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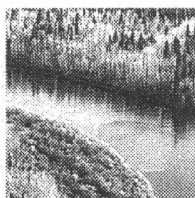


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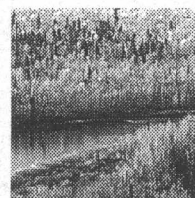
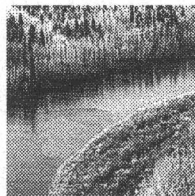
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by
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NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 13
**ANALYSES AND INTERPRETATION
OF STEROID HORMONES AND
GONAD MORPHOLOGY IN FISH
UPPER ATHABASCA RIVER, 1992**

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THARRELL

PREFACE:

The Northern River Basins Study was initiated through the "Canada-Alberta-Northwest Territories Agreement Respecting the Peace-Athabasca-Slave River Basin Study, Phase II - Technical Studies" which was signed September 27, 1991. The purpose of the Study is to understand and characterize the cumulative effects of development on the water and aquatic environment of the Study Area by coordinating with existing programs and undertaking appropriate new technical studies.

This publication reports the method and findings of particular work conducted as part of the Northern River Basins Study. As such, the work was governed by a specific terms of reference and is expected to contribute information about the Study Area within the context of the overall study as described by the Study Final Report. This report has been reviewed by the Study Science Advisory Committee in regards to scientific content and has been approved by the Study Board of Directors for public release.

It is explicit in the objectives of the Study to report the results of technical work regularly to the public. This objective is served by distributing project reports to an extensive network of libraries, agencies, organizations and interested individuals and by granting universal permission to reproduce the material.

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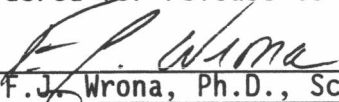
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Whereas the above publication is the result of a project conducted under the Northern River Basins Study and the terms of reference for that project are deemed to be fulfilled,

IT IS THEREFORE REQUESTED BY THE STUDY OFFICE THAT;

this publication be subjected to proper and responsible review and be considered for release to the public.


(Dr. F.J. Wrona, Ph.D., Science Director)

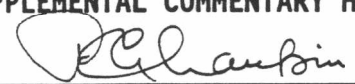
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Whereas it is an explicit term of reference of the Science Advisory Committee "to review, for scientific content, material for publication by the Board",

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this publication has been reviewed for scientific content and that the scientific practices represented in the report are acceptable given the specific purposes of the project and subject to the field conditions encountered.

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(Dr. P. A. Larkin, Ph.D., Chair)

11 June 93
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Whereas it is the duty of the Operations Committee to attend to the day-to-day management of the Study on behalf of the Study Board,

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THIS PUBLICATION HAS BEEN REVIEWED BY THE HEALTH ASSESSMENT COMMITTEE AND SUBSEQUENTLY FORWARDED TO APPROPRIATE HEALTH AUTHORITIES: ☐ Yes ☐ No

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this publication be released to the public, and that this publication be designated for: ☐ STANDARD AVAILABILITY ☐ EXPANDED AVAILABILITY


(Bev Burns, Co-chair)

10/6/93
(Date)


(Peter Melnychuk, Co-Chair)

22/06/93
(Date)

**ANALYSES AND INTERPRETATION OF STEROID HORMONES
AND GONAD MORPHOLOGY IN FISH
UPPER ATHABASCA RIVER, 1992**

STUDY PERSPECTIVE

The Northern River Basins Study is an investigation into the health of the Peace, Athabasca and Slave river aquatic ecosystem. Baseline information is scant and often times more qualitative than quantitative. Such is the case with the issue of fish health. Until recently, most attention has focused on the more visible and acute symptoms of fish health (eg., external condition - lesions and tumours), behaviour, and colour. These characteristics are less than ideal for monitoring the sub-lethal effects of contaminants and other stressors on fish.

New research has provided techniques for measuring and monitoring fish physiology in relation to different stressors (eg., contaminants) and habitat changes. Hormone levels associated with fish sexuality and reproduction have been shown to be sensitive indicators of stress from pulp mill effluents.

This report describes the results and interpretation of analytical findings of sex hormone levels and gonad maturation in four species of fish collected in the spring and fall of 1992 from the upper Athabasca River. The investigators' findings suggest that there are indications that mountain whitefish, northern pike, and suckers are exhibiting some signs of altered fish physiology. However, results are inconclusive and more work is recommended to confirm or refute these initial findings. Recommendations on the need to compare the project's findings with other investigations on the same fish specimen (eg., contaminant body burdens, liver enzyme activity) are being followed-up. On completion these additional comparisons will be used to guide any future collection and analysis to better understand the general health of the river fish populations and the likely effects this may have on the continued viability of fish populations within the Study area.

Related Study Questions

- 1a) *How has the aquatic ecosystem, including fish and/or other aquatic organisms, been affected by exposure to organochlorines or other toxic compounds?*
- 4a) *What are the contents and nature of the contaminants entering the system and what is their distribution and toxicity in the aquatic ecosystem with particular reference to water, sediments and biota?*
- 6) *What is the distribution and movement of fish species in the watersheds of the Peace, Athabasca and Slave river? Where and when are they most likely to be exposed to changes in water quality and where are their important habitats?*
- 8) *Recognizing that people drink water and eat fish from these river systems, what is the current concentration of contaminants in water and edible fish tissue and how are these levels changing through time and by location?*
- 12) *What native traditional knowledge exists to enhance the physical science studies in all areas of enquiry?*
- 13b) *What are the cumulative effects of man made discharges on the water and aquatic environment?*

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INTRODUCTION

Several recent studies indicate possible reproductive problems in fish exposed to bleached kraft pulp mill effluent (McMaster et al. 1991; Munkittrick et al. 1991, 1992a,b; Van der Kraak et al. 1992). These fish typically display lower circulating levels of steroids, reduced gonadal size, fewer secondary sexual features and delayed sexual maturity. The aquatic fauna in the Upper Athabaska River are also exposed to bleached kraft mill effluent from a mill located at Hinton, Alberta. The objective of the reported analyses was to examine reproductive indices in fish collected at sites upstream, near and downstream of effluent from the Hinton mill.

In accordance with the terms of reference for Project 2352-B1, fish samples from the 1992 Special Fish Collections in the Upper Athabaska River (Barton et al. 1992a,b) were analyzed for steroid hormones found in females (17 β -estradiol & testosterone) and males (testosterone & 11-ketotestosterone). For maturity, microscope slides of gonad tissues were prepared, gonads were staged and oocyte size-frequency histograms prepared. The numbers of vitellogenic oocytes in formaldehyde-fixed gonads were used to provide estimates of fecundity. This objective biochemical and histological approach provides sufficient detail such that subtle gonadal changes likely to be found over time in contaminated environments can be detected. The premise behind the monitoring approach is for early detection of pollutant effects.

