	0.76	1.90	1.62	4	1	9	A
15-Jul	1.26		2.78	4.87	55.0			0.83	4	1	9	A
16-Jul	0.95		1.45	1.02	58.0	0.31	1.42	0.89	4	1	8	A
16-Jul	3.29		0.11	0.12	60.0	0.37	1.50	0.86	4	1	8	AB
16-Jul	1.38		2.26	5.31	58.0	0.51	1.61	0.89	3	1	9	A
17-Jul	2.70		4.37	7.84	56.0	1.30	2.29	0.92	4	1	9	A
17-Jul	0.27		0.13	0.40	57.0	0.27	1.25	0.93	3	1	7	AB
17-Jul				1.46	53.5	0.60	1.79	0.77	3	1	9	A
17-Jul	0.57		1.08	6.42	55.0	0.46	1.59	0.92	4	1	8	A
19-Jul	0.37		0.07	0.17	55.0	0.12	0.74	0.62	3	1	7	B
19-Jul	0.81		0.85	2.14	56.5	0.43	1.34	0.82	4	1	8	A
24-Jul	1.16		1.15	3.82	56.0	0.39	1.19	0.88	3	1	8	A
24-Jul	1.47		2.65	12.35	53.0	1.13	1.94	0.85	4	1	9	A
24-Jul	1.14		1.88	8.78	58.0	1.14	2.18	0.98	3	1	9	A
04-Aug					63.0	0.10	1.52	0.83	4	1	6	B
MEAN	1.18	0.03	1.53	4.17	57.26	0.55	1.58	0.92	3.6	.	.	.
STD	0.82	.	1.20	3.56	3.10	0.36	0.40	0.20	0.6	.	.	.
N	15	1	14	15	17	16	16	17	17	17	17	17

c) Sampling Location: St. Barbe Bay, 1990

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Ovary Stage	Stain
29-Jun	3.22		12.75	2.05	58.0	1.37		0.81	5	1	10	A
30-Jun	3.34		94.00	1.81	56.0	1.05		0.88	4	1	10	A
30-Jun	1.59		10.58	3.03	57.5	0.87		0.90	4	1	10	A
30-Jun	2.41		13.01	3.72	52.2	0.74		0.83	3	1	9	A
02-Jul	0.29		7.64	1.82	56.0	0.90		0.80	3	1	10	A
02-Jul	1.68		15.73	3.59	57.0	0.85		0.81	4	1	10	A
02-Jul	1.10		15.13	3.92	59.2	1.24		0.94	5	1	10	A
02-Jul	6.03		14.16	2.85	61.1	0.63		0.95	5	1	10	A
02-Jul	1.59		11.68	0.55	53.0	0.57		0.74	4	1	9	A
02-Jul	0.80		10.77	0.68	49.5	0.63		0.58		1	9	A
04-Jul	1.47		11.98	4.87	52.4	0.81		0.82	3	1	10	A
05-Jul					54.7	0.86		0.84	4	1	10	A
09-Jul	2.17		19.62	3.00	48.5	0.51		0.67	3	1	9	A
10-Jul	0.75		13.77	2.28	51.3	0.65		0.71	3	1	10	A
10-Jul	7.18		17.25	0.42	56.4	0.93		0.80	3	1	10	A
10-Jul	5.59		13.24	3.22	55.0	0.69		0.78	3	1	10	A
10-Jul	1.46		13.09	1.56	59.3	0.63		0.86	3	1	10	A
10-Jul	0.29			1.70	55.2	0.60		0.81	4	1	9	A
10-Jul	6.76		17.22	3.02	53.6	0.79		0.68	3	1	10	A
11-Jul	2.45		14.06	2.66	61.5	1.57		0.97	5	1	10	A
11-Jul	4.35		12.38	2.65	59.0	0.85		0.81	3	1	10	A
13-Jul	1.34			1.62	50.3	0.52		0.68	3	1	8	A
14-Jul	1.48		11.81	0.97	57.0	0.25		0.81	3	1	10	A
MEAN	2.61	.	17.49	2.36	55.38	0.80	.	0.80	3.6	.	.	.
STD	2.08	.	18.20	1.17	3.60	0.29	.	0.10	0.8	.	.	.
N	22	0	20	22	23	23	0	23	22	23	23	23

d) Sampling Location: St. Barbe Bay, 1991

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Ovary Stage	Stain
23-Jul	2.42		2.73	7.55	52.5	1.01	1.78	0.90	4	1	10	A
25-Jul	0.72		0.53	2.12	55.0	0.66	1.94	0.99	3	1	9	A
25-Jul	8.29		7.55	11.07	51.0	1.18	2.25	0.93	4	1	9	A
MEAN	3.81	.	3.61	6.91	52.83	0.95	1.99	0.94	3.7			
STD	3.97	.	3.59	4.51	2.02	0.27	0.24	0.04	0.6			
N	3	0	3	3	3	3	3	3	3			

