Project 109-731

COLLECTION, PROCESSING AND STORAGE OF SALMON PITUITARIES

Prepared for

International Development Research Center

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SUMMARY

Pituitary glands were collected from commercially caught Pacific salmon on a commercial scale during the 1975 fishing season. A total of 19 kg of pituitaries, representing about 269,000 fish were collected. Chum and Chinook pituitaries were the most economical to collect due to their large size and the large size of the fish heads. The heads of Coho and Pinks were much smaller, thus harder to handle and the glands more costly to collect. Moreover, the pituitaries of Pink salmon had very low levels of gonadotropin activity.

Based on the cost of this study it was estimated that it would cost about $2.50 (1975 $) per gram to pick and freeze salmon pituitaries on a commercial scale. However if only Chum and Chinook glands were processed the cost might be lower.

In general Chinook pituitaries had the highest hormone activity (2.5 - 4 g/kg of pituitaries) followed by Chum at 2.4 - 3.8 g/kg and Coho at 1.5 - 3.2 g/kg. The concentration of gonadotropin in Pink pituitaries was 0.4 - 0.7 g/kg. The concentration of hormone increased as the fishing season progressed.

Frozen pituitaries were processed into simple extracts, partially purified extracts, acetone powders and freeze dried extracts and partially purified extracts. Purification procedures resulted in significant losses of activity and the liquid preparations were probably too dilute to be practical. Freeze drying involved several additional processing steps and additional losses but produced a good product. Simple acetone powders appeared to be the most satisfactory form for preserving and using the pituitaries. Limited storage tests indicated no marked deterioration when freeze dried and acetone preps were stored at room temperature or when liquid preparations were stored at -29°C.

It was concluded that Chum and Chinook pituitaries are the least expensive to collect and have the highest activity. The production of acetone powders is the simplest processing procedure and results in the lowest processing losses. Such powders are easy to handle, store and ship. If suitable biological activity is demonstrated the production of acetone powders would be the preferred route for introducing salmon gonadotropin to the commercial market.

INTRODUCTION

OBJECTIVE

To collect, process and store salmon pituitaries for ultimate use as a source of gonadotropin, a hormone used to induce spawning in fish.

BACKGROUND

A hormone known as gonadotropin and found in the pituitary of various fish has been found to play a role in spawning. IDRC is sponsoring programs in various countries concerned with artificially spawning carp and other fishes. In many areas carp do not spawn naturally and the Centre feels that the injection of gonadotropin (either purified or crude) or even pituitary extracts, may induce spawning.

Preliminary tests with the hormone have indicated it may be successful but the amount of hormone available is very limited. If large amounts are to be made available, it will be necessary to establish a reliable source that lends itself to large-scale, and hopefully inexpensive collection techniques.

One such source is Pacific salmon which are caught in large numbers each fall as the mature fish return to their home rivers. Small collections have been made at salmon hatcheries (1) but widespread utilization of the hormone would require more efficient procedures. The obvious solution to the problem is to collect the glands from commercially caught salmon at the processing plants. In order to assess this possibility IDRC sponsored this project to demonstrate the practicality of collecting pituitaries at a commercial processing plant during the annual salmon run. In order to maximize the information gained, the study was set up in such a way that the gonadotropin content of the collected pituitaries could be correlated with species of fish, time of the year, and area of catch.

Following collection of the pituitaries studies were undertaken to process the pituitaries in various ways in order to establish what would be the most economical and effective form in which to utilize the glands for fish spawning. In addition storage studies were carried out on various prepared fractions.

Early in the investigation it became apparent that the field trials carried out in various foreign countries under IDRC sponsorship were not going to provide the required answers with regard to the effectiveness of the hormone, and so it was decided to curtail the amount of work done at B. C. Research and expend about 40% of the contract funds on a laboratory study with Dr. David Idler at the Marine Science Research Laboratory of Memorial University, St. Johns, Newfoundland. Dr. Idler had the techniques

and capabilities of assessing the cross reactivity of salmon gonadotropin to carp. In addition, Dr. Idler had the facilities to assay the various preparations prepared as part of this program. The work carried out by Dr. Idler was undertaken along the lines of the proposal contained in Appendix I. The results of Dr. Idler's assays have been incorporated into this report, whereas his cross reactivity studies are being reported directly to IDRC.

EXPERIMENTAL PROCEDURES

COLLECTION OF PITUITARIES

Arrangements were made with B. C. Packers Limited, Steveston, B. C., to set up a pituitary collecting line on an at cost basis using Imperial Cannery employees during the fall of 1975. The procedure was based on a previous study by Blackburn and Donaldson (1). Heads from fish being processed for the frozen fish market, i.e. the fresh fish line as opposed to the canning fish line, were used since these were the better quality fish. A bin of heads (usually of known species, catch date and catch area) were transported to a processing area where they were dumped on a conveyor belt that distributed them to bandsaw operators (Photograph 1) who sliced off the top of the head. During this study 4 - 6 bandsaws were used. The sawing procedure was to hold the fish head by placing the thumb in the mouth and then slice off the top of the head starting just above the eyes (Photograph 2), exposing the optic nerve. The cut heads were dumped into baskets (Photograph 3) in front of the pickers (two per saw) who picked up a head, flipped up the optic nerve (Photograph 4) and picked out the pituitaries with a pair of tweezers. The pituitary was dropped on to a collection dish sitting on a block of dry ice (Photograph 5) and the head was discarded to a collection bin (Photograph 6) and sent to the reduction plant, its normal destination. The collected pituitaries were bagged in plastic, placed in jars, sealed and stored over dry ice (Figure 7).

The relative size of the fish heads used is shown in Figure 8.

PROCESSING PITUITARIES

Crude extracts of frozen pituitaries were made using the buffer B of Idler *et al*. (2) or 1.25% sodium chloride (3). Acetone powders were produced by homogenizing the frozen pituitaries in 4 volumes of acetone (w/v) at -10°C for 5 minutes in an Omnimixer followed by centrifugation at 2000 g for 10 minutes (-20°C). The supernatant was discarded and the procedure repeated. The dried pituitaries were collected by vacuum filtration, washed with acetone and then with 50 ml of ether (-10°C) and finally desiccated at 2°C to constant weight.

Semipurified gonadotropin preparations were prepared by aqueous extraction and column chromatography (Procedure B of Idler et al. (2)). The Con A II fraction was collected as the final product and frozen. In some instances the Con A II fraction was concentrated using a diaflow cell; desalted by dialysis against Buffer B or passage through G-25 sephadex; and then frozen or freeze dried.

All fractions were assayed by the staff of the Marine Science Research Laboratory at Memorial University using the Radioimmunoassay Technique (4).

STORAGE STUDIES

Various frozen or freeze dried buffer extracts and Con A II fractions were stored at 37°C, 20°C with and without desiccant, 5°C, -29°C and -70°C in sealed glass vials for up to seven months, and then assayed (4) in order to estimate the shelf life.

RESULTS

COLLECTION OF PITUITARIES

Pituitaries were collected during September and October, 1975 at the Imperial Cannery of B. C. Packers, Steveston, B. C., primarily using the better quality fish fed to the "fresh fish" line. A total of 18,069.5 g of pituitaries, comprising about 269,000 glands, were collected (Table 1). About half the total weight was Chum pituitaries, which were the most economical to collect due to their large size (85 mg). Chinook pituitaries were also large (90 mg), but during the 1975 campaign, very few Chinook were caught. Coho and Pink pituitaries were much smaller and thus were more costly per gram to collect. The Pink heads, as shown in Figure 8, were small and hard to handle, and as will be shown later, the gonadotropin content of their pituitaries was very low. Pinks are uneconomic as a source of gonadotropin when compared to Chum. Very few Sockeye were available during the 1975 campaign. Their pituitaries were also very small.

The total cost of the collection, including B. C. Research costs for planning, supervision and liaison, was $31,599, broken down as shown in Table 2. The cost of labour was $1.06 (1975 dollars) per g of picked pituitaries (excluding setup and maintenance costs). Using an average figure of 15 pituitaries per g, this represents a cost of about $.07 per fish head handled. The figures do not include any charges for the heads, for equipment, supervision, company overhead, profit margin, or freezing, packaging and storing the glands.