METHODS

1. Fish Samples and Collection Sites

Plasma and gonad samples of mountain whitefish (*Prosopium williamsoni*), longnose sucker (*Catostomus catostomus*), white sucker (*Catostomus commersoni*) and northern pike (*Esox lucius*) were obtained from preselected sites on the Upper Athabaska River by Environmental Management Associates (EMA), Calgary, Alberta. Frozen plasma samples and preserved gonad tissues were subsequently sent to the Freshwater Institute for analyses of steroid hormones (females, 17 β -estradiol & testosterone; males, testosterone & 11-ketotestosterone) and histological assessments of sexual maturity and female fecundity.

Collection sites were preselected by the Northern River Basins Study Board and descriptions are detailed in the Special Fish Collection Reports (Barton et al. 1992a,b) provided by EMA. Designated sites were as follow:

1. Spring site A/fall site G - Near Entrance
2. Spring site B/fall site H - Weldwood Haul Bridge at Hinton
3. Spring site C/fall site J - Obed Mountain Coal Bridge

4. Spring site D/fall site K - Emerson Lakes Bridge
5. Spring site E/fall site L - Below Berland River confluence
6. Spring site F/fall site M - Windfall Bridge.

We analyzed samples of mountain whitefish and northern pike from both spring and fall collections. Longnose sucker and white sucker were only from the fall collection. Northern River Basins study sample numbers have been used in tables listing data. However, to avoid any confusion regarding location only the spring coding (A-F) has been retained to describe the collection sites in both spring and fall samples.

2. Steroid Hormone Assays

Prior to assay, duplicate plasma samples (250 μ L) were extracted in 2.5 mL of ethyl acetate:hexane (3:2, v/v). The dried extracts were redissolved in assay buffer (250 μ L). After appropriate dilution, aliquots of this redissolved extract were then used for either 17 β -estradiol, testosterone or 11-ketotestosterone analysis (see below). The percent recovery of hormones from each extracted sample was determined by addition of a mixture of 3 H-labelled steroid tracers (1500 cpm each of 17 β -estradiol, testosterone & 11-keto-testosterone) to every sample and counting an aliquot (25 μ L) of the redissolved extract by liquid scintillation counting. We have previously demonstrated that each hormone is extracted with nearly identical efficiency (We have also set aside a portion of the extract and if required we can confirm this chromatographically for any of the Special Fish Collection samples). Extraction efficiencies were $78.4 \pm 1.4\%$ (mean \pm SE) for the samples processed. Extraction efficiency did not differ between species or times. For calculating the final hormone concentration the extraction efficiency for each individual sample was used to correct for losses.

2.1. Plasma 17 β -Estradiol

An enzyme-immunoassay (EIA) was used to assess plasma estradiol. The coefficient of reactivity at 50% displacement (CR50%) of estradiol tracer was determined for each of 8 steroids (17 β -estradiol, 17 α -estradiol, estrone, estriol, progesterone, 17 α ,20 β -dihydroxy-4-pregnen-3-one, testosterone and cortisol). Steroids giving greater than 0.1 CR% with the estradiol antibody were: 17 β -estradiol (100), estrone (1.7), 17 α ,20 β -dihydroxy-4-pregnen-3-one (0.3) and testosterone (0.1). Intraassay coefficient of variation (CV), from 10 duplicate analysis of the same sample was 6.9%. Interassay CV of duplicate analysis from 10 assays was 9.8%. Recoveries of estradiol (0.25 - 2.0 ng/mL) added to mountain whitefish or longnose sucker plasma was $101.2 \pm 2.6\%$ (mean \pm SE). The minimum level of sensitivity, defined as that dose level 2 standard deviations away from the 0 dose measurement, averaged 0.004 ng/mL over 7 assays. Serial

dilutions of plasma extracts were parallel to the standard curves and gave estimates of hormone concentrations within 7%.

2.2 Plasma Testosterone

An enzyme-immunoassay (EIA) was used to determine plasma testosterone levels. The coefficient of reactivity at 50% displacement (CR50%) of testosterone tracer was determined for each of 8 steroids (11-ketotestosterone, testosterone, 11 β -hydroxytestosterone, androstenedione, cortisol, progesterone, 17 α ,20 β -dihydroxy-4-pregnen-3-one and estradiol). Steroids giving greater than 0.1 CR% with the testosterone antibody were: testosterone (100) 11-ketotestosterone (5.1), androstenedione (3.6) and 11 β -hydroxytestosterone (1.2). Intraassay coefficient of variation (CV), from 10 duplicate analysis of the same sample was 8.8%. Interassay CV of duplicate analysis from 10 assays was 10.9%. Recoveries of testosterone (0.63-2.5 ng/mL) added to fish plasma ranged from 91.7 to 103.8%. The minimum level of sensitivity, defined as that dose level 2 standard deviations away from the 0 dose measurement, averaged 0.002 ng/mL over 13 assays. Serial dilutions of plasma extracts were parallel to the standard curves and gave estimates of hormone concentrations within 5%.

2.3. Plasma 11-ketotestosterone

A radioimmunoassay (RIA) was used to assess plasma 11-ketotestosterone. RIA antibody was obtained from Helix Biotech and ³H-labelled 11-ketotestosterone was synthesized in-house from ³H-cortisol (Truscott 1981). The prepared 11-ketotestosterone tracer was purified by high-performance liquid chromatography prior to use.

The coefficients of reactivity at 50% displacement (CR50%) of 11-ketotestosterone tracer was determined for each of 8 steroids (11-ketotestosterone, testosterone, 11 β -hydroxytestosterone, androstenedione, cortisol, progesterone, 17 α ,20 β -dihydroxy-4-pregnen-3-one and estradiol). Steroids giving greater than 0.1 CR% with the 11-ketotestosterone antibody were: 11-ketotestosterone (100), testosterone (7.0%), 11 β -hydroxytestosterone (4.8%) and androstenedione (4.6%). Intraassay coefficient of variation (CV), from 10 duplicate analysis of the same sample was 9.2%. Interassay CV of duplicate analyses from 5 assays was 12.8%. Recoveries of 11-ketotestosterone (2.5-5.0 ng/mL) added to fish plasma ranged from 93.2 to 107.6 %. The minimum level of sensitivity, defined as that dose level 2 standard deviations away from the 0 dose measurement, averaged 0.35 ng/mL over 5 assays. Serial dilutions of plasma extracts were parallel to the standard curves and gave estimates of hormone concentrations within 6%.