(continued next page)

Appendix 13 continued

e) Sampling Location: Twillingate, 1991

Sample date	T ng/ml	11-KT ng/ml	B2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Ovary Stage	Stain
24-Jun					47.0	0.49	1.65	0.78	3	1	8	A
24-Jun					54.0	0.94		0.87	4	1	9	A
24-Jun					55.0	0.30		0.97	3	1	6	AB
24-Jun					57.0	0.68		1.01	3	1	9	A
24-Jun					52.0	0.45	0.97	0.96	4	1	8	A
24-Jun					53.0	0.49		0.90	3	1	8	A
25-Jun					48.0	0.45	1.46	0.95	2	1	8	AB
25-Jun					53.0	0.44	1.79	0.91	4	1	8	A
25-Jun					59.0	0.89	2.06	0.81	5	1	8	A
26-Jun					56.0	0.87	1.52	0.95	4	1	9	A
26-Jun					56.0	0.41	1.45	0.90	4	1	8	A
01-Jul					58.0	0.65	1.49	0.98		1	9	A
01-Jul					69.0	0.57	1.18	2.16	4	1	7	A
01-Jul					50.0	0.51	1.38	0.81	3	1	8	A
01-Jul					52.0	0.64	1.36	0.80	3	1	8	A
02-Jul					52.0	0.33		0.91	3	1	7	AB
03-Jul					51.0	0.25		0.87	3	1	7	B
03-Jul					56.0	0.52		0.88	3	1	8	A
03-Jul					53.0	0.26		0.86	3	1	7	AB
04-Jul					53.0	0.36		0.92	3	1	8	A
04-Jul					46.0	0.37		0.97	3	1	8	AB
01-Jul					59.0	0.55		0.94	3	1	8	A
MEAN	.	.	.	.	54.05	0.52	1.48	0.96	3.3	.	.	.
STD	.	.	.	.	4.89	0.20	0.29	0.28	0.7	.	.	.
N	0	0	0	0	22	22	11	22	21	22	22	22

Appendix 14. Parameter values measured in blood and testis samples from wild male Atlantic salmon at three commercial fishery locations in 1990 and 1991. 'CF' refers to Condition Factor.