The actual cost of a commercial operation is hard to predict, but it would probably be about 2½ times the cited figures. There would possibly be some economy if a full scale operation was undertaken during a normal fishing year (1975 was a very poor year, in part due to a shortened fishing season due to labour strife).

SOURCE OF PITUITARIES AND THEIR GONADOTROPIN CONTENT

During this study pituitaries were collected from the heads of four species of salmon: Chum (Oncorhynchus keta); Coho (O. kisutch); Chinook (O. tschawytscha); and Pink (O. gorbuscha). The fish were being processed on the fresh fish line but some heads were collected from the "iron chink" which cleans and beheads fish for canning. At the point of collection these latter fish were not segregated as to species and so are classed as mixed in the accompanying tables and Discussion. The fish were caught in four areas: Johnston Straits, which lies between Vancouver Island and the Mainland near Campbell River; San Juan, which is off the west coast of the southern end of Vancouver Island; West Coast, which is off the west coast of the northern half of Vancouver Island; and River, the mouth of the Fraser River.

The contribution of the various species and areas to the total collection is shown by date in Table 1. The collected pituitaries were batched into approximately 200 g lots of the same species, catch area and catch date. These lots, referred to by a code number, were then processed or distributed in various ways (see Table 3).

When representative portions of the pituitaries were assayed, the gonadotropin content shown in Table 3 and Figure 1 was obtained. The data shows quite clearly that pituitaries from the Pinks and the Mixed (mostly Pinks) had the lowest gonadotropin content with the usual content being between 0.4 and 0.7 g/kg of pituitaries (wet weight). Codes 32 and 42 gave anomalously high results; the former were supposed to be River Pinks but this would appear to be doubtful, whereas the latter were Coho. Since these pituitaries were also the smallest in weight and the Pink heads were hard to handle, the cost per gram of gonadotropin is by far the highest and Pinks are probably not a reliable source of hormone.

Pituitaries from the other species had much higher gonadotropin levels. The Chinook pituitaries appear to have the highest content although the number collected was small. Except for two samples from the West Coast (Code 69 and part of Code 70) and one sample from San Juan (Code 66) the Chinook pituitaries contained between 2.5 and 4 g of gonadotropin per kg. Samples taken from the River area late in the season contained more hormone than samples collected earlier from Johnstone Strait.

Most of the Coho were collected from the San Juan area and in general the hormone level increased with time. Those caught in September had pituitaries that contained between 1.5 and 2.5 g of gonadotropin per kg, whereas in early October the quantity ranged from 2.2 to 3.2 g/kg.

Chum from all areas (except the West Coast) were examined, and again the gonadotropin content of the pituitaries appeared to increase with time. The sample collected in the Johnstone Strait area in early September had an assay value of 2.4 g/kg, whereas by late October samples from the same area gave values ranging from 3 to 3.8 g/kg. Chum taken from the River area showed the same trend but gave higher values.

PURIFYING SALMON PITUITARIES

Simple Extracts

Frozen pituitaries were extracted in either the Buffer B of Idler (2) or the sodium chloride buffer of Schmidt et al. (3). The data in Table 4 shows that the Schmidt and Idler procedures indicated comparable levels of activity with Chum salmon, although the former procedure gave a more concentrated extract. With Coho and Pink salmon pituitaries the Schmidt extract was more concentrated and in addition, seemed to extract more hormone, i.e. indicates a higher gonadotropin content in the original pituitary. The apparent superiority of the Schmidt buffer will have to be evaluated in more detail if it is decided to produce extract on a commercial basis. However, since subsequent results have indicated that the production of extracts and semi purified gonadotropin does not appear to be as economical as the production of acetone powders, this point was not investigated further at this time.

Partially Purified Extracts

At the time this investigation was initiated there were two procedures available for purifying gonadotropin from salmon pituitaries (2, 5). The procedure of Idler (2) was selected as the most practical and low cost procedure for partially purifying large quantities of gonadotropin. Since the procedure had not been carried out on a large scale in the past, it was necessary to determine the recoveries obtainable at various stages.

Initial extraction

A 175.2 g portion of San Juan Chum pituitaries collected on September 18 and 19, 1975 (Code 13) was extracted three times in Buffer B and the three extracts assayed. The results were as follows:

Extraction	Lowry Protein (mg)	Gonadotropin (mg)	Specific Activity Gonadotropin/Protein
First Extraction	11,070	333	0.030
Second Extraction	2,110	65.4	0.031
Third Extraction	412	20	0.048

It was concluded from the above that two extractions were sufficient to obtain most of the gonadotropin available in the extracts.

Recovery from Concanavalin A Sepharose columns

In the Idler procedure the gonadotropin is held on Concanavalin A while most of the extraneous protein and other material passes through. The recovery of gonadotropin following this procedure was usually less than 50% (Table 5). The only high extraction occurred with Sample Code 35, which had a very low initial level of activity.

Although the specific activity of the Con A II fraction was usually increased 10 fold over the original extract, the actual activity in mg/ml of solution was usually lower. This made it necessary to inject very large volumes into spawning carp, and indicated that the utilization of a Con A II fraction in its "as produced" form was not practical. Field trials confirmed this.

The effect of concentrating the Con A II fraction using an Amicon diaflow cell with a 76 mm PM-10 filter was investigated. A 5 fold increase in concentration was achieved with a 10 - 20% loss in activity. However if the concentration was held to 2 or 3 fold, there was no significant loss in activity.

In one experiment a Schmidt extract of Johnstone Strait Chum (Code 4) was passed through the Con A column and the recovery was 89%. The loss on concentration in the diaflow cell was 20%. The apparent improved recoveries of the Schmidt extract were not examined in more detail because it was concluded that the production of a purified gonadotropin was probably not a practical procedure when compared to the preparation of acetone powders.

Freeze Dried Preparations

Attempts were made to freeze dry original extracts as well as Con A II fractions. However the high salt content of the buffers resulted in an unsatisfactory product. To overcome this problem, the material was desalted either by dialysis or on a G-25 Sephadex Column. Both procedures resulted in a 15 - 30% loss of gonadotropin. However, the freeze dried product was a light fluffy powder which was easy to handle and was quite suitable for shipping and redissolving.

The loss of activity on freeze drying Con A II fractions ranged from 10 - 50% with no correlation between percentage loss and species. When extracts were freeze dried the loss of activity was in the same range.

A freeze dried portion of the Code 22 Con A II fraction that had been desalted on Sephadex was assayed using the chick assay (E. M. Donaldson, personal communication) and found to have 1/8 the activity of an SG-G-100 prep (1973 - 1974 IDRC). The Code 22 material had an assay value of 78.1 mg/g (Table 3) at that time.

Acetone Powders

When it became evident that partial purification of the gonadotropin by chromatography was producing dilute solutions and that activity losses were high, it was decided to produce acetone powders. The data in Table 6 shows that the loss of activity in producing these powders ranged from 0 - 47%.

The yields of acetone powders produced from the various samples are also shown in Table 6. The yields ranged from 8.8 to 14.6%, with the mean being 11.5%. Chum pituitaries gave the highest yield and the acetone powder with the highest activity. This was consistent with our other data that show that the level of gonadotropin in Chum pituitaries was higher than in most other species.

STORAGE STUDIES

In order to get some assessment of the shelf life of the various products a brief storage study was undertaken. The results in Table 7 show that the frozen liquid preparations kept better at -29°C than they did at -70°C. Inevitably the assay values obtained after either 4 or 7 months storage at -29°C were higher than the original assays as well as the samples stored at -70°C. Idler cautions that the assay procedure is not so precise that exact comparisons can be made between assays conducted on different days. Thus caution must be applied when interpreting the differences between the storage and original values. However it does appear there was no marked deterioration. All the storage samples were assayed at the same time so the difference between -29°C and -70°C samples is real.

When the freeze dried preparations were examined (Table 8) it was evident that storage at -29°C was the most satisfactory procedure but that the amount of deterioration occurring when samples were stored dry at 20°C was not great, i.e. storage at 20°C probably resulted in a 10 - 20% loss in activity compared to storage at -29°C.

One sample of acetone powder was stored for 4 months. Both storage samples assayed higher than the original sample (Table 8) and the one stored at 20°C was the most active.