3. Histology

Davidson's fixed tissues were dehydrated in n-butanol and embedded in paraffin. Tissue sections were cut at 8 μm and stained with Harris' hematoxylin and eosin. Testes were staged using a light microscope. Each ovary was also placed into one of five categories. For comparative purposes, gonadosomatic index (GSI) for each fish was calculated:

$$\text{GSI} = 100 * \text{GONAD WEIGHT} / [\text{TOTAL FISH WEIGHT} - \text{GONAD WEIGHT}]$$

3.1. Female Fish

The ovaries were categorized into one of five Groups (7 - 11) which are also found under the 'MATURITY INDEX' column in Tables 1 - 12. The groups are described below:

- Index 7 - those with only pre-vitellogenic oocytes, the largest having reached the yolk vesicle stage. In our histograms the frequency mode from 0 - 500 μm for whitefish, 0 - 700 μm for longnose sucker, 0 - 450 μm for white sucker and 0 - 300 μm for northern pike egg diameter corresponds to pre-vitellogenic oocytes (e.g. H-I-7, K-I-7, M-IV-1, M-IV-3, K-IV-8).
- Index 8 - those with only pre-vitellogenic oocytes, the largest at the yolk vesicle stage, plus a remarkable number of large resorbing eggs (e.g. H-I-8, H-I-10, K-I-3, K-I-4, L-IV-3, L-IV-7)
- Index 9 - those samples with a distinct vitellogenic clutch of developing oocytes plus a core of pre-vitellogenic resting oocytes
- Index 10 - those samples with a distinct vitellogenic clutch of mature oocytes plus a core of pre-vitellogenic resting oocytes
- Index 11 - ovulated fish, samples comprised almost exclusively of loose clutch oocytes; therefore clutch proportions are skewed

Fecundity estimates. Between 60 and 100 formaldehyde-fixed vitellogenic oocytes were teased out of the ovary tissue, lightly blotted and weighed. The associated connective tissue and pre-vitellogenic oocytes were also weighed to estimate their contribution to overall gonad weight. Absolute fecundity (number of eggs per fish) was estimated as:

$$\text{ABSOLUTE FECUNDITY} = [\text{GONAD WEIGHT} * \text{PROPORTION OF GONAD REPRESENTED BY VITELLOGENIC EGGS}] / \text{AVERAGE EGG WEIGHT}$$

Relative fecundity (eggs per gram of fish) was calculated as:

$$\text{RELATIVE FECUNDITY} = \frac{\text{ABSOLUTE FECUNDITY}}{[\text{TOTAL FISH WEIGHT} - \text{GONAD WEIGHT}]}$$

Oocyte diameters. The microscopic image of each ovary was projected onto a digitizing tablet and two diameter measurements were made on each oocyte to obtain an average diameter. Depending on oocyte size and variety within the ovaries, 75 to 250 eggs were measured for each fish. The mean diameters for the clutch oocytes were calculated from these measurements. Frequency distribution of oocyte diameters were prepared on histograms (e.g. Mayer et al. 1990) and plotted (see Appendices 1-6). From this data the percent of oocytes representing the clutch was calculated. For some samples, in which there were only large ovulated eggs (therefore no differential count), 50 caliper measurements were done on the formaldehyde fixed eggs and a correction factor applied for shrinkage associated with the embedding process.

The mean diameter of the clutch oocytes as measured from Davidson's fixed histological preparations was 70% the diameter of fixed (Davidson's or 5% formalin) but unprocessed eggs measured with Vernier calipers. The heat required to embed tissues in paraffin, and exposure to alcohol during dehydration combine to cause shrinkage. Our experience has shown that caliper measured, fixed white sucker oocytes are about 94% the value of fresh eggs (approx. 2.0 mm in diameter) measured in the field.

This translates into the presented clutch oocyte diameters being an estimated 66% of actual values (if fixation shrinkage is similar). For example our measured clutch diameters for fall mountain whitefish ranged from 1900 - 2222 μm . This would translate into actual diameters of 2.9 - 3.4 mm. Water-hardened eggs (which are increased in size over fresh eggs) from mountain whitefish in Montana averaged 3.7 mm (Brown 1952, cited in Scott and Crossman 1973).

3.2. Male Fish

The classification of fish testes using histological parameters determines the relationship between the maturation stage (6-10) assessments on whole organs determined by Barton et al. (1992a, 1992b) and the range of gonad organization (Stage 1 - 7). The histological stages we used are based on those for herring (*Clupea harengus* L.) as outlined by Bowers and Holliday (1961). Each testis is classified in the Tables 13-24 under the 'MATURITY STAGE' column.

In brief, the histological stages as applied to Northern River Basins fish can be described as follows:

- Stage 1
 - numerous large, spherical, primary germ-cells lying singly or in small groups
 - solitary germ cells about 15 μm in diameter
 - germ cells in groups are smaller
 - fibrous connective tissue organizing around the germ cells to form lobules
- Stage 2
 - the tunica is clearly defined
 - lobule formation is complete
 - groups of primary germ cells become progressively less common
 - primary and secondary cysts comprised of spermatogonia occurring in large numbers
 - cysts containing spermatocytes, spermatids and spermatozoa may be present
- Stage 3
 - all cell types mentioned above are present
 - relative numbers differ from 2, more cysts containing spermatocytes, spermatids and spermatozoa are present
 - lobules are wider than stage 2
- Stage 4
 - within sperm cysts spermatocytes mostly replaced by spermatids and spermatozoa
- Stage 5
 - lobules are tightly packed with spermatozoa, no cysts spermatocytes or spermatids present
- Stage 6
 - the 'ripe' or 'running' testis
 - absence of sperm from some lobules, walls thickened
- Stage 7
 - fibrous connective tissue thickened by contraction
 - tunica is thick and folded
 - distorted and collapsed lobules
 - relic sperm and cell debris in lobules

4. Statistics

Differences between groups of fish collected at each site for any given parameter were tested by one-way analysis of variance (ANOVA) computed using the Systat statistical package (Wilkinson et al. 1992). Comparison between gonad weight, egg weight, egg size, and absolute fecundity estimates were tested using analysis of covariance, with adjusted body weight (total weight - gonad weight) as the covariate. Pairwise comparisons were conducted by applying Tukey or Dunnett tests. A probability level of <0.05 was considered significant. Bartlett's test was applied to test for homogeneity of variance and, where necessary, data were log transformed to obtain more uniform variances. However, for clarity of presentation arithmetic means with standard errors have been used in the tables.

