An Investigation of the Regulation of Reproductive Development in Pink Salmon (*Oncorhynchus gorbusha*) in Relation to the Initiation of an Off-year Pink Salmon Run

by

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B.Sc. University of British Columbia, 1974

A Thesis Submitted in Partial Fulfilment
of the Requirements of the Degree of
Master of Science
in the Department of
Zoology

We accept this thesis as conforming to the required standard.

The University of British Columbia

December, 1976

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Date April 14/1977

ABSTRACT

This study was undertaken to determine whether gonads of pink salmon (Oncorhynchus gorbuscha) could be stimulated by pelleted or injected salmon gonadotropin to reproductive maturity one year earlier than normal. This procedure, if successful, might be used in attempting to populate an "off" year cycle of pink salmon.

In juvenile male pink salmon complete maturity was attained by September in the year of hatching with both pellet implantation (1x/3 weeks) and injection (thrice weekly) of 1.0 micrograms of chinook salmon (Oncorhynchus tshawytscha) gonadotropin per gram body weight. Time of onset of mitotic division of spermatogonia and rate of spermatogenesis were accelerated in the precociously mature testes. Similar doses of salmon gonadotropin injected at longer time intervals (1x/week, and 1x/2 weeks) resulted in a slower maturation.

In females, acceleration of maturation was achieved in immature pink salmon by injection and pellet implantation of salmon gonadotropin. The primary yolk vesicle stage was achieved after 4 months of treatment with thrice weekly injections and 1x/3 week pellet implantations of 1.0 μg/gm body weight of salmon gonadotropin. Similar doses of salmon gonadotropin injected at longer time intervals (1x/week and 1x/2 weeks) resulted in a reduction in the rate of maturation. Large numbers of

preovulatory corpora atretica were observed in all treated fish.

The knowledge developed by this research formed part of a large scale co-operative program with two objectives. The pragmatic objective is to develop an "off" year pink salmon spawning population in Bear River which has no spawners in odd-numbered years. The scientific objective is to assess the value of adding "home stream" genetic material to the transplanted embryos and to evaluate two experimental techniques as to their effectiveness in providing "home stream" genes to the transplanted population. The approach was to fertilize eggs taken from fish of another river (Glendale River) with 1) spermatozoa from precocious Bear River males, 2) cryopreserved spermatozoa from Bear River males, and 3) spermatozoa from Glendale River males (control transplant group). The fertilization rate among eggs of the precocious Bear River male x donor Glendale female group was equal to the control group (Glendale River male x Glendale River female) and twice that of the cryopreserved Bear River male x Glendale female transplant group. The control group exhibited a higher developmental index (K_D) and migrated about 6 days earlier than the other two groups which had 50% of their genetic complement from the Bear River stock.

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ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my thesis supervisor, Dr. E.M. Donaldson, for suggesting the problem and for his guidance and assistance throughout the study.

Appreciation is also extended to my academic supervisor, Dr. W.S. Hoar, for his advice during the study.

The technical assistance of Ms. H. Dye in preparation of salmon pituitary gonadotropin, Mrs. K. Kramer in paraffin embedding specimens, and Dr. D. Higgs and Mr. J. McBride for helpful suggestion during the course of the study.

My appreciation is also extended to Mr. F.C. Withler and Mr. R. Morley of the Nanaimo Biological Station who so generously supplied samples and data from the Bear River Transplant Program, and to Mr. A. Lamb and Mr. J. Heading for help in caring for the fish.

I further wish to acknowledge Mrs. M. Young for typing of the manuscript and Mr. Steve Borden for his statistical advice.

GENERAL INTRODUCTION

Throughout the geographic range of pink salmon (Oncorhynchus gorbuscha) there are rivers in which there is a pronounced difference in abundance between even and odd numbered years (Ricker, 1962; Neave, 1965). The major areas which currently display this pronounced disparity have done so since before white men began to fish salmon intensively on the North American Coast. In the Fraser River-Puget Sound-Howe Sound region, for example, even-year fish are virtually absent, whereas the rivers on the Queen Charlotte Islands are characterized by a lack of pink salmon spawning in the odd-numbered years (Neave, 1962). If such disparities between even and odd years could be eliminated by repopulating the empty pink salmon cycles, there would be a major increase in the size and stability of the resource base and commensurate economic benefits to the fishing industry.

The results of transplanting pink salmon to rivers where there are no spawners have been highly inconsistent. Excellent reviews by Ricker (1972) and Neave (1965) reveal that there have been many instances where transplantations failed to produce evidence of returning adults. In cases where adults did return, it was not uncommon for the runs to decline and eventually disappear. One successful transplantation of pink salmon (O. gorbuscha) occurred in Alaska in 1964 when 1,800 mature adults were moved 30 km from a donor stream and released to spawn naturally in Saskin Creek (McNeil, et al., 1969). Returning runs of adults from this transplant have returned to Saskin Creek in numbers

varying between 6,000 and 13,000 annually between 1966 and 1974 (personal communication, McNeil). Technical problems interfered with the first introduction of pink salmon (O. gorbuscha) to the Barents and White Seas from eastern U.S.S.R. in 1956 and 1957, but in 1960 more than 100,000 adult pink salmon returned to northwest U.S.S.R. streams from a release of 15.3 million juveniles. The runs declined to much lower levels for a period after the large return in 1960, but Kamyshnaya and Smirnov (1968) felt that naturally reproducing populations may have become established. This conclusion is reinforced by more recent reports (Thurow, 1974) that about 200,000 adult pink salmon returned to northwest U.S.S.R. in 1973.

Recently an appreciation has developed for the possibility of genetic barriers to successful transplantation. The opinion has arisen gradually that each salmon stock is, through selection over a long period, quite finely tuned to the particular conditions which prevail in a particular spawning or rearing area, and that the necessary characteristics were hereditary (Calaprice, 1969; McNeil et al., 1969). Considering the evidence that "homing" in salmonids is partially inherited (Bams, in press; Brannon, 1972; Raleigh, 1971) it would appear that genetic factors play a most important role in determining whether a donor stock will survive in the new system. It follows that in attempting introductions care must be taken to choose stocks whose heredity is especially appropriate to the area to be populated.

It is known that pink salmon are characterized by such a rigid two-year life cycle that populating a barren cycle naturally

from fish in the "on" year stock is highly improbable (Funk and Donaldson, 1972). Recent studies by MacKinnon and Donaldson (1976) and Ivankov et al.(1975) have shown instances of male pink salmon reaching maturity at an age of one year without exogenous hormonal treatment, but the proportion of these naturally occurring precocious males is extremely small. Another means of employing the genetic complement of home stream fish would be to manipulate the reproductive cycle of pink salmon such that they spawn exactly one year earlier than normal. Previous work (Funk and Donaldson, 1972) has shown that only the males of pink salmon can be matured one year earlier, by injection of a preparation of spring salmon (O. tshawytscha) gonadotropin. This work led to the proposal that a pelleted preparation of spring salmon gonadotropin be used in an attempt to accelerate the development of the gonads of both male and female pink salmon to sexual maturity by September of the year of hatching.

To date Fraser River male pink salmon have been accelerated to sexual maturity one year earlier than normal by thrice weekly injections of salmon gonadotropin over a period of 2-3 months (Funk and Donaldson, 1972). Spermatozoa from these accelerated males were used to fertilize ova obtained from wild females from Northern British Columbia. The eggs hatched to produce fry having 50% of their genetic complement from the Fraser River stock (Donaldson, et al., 1972).

This paper reports on certain aspects of a Federal-Provincial Salmonid Enhancement project aimed at developing effective methods for introducing self-sustaining runs into barren cycles of pink

salmon streams having pronounced "on-off" characteristics. Currently, the objective of the study is to determine whether an infusion of "homestream" genes from the male component of the "on-year" run into ova transplanted from a donor stream will significantly increase the return of adults to the recipient stream. The success of the technique will be assessed by comparing the number of returns thus obtained with the returns obtained from similar eggs fertilized by males from the donor stream (representing a traditional transplant of entirely "foreign" origin).

The project is a joint undertaking by Mr. F.C. Withler of the Pacific Biological Station and Dr. E.M. Donaldson of the Vancouver Laboratory, both of the Research and Resource Service of the Department of Fisheries and Environment. My part of the project involved testing methods of bringing male and female pink salmon to maturity at about one year of age by gonadotropin injection, and controlling the rate of maturation so that it was achieved at the same time as that of the natural donor stock providing gametes for the transplant test. Further, this report contains preliminary results of fertilizing donor ova with sperm from precocious males, by comparing their developmental states and times of emergence with fry of different parental origin.

The three facets of this research are reported in the three chapters to follow. Chapter one describes the attempts to regulate the reproductive development of the male pink salmon, Chapter two describes the attempts to regulate the reproductive development of the female pink salmon, while Chapter three describes the application of this work to an actual field project.

CHAPTER I

The Testis

INTRODUCTION

In common with other vertebrates the testes of teleost fishes require the support of an intact and functioning pituitary gland for their development and maintenance. Gonadotropin withdrawal, either by hypophysectomy or methallibure treatment, has demonstrated that the pituitary gland and, specifically, gonadotropin(s) are essential for normal spermatogenesis in teleosts (Barr, 1963; Lofts, et al., 1966; Hoar, Wiebe and Wai, 1967; Sundararaj and Nayyar, 1967; Yamazaki and Donaldson, 1968; Pandey, 1969; Wiebe, 1969).

Previous research on juvenile salmonids has demonstrated a stimulation of testicular development with heteroplastic pituitary preparations. Administration in cholesterol pellets of a preparation from lyophilized pituitaries of spawning salmon (Oncorhynchus tshawytscha and O. keta) brought about active spermatogenesis and the shedding of motile spermatozoa in immature rainbow trout (Salmo gairdnerii) (Robertson and Rinfret, 1957). Pituitary gland extracts from chinook salmon injected into immature rainbow trout for a period of two weeks brought about an acceleration in spermatogenesis (Schmidt et al., 1965). Pituitary homogenates from spawning adult coho salmon (O. kisutch) injected into fingerling coho salmon stimulated the mitotic division of the spermatogonia (Chestnut, 1970). Further purification of

the pituitary extract used by Schmidt et al.(1965) which involved gel filtration on Sephadex G-100, culminated in a partially purified preparation of O. tshawytscha gonadotropin (SG-G100) (Donaldson and Yamazaki, 1968; Donaldson et al., 1972). When Funk and Donaldson (1972) injected this partially purified salmon gonadotropin (SG-G100) into male pink salmon, they completely matured within 98 days of treatment and within the year of their hatching.

The present study was undertaken to determine whether the time of sexual maturity in pink salmon could be manipulated precisely enough to be made to coincide with the sexual ripening of a particular natural stock of pink salmon. In addition the study was aimed at determining optimal dosages, modes of administration of gonadotropin, and the use of elevated water temperatures for induction of maturation in male pink salmon.

Two experiments were proposed, 1) to investigate the dose-response effect that pellet-implanted gonadotropin has on gonadal maturation in the male pink salmon, and 2) the effects on maturation of male pink salmon treated with pelleted gonadotropin compared with male pink salmon treated with gonadotropin injected at varying time intervals.

MATERIALS AND METHODS

Origin of Test Animals

The pink salmon fry employed in the first experiment were obtained from Mr. F.C. Withler, Research and Resource Services Directorate, Fisheries and Marine Service, Nanaimo, B.C., in early February, 1974. They had been hatched and reared at an elevated water temperature (12C) at the Pacific Biological Station from eggs taken at Jones Creek (near Hope, B.C.) in October, 1973. Upon arrival at the West VancouverLaboratory the newly hatched fish were placed outdooors under a natural photoperiod regime in 4,000 litre saltwater tanks where flow and temperature were maintained at 30 litres/min. and 12C.

Feeding commenced January 3, using frozen brine shrimp. Beginning January 9, wet food (Donaldson and McBride, 1967) was included in the diet with brine shrimp being deleted on January 18. Commencing January 28, these fish were fed to satiation 2 times/day on a diet consisting of Oregon Moist Pellets of an appropriate size (Westgate et al. 1964)

From the original stock of fish 160 were divided into 4 groups of 40. These fish were kept outdoors in 450 litre fiberglass aquaria and subjected to hormonal treatments as described in the following section.

The pink salmon fry used in the second experiment were obtained in late March, 1975. They were hatched and reared at the Pacific Biological Station but originated from eggs taken at Bear River (near Campbell River, B.C.) in October 1974. They were gradually acclimated to saltwater between February 20 and March 1 before being transferred to the West Vancouver Laboratory. Upon arrival at West Vancouver the fish were placed outdoors under a natural photoperiod and temperature regime in 4,000 litre, saltwater tanks where flow and temperature were maintained at 30 litres/min. and 12C.

Feeding commenced February 15 using frozen brine shrimp. Beginning February 21, wet food (Donaldson and McBride, 1967) was included in the diet with brine shrimp being deleted on March 15. Commencing March 21, these fish were fed to satiety on a diet consisting of Oregon Moist Pellets of an appropriate size.

From the original stock of animals, 392 fish were divided into 7 groups of 56, kept in separate 450 litre, fiberglass aquaria and subjected to hormonal treatments as described below.

Experimental Design and Treatments

The first or pilot experiment consisted of four groups of 40 fish: a cholesterol only implanted pellet control group and three treatment groups receiving a partially purified pelleted preparation of chinook salmon gonadotropin (SG-G100) (Donaldson and Yamazaki, 1968; Donaldson, et al., 1971) bound with cholesterol (Robertson and Rinfret, 1957) at doses of 0.05, 0.5 and 5.0 micrograms SG-G100 per gram body weight.

After a control (untreated) sample had been taken, treatment commenced November 1, 1974. Samples were taken on November 13, 1974 and January 24, 1975.

The second experiment consisted of seven treatment groups of 56 fish. All groups treated with pituitary gonadotropin received the same amount of salmon gonadotropin per unit time. Only the method of administration differed in each treatment group. The seven groups consisted of:

1. untreated control
2. saline injected control, 0.05 ml saline 1 time/week
3. 1.0 µg/gm $\overline{B.W.}$ salmon gonadotropin injected 3 times/week
4. 3.0 µg/gm $\overline{B.W.}$ salmon gonadotropin injected 1 time/week
5. 6.0 µg/gm $\overline{B.W.}$ salmon gonadotropin injected 1 time/2 weeks
6. cholesterol only control pellet implanted 1 time/3 weeks
7. 9.0 µg/gm $\overline{B.W.}$ of pelleted salmon gonadotropin implanted 1 time/3 weeks

The fish were sampled at 4 week intervals. The pre-treatment control sample was taken on June 17, 1975. Treatments were continued until the final sampling on October 7, 1975.

Experimental Techniques

(i) Hormone administration

Prior to hormone administration the fish were netted and transferred to an aerated bucket where they were anesthetized with 2-phenoxyethanol (0.5 ml/litre seawater). Removal of the fish from the bucket was followed quickly by either intraperitoneal injection of a test solution or the implantation of a prepared pellet. For the injection treatments the saline control fish received the same

volume (0.05 ml) of solution (saline 0.7% NaCl) as the experimental fish, but without the added hormone. Injection was directed into the peritoneal cavity at a site immediately anterior to the pelvic fin. To facilitate handling large numbers of fish a Repette Syringe (Jencons Scientific Ltd.) fitted with a 27 guage, 1/2 inch disposable needle was employed. The animals revived within approximately one minute when returned to saltwater.

In preparing the pellets, salmon gonadotropin was mixed homogenously with cholesterol (BDH Chemicals Canada Ltd). Then the mixture was pressed into pellet form by placing a weighed amount of the mixture into the die (3mm diameter) of a hand press (Parr Instrument Co., Moline, Illinois) and applying pressure.

The proportion of salmon gonadotropin by weight depended upon the dose (treatment range, 0.3%-30.0%). Pellets for control fish were composed of cholesterol only and were of similar size to those containing both salmon gonadotropin and cholesterol. Initially the pellets weighed 6 mg; their final weight was 13.6 mg + 5%.

Implantation of the pellet into the anesthetized fish was accomplished in the following manner. First a small incision along the midventral line between the pelvic and pectoral fins was made. A trocar was used to channel a hole into the peritoneal cavity. By using a pair of curved needle forceps, it was possible to implant a pellet in the incision and with the use of a polished glass probe to push the pellet into the peritoneal cavity. One drop of terramycin (50 mg/ml) from a 27 guage needle was applied to the wound, which was subsequently closed with a drop of dental

adhesive (Eastman 910, Adhesives). The animals revived within 5 minutes when returned to saltwater. For both pellet implantation and injection, opposite sides of the fish were used for each succeeding treatment.

(ii) Sampling technique

Before sampling the fish were anesthetized in 2-phenoxy-ethanol (0.5 ml/litre) and then killed by decapitation. Excessive moisture was blotted from each fish and the body length (measured to the nearest mm), body weight (measured to the nearest 0.1 gr), gonad appearance and condition of the fish were recorded. Both gonads were removed, one gonad was fixed immediately in Bouin's solution while the other was weighed to the nearest 0.001 gr.

Prior to the start of each experiment or treatment, a sample was taken to determine the initial state of gonadal development.

(iii) Histological techniques

Following fixation in Bouin's solution for a minimum of 24 hours, the testis was washed, dehydrated and embedded in para-plast m.p. 56-57C (Culling, 1963). The specimens were then sectioned at 6 microns, and stained with Mayer's haematoxylin and eosin.

(iv) Analysis of the histological results

The gonadosomatic index was calculated for each fish from the formula:

$$GSI = \frac{(\text{weight of one testis}) \times 2}{\text{body weight}} \times 100$$

(v) Histological analysis

The stage of sexual maturity of the testis was determined from examination of a single median saggital section from each specimen

and rating it as one of the following stages of testicular development (Funk and Donaldson 1972):

1. Spermatogonia only are present.
2. Spermatogenesis has been initiated. Predominant cell types are primary spermatocytes.
3. Primary spermatocytes, secondary spermatocytes, and spermatids are all present.
4. Spermatogenesis is proceeding actively; spermatozoa are present in the lobule lumina. In this stage the testis reaches its maximum size.
5. The testis is now "functionally" mature. There is an extensive breakdown of the testicular morphology, and spermatozoa lie free in the sperm duct. Semen can be stripped from the animal at this stage.
6. The testis is completely regressed.

(vi) Statistical analysis

The unbalanced data were randomly reduced to equal sample sizes and subjected to balanced analysis of variance using ANOVAR (U.B.C.). The Student Newman Keuls test was performed on data where F in the Anova was significant at the 0.05 level.

A water supply problem resulted in the mortality of the injection 1x/2wk groups in experiment 2. Therefore it was necessary to use an average value for the last sampling period in this group to balance the analysis of variance.

(vii) Disease treatment

The fish employed in experiment 1 suffered from a high mortality and were diagnosed as having kidney disease. To control the extent of the kidney disease the antibiotic erythromycin was incorporated in the diet of all groups at a dose of 100 mg erythromycin/Kgm - fish/day for 21 days beginning October 7 and ending October 31.

RESULTS

All of the male pink salmon in both experiments that had been treated with salmon gonadotropin showed a stimulation of spermatogenesis. In experiment 1 the functionally mature stage 5 state (Fig. 4) was attained in both the large dose (5.0 ug/gm $\overline{B.W.}$) and medium dose 0.5 ug/gm $\overline{B.W.}$) after 12 weeks of treatment (Fig. 7). The functionally mature stage 5 state in experiment 2 was attained in the pelleted gonadotropin group and the 3x/week gonadotropin injection group between 8 and 12 weeks after commencing treatment. Only one fish from the large dose group (5.0 ug/gm $\overline{B.W.}$) of experiment 1 underwent the complete cycle of gonad development ending in stage 6-testicular regression (Fig. 7). Testicular regression for this fish was attained after 12 weeks of treatment.