SPAWNING DATES

Since the fish were caught in open waters little knowledge existed as to their state of sexual maturity or their home rivers. In an attempt to gain some information the biologists responsible for management of the fishery were contacted to see if some correlation could be developed between tagged fish recovery, river of origin and spawning times.

Tagged fish come from the locations shown in Figure 2. The data presented in Table 9 show that the peak spawning period for Canadian Coho was in November, whereas most of the Chinook spawned in the last half of October. No data were available for Chum.

Table 10 shows the percentage distribution by spawning stream of nose tagged Coho for the 1975 fishery. The data is only a guide at best to the state of sexual maturity of the fish used as a source of pituitary glands since there is no information available as to the percentage of the catch coming from spawning grounds having a tagging program. However with this limitation in mind, and realizing the small number of tagged fish which were recovered, it would appear that most of the Coho caught in the Johnstone Strait area were from the Big Qualicum River where the peak spawning dates were late November. The next largest source was Rosewall Creek where the fish spawn in early December.

Tagged Coho taken in the San Juan and West Coast fisheries were primarily U.S. stocks. The most common Canadian stock was Robertson Creek for the West Coast, especially during statistical week 92, and Big Qualicum River for the San Juan. Robertson Creek spawning dates encompassed the Big Qualicum dates.

Similar data for Chinook are presented in Table 11. For the Johnstone Strait fishery the Big Qualicum and Capilano Rivers were the major home streams and since the Capilano fish were originally Big Qualicum stock, their spawning dates are the same; the last half of October. The fish caught in the San Juan and West Coast fishery were mostly U.S. stocks with the main Canadian source being Robertson Creek for the West Coast and Big Qualicum for San Juan. The spawning dates for the former were the first half of November.

DISCUSSION

The results of this study have shown that it is practical to collect salmon pituitaries on an industrial scale from commercially caught fish. The pituitaries of Chum, Coho and Chinook salmon contain sufficient gonadotropin to make the collection worthwhile. However, Pink salmon pituitaries appear to contain a very low level of hormone and because of their small size they are expensive to collect. Insufficient fish were available during 1975 to assess the potential of Sockeye (O. nerka).

The collection procedure employed, which consists of slicing off the top of the head, displacing the optic nerve and removing the pituitary with tweezers, is effective but labour intensive. Productivity depended primarily on the sawyer who had the most tedious and dangerous job in the operation. The sawyer could probably be displaced by some sort of an automatic coring device providing that the size of the fish heads being processed remained relatively constant. However, hand labour would still be required to remove the pituitary from the core. Barring such a technological development, it may cost as much as 18¢ each (based on 1975 dollars) to harvent pituitaries on a commercial scale.

The gonadotropin content of the salmon appeared to be a function of species and time of year rather than the area where the fish were caught. As mentioned above, Pink pituitaries had a very low content of hormone compared to Chum, Coho and Chinook. Chum and Chinooks had the highest content and since their pituitaries were the largest the unit cost of production was lowest for these species.

The gonadotropin content of the pituitaries increased as the fishing season progressed, indicating that the fish were approaching the point of spawning. Thus if it was desired to obtain only limited quantities of hormone, then the collection should occur in the latter part of the

fishing season. However, commercial fishing is an irregular business and there cannot be any real guarantee that fish will be caught at any particular time.

One factor that was not established during this study was the change, if any, in the size of the pituitary gland with time.

The collected pituitaries were processed by a variety of procedures to obtain preparations of various degrees of purity. Buffer extracts and partially purified preparations (Con A II fractions) were found to have low levels of activity per ml and initial reports from the field indicated that it was necessary to administer large volumes which was difficult. Moreover, the losses incurred in producing partially purified extracts were high and it was expensive to ship and store frozen preparations in tropical climates.

Freeze drying extracts was expensive due to the large volumes of water that had to be removed. Con A II preparations could not be freeze dried without a prior desalting step which resulted in additional product losses. Batches of various freeze dried preparations were sent out for field trials and the results of such trials will be reported to IDRC. However, one preliminary report indicated that the material was not biologically active.

The major reason for trying to prepare partially purified preparations was to eliminate any problems due to injecting large quantities of foreign protein into the test fish. To determine if this was a problem acetone powders were prepared and samples sent for field trials. The results will be reported to IDRC, but one trial using these powders to spawn Milkfish has apparently been successful. The loss of activity in producing acetone powders appears to be lower than the losses incurred in producing partially purified preparations and limited storage tests indicate that they are stable. Since the preparation of acetone powders is relatively simple and low cost, it could be handled by a commercial operation, and thus seems to be the most satisfactory form for introducing salmon gonadotropin to the commercial market. The commercial product would need to be sold either at a fixed strength or with an assay value provided so that the potential user would be able to estimate the dosage required to spawn a particular fish.

Should the field trials being conducted by IDRC indicate that the large scale utilization of salmon gonadotropin is successful and practical, the information in this report should form the groundwork for the establishment of a commercial operation to increase the utilization of a Canadian resource.

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TABLE 1

LOCATION OF CATCH, DATE OF COLLECTION, AND WEIGHT OF COLLECTED PITUITARIES

Collection Date 1975	Statistical Week	Species											
		Coho		Chum		Pinks		Chinook		Mixed		Other	
		Location	Grams	Location	Grams	Location	Grams	Location	Grams	Location	Grams	Location	Grams
September 9 (9 - 19 for mixed)	92	West Coast San Juan	374.0	West Coast	13.9	?	36.1	West Coast	28.9	Johnstone San Juan	172.2		
10	92	West Coast	161.5	West Coast	12.7	?	61.2	West Coast	122.9	West Coast	208.0		
11	92			Johnstone	58.7	Johnstone	287.2	West Coast	36.3	West Coast	39.6		
12	92	Johnstone	9.8	Johnstone	89.6	Johnstone	349.2	Johnstone	11.5	Johnstone	476.9		
13	92	Johnstone	268.0	Johnstone	16.4	Johnstone	93.3	Johnstone	41.3	Johnstone	404.8		
16	93	San Juan	87.5	West Coast	67.7	San Juan River	343.1	West Coast San Juan	38.2	West Coast River	490.3		
17	93	San Juan	122.5	San Juan	96.6	San Juan River	481.6			San Juan	211.7		
18	93	San Juan	321.2	San Juan	186.5	San Juan	242.2	Johnstone	20.0	San Juan	273.0		
19	93	San Juan	322.8	San Juan	150.5	San Juan	53.4			San Juan	263.2		
25	94	San Juan	611.9	West Coast	44.0							San Juan	98.2 Sockeye
26	94	San Juan	312.3					San Juan West Coast	45.2	San Juan	41.9		5.6 Ling Cod

TABLE 1 (continued)

LOCATION OF CATCH, DATE OF COLLECTION, AND WEIGHT OF COLLECTED PITUITARIES

Collection Date 1975	Statistical Week	Species											
		Coho		Chum		Pinks		Chinook		Mixed		Other	
		Location	Grams	Location	Grams	Location	Grams	Location	Grams	Location	Grams	Location	Grams
October 1	101	San Juan West Coast River	252.9	Johnstone River	810.4			River	88.0	River Johnstone	180.9		
2	101	San Juan	291.9	Johnstone	964.2								
3	101	San Juan West Coast	68.8	Johnstone West Coast	1545.4					Johnstone	30.8		
7	102			River	414.4			River	50.4				13.7 Perch 6.1 Grey Cod
9 13	102	San Juan	665.1	San Juan San Juan	178.9 59.7			River	41.3				
16	103	San Juan	40.8	Johnstone	966.1								
24	104	River	41.2	River Johnstone	1135.6			River	11.7				
25	104			Johnstone	1201.2								
27	105			Johnstone	704.9								
Total Weight (g)		3952.2		8717.4		1947.3		535.7		2793.3		98.2*	
Average Weight of Individual Pituitary (mg)		53.0		84.6		50**		90		65**		40*	
Estimated Number of Pituitaries		74,570		103,042		38,946		5,952		42,973		3,090	