RESULTS

1. Female Fish

1.1. Longnose Suckers -- Fall Sample (Table 1 & 2; Appendix 1)

Results obtained for each female longnose sucker collected (N=31) are outlined in Table 1. The sample contained 6 female fish (H-IV-1, K-IV-8, L-IV-3, L-IV-7, M-IV-1 & M-IV-3) without clutch eggs (no vitellogenic oocytes) in the sample. These fish will not spawn in the spring and were omitted from statistical analyses. For maturing females site specific means and standard errors are summarized in Table 2.

There are very few comparative data for plasma steroid hormone levels in longnose suckers and as yet there has been no complete seasonal study. The plasma estradiol and GSI were similar but plasma testosterone levels we measured are somewhat higher than those reported by Munkittrick et al. (1992a) for longnose sucker collected in late September from Lake Superior.

Plasma estradiol was lower in fish from all sites downstream of site A. When compared to levels found in fish from site A, plasma testosterone values were lower in fish from site E.

The previtellogenic oocytes including yolk vesicle stage oocytes were usually <700 μ m in diameter. These oocytes and connective tissue portion of the ovary comprised 11% of total gonad weight and therefore a correction factor (clutch oocytes ranged from 85 to 93% of gonad weight) was applied for fecundity estimates. Absolute fecundity estimates for longnose sucker averaged $19,747 \pm 755$ (mean \pm SE) eggs per female (Table 1). This falls near the low end of the range (17,000 to 60,000 eggs per female) reported by Scott and Crossman (1973). The decline in egg size with downstream progression (site A to site F) was not statistically significant. Also, other reproductive parameters (GSI, egg weight, percent clutch eggs & fecundity estimates) did not differ between sites (Table 2).

Table 1. Physical characteristics (length, weight & gonad weight), plasma steroid hormones (E2 & Test) and reproductive indices (GSI, clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of each female longnose sucker collected during the fall 1992 Special Fish Collection. GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

Longnose Sucker Females -- Fall Sample													
Sample	Site	Length (mm)	Weight (g)	Gonad (g)	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (μ m)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (eggs/g)	Maturity Index
G-IV-1	A	375	690	53.7	8.44	1.01	0.57	2.3	1197	26.4	20780	32.7	10
G-IV-2	A	416	820	58.8	7.72	1.17	1.33	3.5	1352	28.3	14952	19.6	10
G-IV-5	A	412	870	84.6	10.77	1.13	9.09	3.1	1296	21.4	24288	30.9	10
G-IV-6	A	426	940	54.8	6.19	1.57	1.11	2.0	1134	29.1	24386	27.5	10
G-IV-8	A	388	760	54.8	7.77	0.96	9.62	2.7	1283	29.5	18064	25.6	10
G-IV-9	A	400	870	79.2	10.02	1.08	11.19	3.4	1252	39.5	20732	26.2	10
G-IV-10	A	378	630	45.9	7.86	1.05	9.90	1.8	1047	19.8	22695	38.9	10
H-IV-1	B	414	880	19.0	2.21	0.12	0.25	*	*	*	*	*	7
H-IV-5	B	412	960	74.1	8.36	1.02	1.89	2.8	1204	40.8	23553	26.6	10
H-IV-7	B	400	710	50.5	7.66	0.51	2.47	3.5	1232	17.0	12841	19.5	10
H-IV-8	B	400	830	60.9	7.92	0.28	1.02	3.0	1213	40.0	18067	23.5	10
J-IV-1	C	376	680	54.4	8.70	0.30	1.99	3.0	1176	28.5	16139	25.8	10
J-IV-2	C	406	835	76.9	10.14	0.64	3.37	2.7	1213	25.9	25349	33.4	10
J-IV-6	C	386	750	48.4	6.90	0.83	1.90	2.3	1158	23.3	18729	26.7	10
J-IV-10	C	408	810	75.4	10.26	0.09	3.59	2.9	1198	34.8	23140	31.5	10
K-IV-7	D	372	650	48.8	8.12	0.59	3.62	2.5	1065	15.3	17373	28.9	10
K-IV-8	D	374	640	9.7	1.54	0.04	0.03	*	*	*	*	*	7
L-IV-1	E	384	690	46.1	7.16	0.44	2.85	2.6	1078	26.9	15780	24.5	10
L-IV-2	E	442	1060	80.4	8.21	0.36	2.56	4.7	1411	20.3	15225	15.5	10
L-IV-3	E	380	620	6.6	1.08	0.09	0.02	*	*	*	*	*	8
L-IV-4	E	423	910	72.3	8.63	1.35	3.65	2.7	1178	28.7	23832	28.4	10
L-IV-7	E	385	680	7.8	1.16	0.06	0.02	*	*	*	*	*	8
L-IV-8	E	399	760	49.5	6.97	0.49	2.74	2.9	1202	39.2	15191	21.4	10
L-IV-9	E	412	800	46.8	6.21	0.51	0.81	2.4	1119	26.4	17355	23.0	10
L-IV-10	E	414	810	41.4	5.39	0.43	0.95	1.7	1044	16.5	21674	28.2	10
M-IV-1	F	407	830	7.2	0.88	0.08	0.06	*	*	*	*	*	7
M-IV-3	F	409	880	11.7	1.35	0.03	0.03	*	*	*	*	*	7
M-IV-4	F	403	940	77.9	9.04	0.18	5.10	3.2	1212	19.6	21666	25.1	10
M-IV-5	F	417	840	55.2	7.03	0.25	1.40	3.0	1110	28.0	16376	20.9	10
M-IV-6	F	376	730	53.4	7.89	0.54	4.46	2.4	1100	32.1	19803	29.3	10
M-IV-7	F	427	975	99.1	11.31	0.20	4.14	3.4	1177	36.3	25941	29.6	10
* - Immature, no clutch eggs present													

Table 2. GSI, plasma steroid hormones (E2 & Test) and reproductive indices (clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of female longnose sucker collected during the fall 1992 Special Fish Collection. Values represent mean and SE of mature fish from each site. Shaded cells indicate means significantly different from the corresponding value for fish from site A (Dunnett, $P < 0.05$). GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

Longnose Sucker Females -- Fall Sample										
Site	N	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (#/g)	Maturity Index *
A	7	8.40	1.14	6.12	2.69	1223	27.7	20842	28.8	10
		0.58	0.08	1.82	0.26	40	2.4	1294	2.3	
B	3	7.98	0.60	1.79	3.10	1216	32.6	18154	23.2	10
		0.21	0.12	0.42	0.21	8	7.8	3093	2.1	
C	4	9.00	0.47	2.71	2.73	1186	28.1	20839	28.6	10
		0.79	0.17	0.45	0.15	12	2.5	2085	2.4	
D	1	8.12	0.59	3.62	2.50	1065	15.3	17373	28.9	10
E	6	7.09	0.60	2.26	2.83	1172	26.3	18176	23.5	10
		0.49	0.15	0.46	0.41	54	3.2	1508	2.0	
F	4	8.82	0.29	3.78	3.00	1150	29.0	20946	26.2	10
		0.93	0.08	0.82	0.22	27	3.6	1993	2.1	
* - Immature fish not included.										