GSI measurements for experiment 1 revealed that the three dose groups that received pelleted salmon gonadotropin were a homogeneous subset showing greater GSI than the pellet control (Table B). The first two time period groups (0 and 6 weeks) were a homogeneous subset displaying a lesser GSI than the 12 week period group (Table 8). There was however a significant interaction between dose and time for GSI in experiment 1. Graphical display of this interaction (Fig. 5) showed that there was no increase over time in the control group and that a large GSI increase occurred between 6 and 12 weeks in the low dose group (0.05 μg/gm $\overline{B.W.}$) Similarily for experiment 2,

homogeneous subsets were the three groups that received no salmon gonadotropin (0 µg/g/$\overline{B.W.}$ control pellet, 0 µg/g/$\overline{B.W.}$ injection control, untreated) and the three groups that received salmon gonadotropin (pellet 1x/3wk injection 1x/wk, injection 1x/2wk)(Table 8). The later three groups that received salmon gonadotropin showed a significantly greater GSI than did the three groups that received no salmon gonadotropin, while the group that received salmon gonadotropin injected 3x/wk displayed a significantly greater GSI than did all other groups. The second and third periods (4 and 8 weeks) were a homogeneous subset with GSI significantly increasing in 12 weeks and 16 weeks (Fig. 6). However a significant interaction over time occurred between the groups in experiment 2. Graphical display of the GSI interaction (Fig. 6) showed that there was no increase in GSI over time in the injection control, pellet control or untreated groups. Two groups receiving salmon gonadotropin (pellet 1x/3wk, injection 3x/wk) showed a sharp rise in GSI from 0 to 8 weeks with a tapering off in GSI from 8 to 16 weeks, while the other two salmon gonadotropin groups (injection 1x/wk, injection 1x/2wk) showed a steady rise in GSI from 0 to 16 weeks.

Secondary Sexual Characteristics

The characteristic humping of the back and the brownish-red coloration of the sides of the male pink salmon approaching sexual maturity were apparent in the males injected with salmon gonadotropin, making it possible to distinguish between males and females within a treatment group. The male secondary sexual characteristics were more prominent and appeared earlier in the groups receiving the greater gonadotropin dosage in experiment 1, and in the pellet implanted and 3x/wk injected salmon gonadotropin groups in experiment 2.

DISCUSSION

The testis of the gonadotropin-treated pink salmon passed through all stages of gonad maturation, including testicular regression in some fish. Semen could be stripped from the testes of mature fish by stroking in a posterior direction. The results indicate that the time of sexual maturity of males could be manipulated to coincide with that of a particular natural stock of spawning fish. This was an important conclusion for the experimental transplant program described in Chapter III.

A relationship existed between the method of administration of a constant dose of gonadotropin per time period and the rate of maturation. The group receiving a small dose of gonadotropin injected at a shorter time interval (injection 3x/wk) matured faster than did those groups receiving larger doses of gonadotropin at longer intervals (injection 1x/wk, injection 1x/2wk). On the other hand the pellet implanted gonadotropin group (1x/3wk) matured almost as quickly as the 3x/wk gonadotropin group. Because the concentration of a circulating hormone depends upon the rate of secretion into, and clearance from the plasma, it may be postulated that, when the interval between injections is longer (as in the 1x/wk and 1x/2wk groups), the metabolic clearance may reduce the gonadotropin to an ineffective level for part of the interval, thus retarding maturation. In the case of the pelleted gonadotropin group, gonadotropin was being leached constantly from the pellet over a period of three weeks. With regard to the plasma GTH

concentration, there is probably a steady state interaction in which the rate of leaching is in equilibrium with the metabolic clearance rate. This may explain why the pelleted gonadotropin group matured almost as quickly as the 3x/wk salmon gonadotropin injected group.

The GSI measurements and testicular stage data both suggest that onset of mitotic division to form primary spermatocytes and the process of active spermatogenesis were accelerated in fish treated with salmon gonadotropin. Maturity was advanced 9 months in experiment 1 and 13 months in experiment 2 prior to the appearance of sexual maturity in natural stocks of pink salmon. The progress of active spermatogenesis to the production of mature spermatozoa in one-half the normal time in the precociously developed pink salmon might not be due directly to injected gonadotropin. While some aspects of spermatogenesis may be under direct control by pituitary gonadotropins, there are strong indications that androgens may be the ultimate controlling agents for at least some of the spermatogenetic events (Dodd, 1972). It has been shown that spermatogenesis can be induced by androgen treatment (testosterone, dehydro-epiniandrosterone, methyltestosterone) in hypophysectomized Fundulus heteroditus (Lofts, et al., 1966), Heteropneutes fossilis (Sundararaj and Nayyar, 1967; Sundararaj et al., 1971), Carassius (Yamazaki and Donaldson, 1969; Billard, 1974). In addition methyltestosterone is also a potent agent in activating spermatogenesis in Mugil cephalus (Shedadeh et al, 1973). However, testosterone

failed to activate spermatogenesis in methallibure-treated Cymatogaster (Weibe, 1969) and Tilipia (Hyder, 1972). Yamazaki (1972) reported that methyltestosterone suppressed spermatogenesis and caused degeneration of spermatogonia in the salmonids Oncorhynchus gorbuscha and O. nerka. However, Pandey (1968) also reports the lack of effect of methyltestosterone on the testis of juvenile Poecilia: there is no stimulation of any stage of spermatogenesis. Pandey (1968) suggests that the gonadal tissues must first be primed by gonadotropin before they are reactive to steroids. In any case, these facts demonstrate that in pink salmon as well as other teleosts the pituitary gland and especially gonadotropin(s) are essential for normal spermatogenesis.

The rate of induction of active spermatogenesis and the time of appearance of spermatozoa in the gonadotropin-treated juvenile pink salmon compared favourably with similar treatment of other salmonids. Schmidt et al. (1965) found after two weeks of injection of extracts from O. tshawytscha pituitaries into immature rainbow trout that spermatogenesis had been accelerated to the primary and secondary spermatocyte stage. Injection of fingerling coho salmon for three weeks with a pituitary homogenate from spawning adult coho stimulated the mitotic division of the spermatogonia; however no spermatocytes could be found (Chestnut, 1970). Administration of an extract from the pituitaries of spawning salmon (O. keta and O. tshawytscha) in cholesterol pellets to six month old rainbow trout resulted in the shedding of spermatozoa two months later. Injection of chinook salmon gonadotropin into

juvenile pink salmon resulted in male pink salmon passing through all stages of gonad maturation including terminal testicular regression in about three months (Funk and Donaldson, 1972).

In contrast to the findings by Funk and Donaldson (1972) the secondary sex characteristics typical of spawning adult male pink salmon were evident in the precocious males produced in this study. Such sexual dimorphism is a result of androgen biosynthesis in the testis and demonstrates the potential of the testes for producing sex hormones while under gonadotropin administration.

CHAPTER II

The Ovary

INTRODUCTION

Earlier work has shown that the development (maturation and growth) of the ovary in young teleosts is dependent upon the pituitary. However, the experimental evidence related to the nature of the pituitary-ovarian relationship is contradictory and difficult to evaluate (Dodd, 1972).

In line with evidence from studies of amphibians (Schuetz, 1974), studies of teleosts both *in vitro* and *in vivo* have shown that the gonadotropins play a significant role in regulating gametogenesis. All stages of reproductive maturity, including vitellogenesis and ovulation, were reinduced by replacement therapy with chinook salmon gonadotropin (SG-G100) following pituitary removal in adult *Carassius auratus* (Yamazaki and Donaldson, 1968), *Poecilia reticulata* (Liley and Donaldson, 1969), and *Heteropneustes fossilis* (Sundararaj *et al.*, 1972). Salmon gonadotropin treatment has accelerated vitellogenesis and induced ovulation in maturing *Mugil cephalus* (Donaldson and Shehadeh, 1972; Shehadeh *et al.*, 1972; Shehadeh and Kuo, 1972). Among salmonids, Schmidt *et al.* (1965) found a small increase in ovarian size in immature rainbow trout injected for 2 weeks with an extract of chinook salmon pituitaries. Oocytes in fingerling coho salmon matured slowly when treated for 3 weeks with a homoplastic preparation of pituitary glands from spawning adults (Chestnut, 1970). Funk and Donaldson (1972) found

a stimulation in the development of previtellogenic stages and the onset of vitellogenesis by salmon gonadotropin (SG-G100) therapy in pink salmon (O. gorbuscha).

Jalabert et al. (1973) have shown that oocyte final maturation can be induced in in vitro ovarian fragments of rainbow trout by purified carp gonadotropins or progesterone, but not by estogens or corticosteroids. These investigators (Jalabert et al., 1972) suggest that gonadotropins stimulate the ovary to produce progestogens which in turn stimulate final maturation of oocytes. Fostier et al. (1973) found that 17 hydroxy 20 B dihydroprogesterone induces maturation in vitro in oocytes of rainbow trout, and of all of the steroids tested he found it to be the most active. It is interesting to note that this steroid has been isolated from the plasma of salmon by Idler et al. (1960) and that it exerts an influence at doses lower than those found in vivo before spawning in S. salar (Schmidt and Idler, 1962). One may surmise that the gonadotropic hormone(s) act on the ovary by provoking the synthesis and/or release of steroid(s) which in turn induce final maturation. In addition Jalabert (1975) has shown that cortisol and cortisone increase the effectiveness of gonadotropin on intrafollicular maturation in vitro of the oocytes of trout rendering 17 hydroxy-20 B dihydroprogesterone even more effective. However, the injection of a preparation of gonadotropin from the chinook salmon into gonadectomized sockeye salmon (O. nerka) causes no increase in plasma cortisol or cortisone and does not stimulate the activity

of interrenal tissue. These results suggest that certain actions of salmon gonadotropin in the genital sphere which could be ascribed to certain corticosteroids, cannot be explained by interrenal corticosteroidogenesis caused by this gonadotropin (Donaldson and McBride, 1974).

Concerning oocyte growth, observations indicate that in teleosts estrogen stimulates the development of phospholipid proteins in the liver that are essential in building vitellus. Idler et al. (1961) obtained some evidence that estradiol increased the mass of female gonads in sockeye salmon. Aida et al. (1973) injected estradiol-17B into the Ayu (Plecoglossus altivelus) and found that the amount of yolk material which appeared in the blood serum increased in proportion to the amount of estradiol 17B injected. A still more complex picture prevails regarding the action of oocytes with incomplete vitellogenesis (Sakun, 1970). In most fishes, the transition to maturation becomes possible at the end of vitellogenesis. For example, Sakun (1975) has shown this to be the case in whitefish (Coregonus lavaretus pidschian). However, in the Atlantic salmon (S. salar), the pink salmon, and the rainbow trout, the maturation of oocytes can be induced long before the completion of vitellogenesis. Maturation of oocytes long before completion of vitellogenesis is probably the reason for the variability between the periods of maturation and the sizes of ripe eggs observed in salmon (Sakun, 1975).

In any case, evidence appears to indicate that steroids, both estrogenic and progestional compounds, are synthesized by the salmonid ovary under gonadotropic stimulation, and are involved in intra and extra-ovarian processes related to follicular and oocyte maturation.

Thus it appears that treatment consisting of physiological dosages of salmon gonadotropin alone would be most likely to accelerate the maturation in the ovary of immature pink salmon. Funk and Donaldson (1972) did not obtain maturity in the year of hatching in female pink salmon treated with salmon gonadotropin (SG-G100) alone and in combination with estradiol-17B. However, they did obtain female pink salmon that approached sexual maturity in January (5 months later) of the year in which they would normally spawn.

After considering this information and the size-fecundity relationship in pink salmon (Foerster and Pritchard, 1941) it was proposed that the larger pink salmon occurring under experimental rearing conditions (see Chapter I) be subjected to gonadotropin treatment. Two experiments were proposed; 1) to investigate the dose-response effect that pellet implanted gonadotropin has on ovarian maturation in the female pink salmon, and 2) to compare the effects on ovarian maturation of pellet implanted gonadotropin with the effects of injected gonadotropin administered at varying time intervals.

If reproductive maturity were stimulated it might be possible

to fertilize the eggs these precocious female pink salmon with sperm from precocious male pink salmon and possibly to populate "off" year cycles of pink salmon in British Columbia with fish containing a 100% home stream genetic complement.

METHODS AND MATERIALS

Origin of Test Animals

The females were from the same stock and were maintained under the same conditions as described for males in the materials and methods section of Chapter 1.

Experimental Design and Treatments

The design and treatments for the observations on the female pink salmon were the same as those conducted on the male pink salmon as described in Chapter 1. Briefly they were:

(1) November 1974 to January 1975 - Examine the effect of various dosages (0.05, 0.5, 5.0 μg/gm $\overline{B.W.}$) of chinook salmon gonadotropin (SG-G100) administered in pelleted form on development of the pink salmon ovary.

(2) July 1975 to October 1975 - Examine the effect of various modes of administration of a constant dosage of salmon gonadotropin on development of the pink salmon ovary.

Experimental Techniques

(i) Hormone Administration. As in chapter 1

(ii) Sampling Technique. As in chapter 1

(iii) Histological Technique. As in chapter 1

(iv) Analysis of the histological results

(a) Measurements

The gonadosomatic index was determined as follows:

$$GSI \quad \frac{\text{weight of one ovary X 2}}{\text{body weight}} \times 100$$

The mean oocyte diameter was obtained from an average of the diameter of 30 oocytes per ovary, measured with an ocular micrometer. Only oocytes containing the greatest part of the nucleus were measured. The value for ovoid oocytes was calculated according to Braekevelt and McMillain (1967) from the formula:

$$\text{oocyte diameter} = K \sqrt{\text{greatest diameter} \times \text{least diameter}}$$

where K is the factor which converts the ocular micrometer measurements of the greatest and least oocyte diameter into millimeters.

b) Histological analysis

The composition of each ovary was determined by counting all the nucleated oocytes in a median sagittal section, and expressing the value for each type as a percentage of the total. This information was then averaged for each treatment and plotted graphically.

The characteristics for each type of oocyte were those employed by Funk and Donaldson (1972) which were similar to those described by Yamazaki (1965) in his study of the ovary of the goldfish (Carassius auratus L.). They were as follows:

(1) Early perinucleolus stage

Several nucleoli are apparent near the nuclear membrane. The cytoplasm has a marked affinity for haematoxylin; certain basophilic, clumped, granular portions are referred to as a palliar layer by Kudo in studies on Plecoglossus altivelus (1969a) and Pseudorasbosa pimula (1969b). The cytoplasm and nucleus increase greatly in size. The oocyte varies from 40 to 250 microns in diameter. During this stage, the oocytes become surrounded by squamous cells from the interstitial region to form a follicular layer one cell thick. The zona radiata is not yet apparent.

(2) Late perinucleolus stage

Nucleoli are still present at the periphery of the nucleus. The ooplasm has lost its affinity for haematoxylin. The pallial layer has condensed to form a yolk-nucleus at the periphery of the cytoplasm. The oocyte has increased in size, but not as markedly as in the previous stage. The diameter of the oocytes vary from 180 to 320 micra. There is a division of the cells from the interstitial region to form a second layer. The zona radiata is vaguely apparent.

(3) Yolk vesicle stage

Cortical alveoli (or vesicles) appear in the periphery of the ooplasm as a single layer. The size of the oocytes varies from 260 to 900 micra. As the oocytes increase in diameter, the yolk vesicles accumulate toward the nucleus, and contain intravesicular eosinophilic granules. The nucleus becomes irregular in outline, it still contains peripheral nucleoli. The follicular epithelium is composed of a single layered zona granulosa of mitotically dividing squamous and ovoid cells, and an overlying theca of one to several layers of cells. The zona radiata is thin at the beginning of this stage and increases in thickness and eosin affinity toward the end.

(4) Primary yolk stage

At the beginning of this stage, oil globules are present near the nucleus, and small yolk granules are present at the periphery of the ooplasm. The yolk granules coalesce to form

large globules, and move throughout the cytoplasm of the oocyte. The zona granulosa is composed of cuboidal cells, while the overlying layer consists of squamous cells. The thecal layer thickens, the cells which compose it are all squamous. The zona radiata stains intensely with eosin, but radial striations are only vaguely apparent. The oocytes in this stage range in diameter from 550 to 1,500 micra.

(5) Post primary yolk stage

This stage consists of all those oocytes larger than 1.0 mm and more advanced than in the primary yolk stage.

(v) Statistical analysis

- see Chapter 1

(vi) Disease treatment

- see Chapter 1.

RESULTS

Measurements

The development of the ovary in pink salmon juveniles injected with various doses of pelleted gonadotropin in experiment 1 and by various modes of administration in experiment 2 were compared with each other and the control groups.

i) Gonadosomatic index (GSI)

GSI measurements in experiment 1 revealed that the three dose groups that received pelleted salmon gonadotropin (0.05, 0.5, 5.0 µg/gm $\overline{B.W.}$) were a homogeneous subset having a lesser GSI than the pellet control group (Table 22). The groups in the first two time periods (0 and 6 weeks) were a homogeneous subset with a significantly greater GSI than the groups in the 12 week period. There was a significant interaction over time occurring between the groups. Graphical analysis of the interaction (Fig. 15) revealed an increase over GSI in the control group, a sharp drop in GSI in the 5.0 µg/gm $\overline{B.W.}$ treatment group between 0 and 6 weeks, and a sharp drop in GSI in the 0.05 and 0.5 ug/gm $\overline{B.W.}$ treatment groups between 6 and 12 weeks. In experiment 2, homogeneous GSI subsets were (inj. SG-G100 3x/wk, inj. SG-G100 1x/wk, SG-G100 pellet implant), (inj. SG-G100 1x/wk, SG-G100 pellet implant, pellet control, inj. SG-G100 1x/2wk, untreated), and (pellet control, inj. SG-G100 1x/2wk, untreated, inj. control) (Table 22). The last three time periods (8, 12, and 16 weeks) were a homogeneous subset with GSI greater in these periods than in the 4 week period. No significant

interaction occurred between time and groups in experiment 2.

ii) Oocyte diameter

In experiment 1, oocyte diameter decreased over time in all three salmon gonadotropin treated groups, the greatest decrease occurred between commencement of treatment and 6 weeks (Fig. 17). Oocyte diameter in the control group remained almost unchanged over the course of the experiment. In experiment 2, oocyte diameter increased significantly over time in the injection 3x/wk and pellet implanted salmon gonadotropin groups reaching 1mm at 16 weeks (Fig. 18). The injection 1x/wk and inj. 1x/2wk salmon gonadotropin groups displayed a slight rise in oocyte diameter over time similar to the untreated and control groups.

iii) Oogenesis

Figure 19 shows the results of experiment 1 on the effect of various dosages of pelleted gonadotropin on development of the oocytes in immature pink salmon treated during the winter of 1974. In all three salmon gonadotropin treated groups the ovarian stage regressed over the course of the experiment while the ovarian stage of the control group remained relatively unchanged. At the end of 12 weeks of treatment the 5.0 ug/gm $\overline{B.W.}$ group regressed from oocytes mainly in the yolk vesicle stage to oocytes in the early perinucleolus stage (Fig. 19), the 0.5 ug/gm $\overline{B.W.}$ group regressed from oocytes mainly in the yolk vesicle stage to oocytes in the late perinucleolus stage (Fig. 19), and the 0.05 ug/gm $\overline{B.W.}$ group regressed from oocytes mainly in the yolk vesicle stage to oocytes mainly in the late perinucleolus stage. In experiment 2, treatment was

initiated at a slightly earlier ovarian stage. Figure 20 showed that the injection 3x/wk and pellet implanted salmon gonadotropin groups advanced after 16 weeks of treatment from oocytes mainly in the late perinucleolus stage to oocytes mainly in the primary yolk vesicle stage. The 1x/wk and the 1x/2wk salmon gonadotropin groups, the control groups, and the untreated group showed no advance in ovarian stage after 16 weeks of treatment. There were primary yolk vesicle stage oocytes present in the injection 1x/wk and inj. 1x/2wk salmon gonadotropin groups at 8 weeks (Fig. 20). However no evidence of primary yolk stage vesicles were apparent at later periods of 12 and 16 weeks in these two groups.

In both experiments ovarian atresia was found in fish from gonadotropin treatment groups and to a lesser extent in control groups in the yolk vesicle and the primary yolk vesicle stage (Tables 17, 18 Fig. 14). Oocytes in both the early and late perinucleolus stages showed little sign of atresia.

Appearance

No secondary sexual characteristics were observed in any of the treated or untreated female pink salmon.

Kidney disease killed many fish in experiment 1.

DISCUSSION

Ovarian maturation in juvenile pink salmon was stimulated with salmon gonadotropin in experiment 2, while in experiment 1 ovarian maturation was retarded by salmon gonadotropin. Although it is conceivable that salmon gonadotropin may have caused the retardation of maturation in experiment 1, the results should be discounted because of the presence of kidney disease in the fish and the subsequent treatment with antibiotics, which may affect pituitary gonadotropin induced maturation in oocytes by inhibiting RNA and protein synthesis (Goswami and Sundararaj, 1973; Jalabert, 1976)

The pituitary-ovarian relationship in terms of growth and development of oocytes in the preyolk vesicle stage has had little study in teleosts. In addition the results are contradictory and therefore difficult to evaluate (Bullough, 1942; Barr, 1963; Vivien, 1938, 1941; Yamazaki, 1965). In the present study conversion of late perinucleolar stage oocytes to yolk vesicle oocytes occurred earlier in juvenile pink salmon treated with salmon gonadotropin than in the controls. This observation agrees with that of Funk and Donaldson (1972) in that there is a stimulation of the development of previtellogenic stages by pituitary gonadotropin.