* Sockeye
** Estimated

TABLE 2

COST OF COLLECTING PITUITARIES

	September 8-21	September 12 October 19	October 19-27	Total
Days worked	9	7 1/2	2 1/4	18 3/4
Setup, Cleanup and Maintenance				
Labour $	1,240.72	202.51	273.71	1,716.94
Material $	57.96	534.54	346.19	938.69
Forklift $	472.50		381.38	853.88
SUBTOTAL:	1,771.18	737.05	1,005.28	3,509.51
Picking Labour				
Hours	1,239.4	894.5	-	-
$	9,287.29	6,450.68	3,440.34	19,178.31
Total	11,058.47	7,187.73	4,441.62	22,687.82
Man days (8-hr)	154.9	111.8	59.6*	
Average crew	17	15	24	
B.C. Research Costs				8,911.18
TOTAL COST				$31,599.00

Cost per fish (total)	$0.117	
Cost per fish (Picking Labour)	$0.071	
Cost per gram (total)	$1.75	Average 15 pituitaries per gram
Cost per gram (Picking Labour)	$1.06	

* Assuming 7.21/hr average wage

TABLE 3

UTILIZATION OF PITUITARIES

Code Number	Bag Numbers	Total Weight (g)	Date Caught	Source	Species	Processed To	Gonadotropin Content of Pituitaries g/kg (wet weight)
1	35, 37, 56, 59	148.3	Sept. 11	Johnstone	Chum	Con A II	2.357
2	215, 216, 217, 220	190.4	Oct. 1	Johnstone	Chum	Fresh to Malaysia	
3	218, 222, 225, 227	191.3	Oct. 1	Johnstone	Chum	Lost	
4	228, 229	84.3	Oct. 1	Johnstone	Chum	Schmidt Extract	2.832
5	230, 231, 233, 234	195.5	Oct. 1	Johnstone	Chum	Idler Extract	Not Assayed
6	235, 236, 238, 239, 240	212.5	Oct. 2	Johnstone	Chum	Con A II	Not Assayed
7	247, 251, 252, 254	188.8	Oct. 2	Johnstone	Chum	Idler Extract and Freeze Dried	3.198
8	255, 256	94.5	Oct. 2	Johnstone	Chum	Lost	
9	396, 397, 398, 399	170.3	Oct. 25	Johnstone	Chum	Fresh to Idler	
10	400, 401, 402, 403, 404	197.1	Oct. 25	Johnstone	Chum	Con A II and Freeze Dried	3.365
11	405, 406, 407, 408	158.1	Oct. 25	Johnstone	Chum	Acetone Powder	3.251
12	409, 410	86.5	Oct. 25	Johnstone	Chum	Schmidt Extract	3.044
13	140, 144, 151, 156	175.3	Sept. 18	San Juan	Chum	Idler Extract and Freeze Dried	2.274
14	107, 113, 137, 139	190.6	Sept. 17	San Juan	Chum	3/4 to Con A II	2.545
15	159, 160	68.0	Sept. 19	San Juan	Chum	Schmidt Extract	3.452
16	337; 341	83.5	Oct. 9	San Juan	Chum	Fresh to India; Acetone	
16	336; 338	95.4	Oct. 9	San Juan	Chum	Fresh to Malaysia; Acetone	
17	369, 370, 372, 373	222.1	Oct. 24	River	Chum	Fresh to Idler	
18	374, 380, 381, 382	104.7	Oct. 24	River	Chum	Con A II	3.735
19	387	46.6	Oct. 24	River	Chum	Schmidt Extract	4.135
20	200, 202, 205, 211	185.6	Oct. 1	River	Chum	Con A II and Freeze Dried	3.705
21	307, 308, 311, 312, 313	170.2	Oct. 7	River	Chum	Con A II to Idler	3.553
22	303, 304, 305, 309, 316	244.2	Oct. 7	River	Chum	1/2 to Con A II	3.505
23	33, 34, 36	181.7	Sept. 11	Johnstone	Pink	Idler Extract	0.456
24	38, 39	147.0	Sept. 11	Johnstone	Pink	Con A II and Freeze Dried	0.392
25	47, 48, 49	150.7	Sept. 12	Johnstone	Pink	Con A II and Freeze Dried	0.384

TABLE 3 (continued)

UTILIZATION OF PITUITARIES

Code Number	Bag Numbers	Total Weight (g)	Date Caught	Source	Species	Processed To	Gonadotropin Content of Pituitaries g/kg (wet weight)
26	50, 51	103.5	Sept. 12	Johnstone	Pink	Schmidt Extract	0.819
27	53, 68, 78	146.8	Sept. 12	Johnstone	Pink		
28	96, 97, 105	151.9	Sept. 16	San Juan	Pink	Idler Extract	0.609
29	108, 109, 112, 114	238.4	Sept. 17	San Juan	Pink	Con A II and Freeze Dried	0.560
30	115, 116, 120, 121, 125	253.1	Sept. 17	San Juan	Pink	Con A II and Freeze Dried	0.492
31	131, 132, 133, 161	199.0	Sept. 18	San Juan	Pink	Fresh to Idler	
32	81, 84, 88	123.5	Sept. 16	River	Pink	Idler Extract	3.089
33	93, 101, 102	154.4	Sept. 16	River	Pink	Con A II	0.404
34	40, 42, 43	127.7	Sept. 12	Johnstone	Mixed	Idler Extract	0.952
35	44, 45, 46, 54	251.0	Sept. 12	Johnstone	Mixed	Con A II	0.211
36	55, 57	98.2	Sept. 12	Johnstone	Mixed		
37	60, 61, 62	183.0	Sept. 13	Johnstone	Mixed	Con A II and Freeze Dried	Not Assayed
38	63, 64, 65, 75, 79	249.3	Sept. 13	Johnstone	Mixed	Idler Extract	Lost
39	224, 268	69.5	Oct. 1	Johnstone	Mixed	Lost	
40	110, 111	109.7	Sept. 17	San Juan	Mixed	Con A II	0.479
41	141, 142	81.9	Sept. 18	San Juan	Mixed	Con A II and Freeze Dried	0.379
42	117, 118, 119	102.0	Sept. 17	San Juan	Mixed	Idler Extract and Freeze Dried	3.027 (coho)
43	145, 146, 147, 148	178.8	Sept. 18	San Juan	Mixed	Con A II and Freeze Dried	0.714
44	122, 123, 124	126.2	Sept. 18	San Juan	Mixed		
45	149, 164, 192	191.2	Sept. 19	San Juan	Mixed	Con A II and Freeze Dried	0.751
46	82, 83, 85, 86	193.1	Sept. 16	River	Mixed	Idler Extract	0.421
47	87, 89, 91, 92, 99, 100	269.7	Sept. 16	River	Mixed	Con A II and Freeze Dried	0.742
48	210, 213, 306	89.7	Oct. 1	River	Mixed		
49	22, 23, 26, 31	120.9	Sept. 10	West Coast	Mixed		
50	27, 28, 29, 30	126.7	Sept. 10	West Coast	Mixed		

TABLE 3 (continued)

UTILIZATION OF PITUITARIES

Code Number	Bag Numbers	Total Weight (g)	Date Caught	Source	Species	Processed To	Gonadotropin Content of Pituitaries g/kg (wet weight)
51	52, 66, 69	125.3	Sept. 12	Johnstone	Coho	Acetone Powder	2.534
52	71, 72, 73, 76	152.5	Sept. 13	Johnstone	Coho	Acetone Powder	
53	1, 2, 3, 4, 7	206.0	Sept. 9	San Juan	Coho	Idler Extract	2.207
54	94, 98, 103, 104, 106	210.0	Sept. 16	San Juan	Coho	Idler Extract	1.901
55	127, 128, 129, 130, 134, 135	185.5	Sept. 18	San Juan	Coho	Con A II	1.676
56	136, 138, 143, 150, 152	182.2	Sept. 18	San Juan	Coho		
57	153, 154	83.8	Sept. 19	San Juan	Coho	Schmidt Extract	3.181
58	219, 223, 226, 232	96.2	Oct. 1	San Juan	Coho	1/2 Fresh to Malaysia	
59	237, 241, 242, 243, 244, 245	176.3	Oct. 2	San Juan	Coho	Idler Extract and Freeze Dried	2.723
60	246, 248, 249, 250, 273, 295	184.4	Oct. 2	San Juan/ West Coast	Coho	Acetone Powder	
61	320, 322, 323, 324, 325	149.1	Oct. 9	San Juan	Coho	Acetone Powder	
62	326, 327, 328, 329, 330, 331	174.2	Oct. 9	San Juan	Coho	Assayed	3.053
63	8, 18, 25	129.1	Oct. 10	West Coast	Coho	Assayed	2.219
64	207, 321, 339	100.2	Oct. 1	West Coast	Coho	Assayed, Acetone Powder	1.690
65	41, 70, 71, 74, 126	72.8	Sept. 12	Johnstone	Chinook	Con A II	2.571
66	95, 198	33.3	Sept. 16	San Juan	Chinook	Con A II	1.481
67	201, 212, 214	68.5	Oct. 1	River	Chinook	Con A II	3.868
68	315, 318, 319, 371	59.7	Oct. 7	River	Chinook	Con A II	4.070
69	6, 32, 90	85.4	Sept. 9	West Coast	Chinook	Con A II	1.375
70a	20, 24	42.8	Sept. 10	West Coast	Chinook (white)	Acetone Powder	2.669
70b	196	29.9	Sept. 25	West Coast	Chinook	Acetone Powder	0.837
70c	310	43.7	Oct. 7	River	Chinook	Acetone Powder	3.705