1.2. Mountain Whitefish -- Fall Sample (Table 3 & 4; Appendix 2)

Analytical results for each female mountain whitefish collected (N=45) are detailed in Table 3. There were 6 immature fish (H-I-7, H-I-8, H-I-10, K-I-3, K-I-4 & K-I-7) lacking clutch eggs. There were 8 fish collected at sites E and L which had ovulated and contained loose eggs. In these fish, accurate estimates of percent clutch and fecundity cannot be obtained because possible spawned eggs or those lost due to handling are unaccountable. As only unovulated fish were collected at upstream site A, only prespawning fish were used in statistical comparisons. The immature and ovulated fish were not uniformly distributed between sites and lack sufficient numbers for statistical analysis. Site specific means and standard errors for preovulatory fish are summarized in Table 4.

The only steroid hormone measurements we are aware of for mountain whitefish are unpublished data from the Procter and Gamble study on the Smokey/Wapiti system (Klopper-Sams and Benton 1992). The samples were obtained in May-June and are not comparable to fish sampled in the fall. Plasma steroid levels in the near spawning mountain whitefish collected in the fall are somewhat higher than those reported for lake whitefish collected in August (Munkittrick et al. 1992b).

When compared to fish collected from site A, plasma estradiol levels were depressed in fish obtained from the downstream sites C, D, E and F. However, conclusions about the cause of the reduced estrogen levels must be tempered because hormone levels are much lower in ovulated fish (Table 3).

Table 3. Physical characteristics (length, weight & gonad weight), plasma steroid hormones (E2 & Test) and reproductive indices (GSI, clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of each female mountain whitefish collected during the fall 1992 Special Fish Collection. GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

Mountain Whitefish Females -- Fall Sample													
Sample	Site	Length (mm)	Weight (g)	Gonad (g)	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (eggs/g)	Maturity Index
G-I-3	A	383	740	117.1	18.80	3.958	17.13	12.66	1994	52.7	9250	14.8	10
G-I-5	A	360	510	53.7	11.77	2.986	27.51	11.64	1928	56.1	4613	10.1	10
G-I-6	A	366	580	73.0	14.40	1.317	27.07	12.82	2082	38.8	5694	11.2	10
G-I-8	A	325	390	56.3	16.87	0.529	22.84	16.58	2088	45.1	3396	10.2	10
G-I-9	A	405	695	75.6	12.21	3.787	23.89	11.08	1997	40.7	6823	11.0	10
H-I-1	B	382	720	161.7	28.96	2.793	24.61	13.26	1987	43.9	12195	21.8	10
H-I-2	B	401	790	59.2	8.10	0.733	19.88	13.24	1965	40.6	4471	6.1	10
H-I-3	B	440	1100	158.4	16.82	1.216	19.56	12.28	2063	42.2	12899	13.7	10
H-I-4	B	413	820	111.4	15.72	0.557	7.96	14.46	2185	49.2	7704	10.9	10
H-I-5	B	402	870	109.0	14.32	1.165	13.90	13.48	2075	41.2	8086	10.6	10
H-I-6	B	314	360	4.9	*	2.153	9.77	12.16	1907	*	*	*	10
H-I-7	B	321	375	0.7	0.19	0.029	0.01	**	**	**	**	**	7
H-I-8	B	315	360	2.1	0.59	0.028	0.01	**	**	**	**	**	8
H-I-10	B	469	1220	3.9	0.32	0.030	0.04	**	**	**	**	**	8
J-I-2	C	377	675	99.9	17.37	0.237	18.94	18.44	2200	27.9	5418	9.4	10
J-I-4	C	385	745	110.9	17.49	0.429	16.56	13.98	1957	66.7	7933	12.5	10
J-I-5	C	358	570	67.9	13.52	1.295	27.68	14.28	1993	40.5	4755	9.5	10
J-I-6	C	353	580	36.5	6.72	0.184	12.98	15.16	2135	15.5	2408	4.4	10
J-I-7	C	344	480	46.1	10.62	0.515	41.84	15.7	2051	30.3	2936	6.8	10
J-I-8	C	416	900	110.6	14.01	2.447	13.52	12.48	1933	49.5	8862	11.2	10
J-I-9	C	315	365	40.1	12.34	1.193	7.73	13.38	1992	44.3	2997	9.2	10
J-I-10	C	340	510	56.7	12.51	0.537	24.69	16.02	2054	35.3	3539	7.8	10
K-I-1	D	381	740	85.1	12.99	0.334	42.57	17.34	2000	40.5	4908	7.5	10
K-I-2	D	329	430	55.6	14.85	0.196	7.89	15.98	2222	43.2	3479	9.3	10
K-I-3	D	324	380	1.4	0.37	0.004	0.25	**	**	**	**	**	8
K-I-4	D	379	640	1.8	0.28	0.002	0.03	**	**	**	**	**	8
K-I-5	D	322	425	56.7	15.40	0.458	6.99	12.98	1947	46.7	4368	11.9	10
K-I-6	D	444	1160	192.2	19.86	0.138	13.39	17.32	2110	60.6	11097	11.5	10
K-I-7	D	358	530	1.7	0.32	0.015	0.05	**	**	**	**	**	7
K-I-9	D	346	540	89.0	19.73	0.314	4.34	15.28	2029	48.7	5825	12.9	10
K-I-11	D	352	600	72.3	13.70	0.178	47.69	17.46	2109	40.2	4141	7.8	10
L-I-2	E	319	450	41.9	10.27	0.049	0.56	14.5	1995	***	***	***	11
L-I-3	E	382	710	74.6	11.74	0.022	0.34	16.3	2094	***	***	***	11
L-I-4	E	423	860	104.9	13.89	0.002	0.52	14.3	1994	***	***	***	11
L-I-5	E	379	720	111.5	18.32	0.393	5.55	17.36	1912	43.0	6423	10.6	10
L-I-6	E	415	805	58.6	7.85	0.019	0.19	13.94	1952	***	***	***	11
L-I-7	E	426	1010	110.5	12.28	0.072	5.26	17.88	2184	33.0	6180	6.9	10
L-I-8	E	407	790	109.9	16.16	0.189	17.34	17.88	2012	35.1	6147	9.0	10
L-I-9	E	409	860	124.8	16.97	0.002	0.31	18.22	2185	***	***	***	11
L-I-10	E	338	485	55.7	12.97	0.013	0.60	15.78	2011	***	***	***	11
M-I-2	F	378	560	33.0	6.26	0.017	0.29	17.54	2089	***	***	***	11
M-I-4	F	399	680	40.0	6.25	0.019	0.22	15.84	2043	***	***	***	11
M-I-7	F	400	840	131.2	18.51	0.500	9.73	17.46	2074	50.9	7514	10.6	10
M-I-8	F	450	1280	211.1	19.75	0.626	4.72	19.48	2016	48.1	10837	10.1	10
M-I-9	F	362	625	72.6	13.14	0.223	6.90	17.96	1949	31.8	4042	7.3	10
* - Gonad weight appears incorrect in Fish Collection Report.													
** - Immature, no clutch eggs present.													
*** - Because these fish had ovulated, accurate estimates of % clutch and fecundity cannot be obtained.													