In experiment 2, oocytes in the primary yolk stage were first observed in pink salmon injected 3x/wk, 1x/wk and 1x/2wk

with salmon gonadotropin after 8 weeks of treatment. At 12 and 16 weeks the inj. 3x/wk and the pellet implanted salmon gonadotropin groups were the only groups exhibiting primary yolk stage oocytes. It should be noted that in the 1x/wk and 1x/2wk salmon gonadotropin groups the primary yolk stage oocytes which occurred in the 8 week period were not present in the 12 and 16 week periods. Considering that the concentration of a circulating hormone results from the rate of secretion or injection into, and the clearance from the plasma, it may be postulated that the gonadotropin level in the greater dose/longer time interval between injection groups (1x/wk, 1x/2wk) is not effective enough to maintain the vitellogenic stage. It is possible that either a change in the clearance rate of gonadotropin from the plasma or an increase in the demands of the ovary for gonadotropin occurs. In the injection 3x/wk and pellet implant salmon gonadotropin groups, primary yolk oocytes were maintained and increased in number during the course of the experiment. This suggests perhaps that the smaller dose/smaller time interval between injections and the constant leaching of gonadotropin from the pellet may maintain a sufficient gonadotropin concentration in the plasma for oocyte development. It appears then that the gonadotropin demands of the ovary increase with development of the ovary. This observation agrees with that of Crim et al. (1975) who found that female plasma gonadotropin titers are low at the early stages of ovarian growth, while further development of the oocyte including the vitellogenic phases, is associated with rising gonadotropin values.

Gonadotropin extracted from teleost pituitaries has been found to be effective as a stimulant for ovary development in hypophysectomized adult teleosts. All stages of reproductive maturity, including vitellogenesis and ovulation were reinduced by replacement therapy with salmon gonadotropin (SG-G100) following pituitary removal in Carassius auratus (Yamazaki and Donaldson, 1968), Poecilia reticulata (Liley and Donaldson, 1969), and Heteropneustes fossilis (Sundararaj et al., 1971). SG-G100 treatment accelerated vitellogenesis and induced ovulation in Mugil cephalus (Donaldson and Shehadeh, 1972; Shehadeh et al., 1972; Shehadeh and Kuo, 1972). Concerning salmonids, Schmidt et al. (1965) found a small increase in ovarian size in immature Salmo gairdnerii injected for 2 weeks with an extract of O. tshawytscha pituitaries. Oocytes in fingerling O. kisutch matured slowly when treated for 3 weeks with a homoplastic preparation of pituitary glands from spawning adults (Chestnut, 1970). In comparison with the results of Funk and Donaldson (1972) the primary yolk vesicle stage reached after 16 weeks of treatment in this study was more advanced than the gonadotropin injected groups, but similar to the gonadotropin-estradiol injected groups of Funk and Donaldson (1972) after 16 weeks of treatment in the pink salmon. However, this difference in maturation may be attributed to the fact that the fish employed in experiment 2 in this study were reared at a higher temperature and were significantly larger than those fish used by Funk and Donaldson (1972) in their study.

In this study no secondary sexual characteristics developed in the females.

Atresia of oocytes undergoing vitellogenesis was first

observed in experiment 1 in treatments injected with salmon gonadotropin. However, considering the fact that the fish suffered from kidney disease and a high mortality rate the impression that salmon gonadotropin administration caused the atresia should be discounted. In experiment 2 atresia increased with ovarian maturation. More than one third of the oocytes had become atretic, developing into preovulatory corpora atretica at 16 weeks in the injection 3x/wk and pellet gonadotropin groups. This phenomenon is not unusual, as the ovary of every reproductively mature fish contain regressed oocytes (Ball, 1960; and Yamazaki, 1965). The stages of atresia followed the pattern described by Bretschneider and deWit (1947) and Beach (1959) and was confined to those oocytes in the yolk granular stage (vitellogenic stage).

Much attention has been given to the mode of formation, function and endocrine status of these atretic oocytes and the subject has been reviewed repeatedly, though the question of function has not yet been resolved (Dodd, 1955; Pickford and Atz, 1957; Dodd, 1960; Ball, 1960; Hoar, 1965; Hoar, 1969).

In summary, although ovarian maturation was induced by gonadotropin treatment it was not possible with these treatments to obtain mature female pink salmon in the year of hatching.

CHAPTER III

Aspects of the Bear River Pink Salmon Transplant Program

INTRODUCTION

The rigid 2-year life cycle of pink salmon in nature prevents the possibility of populating a stream's barren cycle directly from that stream's "on-year" stock. Attempts to populate barren cycles with eggs transplanted from other streams have so far been largely unsuccessful. However, the ability to produce fertile 1-year-old males by gonadotropin injections (as described earlier) makes it possible to transfer one-half of a stream's on-year gene pool into the barren cycle, by fertilizing eggs from another stream with males derived from the on-year run (Fig. 21). A test to determine whether an infusion of a stream's male on-year genes into transplanted ova will significantly increase the return of the resulting adults to the recipient stream is being carried out on Bear (Amor de Cosmos) River on northeast Vancouver Island.

The test is being accomplished by fertilizing donor eggs from Glendale River (Knight Inlet, B.C.) with: 1) stored frozen "on-year" sperm from the recipient river (Bear River), 2) sperm from precociously matured "on-year" males of the recipient river, and 3) sperm from males of the donor river (Glendale River).

Fry produced from eggs of the 3 different parental origins were marked distinctively and released in the spring of 1976 to go to sea. The effectiveness of infusing the "on-year" gene pool into transplanted ova will be assessed finally by comparing the numbers of each type of adult salmon returning to the fishery and to the Bear River in 1977.

My part of this study was firstly to create, by gonadotropic hormone treatment, relatively large numbers of precociously matured "on-year" Bear River male pink salmon, whose reproductive development was regulated to provide sperm for fertilizing naturally developed eggs from wild stock females taken at Glendale River. (To date small numbers of Fraser River male pink salmon have been accelerated to sexual maturity 1 year earlier than normal using thrice weekly injections of salmon gonadotropin over a period of 2-3 months (Funk and Donaldson, 1972). Spermatozoa from these accelerated males have been used to fertilize ova obtained from wild females in Northern British Columbia and the eggs hatched to produce fry having 50% of their genetic complement from the Fraser River stock (Donaldson, et al., 1972). Secondly, I was to evaluate other aspects of the test by comparing the fertilizing ability of the sperm from precocious males with that of sperm from wild Bear River males that had been held 1 year by freezing and with sperm from current year wild stock pink salmon from the Glendale River, and by comparing the times of emergence and condition of fry derived from the 3 groups of eggs representing different parental origins.

MATERIALS AND METHODS

i) Transplant Site

The transplant testis being carried out at Bear River, Vancouver Island, B.C. (Fig. 23). Holding and incubating facilities are situated on Bear River near its confluence with Cold Creek. The water supply comes directly from both Cold Creek and Bear River. The water is pumped through sand filters and discharged into a constant level head tank where the two sources of water can be blended if desired.

Stacks of Heath incubators contained the brood to the eyed stage. The brood was then transferred to six 4' x 8' x 4' high gravel incubation boxes equipped with an upwelling water supply of approximately 100 litres/min, leak proof liners of non-toxic formula ABS plastic, and containing a gravel medium of crushed rock of a commercially available size range of 0.75-1.25 inches.

ii) Sexual Product Collection and Transportation

The donor stream was the Glendale River located on Knight Inlet, B.C. (Fig. 23) where a large collecting installation had previously been constructed by the Department of Fisheries and Environment. The existing weir and holding pen on the Glendale River were used to hold the mature pink salmon. Donor females and males were taken from the spawning run during late September and October, 1975. Approximately 1.3 million unfertilized eggs and quantities of milt were transported in insulated boxes by float plane to the transplant site at Bear River where fertilization was carried out.

(iii) Treatment Groups

(1) Control group - sperm from adult donor Glendale River males of 1975 fertilized eggs from adult donor Glendale River females of 1975.

(2) Frozen sperm group - one year old cryopreserved sperm from adult "on year" Bear River males of 1974 fertilized eggs from adult donor Glendale River females of 1975.

(3) Accelerated male group - sperm from precociously matured one year old, "on year" Bear River males fertilized eggs from adult donor Glendale River females of 1975.

The precocious males used for this fertilization were fed, reared and obtained from the same stock of fish described in Chapter I (Experiment 2). 750 juveniles were selected from this stock and held in 4,500 litre self cleaning tanks. These fish ($\overline{B.W.}$ 110.0 g) were weighed and administered, by injection, 1.0 µg salmon gonadotropin 1 gm $\overline{B.W.}$, 3 times/week commencing July 14, 1975. The dosage was recalculated on the new mean body weight of a random sample of 50 fish every three weeks. On August 14, 1975 the males were separated from the females by observing secondary sexual characteristics, with the males continuing receiving injections. The males were placed on a maintenance injection schedule of 1.0 µg salmon gonadotropin/gm $\overline{B.W.}$, 1 time/week commencing August 27, 1975. This procedure was established in order to ensure that the sexual ripening of the precocious males would coincide with the Glendale River egg collection in October, 1975. During the collection, the precocious males were transferred from seawater to freshwater and transported in a chilled (5C) oxygenated tank truck to the Bear River site. There they were held in 2,000 litre self-cleaning tanks with the males continuing to receive a maintenance injection until time of stripping.

The injection procedure was identical to that described in Chapter I under Experimental Techniques-Hormone Administration.

(iv) Spawning Routine

a) Transplant groups

(1) Control (Glendale River males x Glendale River females) -
The spawning routine was to add the milt of about five males into a spawning bucket with the eggs of about five females, mix the sperm into the eggs, wash and water harden the fertilized eggs.

(2) Frozen sperm group (Bear River males x Glendale River females) -
Cryopreserved sperm from the "on year" run at Bear River (1974) and stored in cellophane packets in liquid nitrogen was thawed in a 45°C bath and mixed with the eggs of about five Glendale females. Eggs were washed and then water hardened.

(3) Accelerated male group (Bear River males x Glendale River females) -
The spawning routine followed standard hatchery procedures. The fertilized eggs were then washed and water hardened. All stripped males were returned live to freshwater and retained for observation.

b) General

A total of 1,348,480 eggs taken from the pink salmon spawning run of Glendale River was used in the three experimental groups. The number of eggs fertilized and the concentration of milt used to fertilize the eggs of the experimental transplant groups were as follows: control, 368,460 eggs at 10 cc milt/7200 eggs; frozen sperm,

609,280 eggs at 5 cc milt/7200 eggs; and accelerated males, 370,560 eggs at 10 cc milt/7200 eggs.

During the course of the spawning period, each days egg take was divided and fertilized proportionately among the three transplant groups, according to the ratio; 1 control: 2 frozen: 1 accelerated male.

Once fertilized the eggs of all three transplant groups were placed randomly in darkened Heath incubators (the eggs of approximately five females per tray) and periodic treatments of malachite green were given at 5 ppm for 1 hour. When the eggs had developed to the eyed stage, approximately 6 weeks after fertilization, the dead eggs were removed. The live eggs were placed in the incubation boxes between 3 inch layers of gravel. Eight layers of eggs were placed in each box and two randomly chosen gravel boxes were used for each transplant group.

At the time of egg deposition in the boxes, daily average temperatures for the hatchery water were about 9 C, at time of emergence about 6 C. The boxes were exposed to natural light fluctuation until February 21. From this day on artificial lights were kept on 24 hr/day in order to delay emergence in all groups. Beginning April 11, the lights were turned off for short period of time to permit emergence.

(v) Fry Count

Fry emerged at night and were trapped in troughs attached to the incubation boxes. All treatment fry were counted in the morning following emergence and recorded for that day. A random sample was taken of 10 fry per group for 12 days by successively splitting the

night emergence down to adequate sample size. Samples were processed the next day after preservation, in order of capture. Fork lengths (mm) and total weight (mg), were recorded and stages of development were related to the factor K_D as follows:

$$K_D = \frac{10^3 \text{ W in mg}}{\text{L in mm}} \qquad \text{(Bams, 1970)}$$

Balanced analysis of variance were carried out on the length, weight and K_D data using ANOVAR (UBS computing center). The Student Newman Keuls test was performed on data where F was significant at the 0.05 level.

RESULTS

The male pink salmon treated by gonadotropic injection grew from an average weight of 110.0 grams on July 14 to an average weight of 120.5 grams on October 7. From the 187 males selected for use, 153 produced milt and 34 did not. The 153 males produced a total of 610 cc of milt or approximately 4.0 cc per male. This compares to an average milt production of 25 cc of milt per fish from Glendale River wild stock.

Table 23 demonstrates the comparative survivals from fertilization of "green" (unfertilized) eggs to the eyed egg stage; from the eyed egg stage to the free-swimming fry stage and the overall survival from fertilization to free-swimming fry.

The data showed that the sperm from those fish whose reproductive development had been accelerated by gonadotropic treatment was effective in fertilizing ova. The 96% survival to the eyed embryo compares favourably with the 93% survival of the control group which were fertilized with sperm from untreated wild stock from Glendale River. Less than half of the eggs that were fertilized with sperm from wild stock and held over a year by the freezing technique survived to the eyed stage. The data also shows that fertilized eggs from all three groups that survived to the eyed stage survived well to the fry stage.

The control group fry began migrating earlier than the other groups and maintained the lead throughout the run (Fig. 2). First second (median), and third quartiles of the emergence for all groups are recorded in Table 24. According to the median dates, the control

group emerged earlier than the frozen sperm and accelerated male groups by approximately 6 days. The rate of emergence was very rapid for all groups. Lights had been kept on 24 hr/day since February 21 in order to delay emergence in all groups. Beginning April 11, the lights were turned off for short periods of time in order for emergence to take place.

Mean lengths of the control groups were less than those of the other two groups (Table 25). But the S-N-K test revealed that the mean length of fry from only one control box was significantly different from that of one of the accelerated male boxes (Table 29).

Analysis of variance revealed no significant differences in mean weights between groups.

Mean stage of development (K_D Index) was less advanced (greater) in the control groups. S-N-K test indicates that one control group is significantly less advanced than two frozen sperm groups and one accelerated male group (Table 29).

DISCUSSION

Evaluation to the Fry Stage

In this experiment all three transplant groups were subjected to identical conditions (spawning dates, total thermal units received, egg density, and water flow). Therefore, on the basis of thermal history alone, average developmental stages reached at specific times should have been similar if there were no inherent differences between treatment groups. Comparison of samples for weight, length and stage of development (K_D) at emergence for all three transplant crosses showed that the control fry group(Glendale ♂ x Glendale ♀) were generally shorter and had developed less (K_D Index greater) than the two other groups. In addition, the control fry migrated approximately 6 days earlier than did the other two transplant groups. If average rates of development had been the same in the control groups, allowance of this number of days may have brought the control group fry emergence into phase with the accelerated male (Bear River ♂ x Glendale ♀) and frozen sperm group fry emergence (Bear River ♂ x Glendale ♀).

Survival to planting was greatest in the accelerated male group while the frozen sperm group suffered the highest mortality. The high mortality in the frozen sperm group was probably a result of a reduction in the fertilizing ability of the sperm after storage in liquid nitrogen. On the other hand, the high survival to planting in the accelerated male group was probably a result of the fact that the accelerated males were stripped on site and the sperm had not

been subjected to a 4 hr transportation period. Survivals during the incubation box stage were very high and similar between all three transplant groups indicating that once fertilization mortality has taken place the surviving embryos were equally viable despite the differing sources of sperm.

General and Incidental Comments

Differences in age at migration and differences in inherent rates of development are both known to occur in other salmon stocks (Bams, in press). In this experiment there is some indication that the control cross (Glendale♂ x Glendale♀) displayed a different time of emergence and K_D stage of emergence than the other two crosses (Glendale♀ x Bear♂). Such results suggest that differences in emergence timing exist between different stocks and that the male genetic complement can have a significant influence. Work by Bams (1976) demonstrates the usefulness of the home stream gene pool to enhance the return of transplanted individuals. The differences in K_D and emergence between the Glendale x Glendale group and Glendale and Bear River groups in this experiment may be an indication that the two groups incorporating a 50% genetic input into the transplant from the home stream (Bear River) may be better suited to environmental factors in Bear River.

Additional evidence is mounting that shows that homing in salmonids is partially inherited (Bams, in press; Brannon, 1972; Raleigh, 1971), while final recognition of home waters is in response to environmental cues to which the fish become conditioned early in life (Brett and

Groot, 1963; Sulterlin and Gray, 1973; Veda et al., 1967). If this is the case then the two experimental crosses involving Bear River genetic products may have associated with them a greater tendency to return to Bear River, the recipient stream.

GENERAL SUMMARY

Precocious males obtained by gonadotropin injection are being used in an experiment to determine whether or not an infusion of male genes from the Bear River "on year" run into ova transplanted from a donor stream will significantly increase the return of adults to the Bear River (on Vancouver Island). Preliminary results have demonstrated that sperm from the precocious males fertilized the transplanted ova successfully and that the resultant fry were normal. Comparison of the times of emergence of hybridized fry with those of fry of entirely foreign origin suggested that heritable differences derived from the parental stocks can be detected as early as the emergent fry stage.

Research that is planned to generate knowledge, which may have immediate application, should proceed beyond the controlled laboratory stages to the "pilot plant" application stage. It is rarely possible for a researcher, especially at the graduate student level, to continue his research through the "pilot plant" stage because of the high costs involved. In the case of the research described in Chapters One and Two, it was planned from the outset that if the results were applicable, this student research worker would form part of a research team to carry out a large scale "pilot plant" project funded by the Federal Department of Fisheries and Environment. The Bear River Pink Salmon Transplant is the first attempt to evaluate the concept of "genetic matching" as it may apply to repopulating streams which are barren of spawning pink

salmon every second year.

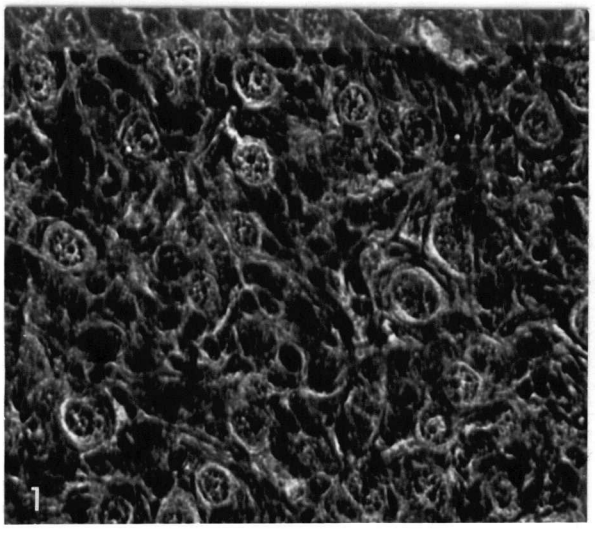
This study has provided evidence that maturation of male and female pink salmon can be accelerated. In juvenile male pink salmon, the cycle of testicular maturation can be manipulated with either pelleted or injected salmon gonadotropin to produce viable spermatozoa one year earlier than normal. Acceleration of ovarian maturation in female pink salmon was achieved either by injection or implantation of salmon gonadotropin; however, full maturity one year earlier than normal was not achieved.

Figure 1 - Cross section of a control testis from an immature (stage 1) pink salmon juvenile. Haematoxylin and eosin x 550.

Figure 2 - Cross section of a stage 3 testis from a pink salmon juvenile. Haematoxylin and eosin x 150.

Figure 3 - Testis of a sexually mature (stage 5) pink salmon juvenile. Haematoxylin and eosin x 30.