TABLE 3 (continued)

UTILIZATION OF PITUITARIES

Code Number	Bag Numbers	Total Weight (g)	Date Caught	Source	Species	Processed To	Gonadotropin Content of Pituitaries g/kg (wet weight)
72	5, 21, 77, 80	110.7	Sept. 9-16	West Coast Johnstone	Chum		
73	177, 215, 217	152.5	Sept. 25-Oct. 1	West Coast Johnstone	Chum		
74	253, 257, 258, 259	211.0	Oct. 2	Johnstone	Chum	Fresh to Idler	
75	260, 261, 262, 263	207.4	Oct. 2	Johnstone	Chum	Fresh to Donaldson	
76	264, 265, 266, 267	194.0	Oct. 3	Johnstone	Chum	Fresh to Donaldson	
77	269, 270, 271, 272, 278	202.7	Oct. 3	Johnstone	Chum	Fresh to Donaldson	
78	274, 275, 276, 280	198.2	Oct. 3	Johnstone	Chum	Fresh to Donaldson	
79	277, 279, 282, 283	195.4	Oct. 3	Johnstone	Chum	3/4 Fresh to Donaldson	
80	281, 284, 285, 286, 289	202.2	Oct. 3	Johnstone	Chum	Fresh to Donaldson	
81	288, 290, 291, 294	202.5	Oct. 3	Johnstone	Chum	Fresh to Donaldson	
82	287, 292, 293, 300	203.2	Oct. 3	West Coast Johnstone	Chum		
83	296, 297, 298, 301, 302	206.9	Oct. 3	West Coast Johnstone	Chum		
84	346, 347, 348, 366	195.2	Oct. 16	Johnstone	Chum		
85	349, 350, 351, 352, 354	197.2	Oct. 16	Johnstone			
86	353, 355, 356, 360	201.5	Oct. 16	Johnstone	Chum	Acetone Powder	
87	357, 358, 361	175.1	Oct. 16	Johnstone	Chum		
88	359, 362, 364, 365	197.0	Oct. 16	Johnstone	Chum		
89	411, 412, 413, 415, 417	199.5	Oct. 25	Johnstone	Chum		
90	414, 416, 421, 423	192.8	Oct. 25	Johnstone	Chum		
91	418, 418, 420, 422, 424	195.1	Oct. 25	Johnstone	Chum		
92	425, 426, 427, 428, 432	208.3	Oct. 27	Johnstone	Chum	Fresh to Idler	
93	429, 430, 431, 433, 441	198.5	Oct. 27	Johnstone	Chum		
94	434, 435, 436, 437, 438	203.1	Oct. 27	Johnstone	Chum		
95	439, 440	95.0	Oct. 27	Johnstone	Chum	Assayed	3.695

TABLE 3 (continued)

UTILIZATION OF PITUITARIES

Code Number	Bag Numbers	Total Weight (g)	Date Caught	Source	Species	Processed To	Gonadotropin Content of Pituitaries g/kg (wet weight)
96	12, 17	97.3	Sept. 9, 10	-	Pink	Fresh to Idler	
97	10, 56, 58, 67, 166	202.2	Sept. 9, 19	Johnstone San Juan	Mixed		
98	206, 208, 209	72.0	Oct. 1	River	Mixed		
99	9, 11, 13, 14, 15	200.4	Sept. 9, 10	-	Coho		
100	155, 157, 158, 162, 163, 165	192.5	Sept. 19	San Juan	Coho		
101	167, 172, 179, 181, 182	205.1	Sept. 25	San Juan	Coho	Assayed, Acetone	2.987
102	171, 173, 174, 175, 178, 185	206.2	Sept. 25	San Juan			
103	180, 183, 184, 169, 170	200.6	Sept. 25	San Juan	Coho		
104	186, 187, 188, 189, 191, 193, 199	196.3	Sept. 26	San Juan	Coho		
105	190, 195, 197	116.0	Sept. 26	San Juan			
106	332, 333, 334, 335, 340; 345	196.4	Oct. 9	San Juan	Coho	Assayed; Fresh to Idler	3,351
107	342, 343, 344, 363	116.6	Oct. 9, 16	San Juan	Coho		
108	168, 176	98.6	Sept. 25	San Juan	Sockeye	Fresh to Idler	
109	16, 209	49.5	Sept. 6/Oct. 1	River	Chinook	Assayed	2.965
Uncoded	375-379, 383-386, 388-395	540.1	Oct. 24	Johnstone	Chum	Fresh to Idler	
Uncoded	203	35.9	Oct. 1	West Coast	Coho	Fresh to Candido/ Smith - UBC	
Uncoded	204	57.5	Oct. 1	River	Coho	Fresh to Candido/ Smith - UBC	
Uncoded	221	32.7	Oct. 1	San Juan	Coho	Fresh to Candido/ Smith - UBC	

TABLE 4

EFFECT OF EXTRACTION BUFFER ON APPARENT GONADOTROPIN LEVEL

Species	Code	Date Caught	Source	Extract	Gonadotropin Activity of Pituitaries g/kg (wet weight)	Activity of Extract mg/ml
Chum	7	Oct. 2	Johnstone	Idler	3.198	0.62
Chum	4	Oct. 1	Johnstone	Schmidt	2.832	0.86
Chum	18	Oct. 24	River	Idler	3.735	0.68
Chum	19	Oct. 24	River	Schmidt	4.135	1.33
Chum	10	Oct. 25	Johnstone	Idler	3.365	0.67
Chum	11	Oct. 25	Johnstone	Idler	3.251	0.46
Chum	12	Oct. 25	Johnstone	Schmidt	3.044	1.04
Chum	13	Sept. 18	San Juan	Idler	2.274	0.41
Chum	14	Sept. 17	San Juan	Idler	2.545	0.44
Chum	15	Sept. 19	San Juan	Schmidt	3.452	0.87
Coho	55	Sept. 18	San Juan	Idler	1.676	0.28
Coho	57	Sept. 19	San Juan	Schmidt	3.181	0.85
Pink	23	Sept. 11	Johnstone	Idler	0.456	0.078
Pink	24	Sept. 11	Johnstone	Idler	0.392	0.069
Pink	25	Sept. 12	Johnstone	Idler	0.384	0.075
Pink	26	Sept. 12	Johnstone	Schmidt	0.819	0.202

TABLE 5

RECOVERY OF GONADOTROPIN AS CON A II FRACTION FROM CONCANAVALIN A COLUMN

Species	San Juan		West Coast		Johnstone		River	
	Code Number	% Recovery	Code Number	% Recovery	Code Number	% Recovery	Code Number	% Recovery
Chum	14	46.8			1	19.8	18	29.7
					10	24.0	22	77.6
							21	44.0
Coho	55	53.0						
Chinook			69	42.4	65	46.0		
Mixed	41	47.4			35	91.5	47	54.0
	43	43.9						
	45	51.0						
Pink	29	30.0			25	58.5		
	30	29.9						