Table 4. GSI, plasma steroid hormones (E2 & Test) and reproductive indices (clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of female mountain whitefish collected during the fall 1992 Special Fish Collection. Values represent mean and SE of mature fish from each site. Shaded cells indicate means significantly different from the corresponding value for fish from site A (Dunnett, $P < 0.05$). GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

Mountain Whitefish Females -- Fall Sample										
Site	N	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (eggs/g)	Maturity Index *
A	5	14.81	2.52	23.69	12.96	2018	46.68	5955	11.48	10
		1.35	0.62	1.87	0.96	30	3.36	1001	0.87	
B	6	16.79	1.44	15.95	13.15	2030	43.42	9071	12.63	10
		3.40	0.35	2.64	0.35	40	1.55	1556	2.60	
C	8	13.07	0.85	20.49	14.93	2039	38.75	4856	8.86	10
		1.24	0.27	3.81	0.65	32	5.46	853	0.89	
D	6	16.09	0.27	20.48	16.06	2070	46.65	5636	10.15	10
		1.22	0.05	7.91	0.71	40	3.11	1138	0.92	
E	3	15.59	0.22	9.39	17.71	2036	37.03	6250	8.82	10
		1.77	0.09	3.98	0.17	79	3.04	87	1.07	
F	3	17.13	0.45	7.12	18.30	2013	43.60	7464	9.35	10
		2.03	0.12	1.45	0.61	36	5.96	1962	1.03	
* - Immature and ovulated fish not included.										

The pre-vitellogenic oocytes were $< 500 \mu\text{m}$ in diameter. These oocytes and connective tissue weight component of fall mountain whitefish ovaries contributed little to overall gonad weight and were considered to be negligible. Therefore no correction factor was applied to fecundity estimates. Reproductive parameters (GSI, egg size, percent clutch eggs & fecundity estimates) did not differ between sites. Averaged over all collection sites (A-F), relative fecundity was 10.2 eggs/g fish. In other studies (cited by Scott and Crossman 1973), relative fecundity estimates for mountain whitefish averaged 11 eggs/g fish while absolute fecundity was 5000 eggs/fish.

1.3. White Sucker -- Fall Sample (Table 5 & 6; Appendix 3)

Analytical results for each female white sucker collected (N=19) are outlined in Table 5. The vitellogenic egg component of these ovaries was identical, 89% of gonad weight, to that of fall longnose sucker and this value was applied to all females for fecundity estimates. There were no gonadal tissue samples for fish H-III-1 and K-III-3. Site specific means and standard errors are summarized in Table 6. Due to the low numbers of fish captured at upstream sites few site specific conclusions are possible. There were no statistical differences between the downstream sites D, E and F.

The observed levels of steroid hormones (Table 6) were similar to those found in fall collections (Sept.) of white sucker from Lake

Superior (Munkittrick et. al. 1992) and from the small pristine lakes at Experimental Lakes Area (ELA) in Northwestern Ontario (Brown and Evans, unpublished). Absolute fecundity of Athabaska River white suckers (24-36,000; Table 6) falls within cited ranges. Scott and Crossman (1973) give an absolute fecundity range of 20-50,000 eggs per female. Relative fecundity at approximately 37-40 eggs/g fish is greater than that reported (25 eggs/g) by Scott and Crossman (1973). White sucker from the Experimental Lakes Area (ELA) in spring have absolute fecundities slightly above 20,000 and relative fecundities around 20.0 eggs/g fish. The pre-vitellogenic oocytes were generally less than 450 μm in diameter. Fall egg diameter from ELA average about 1.0 mm or 1.5 mm actual size after correction for processing shrinkage. This is almost the same size as Athabaska River samples.

Table 5. Physical characteristics (length, weight & gonad weight), plasma steroid hormones (E2 & Test) and reproductive indices (GSI, clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of each female white sucker collected during the fall 1992 Special Fish Collection. GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