Figure 4 - Testis of a sexually mature (stage 5) pink salmon juvenile, but at a higher magnification. Haematoxylin and eosin x 140.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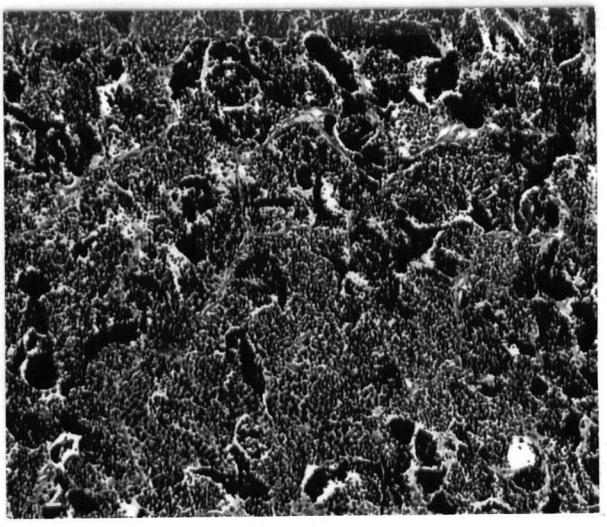
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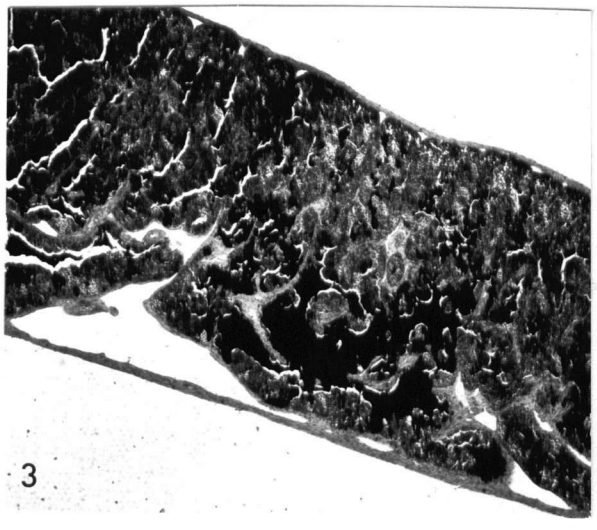
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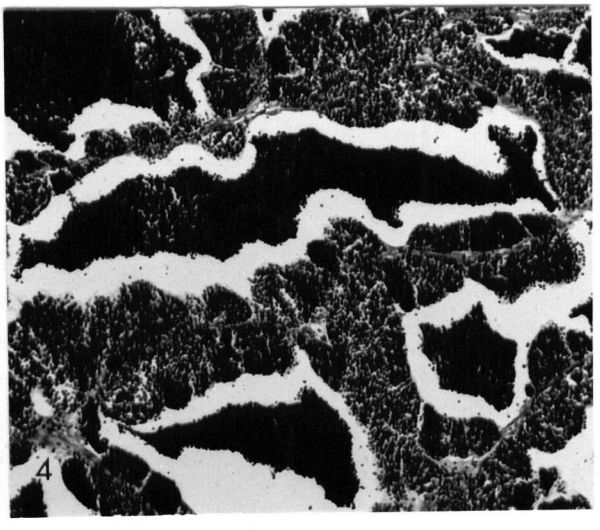

4

FIGURE 5 - Gondosomatic Index (G.S.I.) of male pink salmon implanted intraperitoneally every 3 weeks with various dosages of a salmon gonadotropin:cholesterol pellet. Valves are the means and standard deviations taken from Table 3.

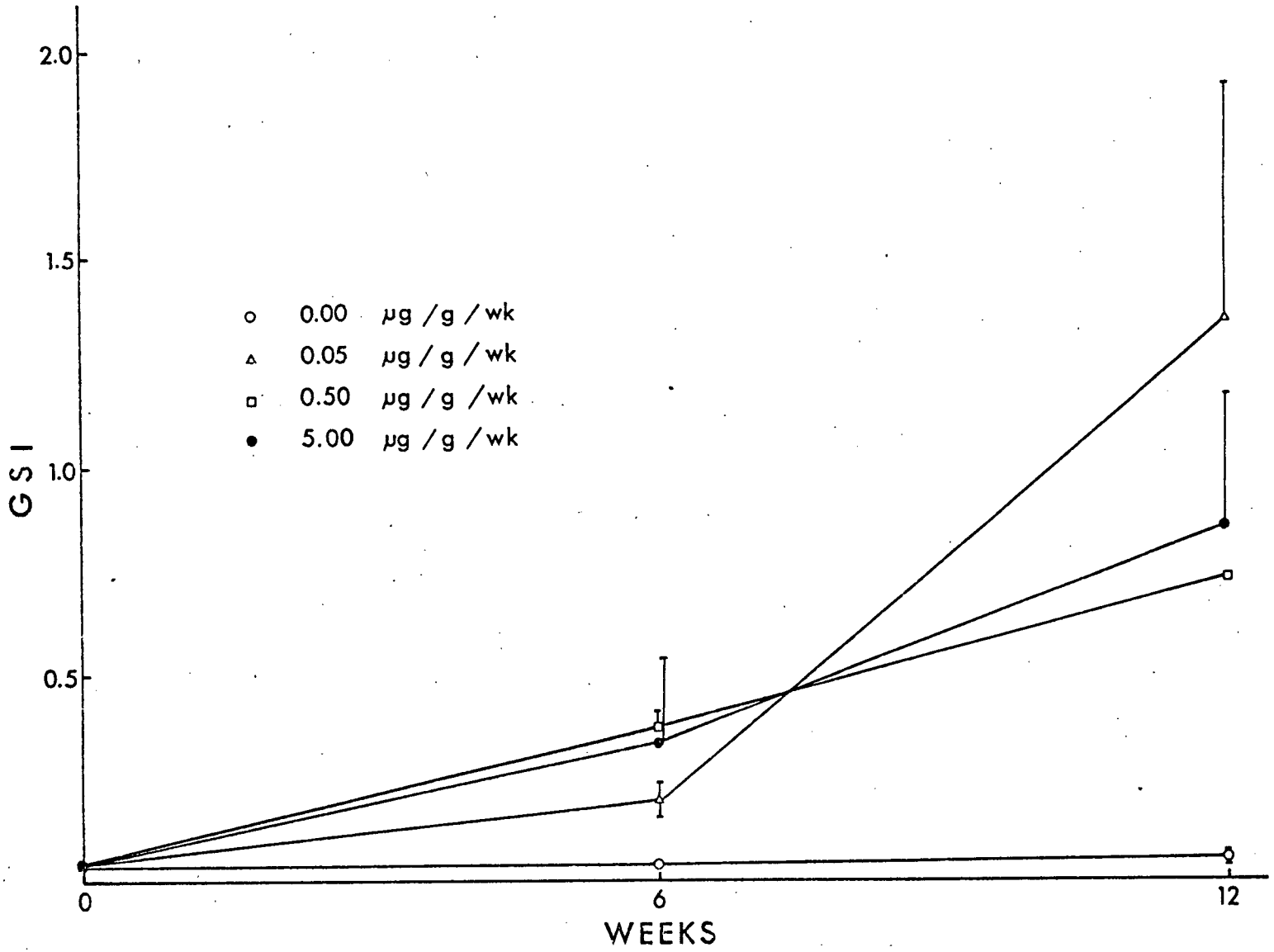

2.0
1.5
1.0
0.5
GSI
0.00 µg / g / wk
0.05 µg / g / wk
0.50 µg / g / wk
5.00 µg / g / wk
0
6
12
WEEKS

Figure 6 - Gonadosomatic Index (GSI) of male pink salmon treated with a constant dosage of salmon gonadotropin per unit time (3 µg/g/wk) applied by pellet implantation and three injection frequencies.

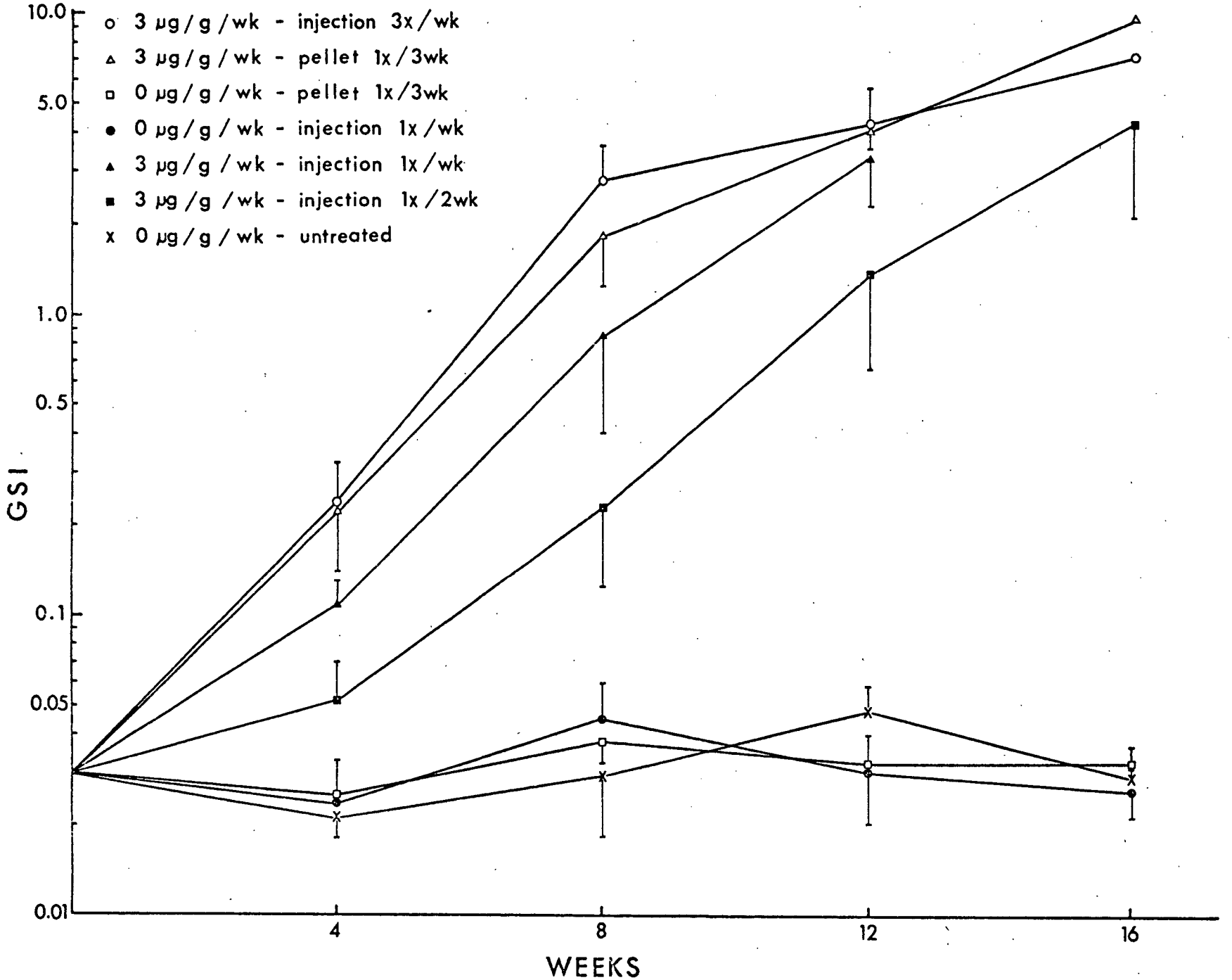

o 3 µg/g/wk - injection 3x/wk
△ 3 µg/g/wk - pellet 1x/3wk
□ 0 µg/g/wk - pellet 1x/3wk
● 0 µg/g/wk - injection 1x/wk
▲ 3 µg/g/wk - injection 1x/wk
■ 3 µg/g/wk - injection 1x/2wk
x 0 µg/g/wk - untreated
10.0
5.0
1.0
0.5
0.1
0.05
0.01
GSI
4
8
12
16
WEEKS

FIGURE 7 - Mean stages of testicular maturity of male pink salmon implanted intraperitoneally every three weeks with various dosages of a salmon gonadotropin:cholesterol pellet. Valves taken from Table 9.

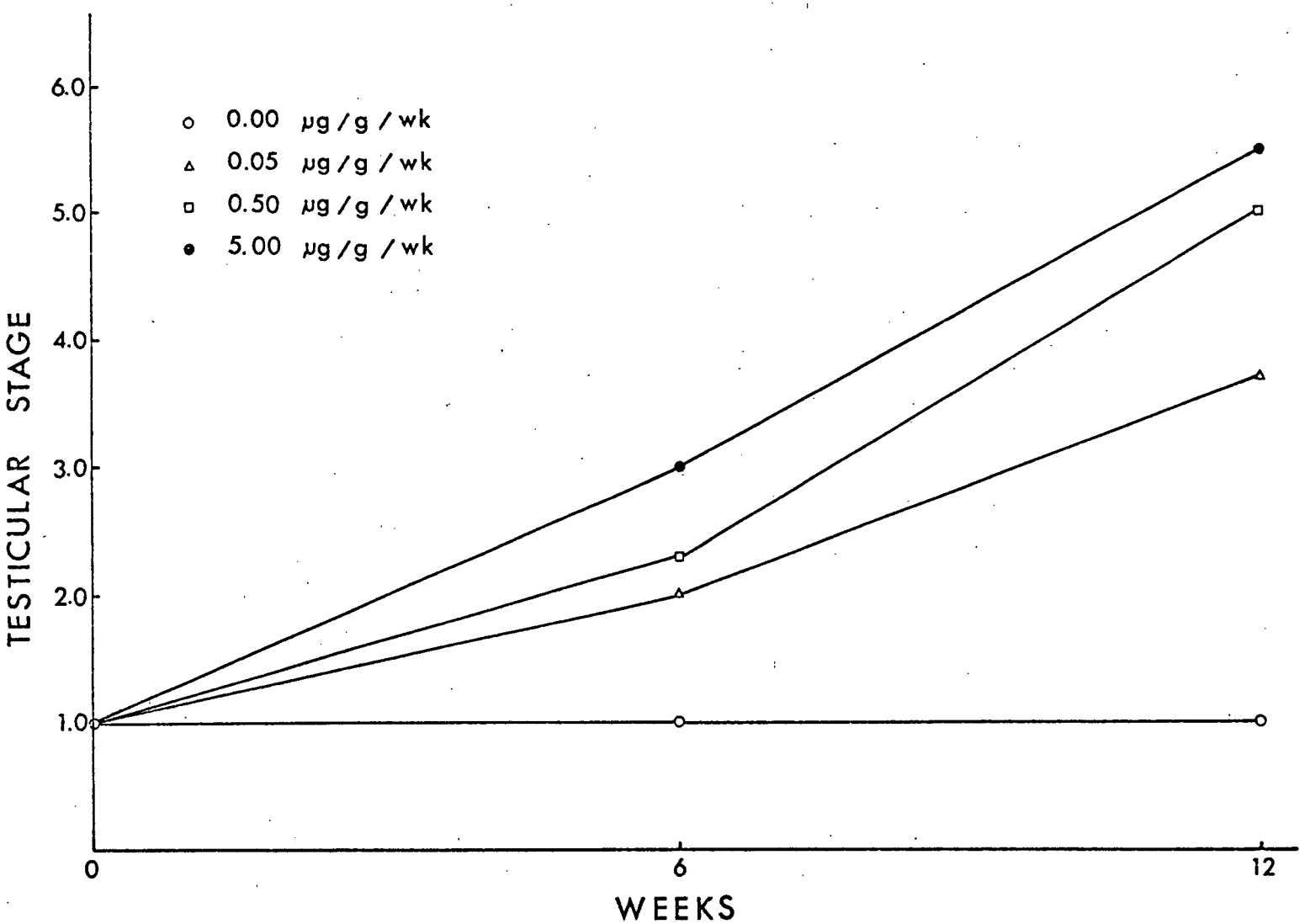

0.00 µg / g / wk
0.05 µg / g / wk
0.50 µg / g / wk
5.00 µg / g / wk
TESTICULAR STAGE
6.0
5.0
4.0
3.0
2.0
1.0
0
6
12
WEEKS

Figure 8 - Mean stages of testicular maturity of male pink salmon treated with a constant dosage of salmon gonadotropin per unit time (3 μg/g/wk) applied by pellet implantation and by injection at three frequencies.

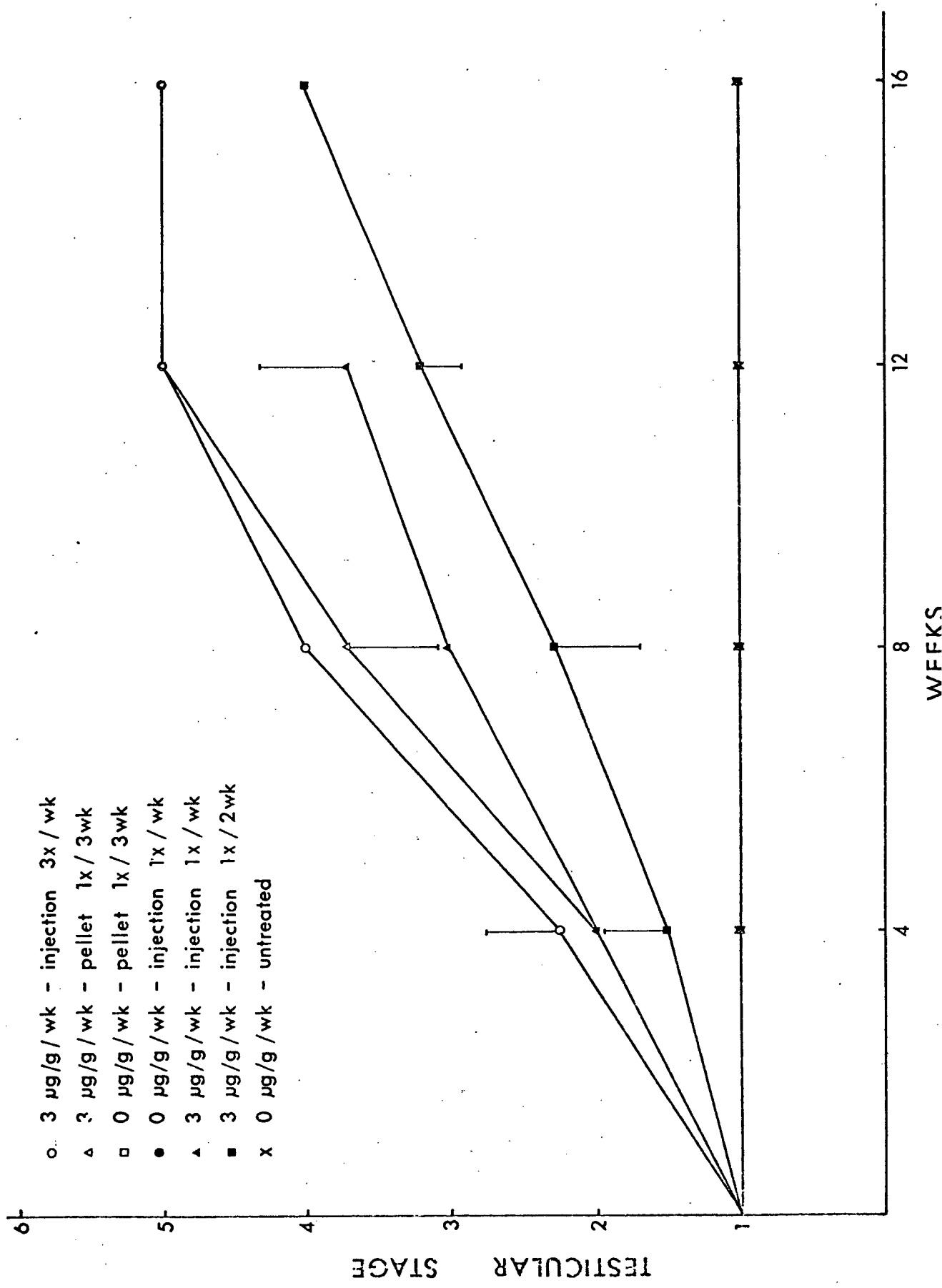

TESTICULAR STAGE
WEEKS
1
2
3
4
5
6
4
8
12
16
○ 3 µg/g/wk - injection 3x/wk
△ 3 µg/g/wk - pellet 1x/3wk
□ 0 µg/g/wk - pellet 1x/3wk
● 0 µg/g/wk - injection 1x/wk
▲ 3 µg/g/wk - injection 1x/wk
■ 3 µg/g/wk - injection 1x/2wk
x 0 µg/g/wk - untreated

Figure 9 - Cross section from an ovary of a juvenile pink salmon injected 1x/wk with 3 µg/g/wk salmon gonadotropin; a) late perinucleolar stage oocyte, b) yolk vesicle stage oocyte, c) atretic oocyte. Haematoxylin and eosin 30x.