TABLE 6

ACTIVITY OF ACETONE POWDERS

Species	Code	Source	Catch Date	Gonadotropin Activity in g/kg Fresh Pituitaries			% Yield of Powder	Activity of Acetone Powder mg Gonadotropin/g
				In Fresh Pituitary	In Acetone Powder	% Loss		
Chum	11	Johnstone	Oct. 25	3.251	4.007	+23	12.4	32.5
Chum	86	Johnstone	Oct. 16	-	4.385		12.9	33.9
Chum	16-338	San Juan	Oct. 9		4.466		14.6	30.5
Chum	16-341	San Juan	Oct. 9		3.575		11.7	30.6
Coho	51	Johnstone	Sept. 12	2.534	2.576	0	12.0	21.4
Coho	52	Johnstone	Sept. 13		1.692		10.9	15.5
Coho	60	San Juan	Oct. 2		2.264		10.9	20.8
Coho	61	San Juan	Oct. 9		1.773		10.5	16.8
Coho	62	San Juan	Oct. 9	3.053	2.239	27	12.7	17.6
Coho	101	San Juan	Sept. 25	2.987	2.449	18	11.9	20.6
Coho	63	West Coast	Sept. 9	2.219	1.353	39	10.7	12.6
Coho	64	West Coast	Oct. 1		1.345		8.8	15.3
Chinook	70	West Coast & River	Sept. 10, 26 Oct. 7	2.811	1.489	47	9.4	15.7

TABLE 7

DETERIORATION OF LIQUID PREPARATIONS ON STORAGE (mg of gonadotropin/ml)

Code Number	12	13	14	20	26	28	33	54	55	66	67
Species	Chum	Chum	Chum	Chum	Pink	Pink	Pink	Coho	Coho	Chinook	Chinook
Source	San Juan	San Juan	San Juan	River	Johnstone	San Juan	River	San Juan	San Juan	San Juan	River
Type of Preparation	Schmidt Extract	Idler Extract	Con A II	Con A II	Schmidt Extract	Idler Extract	Con A II	Idler Extract	Con A II	Con A II	Con A II
Original Assay	1.18	0.38	0.41	1.22	0.18	0.03	0.09	0.35	0.65	0.20	0.26
After 4 months at -29°C				1.40			0.16			0.27	0.43
After 7 months at -29°C	1.65	0.80	0.50		0.14	0.04		0.69	0.52		
After 4 months at -70°C				0.78			0.13			0.17	0.24
After 7 months at -70°C	1.05	0.31	0.50		0.10	0.04		0.35	0.48		

TABLE 8

DETERIORATION OF FREEZE DRIED PREPARATIONS AND ACETONE POWDER ON STORAGE

(mg of gonadotropin/g of freeze dried extract)

Code Number	7	22	59	41	43/45	86
Species	Chum	Chum	Coho	Mix	Mix	Chum
Source	Johnstone	River	San Juan	San Juan		Johnstone
Freeze Dried Material	Idler Extract	Con A II - Sephadex	Idler Extract	Con A II - Sephadex	Con A II - Sephadex	Acetone Powder
Original Assay	21.0	83.3	17.0	1.6	23.5	33.9
After 7 months at 37°C	14.5	69.9	12.1	0.9	24.5	
After 7 months at 20°C	16.8	73.2	13.0	1.2	22.8	
After 7 months at 20°C (desiccated)	20.0	78.1	19.1	0.8	27.0	
After 7 months at -29°C	23.0	108.9	23.9	2.4	27.9	
After 4 months at 20°C (desiccated dark)						48.3
After 4 months at 5°C (desiccated dark)						36.3

TABLE 9

SPAWNING DATES FOR SALMON - 1975

Location	Coho		Chinook			
	Spawning Dates	Peak Dates	Spawning Dates		Peak Dates	
VANCOUVER ISLAND						
Quinsam	Oct. 10 - Jan. 5	Nov. 15	Oct. 15 - Nov. 15		Oct. 30	
Puntleage River	Oct. 1 to Early Jan.	End of Nov.	Summer Oct. 1 - Oct. 20	Fall Run Oct. 1 - Nov. 20	Summer Oct. 15	Fall Oct. 20
Rosewall Creek	All Fish Trapped and Killed	Peak Week of Migration Dec. 5 - 11				
Robertson Creek	Oct. 29 - Dec. 20	Nov. 3 - Dec. 10	Stamp R. Oct. 15 - Nov. 25 Sproat R. Nov. 7 - Nov. 19		Nov. 1 Nov. 19	No Natural Run In Robertson Creek
Big Qualicum	Oct. 20 - Jan. 5	Nov. 20 - Nov. 25	Oct. 15 - 30		Oct. 20 - 27	
Nanaimo River	Oct. 9 - Jan. 10	Nov. 17	June 2 - Nov. 16		Oct. 11	
Cowichan River	Mid Nov.	Dec. 15 - 30	Early Oct. - Early Dec.		Nov. 1 - 20	
MAINLAND						
Tenderfoot Creek	Mid Nov. - Mid Feb.	Mid Jan.				
Meighn Creek	Mid Dec. - Mid Jan.	Dec. 26 - Jan. 4				
Little Stawamus River	Mid Nov. - Feb. 1	Early Jan.				
Capilano	End of Oct. - Early Jan.	Last Week Nov. - First Week Dec.	Oct. 15 - Nov. 5		Oct. 25	Big Qualicum River Stocks Not Native
Harrison River	Oct. - Jan.	Nov.	Oct. - Dec.		Nov.	

TABLE 10

SPAWNING STREAM OF TAGGED COHO CAUGHT DURING COMMERCIAL FISHERY (% DISTRIBUTION)

Statistical Week	92				93				94	101			
Spawn \ Location / Catch	Johnstone	San Juan	West Coast	River	Johnstone	San Juan	West Coast	River	San Juan	Johnstone	San Juan	West Coast	River
VANCOUVER ISLAND													
Quinsam					1						1		
Puntleage River													
Rosewall Creek	16	1			1	5	1	2	5	10	9	9	
Robertson Creek		4	3		1	1	79		1	1		12	
Big Qualicum	73	15	6	3	40	22	16	9	22	76	35	39	50
Nanaimo River													
Cowichan River													
MAINLAND													
Tenderfoot Creek						1		2	1				
Meighn Creek	3	1		3	1			9			1		
Little Stawamus River		1			2		2						
Capilano		1				1							
Harrison River													
Various U.S.	8	77	88	97	54	70	12	78	71	13		40	50
Total Number of Fish in Sample	64	199	35	33	85	354	64	44	354	75	174	33	2

TABLE 10 (continued)

SPAWNING STREAM OF TAGGED COHO CAUGHT DURING COMMERCIAL FISHERY (% DISTRIBUTION)

Statistical Week	102			103			104	
Spawn Location / Catch	Johnstone	San Juan	West Coast	Johnstone	San Juan	West Coast	Johnstone	San Juan
VANCOUVER ISLAND								
Quinsam								
Puntleage River								
Rosewall Creek	21	16		20		50		25
Robertson Creek		2						
Big Qualicum	79	26		75		50	75	75
Nanaimo River								
Cowichan River								
MAINLAND								
Tenderfoot Creek		6		2			25	
Meighn Creek								
Little Stawamus River								
Capilano								
Harrison River								
Various U.S.	2	50	100	3		0	0	0
Total Number of Fish in Sample	24	67	2	60		2		8

TABLE 11

SPAWNING STREAM OF TAGGED CHINOOK CAUGHT DURING COMMERCIAL FISHERY (% DISTRIBUTION)

Statistical Week	92				93				94	101			
Spawn Location / Catch	Johnstone	San Juan	West Coast	River	Johnstone	San Juan	West Coast	River	San Juan	Johnstone	San Juan	West Coast	River
VANCOUVER ISLAND													
Quinsam													
Puntleage River	12									14	8		
Rosewall Creek													
Robertson Creek					5		24						
Big Qualicum	35	8			20					57	15		
Nanaimo River													
Cowichan River													
MAINLAND													
Tenderfoot Creek													
Meighn Creek													
Little Stawamus River													
Capilano	18	8			35					14		6	50
Harrison River	6	4									8	6	
Various U.S.	29	80	100	100	40	100	76	100	100	15	69	88	50
Total Number of Fish in Sample	17	25	14	1	20	6	17	5	8	7	13	16	2

TABLE 11 (continued)