a) Sampling Location: Conche, 1990

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Testis Stage
20-Jun	1.93	4.97			57.0			0.92	3	1	.
20-Jun	3.35	2.26			58.0			0.89	3	1	.
20-Jun	1.00	2.55		0.22	64.0	1.07		0.88	3	1	2
20-Jun	2.43	13.02		0.19	59.0			0.61	3	1	.
20-Jun	7.66	13.45			61.0	1.34		1.00	3	1	2
21-Jun	4.54	17.82			64.0	1.10		0.96	3	1	2
25-Jun	4.07	15.14			55.0	1.09		0.65	3	1	3
26-Jun	3.45	16.30			60.0	1.41		0.88	4	1	4
26-Jun	2.28	9.02		0.21	59.0	1.21		0.81	4	1	3
26-Jun	2.86	0.53		2.52	61.0	1.20		0.92	4	1	3
26-Jun					59.0	1.47		0.98	4	1	3
26-Jun	0.71	1.90			62.0			1.03	3	1	.
26-Jun	3.06	16.76			55.0	0.95		0.59	3	1	3
28-Jun	2.95	13.65			62.5	1.38		0.94	4	1	3
28-Jun	2.34	6.09			59.0	1.06		0.78	3	1	3
28-Jun					66.0	1.03		0.98	4	1	2
30-Jun	5.27	17.84		0.20	56.0	1.33		0.78	3	1	3
30-Jun	3.76	13.08			60.0	1.15		0.89	3	1	2
30-Jun	2.81	6.86		0.03	57.0	1.32		0.82	3	1	.
30-Jun	3.34	13.38			59.0	1.28		0.85	4	1	3
03-Jul	1.98	7.40			61.0	1.05		0.85	4	1	3
03-Jul					57.0	1.53		0.90	3	1	3
03-Jul	2.72	6.35			59.0	1.14		0.73	3	1	3
03-Jul	2.06	10.75			60.0	1.12		0.86	3	1	3
03-Jul	3.69	15.85			63.5	1.08		0.79	4	1	3
03-Jul	2.92	11.43			59.5	1.16		0.86	4	1	2
03-Jul	2.85	17.27			59.5	1.30		0.82	3	1	3
03-Jul	3.46	15.39			57.0	1.43		0.84	3	1	4
03-Jul	2.40	10.81		0.02	59.0	1.21		0.83	3	1	4
04-Jul	0.69	2.93			53.0	1.43		0.80	3	1	3
04-Jul	0.63	2.44			56.5			0.81	2	1	.
04-Jul	1.03	5.56			57.0	1.32		0.81	3	1	.
05-Jul	0.66	2.81			57.0	1.28		0.86	2	1	3
05-Jul	1.64	5.07		0.16	55.0	1.47		0.88	3	1	3
09-Jul	0.99	5.14			62.5	0.92		0.86	2	1	2
09-Jul	3.03	16.76		0.15	58.0	1.33		0.73	3	1	3
10-Jul	1.62	6.72			55.5	1.65		0.82	3	1	4
12-Jul	0.93	2.37			52.0	1.84		0.91	3	1	4
13-Jul					55.5	1.45		0.82	4	1	4
13-Jul	2.53	4.42			61.0	1.29		0.79	3	1	4
19-Jul	4.02	11.42		0.17	66.0	0.83		0.77	3	1	3
20-Jul	1.79	3.60			62.5	1.15		0.85	3	1	3
20-Jul	2.13	6.81		0.24	55.0	1.68		0.87	4	1	3
24-Jul	0.40	0.57	0.16	0.22	59.0			1.02	3	1	.
24-Jul	0.35	0.85		0.15	60.0	1.14		0.93	3	1	5
25-Jul	3.84	15.39		0.20	58.0	1.60		0.87	3	1	4
25-Jul	0.48	0.62	0.43	0.30	62.5			0.87	3	1	.
25-Jul	0.77	0.78	0.37	0.24	58.0			0.96	4	1	.
25-Jul	2.09	7.65		0.20	59.0	1.97		0.85	3	1	3
27-Jul	1.60	0.00		5.36	56.0	1.51		0.83	3	1	4
27-Jul	1.36	1.18			66.0	0.77		0.84	3	1	2
27-Jul	0.41	0.26			57.0	2.06		0.95	3	1	4
30-Jul	1.48	4.52		0.24	52.5	1.66		0.78		1	4
30-Jul	0.72	1.91		0.24	60.0	0.97		0.81	3	1	2
31-Jul	1.00	5.18		0.25	55.5	3.03		0.86	3	1	.
01-Aug					59.0	1.27		0.73		1	4
03-Aug	3.46	6.29		0.27	55.0	1.88		0.79	3	1	3
MEAN	2.30	7.71	0.32	0.54	58.82	1.34	.	0.85	3.2	.	.
STD	1.44	5.76	0.14	1.19	3.26	0.37	.	0.08	0.5	.	.
N	52	52	3	22	57	49	57	57	55	55	55

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b) Sampling Location: Conche, 1991