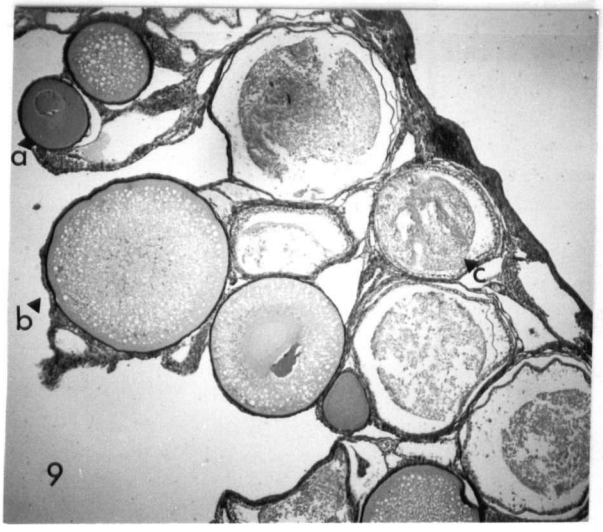
Figure 10 - Oocyte in the yolk vesicle stage. Haematoxylin and eosin 140x.

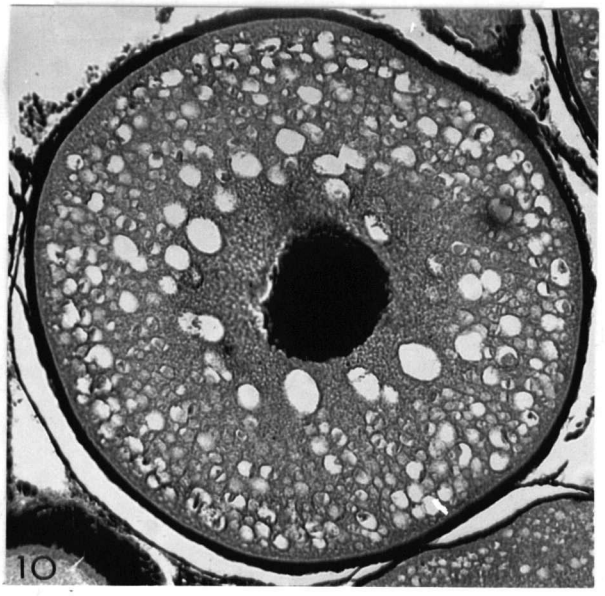
Figure 11 - Follicle and thecal layers from an oocyte in the yolk vesicle stage. Haematoxylin and eosin 550x.

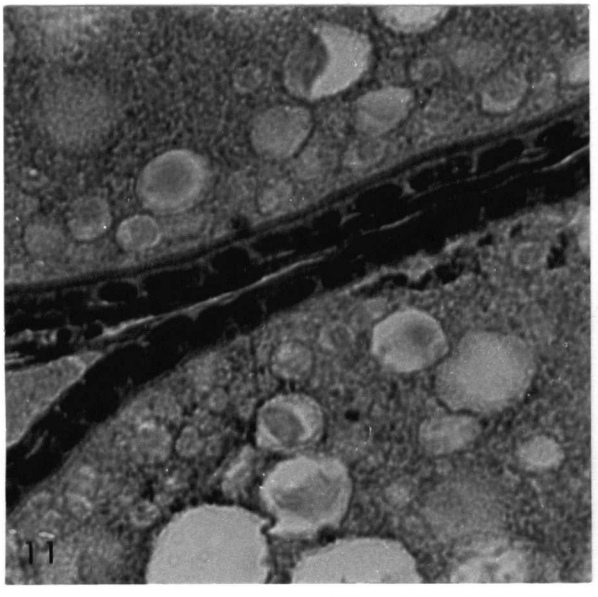
Figure 12 - Primary yolk stage oocyte. Haematoxylin and eosin 30x.

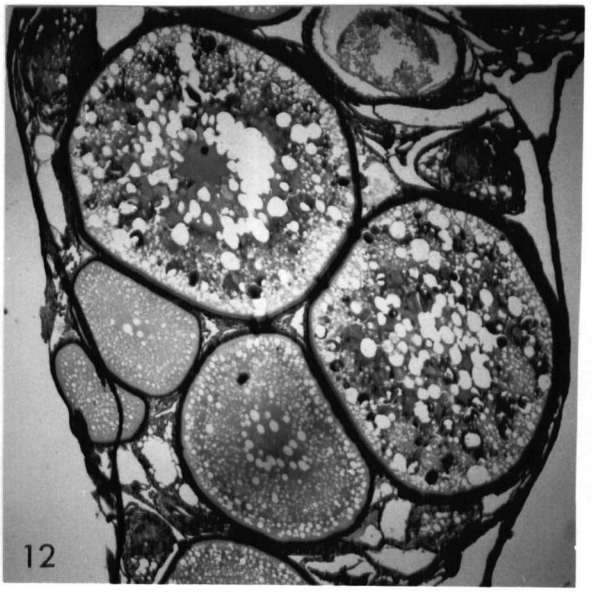
Figure 13 - Follicle and thecal layers from an oocyte in the primary yolk stage. Haematoxylin and eosin 550x.

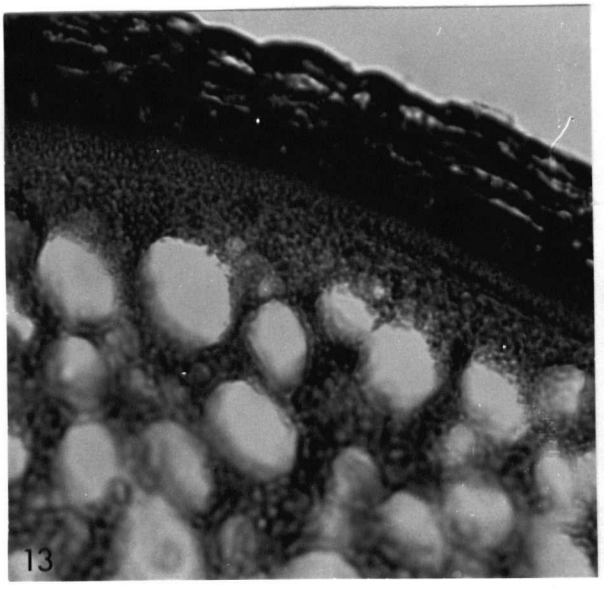
Figure 14 - Atretic oocyte from an ovary of a juvenile pink salmon implanted 1x/3wk with a salmon gonadotropin pellet (3 µg/g/wk). Haematoxylin and eosin 140x.


a
b
c
9


10


11


12


13

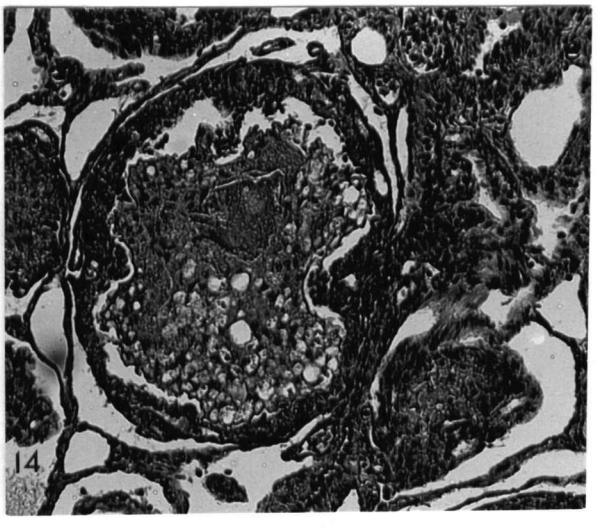

14

FIGURE 15- Gondosomatic Index (G.S.I.) of female pink salmon implanted intrperitoneally every 3 weeks with various dosages of a salmon gonadotropin:cholesterol pellet. Valves are the means and standard deviations taken from Table 13.

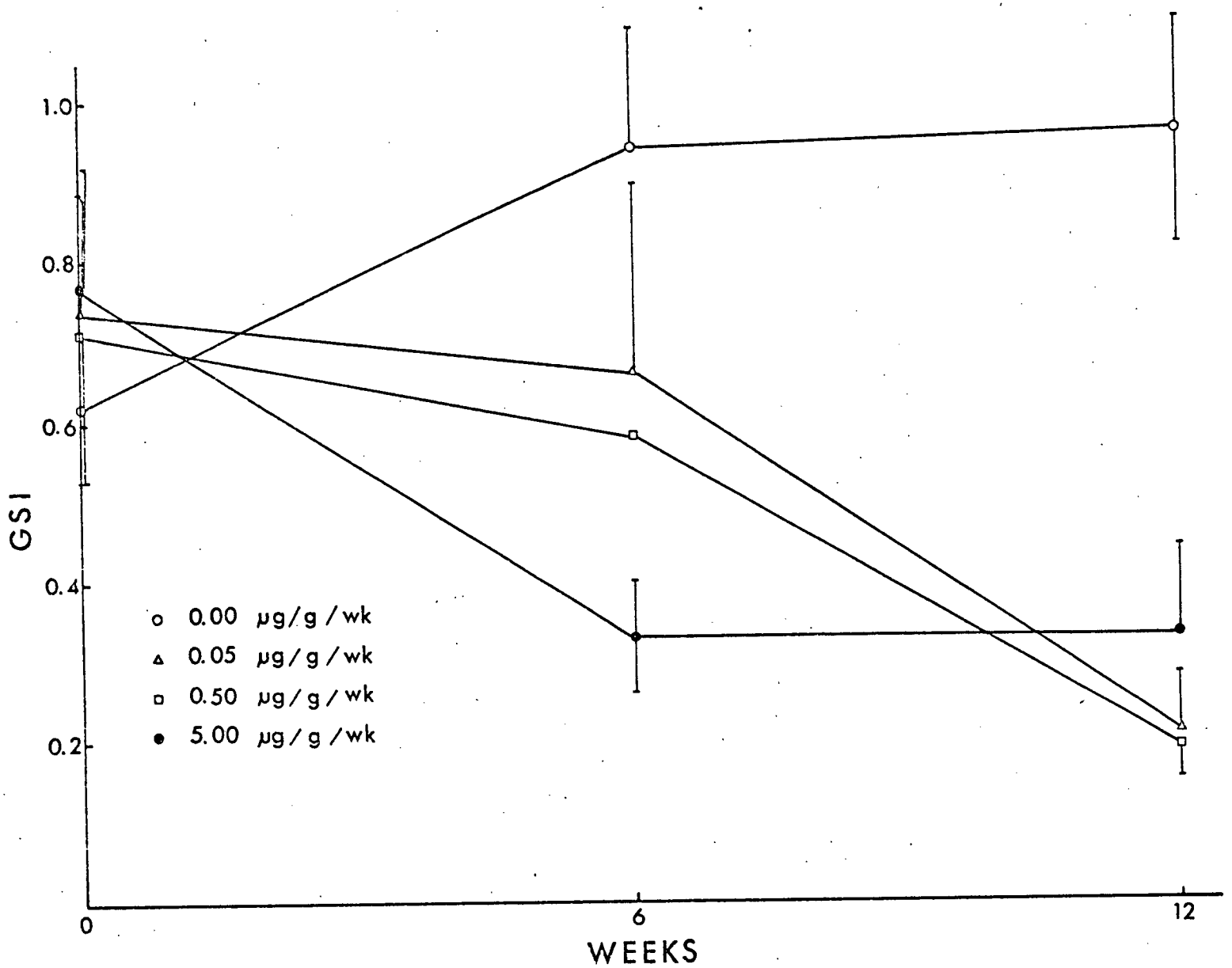
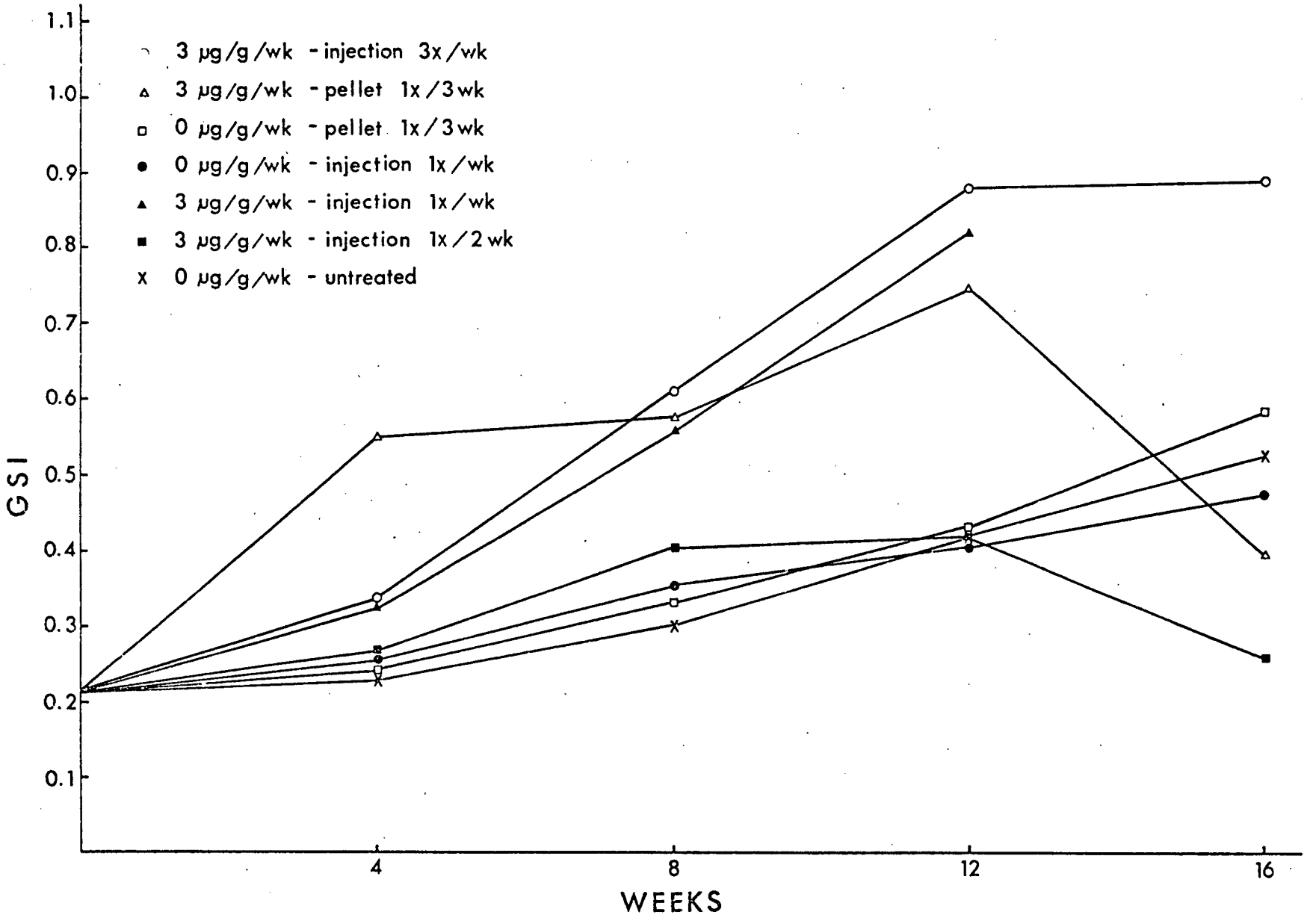

1.0
0.8
0.6
0.4
0.2
GSI
0.00 µg/g/wk
0.05 µg/g/wk
0.50 µg/g/wk
5.00 µg/g/wk
0
6
12
WEEKS

Figure 16 - Gonadosomatic Index (GSI) of female pink salmon treated with a constant dosage of salmon gonadotropin per unit time (3 μg/g/wk) applied by pellet implantation and by injection at three frequencies. Values taken from table 14.


3 µg/g/wk - injection 3x/wk
3 µg/g/wk - pellet 1x/3wk
0 µg/g/wk - pellet 1x/3wk
0 µg/g/wk - injection 1x/wk
3 µg/g/wk - injection 1x/wk
3 µg/g/wk - injection 1x/2wk
0 µg/g/wk - untreated
GSI
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
4
8
12
16
WEEKS

'56 a

FIGURE 17 - Mean oocyte diameter (microns) of female pink salmon implanted intraperitoneally every 3 weeks with various dosages of a salmon gonadotropin:cholesterol pellet. Values are taken from Table 15.

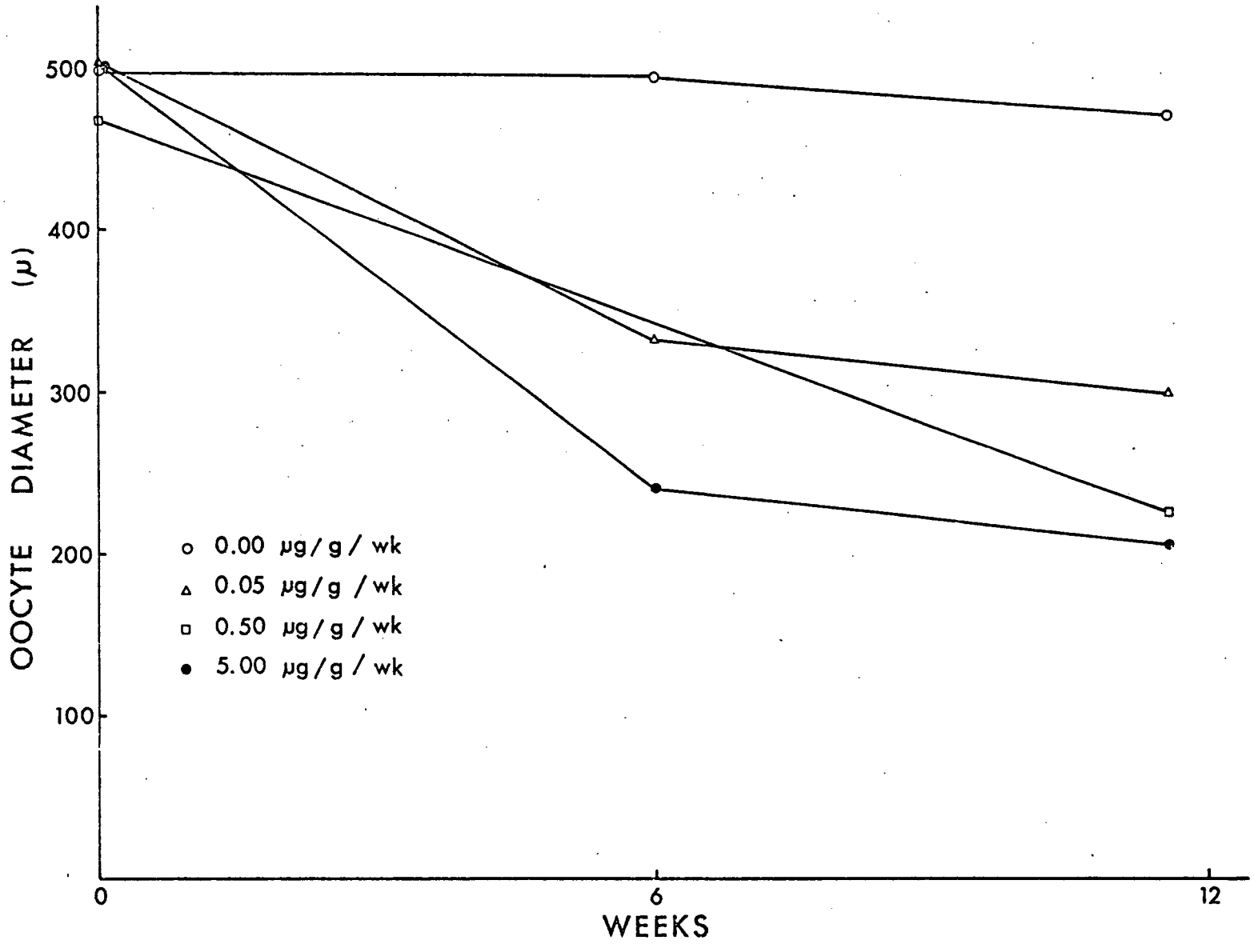

500
400
300
200
100
OOCYTE DIAMETER (μ)
0.00 μg/g/wk
0.05 μg/g/wk
0.50 μg/g/wk
5.00 μg/g/wk
0
6
12
WEEKS

Figure 18 - Mean oocyte diameter (microns) of female pink salmon treated with a constant dosage of salmon gonadotropin per unit time (3 µg/g/wk) applied by pellet implantation and by injection at three frequencies.
Values taken from table 16.