SPAWNING STREAM OF TAGGED CHINOOK CAUGHT DURING COMMERCIAL FISHERY (% DISTRIBUTION)

Statistical Week	102			103		104			105
Spawn Location \ Catch	Johnstone	San Juan	West Coast	Johnstone	West Coast	Johnstone	San Juan	West Coast	West Coast
VANCOUVER ISLAND									
Quinsam									
Puntleage River	25			40		17			
Rosewall Creek									
Robertson Creek									
Big Qualicum	25			30		17	50		
Nanaimo River	25						10		
Cowichan River									
MAINLAND									
Tenderfoot Creek									
Meighn Creek									
Little Stawamus River									
Capilano	25			20		33	20		
Harrison River									
Various U.S.	0	100	100	10	100	33	20	100	100
Total Number of Fish in Sample	4	8	1	10	3	6	10	5	18

Figure 1

GONADOTROPIN CONTENT AS A FUNCTION OF COLLECTION DATE

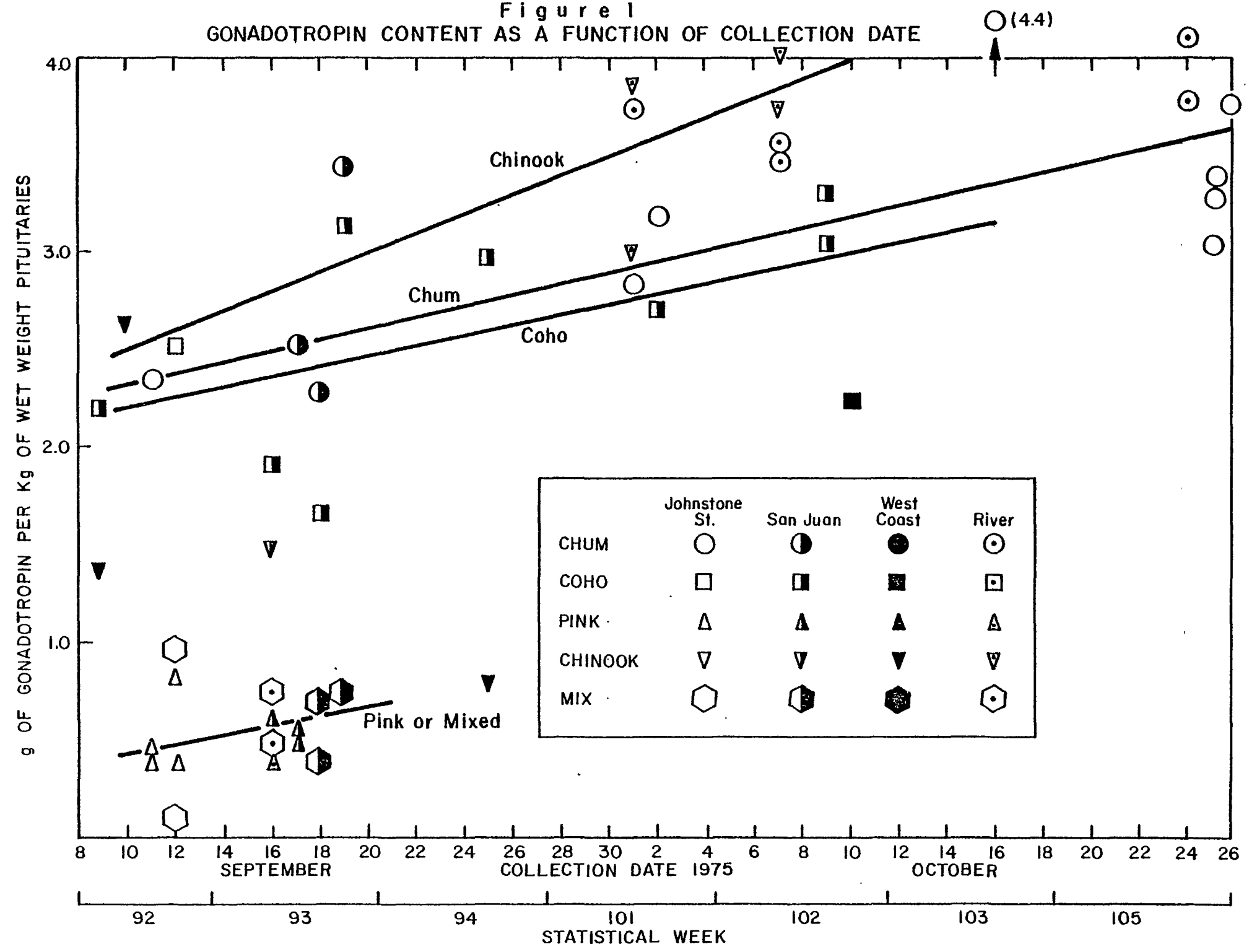
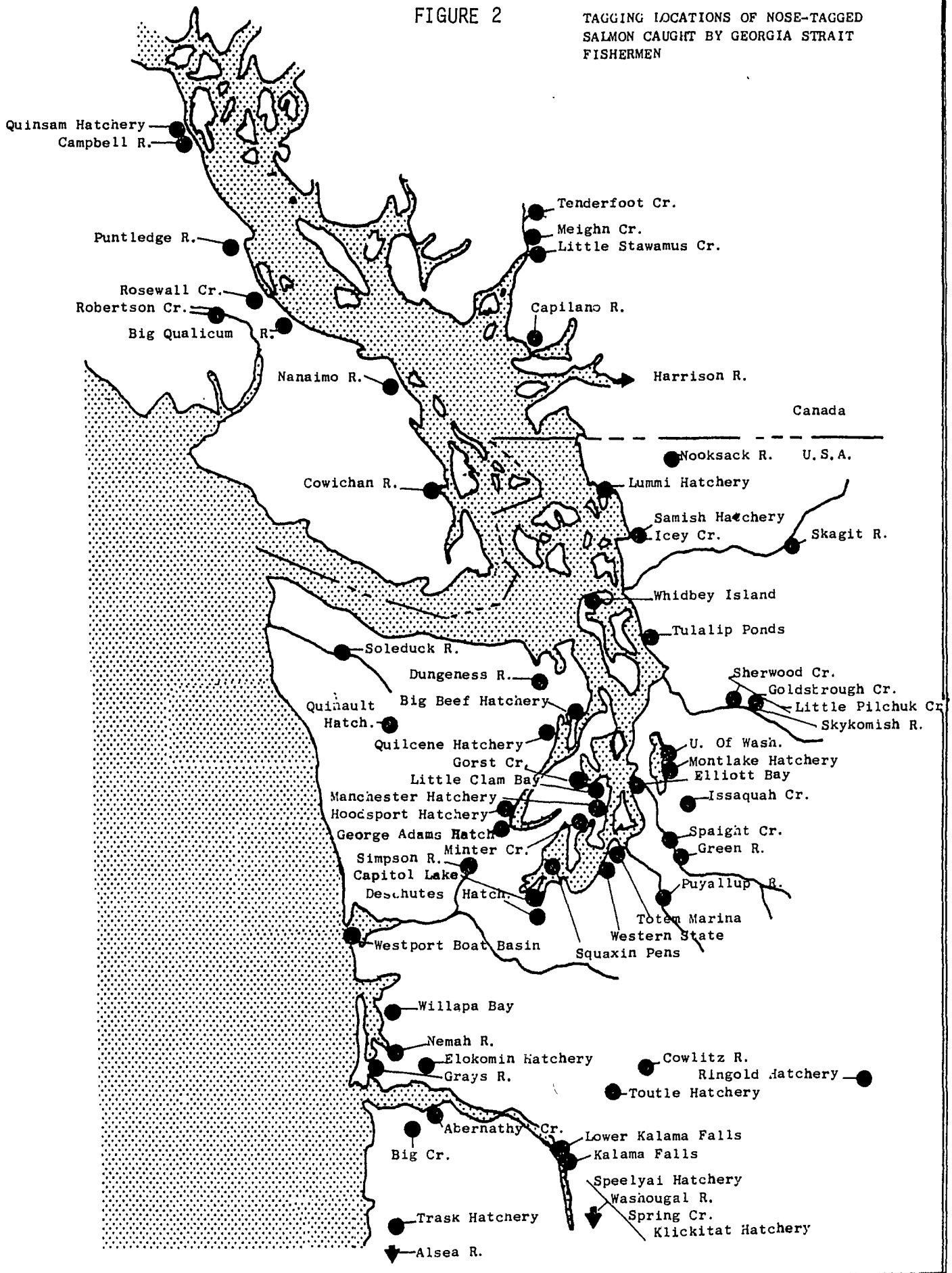
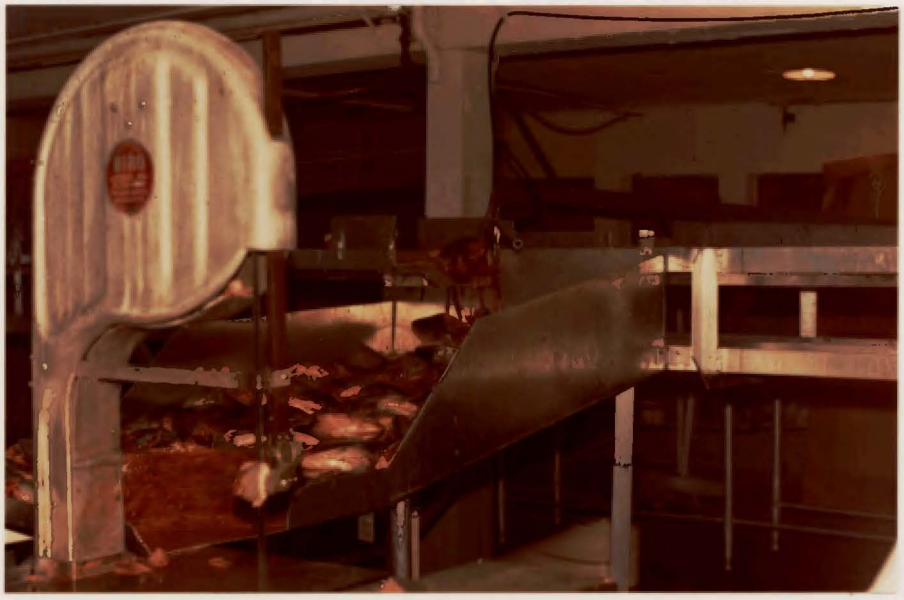