White Sucker Females -- Fall Sample													
Sample	Site	Length (mm)	Weight (g)	Gonad (g)	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (μm)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (eggs/g)	Maturity Index
H-III-2	B	388	960	56.2	6.22	1.186	0.445	1.5	1189	23.5	33796	37.4	10
K-III-1	D	393	825	52.6	6.81	1.442	0.871	1.9	1165	10.4	24769	32.1	10
K-III-2	D	340	550	36.8	7.17	2.658	0.806	1.6	1106	11.6	20217	39.4	10
K-III-4	D	370	650	43.5	7.17	1.342	0.545	1.3	1017	13.0	28892	47.6	10
L-III-2	E	397	820	49.5	6.42	0.622	0.192	1.5	1034	13.6	29567	38.4	10
L-III-3	E	389	820	55.2	7.22	1.507	0.453	1.5	1074	15.4	32972	43.1	10
L-III-4	E	425	1105	48.4	4.58	0.942	0.151	1.5	1055	13.5	28339	26.8	10
L-III-6	E	410	950	57.2	6.41	1.472	0.518	1.5	1039	26.2	33273	37.3	10
L-III-8	E	395	800	57.2	7.70	1.069	0.535	1.9	1196	15.6	26515	35.7	10
L-III-9	E	390	820	57.9	7.60	0.263	0.420	1.6	1064	12.4	33246	43.6	10
L-III-10	E	435	1090	54.7	5.28	1.381	0.313	1.0	909	6.3	47265	45.7	10
M-III-1	F	430	1110	76.7	7.42	0.629	0.105	1.8	1102	16.7	38136	36.9	10
M-III-2	F	408	920	59.8	6.95	1.112	0.155	1.5	1044	20.8	35014	40.7	10
M-III-3	F	399	835	39.3	4.94	0.566	0.115	1.4	1002	18.6	24459	30.7	10
M-III-4	F	380	710	47.2	7.12	0.954	0.149	1.5	1077	22.5	27456	41.4	10
M-III-5	F	406	980	69.0	7.57	0.803	0.437	2.0	1239	31.5	30705	33.7	10
M-III-8	F	431	1020	74.3	7.86	0.733	0.236	1.3	1026	11.9	49720	52.6	10
M-III-9	F	406	960	67.8	7.60	1.213	0.314	1.7	1089	20.8	36351	40.7	10
M-III-10	F	446	1170	103.2	9.67	2.017	0.541	1.9	1159	48.2	48088	45.1	10

Table 6. GSI, plasma steroid hormones (E2 & Test) and reproductive indices (clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of female white sucker collected during the fall 1992 Special Fish Collection. Values represent mean and SE of mature fish from each site. GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

White Sucker Females -- Fall Sample										
Site	N	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (μ m)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (eggs/g)	Maturity Index
B	1	6.22	1.186	0.445	1.48	1189	23.5	33795	37.4	10
D	3	7.05	1.814	0.741	1.62	1096	11.7	24626	39.7	10
		0.12	0.423	0.100	0.16	43	0.8	2505	4.5	
E	7	6.46	1.037	0.369	1.50	1053	14.7	33025	38.7	10
		0.45	0.177	0.058	0.10	32	2.2	2578	2.4	
F	8	7.39	1.003	0.257	1.65	1092	23.9	36241	40.2	10
		0.46	0.165	0.057	0.08	27	4.0	3201	2.4	

1.4. Northern Pike -- Fall Sample (Table 7 & 8; Appendix 4)

Results obtained for each female northern pike collected (N=12) are outlined in Table 7. The sample contained 2 immature female fish (H-II-1 & L-II-1) without clutch eggs (no vitellogenic oocytes) in the sample. These fish will not spawn in the spring. The vitellogenic egg component of maturing ovaries was estimated to be 77.4% (66-84%) of gonad weight and this value was applied to all fish when calculating fecundity estimates. The one exception to this was M-II-4 where a considerable number of large resorbing oocytes (carryovers from spring spawn) increased the non-clutch component to 44% of gonad weight (clutch eggs being 56%). For maturing females site specific means and standard errors are summarized in Table 2.

To our knowledge there are no published data on plasma steroid hormone levels in northern pike from North America. There has been some limited studies on European stains (Simontacchi et al. 1983) but as yet there is no comprehensive information. The plasma estradiol levels were high (>5 ng/mL) indicating that exogenous vitellogenesis was well underway. Plasma testosterone levels were lower than estradiol and similar to that found in white sucker (see Table 6).

The pre-vitellogenic oocytes were generally less than 300 μ m in diameter. Clutch eggs were approximately 1.0 mm at this time. With the exception of the single fish taken at site D, the absolute fecundity estimates were similar to cited levels (32,000; Scott and Crossman 1973). The relative fecundity (approx. 16 eggs/g) of Athabaska River fish is low but near some other reported values (Scott and Crossman 1973; Treasurer 1990).

Table 7. Physical characteristics (length, weight & gonad weight), plasma steroid hormones (E2 & Test) and reproductive indices (GSI, clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of each female northern pike collected during the fall 1992 Special Fish Collection. GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

Northern Pike Females -- Fall Sample													
Sample	Site	Length (mm)	Weight (g)	Gonad (g)	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (μ m)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (eggs/g)	Maturity Index
H-II-1	B	485	825	2.8	0.34	0.323	0.040	*	*	*	*	*	7
K-II-2	D	565	1260	43.3	3.56	7.426	0.349	4.12	1135	17.8	8135	6.7	10
L-II-1	E	438	620	2.4	0.39	0.014	0.425	*	*	*	*	*	7
L-II-3	E	876	5135	286.4	5.91	7.020	0.357	1.95	986	5.6	113679	23.4	10
L-II-7	E	636	2090	66.5	3.29	6.572	0.612	2.07	1124	12.1	24865	12.3	10
L-II-8	E	562	1370	35.4	2.65	7.507	0.250	1.55	892	6.8	17677	13.2	10
M-II-1	F	558	1280	55.4	4.52	8.975	0.266	2.03	1123	7.0	21123	17.2	10
M-II-2	F	766	3450	111.7	3.35	5.101	0.487	1.98	1032	10.1	43665	13.1	10
M-II-4	F	603	1610	55.4	3.56	6.215	0.671	1.20	855	12.7	25900	16.7	10
M-II-6	F	700	2650	84.7	3.30	6.672	0.283	1.92	1075	10.8	34145	13.3	10
M-II-7	F	730	2860	123.1	4.50	1.882	0.295	1.43	948	8.3	66629	24.3	10
M-II-8	F	706	2780	87.2	3.24	13.431	0.494	1.82	946	19.5	37084	13.8	10

* - Immature, no clutch oocytes.

Table 8. GSI, plasma steroid hormones (E2 & Test) and reproductive indices (clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of female northern pike collected during the fall 1992 Special Fish Collection. Values represent mean and SE of mature fish from each site. GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

Northern Pike Females -- Fall Sample										
Site	N	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (μ m)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (eggs/g)	Maturity Index *
D	1	3.56	7.426	0.349	4.12	1135	17.8	8134	6.7	10
E	3	3.95	7.033	0.406	1.86	1001	8.2	52073	16.3	10
		1.00	0.270	0.107	0.16	67	2.0	30872	3.6	
F	6	3.75	7.046	0.416	1.73	997	11.4	38090	16.4	10
		0.25	1.589	0.066	0.14	40	1.8	6579	1.7	

* - Immature fish not included.

1.5. Mountain Whitefish -- Spring Sample (Table 9 & 10; Appendix 5)

Analytical results for each female mountain whitefish collected in the spring (N=46) are outlined in Table 9. The spring sample displayed uniform gonad development and all fish contained developing clutch eggs. Site specific means and standard errors are summarized in Table 10.