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Testis Stage
09-Jul	2.68	5.08			57.0	0.27	1.79	0.93	4	1	2
09-Jul	2.21		0.08	0.21	55.5	0.26	1.47	0.95	3	1	3
09-Jul	0.94	1.92			53.0	0.08	0.92	0.60	3	1	2
09-Jul					57.5	0.27	1.35	0.91	3	1	4
13-Jul	6.02	5.16			55.0			0.95	4	1	3
13-Jul					57.0			0.93	4	1	5
13-Jul					57.0			0.93	4	1	3
13-Jul					54.0			0.79	3	1	3
15-Jul	0.14	0.09			55.0			1.11	3	1	2
15-Jul	1.29	4.79			58.0	0.27	1.45	0.72	3	1	3
15-Jul	4.29	7.77			59.0	0.34	1.21	0.86	3	1	3
15-Jul	0.00	0.50			58.0			0.98	3	1	3
15-Jul	1.43	5.05			55.0			0.95	3	1	3
15-Jul	0.61	2.86			59.0	0.18	1.13	0.82	3	1	2
15-Jul	2.39	3.70			59.5	0.24	1.16	0.84	4	1	3
15-Jul	0.96	1.73			57.0			0.93	3	1	4
15-Jul	0.87	1.57			57.5			0.95	3	1	3
15-Jul	0.73	2.51			60.5	0.36	1.45	0.89	3	1	3
15-Jul	2.02	0.96			51.0	0.19	1.29	0.88	4	1	2
16-Jul	3.47	5.36			61.0	0.44	1.42	0.91	3	1	3
16-Jul	1.44	2.03			51.0	0.25	1.31	0.83	3	1	4
16-Jul	0.93	1.81			55.0	0.37	1.82	0.98	3	1	3
16-Jul	2.59	5.08			56.0	0.29	1.58	0.80	4	1	3
17-Jul					56.0	0.36	1.77	0.88	3	1	4
19-Jul	1.97	4.72			62.0	0.54	1.46	0.88	5	1	5
19-Jul	1.33	3.74			61.0	0.53	1.47	0.78	4	1	4
19-Jul	1.20	2.48			63.0	0.93	1.82	0.83	3	1	4
23-Jul	2.80	4.62			62.0	0.87	0.88	0.82	4	1	4
24-Jul	0.53	0.75			60.0	0.48	1.85	1.08	3	1	3
25-Jul					54.0	0.36	1.59	0.87	3	1	3
25-Jul	2.84	4.38			61.0	0.53	1.84	1.03	4	1	3
01-Aug	0.19		0.04	0.13	53.0	0.44	1.70	0.83	3	1	4
MEAN	1.76	3.11	0.06	0.17	57.20	0.38	1.47	0.89	3.4	.	.
STD	1.38	1.97	0.02	0.06	3.18	0.20	0.29	0.10	0.6	.	.
N	26	24	2	2	32	23	23	32	32	32	32

c) Sampling Location: St. Barbe Bay, 1990

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	Vg mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Testis Stage
29-Jun	1.04	4.09			59.5	0.18		0.88	4	1	3
10-Jul	1.97	5.40			55.2	0.17		0.75	3	1	3
11-Jul	4.30	23.58		0.14	56.2	0.19		0.75	3	1	3
11-Jul	0.70	2.58			55.0	0.33		0.70	4	1	3
11-Jul	0.81	2.47			61.0	0.27		0.78	3	1	3
11-Jul	2.72	10.28			55.5	0.29		0.59	4	1	3
13-Jul	2.06	13.23			55.2	0.40		0.75	4	1	4
14-Jul	2.37	8.26			51.5	0.84		0.70	3	1	3
14-Jul	0.38	0.70			58.5	0.23		0.74	3	1	4
MEAN	1.82	7.84	.	0.14	56.40	0.32	.	0.74	3.4	.	.
STD	1.24	7.16	.	.	2.85	0.21	.	0.08	0.5	.	.
N	9	9	.	1	9	9	.	9	9	9	9

Appendix 14 continued

d) Sampling Location: St. Barbe Bay, 1991

Sample Date	T ng/ml	11-KT ng/ml	E2 ng/ml	V_g mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Testis Stage
23-Jul	.	.	.	.	57.0	0.37	1.46	0.93	4	1	3
MEAN	.	.	.	.	.	.	.	.	.	.	.
STD	.	.	.	.	.	.	.	.	.	.	.
N	.	.	.	.	1	1	1	1	1	1	1

e) Sampling Location: Twillingate, 1991

Sample DATE	T ng/ml	11-KT ng/ml	E2 ng/ml	V_g mg/ml	FLT (cm)	GSI %	HSI %	CF	River Age	Sea Age	Testis Stage
24-Jun					55.0	0.14	1.37	0.87	4	1	2
24-Jun					53.0	0.21	1.67	0.93	3	1	3
24-Jun					56.0	0.15		0.84	3	1	3
25-Jun					56.0	0.18	1.85	0.91	4	1	2
25-Jun					56.0	0.15	1.54	0.84	3	1	2
25-Jun					55.0	0.23	1.44	0.82	3	1	3
01-Jul					55.0	0.23	1.44	0.84	2	1	3
01-Jul					57.0	0.23	1.02	0.94	2	1	3
01-Jul					53.0	0.12	1.87	1.01	2	1	2
02-Jul					58.0	0.25		0.90	3	1	3
02-Jul					54.0	0.19		0.90	4	1	2
03-Jul					58.0	0.20		0.92	2	1	2
04-Jul					56.0	0.40		0.91	3	1	2
MEAN	.	.	.	.	55.54	0.21	1.53	0.89	2.9	.	.
STD	.	.	.	.	1.61	0.07	0.28	0.05	0.8	.	.
N	.	.	.	.	13	13	8	13	13	13	13