FIGURE 2

TAGGING LOCATIONS OF NOSE-TAGGED SALMON CAUGHT BY GEORGIA STRAIT FISHERMEN





PHOTOGRAPH 1

SALMON HEADS BEING DISTRIBUTED TO BAND SAWS (22597.26)

PHOTOGRAPH 2

SAW OPERATOR SLICING OFF THE TOP OF THE HEAD TO EXPOSE THE OPTIC NERVE (22597.10)

PHOTOGRAPH 3

THE PICKING STATION SHOWING BASKETS FOR RECEIVING CUT HEADS, AND THE COLLECTION DISH FOR PICKED PITUITARIES (22507.21 A)

PHOTOGRAPH 4

PICKER PREPARING TO REMOVE PITUITARY LOCATED IN CAVITY BELOW OPTIC NERVE (OPTIC NERVE HAS BEEN FLIPPED FORWARD) (27597.15)

PHOTOGRAPH 5

PITUITARIES IN COLLECTION DISH ON A BLOCK OF DRY ICE (22597.30)

PHOTOGRAPH 6

OVERALL VIEW OF THE COLLECTION OPERATION WITH USED HEADS IN BINS IN THE FOREGROUND (22507.11 A)

PHOTOGRAPH 7

FROZEN PITUITARIES STORED IN GLASS JARS OVER DRY ICE (22597.37)

PHOTOGRAPH 8

RELATIVE SIZE OF HEADS FROM PACIFIC SALMON (22597.6)

APPENDIX 1

1731

INTERNATIONAL DEVELOPMENT RESEARCH CENTRE
CENTRE DE RECHERCHES POUR LE DÉVELOPPEMENT INTERNATIONAL

March 18, 1976

Dr. D. W. Duncan
Associate Head
Division of Applied Biology
B.C. Research
3650 Westbrook Crescent
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V6S 2L2

Fish Pituitary Extracts (B.C. Research) 3-P-75-0103

Dear Doug:

This letter is to serve as confirmation of IDRC acceptance of Dr. David Idler's proposal for the assessment of the quantitative cross reaction and controlled administration of salmon gonadotropin to carp and other species as outlined in his letter of January 5th, to you. The project and funds will be administered by yourself and B.C. Research through the IDRC project, Fish Pituitary Extracts (B.C. Research) centre file # 3-P-75-0103.

Both Bert and I feel that this will be a very useful form of collaboration and look forward to obtaining results in the very near future.

Best Regards.

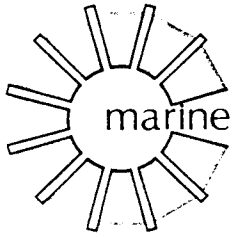
Yours sincerely,

FBD/llp

F. B. Davy
Programme Officer
(Fisheries)

cc: Robin Hallam

David R. Idler, D.F.C., Ph.D., F.R.S.C.
Director



marine sciences
research laboratory

RESEARCH PROPOSAL

ASSESSMENT OF THE QUANTITATIVE CROSS REACTION AND CONTROLLED ADMINISTRATION OF SALMON GONADOTROPIN TO CARP AND OTHER SPECIES

CROSS REACTIVITY OF THE GONADOTROPIN FROM MATURE SALMON (SG) WITH CARP AND OTHER ASIAN SPECIES

I. Recognition of the plasma and pituitary gonadotropin (GTH) of Asian fish species by antibodies directed against salmon and common carp GTH.

Samples required - plasma or serum frozen on dry ice from six fish of each sex (1 ml) plus a pooled sample of male plasma and another of female plasma 6 fish of each sex; total 5-15 ml of each sex. If shipment on dry ice is not possible lyophilized serum or plasma is the second choice. If neither preservation is possible then a preservative-merthiolate or sodium ajide- and chilled shipment is third choice. Require frozen eggs from one mature female carp.

Maturity of fish:

1. The fish should be in an advanced state of sexual maturity. If the fish can be sacrificed the weight of the gonad and the total weight of the fish to be recorded. If sacrifice is not possible the time to spawning should be estimated and size of the fish recorded. [If freezing facilities are available and if the fish can be sacrificed the pituitary glands should be frozen. If the fish can be sacrificed and pituitary glands cannot be frozen then acetone extracted and dry pituitaries is the second choice].

2. The fish be sexually immature - everything else as in (1) above.

Species:

To be selected by IDRC (six species of carp, milkfish etc.).

II. RECOGNITION BY TROUT GONADS OF THE GONADOTROPIN FROM SELECTED SE ASIA SPECIES (cAMP assay)

A. Samples required

Frozen (1st. choice) or acetone dried pituitaries (2nd. choice) collected from individual fish in an advanced state of sexual maturity. Prefer each pituitary be kept separate.

B. Species to be selected by IDAC and the sex and gonadosomatic index of the fish be recorded.

III. RECOGNITION BY GRASS CARP OF SALMON GONADOTROPIN AND CARP GONADOTROPIN

Obtain live immature grass carp and develop quantitative cAMP assay. Compare salmon and carp gonadotropin for relative potencies. Fish will be obtained from Lonoke Arkansas or other appropriate source.

IV. TEST SALMON GONADOTROPIN FOR STIMULATING MATURATION OF S.E. ASIA SPECIES (e.g., grass carp)

This aspect is tentative and will depend on our obtaining sexually mature grass carp.

V. ISOLATE CARP GONADOTROPIN (China Exchange) FROM MATURE AND VITELLOGENIC FISH.

Compare with GTH in salmonid and carp radioimmunoassay; cAMP assay in grass carp and trout; chick assay; test for grass carp ovulation (in vitro or in vivo depending on which proves to be most feasible)

VI. TO DETERMINE APPROPRIATE INJECTION SCHEDULE FOR HORMONE TREATMENT OF GRASS CARP

Grass carp will be injected intraperetonially with salmon gonadotropin and the plasma levels followed by serial blood sampling using the SG radio-immunoassay procedure. The results of these experiments will define how long a specified dose of salmon GTH will maintain plasma GTH at a level comparable to that found in sexually mature carp.