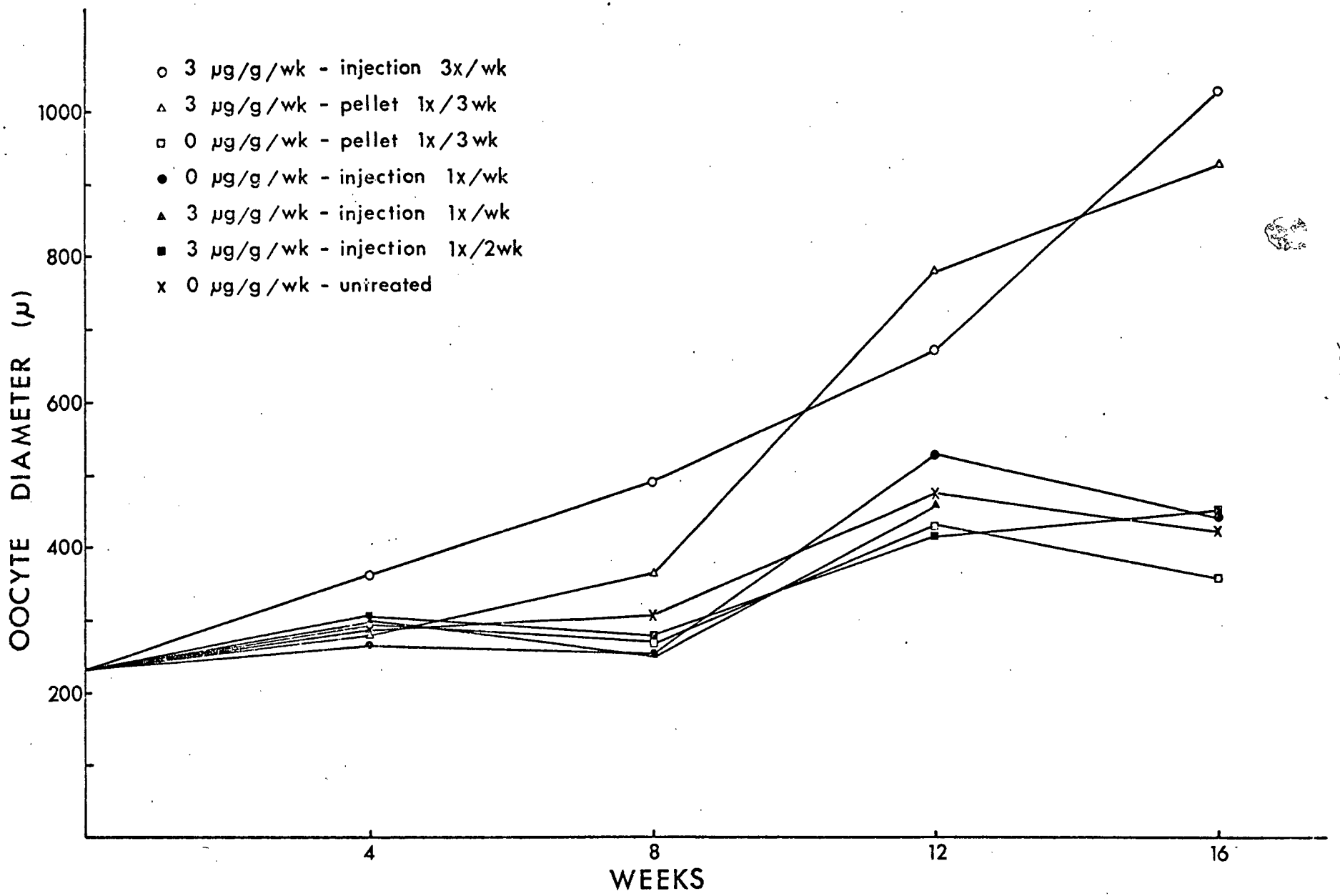

o 3 µg/g/wk - injection 3x/wk
Δ 3 µg/g/wk - pellet 1x/3wk
□ 0 µg/g/wk - pellet 1x/3wk
● 0 µg/g/wk - injection 1x/wk
▲ 3 µg/g/wk - injection 1x/wk
■ 3 µg/g/wk - injection 1x/2wk
x 0 µg/g/wk - unireated
OOCYTE DIAMETER (µ)
1000
800
600
400
200
4
8
12
16
WEEKS

FIGURE 19 - Mean percentages of oocytes which comprise the ovaries of female pink salmon treated with various dosages of pelleted gonadotropin.

1 Early perinucleolar
2 Late perinucleolar
3 Yolk vesicle

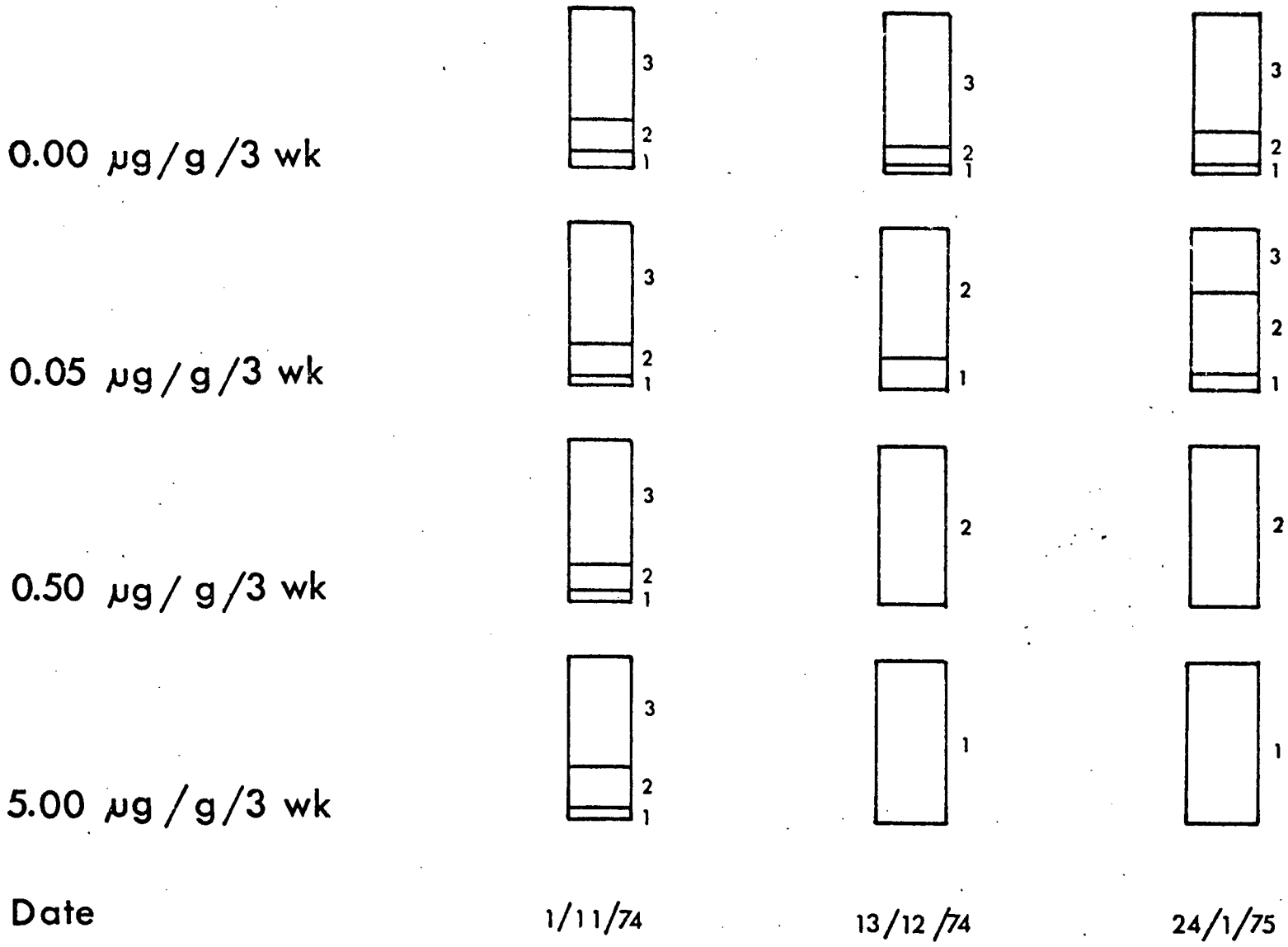

0.00 µg/g/3 wk
0.05 µg/g/3 wk
0.50 µg/g/3 wk
5.00 µg/g/3 wk
Date
1/11/74
13/12/74
24/1/75
3
2
1

FIGURE 20 - Mean percentages of oocytes which comprise the ovaries of female pink salmon treated with a constant dosage of salmon gonadotropin per unit time (3µg/g/wk) applied by pellet implantation and by injection at three frequencies.

1 Early perinucleolar
2 Late perinucleolar
3 Yolk vesicle
4 Primary yolk

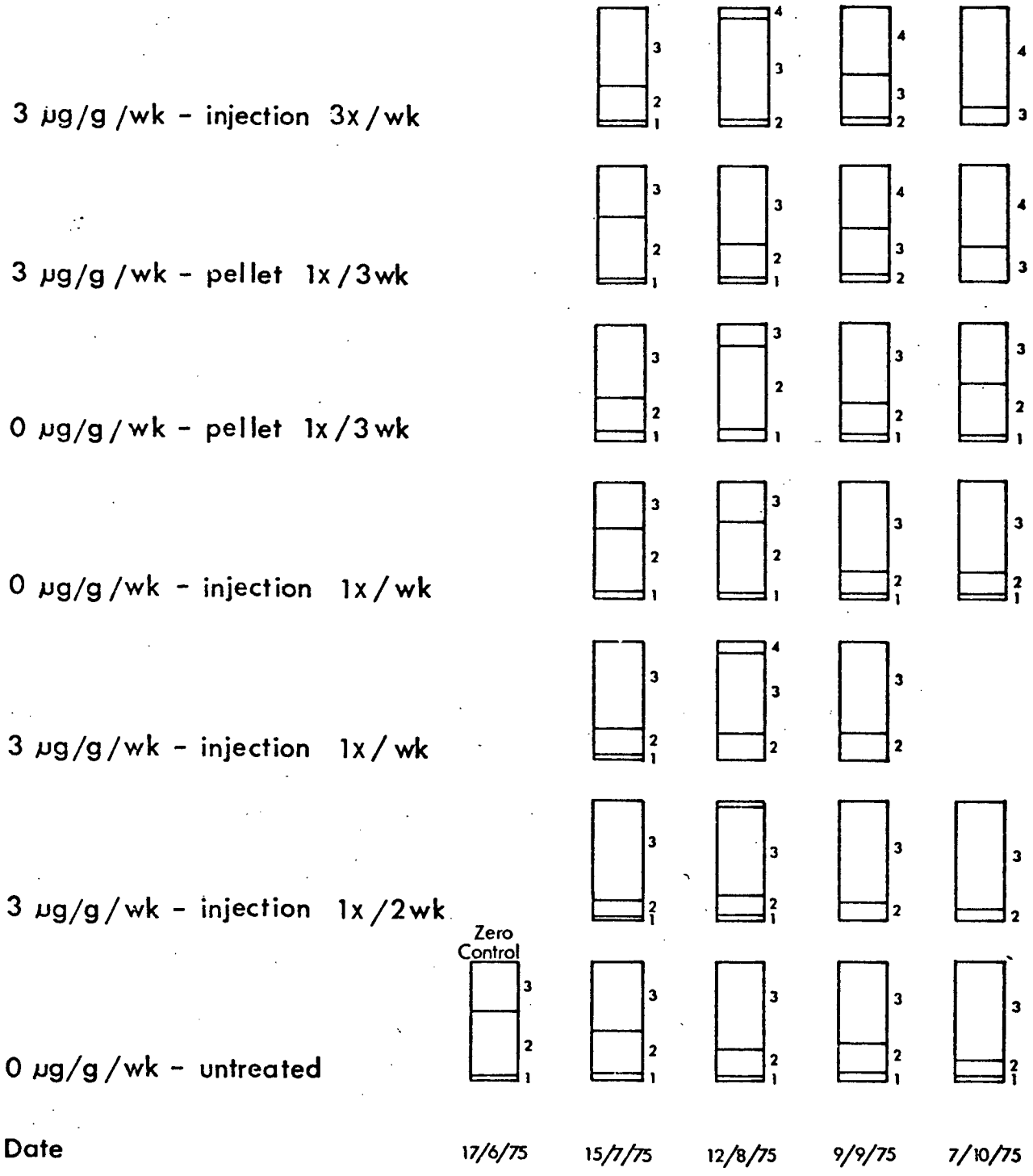

3 µg/g /wk - injection 3x/wk
3 µg/g /wk - pellet 1x/3wk
0 µg/g/wk - pellet 1x/3wk
0 µg/g/wk - injection 1x/wk
3 µg/g/wk - injection 1x/wk
3 µg/g/wk - injection 1x/2wk
Zero Control
0 µg/g/wk - untreated
Date
17/6/75
15/7/75
12/8/75
9/9/75
7/10/75

Figure 21 - Endocrine manipulation used in a proposed test to determine whether or not an infusion of a stream's male on-year genes into transplanted ova will significantly increase the return of the resulting adults to the recipient stream (Bear River).

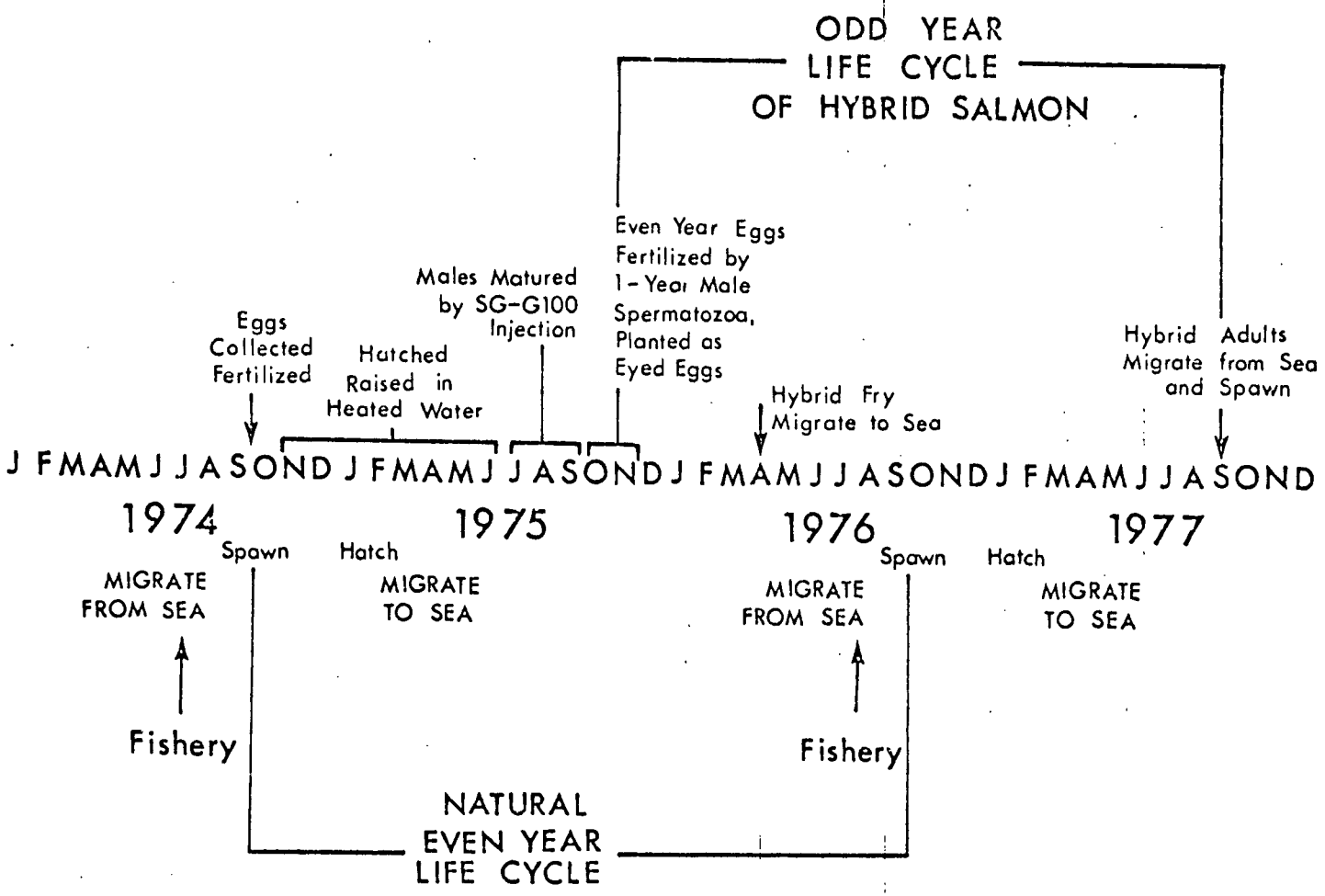

ODD YEAR
LIFE CYCLE
OF HYBRID SALMON
Even Year Eggs
Fertilized by
1-Year Male
Spermatozoa,
Planted as
Eyed Eggs
Males Matured
by SG-G100
Injection
Eggs
Collected
Fertilized
Hatched
Raised in
Heated Water
Hybrid Fry
Migrate to Sea
Hybrid Adults
Migrate from Sea
and Spawn
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1974
1975
1976
1977
Spawn
Hatch
MIGRATE
FROM SEA
MIGRATE
TO SEA
Fishery
NATURAL
EVEN YEAR
LIFE CYCLE

Figure 22 - Pink fry emergence for the three experimental transplant groups in cumulative percentages.

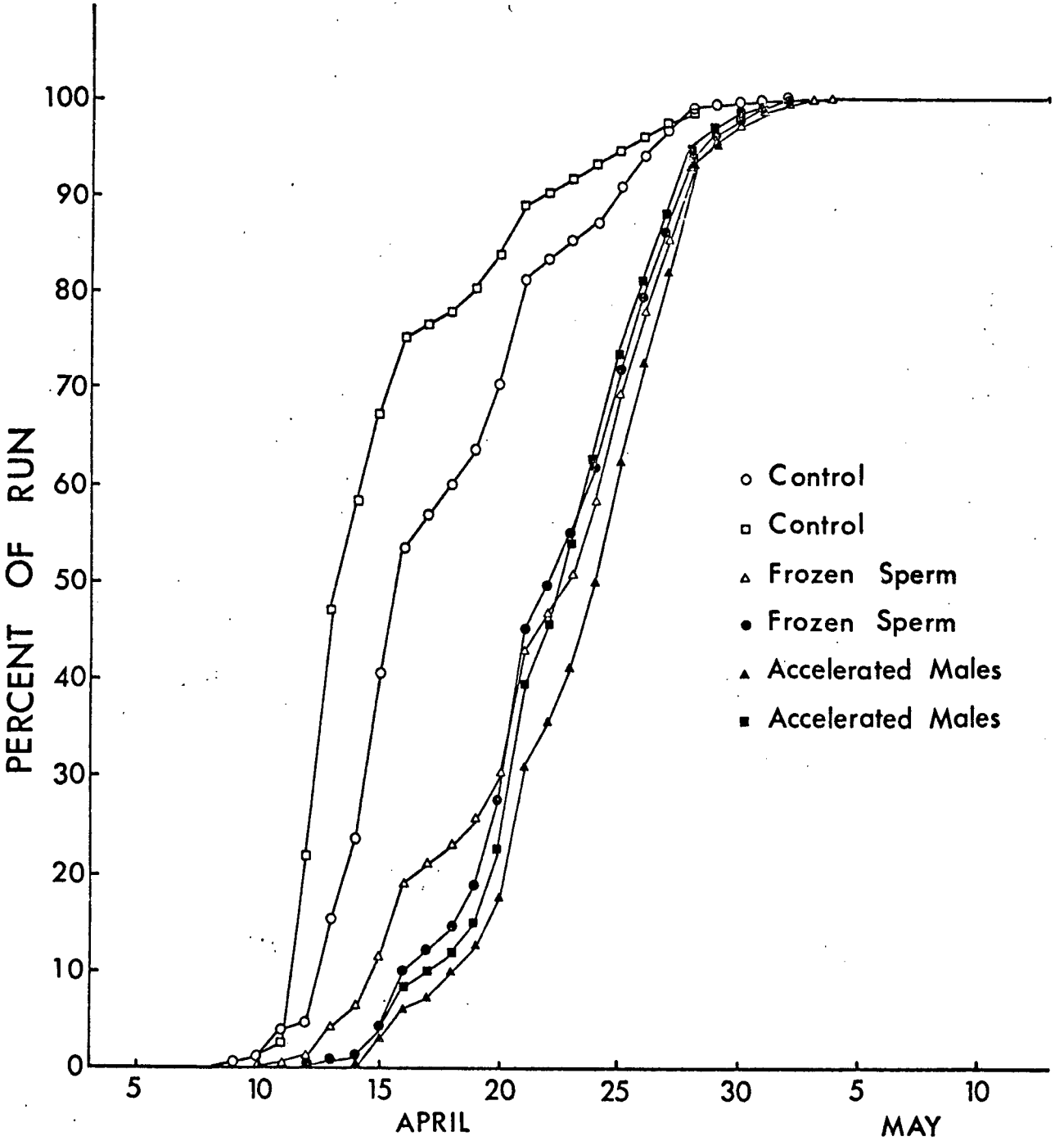

PERCENT OF RUN
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
5
10
APRIL
MAY
Control
Control
Frozen Sperm
Frozen Sperm
Accelerated Males
Accelerated Males

FIGURE 23 - Map of experimental areas showing A, the hatchery site on Bear River; and B, the collection site on the donor stream (Glendale River).

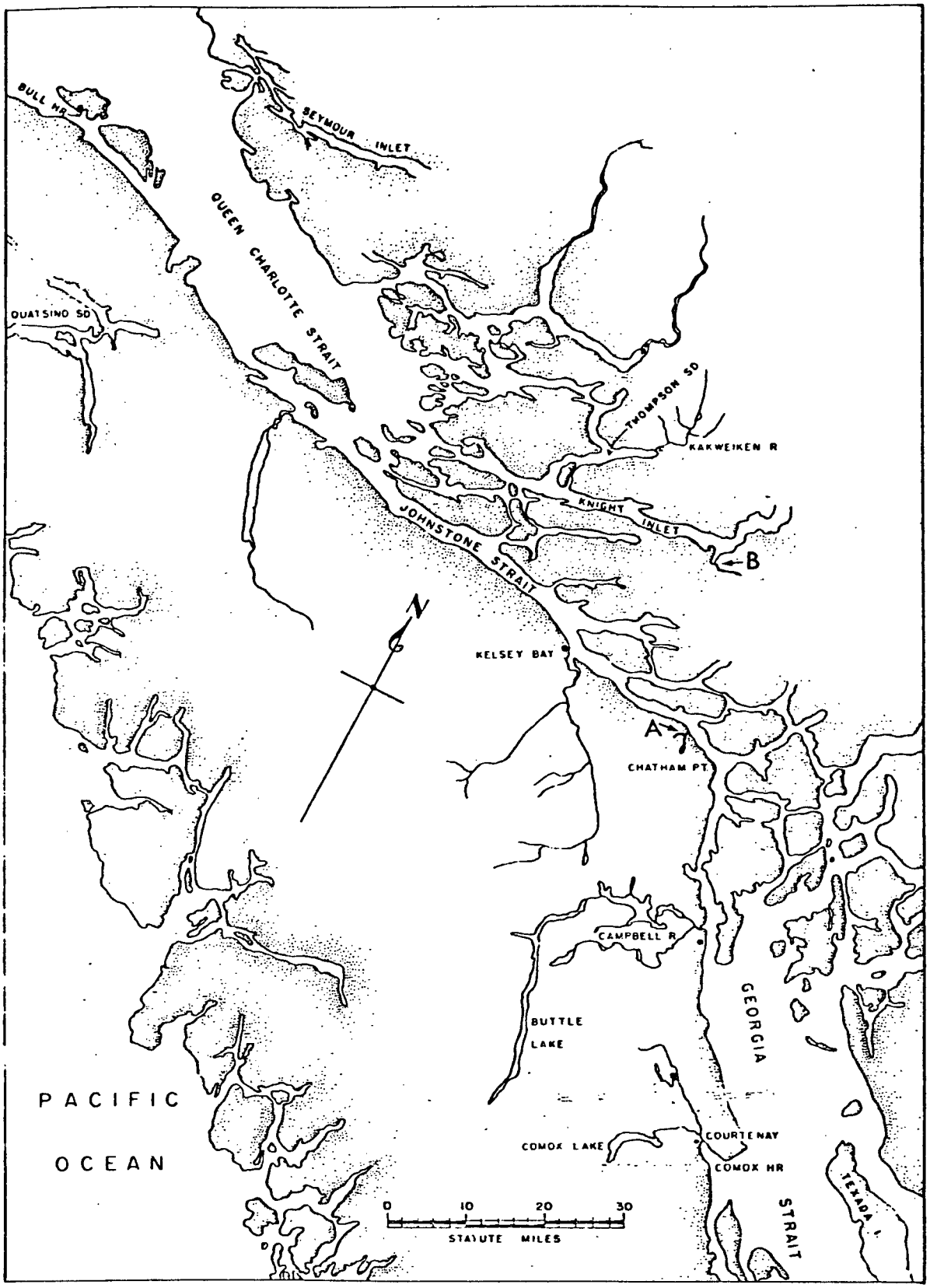

BULL HR
SEYMOUR INLET
QUEEN CHARLOTTE STRAIT
QUATSINO SD
THOMPSON SD
KAKWEIKEN R
KNIGHT INLET
B
JOHNSTONE STRAIT
N
KELSEY BAY
A
CHATHAM PT
CAMPBELL R
BUTTLE
LAKE
GEORGIA
PACIFIC
OCEAN
COMOX LAKE
COURTENAY
COMOX HR
STRAIT
0 10 20 30
STATUTE MILES

TABLE 1 - Male pink salmon treated with various dosages of pelleted salmon gonadotropin - mean body length, mean body weight. μ - mean; S.D. - 1 standard deviation.

Date	Treatment Group	Number of Males	Body Length (cm) μ	Body Length (cm) S.D.	Body Weight (g) μ	Body Weight (g) S.D.
01/11/74	(zero control)					
	0 μg/g/3wk	3	19.0	1.2	94.3	29.4
	0.05 μg/g/3wk	2	18.3	1.0	79.1	21.1
	0.5 μg/g/3wk	3	17.9	0.6	82.8	16.4
	5.0 μg/g/3wk	2	18.5	0.1	87.0	9.8
13/12/74	0 μg/g/3wk	2	19.2	0.4	99.8	3.6
	0.05 μg/g/3wk	3	18.4	0.5	77.3	9.3
	0.5 μg/g/3wk	3	19.4	0.4	95.6	11.4
	5.0 μg/g/3wk	2	20.2	1.9	108.2	31.1
24/01/75	0 μg/g/3wk	2	19.4	0.4	92.8	11.9
	0.05 μg/g/3wk	3	20.0	0.4	99.6	8.3
	0.5 μg/g/3wk	1	16.7	-	54.7	-
	5.0 μg/g/3wk	2	19.3	0.2	81.3	8.6

TABLE 2 - The effect on body length (cm) and body weight (g) of male pink salmon resulting from a constant dosage of salmon gonadotropin per unit time (3 µg/g/wk) applied by pellet implantation and by injection at three frequencies.

Date	Treatment group	Number of males	body length (cm) u	S.D.	body weight (g) u	S.D.
17/6/75	zero control	10	13.0	0.4	29.4	3.4
15/7/75	inj. 3x/wk	7	14.8	0.5	45.8	6.3
	pellet 1x/3wk	7	14.1	0.7	38.8	7.1
	pellet control	7	14.0	0.5	36.1	4.2
	inj. control	3	14.3	0.9	36.8	6.3
	inj. 1x/wk	5	14.5	0.4	41.9	4.4
	inj. 1x/2wk	7	14.5	0.5	43.6	6.4
	untreated	5	14.3	0.7	38.0	7.4
12/8/75	inj. 3x/wk	7	16.8	1.3	75.9	15.9
	pellet 1x/3wk	6	16.1	1.0	71.9	10.1
	pellet control	6	15.5	0.5	53.2	6.1
	inj. control	5	16.1	0.7	63.7	8.2
	inj. 1x/wk	7	16.0	1.4	66.2	21.3
	inj. 1x/2wk	8	16.8	0.9	72.3	11.7
	untreated	5	15.9	0.2	60.0	4.4
9/9/75	inj. 3x/wk	6	16.5	1.1	71.5	11.9
	pellet 1x/3wk	5	15.7	0.5	60.4	6.9
	pellet control	3	16.6	0.6	61.9	6.3
	inj. control	5	16.9	1.2	67.8	11.4
	inj. 1x/wk	6	17.3	0.8	83.2	17.6
	inj. 1x/2wk	7	18.3	0.9	93.0	12.4
	untreated	5	15.9	0.5	55.0	7.6
7/10/75	inj. 3x/wk	2	16.6	1.4	65.0	26.6
	pellet 1x/3wk	6	16.3	1.1	68.7	14.7
	pellet control	5	18.1	1.1	83.5	21.3
	inj. control	3	17.9	1.2	80.5	17.5
	inj. 1x/wk	0	0	0	0	0
	inj. 1x/2wk	6	19.2	1.1	114.9	29.8
	untreated	6	18.9	1.0	92.9	16.0

TABLE 3 - Male pink salmon treated with various dosages of pelleted salmon gonadotropin - mean fixed testis weight, mean gonadosomatic index (G.S.I.) u = mean; S.D. = 1 standard deviation.

Date	Treatment Group	Number of Males	Fixed Testis Weight (g) u	S.D.	G.S.I. u	S.D.
01/11/74	(Zero Control)					
	0 ug/g/3wk	3	0.02	-	0.04	0.02
	0.05 ug/g/3wk	2	0.02	-	0.05	0.01
	0.5 ug/g/3wk	3	0.02	-	0.05	0.01
	5.0 ug/g/3wk	2	0.02	-	0.05	0.02
13/12/74	0 ug/g/3wk	2	0.02	-	0.04	0.01
	0.05 ug/g/3wk	3	0.08	0.01	0.19	0.04
	0.5 ug/g/3wk	3	0.18	0.04	0.37	0.04
	5.0 ug/g/3wk	2	0.16	0.06	0.34	0.21
24/01/75	o ug/g/3wk	2	0.02	0.01	0.05	0.02
	0.05 ug/g/3wk	3	0.68	0.34	1.35	0.57
	0.5 ug/g/3wk	1	0.10	-	0.73	-
	5.0 ug/g/3wk	2	0.34	0.11	0.86	0.36

TABLE 4 - The effect on testis weight (g) and gonadosomatic index (G.S.I.) of male pink salmon resulting from a constant dosage of salmon gonadotropin per unit time (3 μg/g/wk) applied by pellet implantation and by injection at three frequencies.

Date	Treatment group	Number of males	Testis weight (g) u	Testis weight (g) S.D.	G.S.I. u	G.S.I. S.D.
17/6/75	zero control	10	0.004	0.001	0.029	0.005
15/7/75	inj. 3x/wk	7	0.054	0.020	0.237	0.083
	pellet 1x/3wk	7	0.043	0.014	0.223	0.080
	pellet control	7	0.004	0.001	0.025	0.007
	inj. control	3	0.004	0.001	0.024	0.002
	inj. 1x/wk	5	0.022	0.005	0.107	0.022
	inj. 1x/2wk	7	0.011	0.005	0.051	0.019
	untreated	5	0.004	0.001	0.021	0.003
12/8/75	inj. 3x/wk	7	1.080	0.436	2.759	0.787
	pellet 1x/3wk	6	0.633	0.172	1.800	0.581
	pellet control	6	0.010	0.005	0.038	0.010
	inj. control	5	0.015	0.007	0.045	0.014
	inj. 1x/wk	7	0.290	0.191	0.846	0.452
	inj. 1x/2wk	8	0.083	0.035	0.228	0.092
	untreated	5	0.009	0.004	0.029	0.011
9/9/75	inj. 3x/wk	6	1.459	0.289	4.222	1.363
	pellet 1x/3wk	5	1.278	0.549	4.152	1.712
	pellet control	3	0.009	0.002	0.031	0.009
	inj. control	5	0.010	0.003	0.031	0.010
	inj. 1x/wk	6	1.341	0.444	3.322	1.070
	inj. 1x/2wk	7	0.644	0.313	1.375	0.653
	untreated	5	0.013	0.003	0.048	0.010
7/10/75	inj. 3x/wk	2	2.770	3.299	7.036	7.263
	pellet 1x/3wk	6	3.673	4.415	9.721	9.586
	pellet control	5	0.013	0.004	0.032	0.005
	inj. control	3	0.010	0.003	0.026	0.005
	inj. 1x/wk	0	0	0	0	0
	inj. 1x/2wk	6	2.606	1.746	4.230	2.060
	untreated	6	0.013	0.003	0.031	0.011

TABLE 5 - Gonadosomatic index (GSI) of male pink salmon treated with various dosages of pelleted gonadotropin, cell n = 1 (Experiment 1). Data subjected to Analysis of Variance.

Group	WEEKS 0	6	12	Total
Pellet control	0.043	0.040	0.055	0.138
Pellet 0.05 µg/gm SG-G100	0.050	0.197	1.353	1.599
Pellet 0.5 µg/gm SG-G100	0.050	0.370	0.730	1.149
Pellet 5.0 µg/gm SG-G100	0.055	0.335	0.865	1.254
Total	0.200	0.940	3.004	

TABLE 6 - Gonadosomatic index (GSI) of male pink salmon treated with a constant dosage of salmon gonadotropin per unit time (3 µg/g/wk) applied by pellet implantation and by three injection frequencies, cell means (n = 3) (Experiment 2). Data subjected to Analysis of Variance.

Group	WEEKS 4	8	12	16	Total
Inj. 3x/wk	0.293	2.173	3.891	7.618	41.93
Pellet SG-G100	0.292	2.043	1.902	4.069	24.91
Pellet control	0.019	0.049	0.031	0.031	0.38
Inj. control	0.024	0.048	0.029	0.026	0.18
Inj. 1x/wk	0.092	0.667	4.232	2.240	20.93
Inj. 1x/2wk	0.060	0.154	1.225	3.482	20.12
Untreated	0.021	0.032	0.057	0.032	0.19
Total	2.39	15.50	34.10	52.50	

TABLE 7 - Analysis of Variance table of male GSI data (Tables for Experiment 1 and Experiment 2.

Experiment 1

Source	S.S.	dF	M.S.	F	P
Group	8.3637E-01	3	2.7879E-01	5.39	0.00
Time	2.2361	2	1.1180	21.61	0.00
AB	1.1297	6	1.8829E-01	3.64	0.02
Error	8.2760E-01	16	5.1725E-02		
Total	5.0298	27			

Experiment 2

Source	S.S.	dF	M.S.	F	P
Group	1.2565E+02	6	2.0942E+01	13.59	0.00
Time	6.8316E+01	3	2.2772E+01	14.78	0.00
AB	9.4434E+01	18	5.2463	3.40	0.00
Error	8.6286E+01	56	1.5408		
Total	3.7469E+02	83			

TABLE 8 - Student-Newman-Keuls multiple range test of male GSI data (Tables 5 and 6) for Experiment 1 and Experiment 2.

Experiment 1

Homogeneous subsets	
Group	SG-G100 - 0.05, 0.5, 5.0 ug/g/wk
Time	(0 weeks, 6 weeks)

Experiment 2

Homogeneous subsets	
Group	(pellet control, inj. control, untreated), (SG-G100-1x/wk, 1x/2wk, pellet)
Time	(4 weeks, 8 weeks)

TABLE 9 - The testicular stage of male pink salmon treated with various dosages of pelleted salmon gondotropin. μ = mean; S.D. = Standard deviation.

Date	Treatment Group	Number of Males	Testicular Stage μ	S.D.
01/11/74	(zero control)			
	0 μg/g/3wk	3	1.0	0
	0.05 μg/g/3wk	2	1.0	0
	0.5 μg/g/3wk	3	1.0	0
	5.0 μg/g/3wk	2	1.0	0
13/12/74	0 μg/g/3wk	2	1.0	0
	0.05 μg/g/3wk	3	2.0	0
	0.5 μg/g/3wk	3	2.3	0.6
	5.0 μg/g/3wk	2	3.0	0
24/1/75	0 μg/g/3wk	2	1.0	0
	0.05 μg/g/3wk	3	3.7	0.6
	0.5 μg/g/3wk	1	5.0	-
	5.0 μg/g/3wk	2	5.5	0.7

TABLE 10- The effect on testicular stage of male pink salmon resulting from a constant dosage of salmon gonadotropin per unit time (3 μg/g/wk) applied by pellet implantation and by injection at three frequencies.

Date	Treatment group	Number of males	Testicular stage μ	S.D.
17/6/75	zero control	10	1.0	0
15/7/75	inj. 3x/wk	7	2.3	0.5
	pellet 1x/3wk	7	2.0	0
	pellet control	7	1.0	0
	inj. control	3	1.0	0
	inj. 1x/wk	5	2.0	0
	inj. 1x/2wk	7	1.5	0.6
	untreated	5	1.0	0
12/8/75	inj. 3x/wk	7	4.0	0
	pellet 1x/3wk	6	3.7	0.6
	pellet control	6	1.0	0
	inj. control	5	1.0	0
	inj. 1x/wk	7	3.0	0
	inj. 1x/2wk	8	2.3	0.6
	untreated	5	1.0	0
9/9/75	inj. 3x/wk	6	5.0	0
	pellet 1x/3wk	5	5.0	0
	pellet control	3	1.0	0
	inj. control	5	1.0	0
	inj. 1x/wk	6	3.7	0.6
	inj. 1x/2wk	7	3.2	0.3
	untreated	5	1.0	0
7/10/75	inj. 3x/wk	2	5.0	0
	pellet 1x/wk	6	5.0	0
	pellet control	5	1.0	0
	inj. control	3	1.0	0
	inj. 1x/wk	0	0	0
	inj. 1x/2wk	6	4.0	0
	untreated	6	1.0	0

TABLE 11 - Female pink salmon treated with various dosages of pelleted salmon gondotropin - mean body length, mean body weight. μ = mean; S.D. = 1 standard deviation.

Date	Treatment Group	Number of females	Body Length (cm) μ	Body Length (cm) S.D.	Body Weight (g) μ	Body Weight (g) S.D.
01/11/74	zero control					
	0 μg/g/3wk	2	19.0	1.6	99.8	28.9
	0.05 μg/g/3wk	3	18.8	2.3	96.2	27.0
	0.5 μg/g/3wk	3	19.0	1.1	100.1	14.0
	5.0 μg/g/3wk	3	17.9	0.1	80.2	13.3
13/12/74	0 μg/g/3wk	5	19.7	0.5	109.0	20.8
	0.05 μg/g/3wk	2	19.8	0.5	97.3	0.4
	0.5 μg/g/3wk	1	19.5	-	102.5	-
	5.0 μg/g/3wk	3	21.2	1.6	115.5	25.1
24/01/75	0 μg/g/3wk	2	19.5	0.6	93.2	16.5
	0.05 μg/g/3wk	4	19.4	1.0	88.7	14.6
	0.5 μg/g/3wk	5	20.2	1.0	109.3	14.2
	5.0 μg/g/3wk	2	18.8	0.9	81.6	14.8

TABLE 12 - The effect on body length (cm) and body weight (g) of female pink salmon resulting from a constant dosage of salmon gonadotropin per unit time (3 μg/g/wk) applied by pellet implantation and by three injection frequencies.

Date	Treatment group	Number of females	body length (cm) μ	S.D.	body weight (g) μ	S.D.
17/6/75	zero control	11	13.1	0.5	30.5	4.1
15/7/75	inj. 3x/wk	5	14.7	0.8	43.7	8.4
	pellet 1x/3wk	5	14.3	0.5	40.2	4.9
	pellet control	5	13.9	0.5	36.1	4.2
	inj. control	9	14.3	1.0	39.6	7.3
	inj. 1x/wk	7	14.1	0.4	40.2	3.3
	inj. 1x/2wk	5	14.5	0.5	41.2	4.6
	untreated	9	14.0	0.9	36.5	7.2
12/8/75	inj. 3x/wk	5	17.3	0.9	73.2	12.3
	pellet 1x/3wk	6	15.4	0.8	54.2	10.7
	pellet control	6	15.4	0.6	49.4	7.0
	inj. control	8	15.3	1.0	52.8	10.2
	inj. 1x/wk	5	16.2	1.1	60.3	12.4
	inj. 1x/2wk	4	16.6	1.3	65.8	16.2
	untreated	7	15.7	0.7	57.3	8.5
9/9/75	inj. 3x/wk	5	18.7	0.9	85.9	14.1
	pellet 1x/3wk	3	18.4	0.7	92.7	9.3
	pellet control	5	17.8	0.8	78.1	14.9
	inj. control	7	16.4	1.1	61.3	12.2
	inj. 1x/wk	4	17.1	0.8	70.2	11.6
	inj. 1x/2wk	4	18.5	2.1	90.4	30.2
	untreated	6	17.0	0.6	67.2	8.1
7/10/75	inj. 3x/wk	4	21.2	1.6	137.4	28.2
	pellet 1x/3wk	3	20.6	1.4	125.2	26.5
	pellet control	4	18.3	0.6	82.9	9.3
	inj. control	5	18.4	0.8	87.7	9.8
	inj. 1x/wk	0	0	0	0	0
	inj. 1x/2wk	4	18.1	0.9	84.0	7.2
	untreated	5	17.9	1.5	84.3	22.4

TABLE 13 - Female pink salmon treated with various dosages of pelleted salmon gonadotropin - mean Fixed ovary weight, mean gonadosomatic index (G.S.I.). μ = mean; S.D. = 1 standard deviation.

Date	Treatment Group	Number of Females	Fixed Ovary Weight (g) μ	S.D.	G.S.I. μ	S.D.
01/11/74	zero control					
	0 μg/g/3wk	2	0.30	0.04	0.62	0.09
	0.05 μg/g/3wk	3	0.36	0.15	0.74	0.14
	0.5 μg/g/3wk	3	0.35	0.06	0.71	0.18
	5.0 μg/g/3wk	3	0.32	0.11	0.77	0.15
13/12/74	0 μg/g/3wk	5	0.50	0.06	0.94	0.15
	0.05 μg/g/3wk	2	0.32	0.11	0.66	0.23
	0.5 μg/g/3wk	1	0.30	-	0.58	-
	5.0 μg/g/3wk	3	0.19	0.07	0.33	0.07
24/01/75	0 μg/g/3wk	2	0.44	0.01	0.96	0.14
	0.05 μg/g/3wk	4	0.09	0.03	0.21	0.07
	0.5 μg/g/3wk	5	0.11	0.02	0.19	0.04
	5.0 μg/g/3wk	2	0.13	0.06	0.33	0.11

TABLE 14- The effect on ovary weight and gonadosomatic index (G.S.I.) of female pink salmon resulting from a constant dosage of salmon gonadotropin per unit time (3 μg/g/wk) applied by pellet implantation and by three injection frequencies.

Date	Treatment Group	Number of Females	ovary weight (g) u	S.D.	G.S.I. u	S.D.
17/6/75	zero control	11	0.033	0.008	0.216	0.050
15/7/75	inj. 3x/wk	5	0.076	0.028	0.340	0.080
	pellet 1x/3wk	5	0.110	0.020	0.549	0.094
	pellet control	5	0.045	0.008	0.243	0.013
	inj. control	9	0.050	0.014	0.253	0.059
	inj. 1x/wk	7	0.067	0.014	0.337	0.097
	inj. 1x/2wk	5	0.056	0.020	0.269	0.084
	untreated	9	0.044	0.010	0.243	0.047
12/8/75	inj. 3x/wk	5	0.221	0.040	0.612	0.113
	pellet 1x/3wk	6	0.158	0.040	0.577	0.061
	pellet control	6	0.062	0.037	0.331	0.129
	inj. control	8	0.094	0.033	0.354	0.074
	inj. 1x/wk	5	0.161	0.032	0.562	0.198
	inj. 1x/2wk	4	0.122	0.059	0.414	0.279
	untreated	7	0.084	0.013	0.380	0.022
9/9/75	inj. 3x/wk	5	0.395	0.240	0.888	0.436
	pellet 1x/3wk	3	0.332	0.251	0.744	0.585
	pellet control	5	0.172	0.050	0.434	0.068
	inj. control	7	0.123	0.032	0.406	0.102
	inj. 1x/wk	4	0.296	0.121	0.823	0.222
	inj. 1x/2wk	4	0.206	0.134	0.421	0.192
	untreated	6	0.141	0.043	0.423	0.154
7/10/75	inj. 3x/wk	4	0.636	0.408	0.889	0.462
	pellet 1x/3wk	3	0.257	0.228	0.397	0.335
	pellet control	4	0.239	0.032	0.587	0.133
	inj. control	5	0.210	0.051	0.475	0.081
	inj. 1x/wk	0	0	0	0	0
	inj. 1x/2wk	4	0.109	0.060	0.260	0.139
	untreated	5	0.215	0.035	0.527	0.093

TABLE 15 - Mean oocyte diameter and standard deviation of the means for pink salmon treated with various dosages of pelleted gonadotropin.

* total atresia in the Female

\+ total atresia in three of the Females

Date	Treatment Group	Number of Females	Number of Oocytes Measured	Mean Diameter in Microns	+ S.D. of - The Means
01/11/74	(zero control)				
	0 µg/g/3wk	2	60	498.7	31.6
	0.05 µg/g/3wk	3	90	502.7	61.7
	0.5 µg/g/3wk	3	91	467.5	14.5
	5.0 µg/g/3wk	3	90	502.1	26.4
13/12/74	0 µg/g/3wk	5	96	492.8	86.7
	0.05 µg/g/3wk	2	14	338.7	49.0
	*0.5 µg/g/3wk	1	0	0	0
	5.0 µg/g/3wk	3	3	240.7	-
24/01/75	0 µg/g/3wk	2	18	468.6	7.0
	0.05 µg/g/3wk	4	39	299.3	113.1
	+0.5 µg/g/3wk	5	12	224.7	27.6
	5.0 µg/g/3wk	2	9	206.2	9.4

Table 16- Mean oocyte diameter and standard deviation of the means for female pink salmon treated with a constant dosage of salmon gonadotropin per unit time (3 µg/g/wk) applied by pellet implantation and by three injection frequencies.

Date	Treatment Group	Number of Females	Number of Oocytes Measured	Mean Diameter in Microns	+ S.D. of - The Means
17/06/75	zero control	5	170	230.6	47.6
15/07/75	inj. 3x/wk	3	82	363.2	73.0
	pellet 1x/3wk	4	98	281.9	53.1
	pellet control	2	42	289.7	27.8
	inj. control	4	92	268.4	55.8
	inj. 1x/wk	2	48	294.7	36.1
	inj. 1x/2wk	2	48	301.8	23.7
	untreated	3	45	288.8	78.9
12/08/75	inj. 3x/wk	3	60	491.7	58.3
	pellet 1x/3wk	2	43	365.8	55.9
	pellet control	2	46	273.4	88.1
	inj. control	2	20	258.4	43.2
	inj. 1x/wk	2	25	256.5	71.8
	inj. 1x/2wk	2	20	275.2	33.7
	untreated	3	62	302.0	46.2
09/09/75	inj. 3x/wk	3	27	671.3	100.3
	pellet 1x/3wk	2	22	779.0	74.8
	pellet control	2	38	430.7	36.9
	inj. control	2	45	532.0	51.3
	inj. 1x/wk	3	75	460.7	80.7
	inj. 1x/2wk	2	60	420.1	24.8
	untreated	2	60	470.9	30.6
07/10/75	inj. 3x/wk	3	40	1028.2	239.7
	pellet 1x/3wk	2	42	933.8	281.7
	pellet control	2	49	357.2	85.9
	inj. control	2	55	440.9	39.2
	inj. 1x/wk	-		-	
	inj. 1x/2wk	3	48	443.3	107.3
	untreated	2	51	423.4	70.6

TABLE 17 - Percent oocyte atresia and mean percent of oocytes in each stage from ovaries of female pink salmon treated with various dosages of pelleted gonadotropin.

1 Early perinucleolar
2 Late perinucleolar
3 Yolk vesicle

Date	Treatment Group	Number of Females	Number of Oocytes Measured	Mean Percent in Each Stage				Percent Atretic
				1	2	3	4	
01/11/74	(zero control)							
	0 µg/g/3wk	2	60	10	20	70		30
	0.05 µg/g/3wk	3	90	5	18	77		25
	0.5 µg/g/3wk	3	91	5	15	80		40
	5.0 µg/g/3wk	3	90	5	25	70		30
13/12/74	0 µg/g/3wk	5	96	5	10	85		65
	0.05 µg/g/3wk	2	14	20	80			85
	0.5 µg/g/3wk	1	0		100			100
	5.0 µg/g/3wk	3	3	100				95
24/01/75	0 µg/g/3wk	2	18	5	20	75		20
	0.05 µg/g/3wk	4	39	10	50	40		65
	0.5 µg/g/3wk	5	12		100			95
	5.0 µg/g/3wk	2	9	100				95

TABLE 18 - Percent oocyte atresia and mean percent of oocytes in each stage from ovaries of female pink salmon treated with a constant dosage of salmon gonadotropin per unit time (3µg/g/wk) applied by pellet implantation and by injection at three frequencies.

1 Early perinucleolar
2 Late perinucleolar
3 Yolk vesicle
4 Primary yolk

Date	Treatment Group	Number of Females	Number of Oocytes Measured	Mean Percent in Each Stage				Percent Atretic
				1	2	3	4	
17/06/75	zero control	5	170	5	52	43		17
15/07/75	inj. 3x/wk	3	82	4	33	63		32
	pellet 1x/3wk	4	98	6	51	43		11
	pellet control	2	42	8	36	62		10
	inj. control	4	92	11	58	31		10
	inj. 1x/wk	2	48	5	18	77		20
	inj. 1x/2wk	2	48	3	14	83		15
	untreated	3	45	7	37	56		10
12/08/75	inj. 3x/wk	3	60		3	90	7	35
	pellet 1x/3wk	2	43	3	30	67		25
	pellet control	2	46	8	71	21		10
	inj. control	2	20	5	60	35		10
	inj. 1x/wk	2	25		20	70	10	20
	inj. 1x/2wk	2	20	5	15	75	5	20
	untreated	3	60	5	20	75		10
09/09/75	inj. 3x/wk	3	27		5	37	58	35
	pellet 1x/3wk	2	22		5	40	55	32
	pellet control	2	38	5	30	65		10
	inj. control	2	45	3	19	78		10
	inj. 1x/wk	3	75		20	80		25
	inj. 1x/2wk	2	60		15	85		20
	untreated	2	60	5	25	70		10
07/10/75	inj. 3x/wk	3	40			10	90	47
	pellet 1x/3wk	2	42			30	70	35
	pellet control	2	49	5	40	55		25
	inj. control	2	55	2	20	78		10
	inj. 1x/wk	-						
	inj. 1x/2wk	3	48		7	90	3	30
	untreated	2	51	5	10	85		10

TABLE 19 - Gonadosomatic index (GSI) of female pink salmon treated with various dosages of pelleted gonadotropin, cell n = 1 (Experiment 1). Data subjected to Analysis of Variance.

Group	WEEKS 0	6	12	Total
Pellet control	0.615	0.940	0.960	2.514
Pellet 0.05 ug/gm SG-G100	0.743	0.655	0.215	1.614
Pellet 0.50 ug/gm SG-G100	0.707	0.580	0.194	1.482
Pellet 5.0 ug/gm SG-G100	0.773	0.327	0.330	1.431
Total	2.840	2.50	1.70	

TABLE 20 - Gonadosomatic index (GSI) of female pink salmon treated with a constant dosage of salmon gonadotropin per unit time (3 µg/g/wk) applied by pellet implantation and by three injection frequencies, cell means (n = 3) (Experiment 2). Data subjected to Analysis of Variance.

Group	WEEKS 4	8	12	16	Total
Inj. 3x/wk	0.318	0.671	0.671	1.004	7.99
Pellet SG-G100	0.556	0.570	0.744	0.397	6.80
Pellet control	0.249	0.358	0.455	0.557	4.86
Inj. control	0.273	0.406	0.405	0.510	4.79
Inj. 1x/wk	0.269	0.654	0.847	0.590	7.08
Inj. 1x/2wk	0.280	0.486	0.409	0.313	4.47
Untreated	0.248	0.318	0.492	0.559	4.85
Total	6.57	10.39	12.07	11.78	

TABLE 21 - Analysis of Variance table of female GSI data (Tables for Experiment 1 and Experiment 2.

Experiment 1

Source	S.S.	dF	M.S.	F	P
Group	6.2485E-01	3	2.0828E-01	13.32	0.00
Time	4.1251E-01	2	2.0626E-01	13.19	0.00
AB	8.1520E-01	6	1.3587E-01	8.69	0.00
Error	3.5959E-01	23	1.5634E-02		
Total	2.2121	34			

Experiment 2

Source	S.S.	dF	M.S.	F	P
Group	1.0029	6	1.6716E-01	3.57	0.00
Time	9.1290E-01	3	3.0430E-01	6.51	0.00
AB	1.0044	18	5.5803E-02	1.19	0.30
Error	2.6194	56	4.6774E-02		
Total	5.5396	83			

TABLE 22 - Student-Newman-Keuls multiple range test of female GSI data Tables for Experiment 1 and Experiment 2.

Experiment 1

Homogeneous subsets	
Group	S6-6100 - 0.05, 0.5, 5.0 ug/g/$\overline{BW}$
Time	(0 weeks, 6 weeks)

Experiment 2

Homogeneous subsets	
Group	(inj. control, 1x/2wk SG-G100, untreated, pellet control), (1x/2wk SG-G100, untreated, pellet control, pellet SG-G100, inj. 1x/wk SG-G100), (SG-G100-pellet, inj. 1x/wk, inj. 1x/2wk).
Time	(8 weeks, 12 weeks, 16 weeks)

TABLE 23- Mean emergence day, egg and fry numbers, and survival percentages of the three transplant groups.

[a] the survival percentages span three life stages; G = green egg stage; E = eyed egg (planting) stage; and F = emerged fry stage.

[b] eyed eggs for each transplant group were planted in two incubation boxes.

Source	Total eggs fertilized	Mortality to planting	Survival G/E (%) a/	Total eggs planted b/	Fry output	Survival E/F (%) a/	Survival G/F (%) a/
Control	368,640	342,835	93	136,462	131,543	96	89
				136,358	134,129	98	91
Frozen sperm	609,280	274,176	45	135,490	132,965	98	44
				136,090	133,358	98	44
Accelerated males	370,560	355,737	96	136,771	135,019	99	95
				136,559	133,654	98	94

Table 24- First, second (median), and third quartiles of the emergence of the three experimental transplant groups.

Group	Quartile		
	1	2 (median)	3
$Control_1$ box 3	14.1	15.7	20.4
$Control_2$ box 6	12.1	13.3	16.0
$Frozen\ sperm_1$ box 1	18.8	22.8	25.7
$Frozen\ sperm_2$ box 4	19.7	22.1	25.4
$Accelerated\ males_1$ box 2	20.6	24.0	26.3
$Accelerated\ males_2$ box 5	20.1	22.5	25.2

TABLE 25 - Length (cm) of three transplant groups of pink salmon upon emergence in late April 1976, cell means (n = 10). Data subjected to Analysis of Variance.

Source	DAYS												
	1	2	3	4	5	6	7	8	9	10	11	12	Total
Frozen sperm [1/]	3.25	3.28	3.18	3.22	3.22	3.24	3.24	3.24	3.30	3.24	3.28	3.29	389.76
Accelerated males [1/]	3.29	3.16	3.19	3.23	3.24	3.14	3.30	3.21	3.24	3.22	3.34	3.34	389.04
Control [1/]	3.24	3.28	3.19	3.20	3.19	3.19	3.27	3.30	3.22	3.20	3.23	3.23	387.36
Frozen sperm [2/]	3.28	3.21	3.20	3.18	3.25	3.31	3.22	3.32	3.23	3.21	3.26	3.35	390.24
Accelerated males [2/]	3.22	3.20	3.27	3.23	3.26	3.27	3.31	3.29	3.30	3.27	3.37	3.28	392.64
Control [2/]	3.26	3.22	3.19	3.25	3.23	3.18	3.22	3.24	3.19	3.26	3.26	3.32	388.20
Total	195.42	193.50	192.18	193.08	193.92	193.32	195.60	196.02	194.82	193.98	197.40	198.12	

TABLE 26 - Weight (gm) of three transplant groups of pink salmon upon emergence in late April 1976, cell means (n = 10). Data subjected to Analysis of Variance.

	DAYS												
Source	1	2	3	4	5	6	7	8	9	10	11	12	Total
Frozen sperm [1]	0.317	0.315	0.297	0.289	0.286	0.293	0.277	0.281	0.286	0.302	0.286	0.295	35.28
Accelerated males 1	0.307	0.300	0.300	0.297	0.305	0.271	0.299	0.259	0.301	0.322	0.281	0.291	35.28
Control [1]	0.305	0.310	0.314	0.305	0.299	0.294	0.293	0.306	0.291	0.300	0.269	0.282	35.64
Frozen sperm [2]	0.310	0.288	0.300	0.290	0.298	0.312	0.290	0.298	0.273	0.303	0.267	0.301	35.28
Accelerated males 2	0.296	0.298	0.317	0.298	0.294	0.297	0.305	0.285	0.307	0.332	0.282	0.290	36.00
Control [2]	0.312	0.299	0.311	0.300	0.303	0.282	0.290	0.291	0.280	0.318	0.274	0.300	35.64
Total	18.48	18.12	18.36	17.76	17.82	17.46	17.52	17.22	17.40	18.78	16.56	17.58	

TABLE 27- Developmental Index (K_D) of three transplant groups of pink salmon upon emergence in late April 1976, cell means (n = 10). Data subjected to Analysis of Variance.

Source	DAY 1	2	3	4	5	6	7	8	9	10	11	12	Total
Frozen sperm	2.12	2.06	2.10	2.05	2.05	2.05	2.02	2.02	2.00	2.08	2.01	2.03	245.88
Accelerated males	2.06	2.11	2.10	2.07	2.07	2.05	2.03	1.98	2.07	2.13	1.97	1.99	246.24
Control	2.08	2.08	2.12	2.09	2.11	2.08	2.04	2.03	2.06	2.09	2.01	2.02	248.04
Frozen sperm	2.07	2.05	2.09	2.08	2.05	2.04	2.05	2.01	2.01	2.09	1.99	2.02	245.52
Accelerated males	2.05	2.08	2.10	2.07	2.05	2.05	2.02	2.00	2.04	2.12	1.94	2.01	245.28
Control	2.09	2.09	2.13	2.06	2.09	2.05	2.05	2.03	2.04	2.09	2.00	2.03	247.44
Total	124.68	124.68	126.42	124.20	124.20	123.18	122.10	120.72	122.22	126.00	119.22	121.02	

TABLE 28 - Analysis of Variance table of length data (Table 25), weight data (Table 26), and developmental index data (Table 27).

Length

Source	S.S.	dF	M.S.	F	P
Group	1.4296E-01	5	2.8592E-02	2.31	0.04
Time	5.8537E-01	11	5.3216E-02	4.30	0.00
AB	8.6854E-01	55	1.5792E-02	1.28	0.09
Error	8.0130	648	1.2366E-02		
Total	9.6099	719			

Weight

Source	S.S.	dF	M.S.	F	P
Group	3.6228E-03	5	7.2456E-04	0.785	0.56
Time	6.7044E-02	11	6.0950E-03	6.633	0.00
AB	6.0891E-02	55	1.1071E-03	1.205	0.15
Error	5.9544E-01	648	9.1889E-04		
Total	7.2700E-01	719			

Developmental index (K_D)

Source	S.S.	dF	M.S.	F	P
Group	5.3111E-02	5	1.0622E-02	3.21	0.01
Time	8.9794E-01	11	8.1631E-02	24.70	0.00
AB	1.9756E-01	55	3.5919E-02	1.09	0.32
Error	2.1420	648	3.3056E-02		
Total	3.2906	719			

TABLE 29 - Student-Newman-Keuls multiple range test of length data (Table 25), and Developmental Index data (Table 27).

Length

	Homogeneous subsets
Group	(control 1, control 2, accelerated males 1, frozen sperm 1, frozen sperm 2), (control 2, accelerated males 1, accelerated males 2, frozen sperm 1, frozen sperm 2)

Developmental index (K_D)

	Homogeneous subsets
Group	(control 1, control 2, accelerated males 1), (control 2, accelerated males 1, accelerated males 2, frozen sperm 1, frozen sperm 2)

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