The low plasma hormone levels in the fish collected in May were similar to unpublished data from the Procter and Gamble study on the Smokey/Wapiti system (Klopper-Sams and Benton 1992). Also, plasma steroid levels in the Athabaska fish were lower than levels reported for lake whitefish collected in August (Munkittrick et al. 1992b).

Table 9. Physical characteristics (length, weight & gonad weight), plasma steroid hormones (E2 & Test) and reproductive indices (GSI, clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of each female mountain whitefish collected during the spring 1992 Special Fish Collection. GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

Mountain Whitefish Females -- Spring Sample													
Sample	Site	Length (mm)	Weight (g)	Gonad (g)	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (eggs/g)	Maturity Index
A-I-1	A	314	325	3.0	0.93	0.011	0.081	0.37	651	26.2	6122	19.0	9
A-I-3	A	446	841	8.0	0.96	0.096	0.046	0.30	595	53.5	18972	22.8	9
A-I-4	A	292	308	3.0	0.98	0.233	0.120	0.58	708	23.7	4147	13.6	9
A-I-5	A	298	290	3.0	1.05	0.219	0.235	0.56	521	27.2	4891	17.0	9
A-I-6	A	366	531	5.0	0.95	0.326	0.377	0.48	669	48.7	7353	14.0	9
A-I-8	A	413	795	8.0	1.02	0.524	0.101	0.52	700	35.1	13260	16.8	9
A-I-10	A	314	360	4.0	1.12	0.172	0.006	0.66	814	16.8	5505	15.5	9
B-I-1	B	292	309	2.0	0.65	0.077	0.025	0.79	667	25.9	2003	6.5	9
B-I-2	B	285	310	3.0	0.98	0.099	0.036	0.65	782	20.8	3564	11.6	9
B-I-3	B	459	1199	15.0	1.27	0.184	0.140	0.94	923	29.0	13158	11.1	9
B-I-4	B	402	819	13.0	1.61	0.190	0.052	1.04	929	32.5	10331	12.8	9
B-I-5	B	283	291	5.0	1.75	0.146	0.023	0.94	871	31.1	4630	16.2	9
B-I-6	B	372	674	10.0	1.51	0.106	0.057	1.08	869	33.3	8322	12.5	9
B-I-7	B	328	472	6.0	1.29	0.075	0.032	1.19	896	31.1	4768	10.2	9
B-I-9	B	320	400	8.0	2.04	0.509	0.248	0.87	853	25.2	7668	19.6	9
B-I-10	B	375	560	7.0	1.27	0.085	0.009	0.68	692	29.1	7368	13.3	9
B-I-11	B	357	526	8.0	1.54	0.140	0.008	0.77	814	36.4	8013	15.5	9
B-I-12	B	418	961	8.0	0.84	0.049	0.011	0.80	736	23.5	8163	8.6	9
C-I-1	C	401	1029	19.0	1.88	0.386	0.361	1.26	935	40.3	13149	13.0	9
C-I-2	C	420	1030	14.0	1.38	0.318	0.262	0.79	862	46.3	13376	13.2	9
C-I-3	C	421	1060	8.0	0.76	0.249	0.473	0.66	724	40.0	8649	8.5	9
C-I-5	C	385	550	7.0	1.29	0.298	0.270	0.65	793	40.7	7500	13.8	9
C-I-6	C	313	435	7.0	1.64	0.848	0.390	0.95	913	29.4	6250	14.6	9
C-I-7	C	297	341	2.0	0.59	0.022	0.044	0.26	461	19.7	4800	14.2	9
C-I-8	C	385	599	8.0	1.35	0.198	0.198	0.52	686	38.8	12565	21.3	9
C-I-10	C	414	812	12.0	1.50	0.241	0.188	0.66	800	31.0	14907	18.6	9
D-I-2	D	305	361	9.0	2.56	0.198	0.163	0.90	814	35.5	8282	23.5	9
D-I-3	D	329	403	5.0	1.26	0.227	0.168	0.50	699	21.1	7299	18.3	9
D-I-5	D	433	920	12.0	1.32	0.104	0.109	0.84	856	31.1	11250	12.4	9
D-I-6	D	433	1038	11.0	1.07	0.308	0.378	0.74	790	29.6	12360	12.0	9
D-I-7	D	320	415	2.0	0.48	0.023	0.039	0.51	532	22.1	2892	7.0	9
D-I-8	D	395	812	10.0	1.25	0.236	0.200	0.85	746	25.5	9950	12.4	9
D-I-9	D	350	602	8.0	1.35	0.229	0.160	0.99	868	33.1	7385	12.4	9
D-I-10	D	356	570	8.0	1.42	0.134	0.212	0.75	771	24.0	9178	16.3	9
E-I-2	E	453	1173	12.0	1.03	0.178	0.206	0.45	628	16.4	19565	16.9	9
E-I-3	E	342	468	4.0	0.86	0.160	0.194	0.45	689	18.5	6557	14.1	9
E-I-4	E	392	715	5.0	0.70	0.106	0.117	0.51	682	30.0	6565	9.2	9
E-I-5	E	354	491	3.0	0.61	0.027	0.048	0.30	515	14.2	6429	13.2	9
E-I-8	E	412	932	9.0	0.98	0.083	0.751	0.52	636	22.0	13300	14.4	9
E-I-9	E	459	952	21.0	2.26	0.428	0.424	0.66	835	33.9	25050	26.9	9
F-I-2	F	316	428	3.0	0.71	0.039	0.369	0.38	633	11.1	5844	13.8	9
F-I-5	F	333	472	5.0	1.07	0.075	0.593	0.32	585	13.4	8380	17.9	9
F-I-6	F	347	465	3.0	0.65	0.049	0.164	0.33	635	13.5	6923	15.0	9
F-I-7	F	338	497	4.0	0.81	0.031	0.282	0.34	622	15.3	8108	16.4	9
F-I-8	F	302	371	11.0	3.06	0.246	0.389	0.46	693	13.1	14634	40.7	9
F-I-10	F	384	722	4.0	0.56	0.229	0.333	0.31	599	27.5	10526	14.7	9

Table 10. GSI, plasma steroid hormones (E2 & Test) and reproductive indices (clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of female mountain whitefish collected during the spring 1992 Special Fish Collection. Values represent mean and SE of mature fish from each site. Means sharing an alphabetical superscript were not different (Tukey, $P < 0.05$). Shaded cells indicate means significantly different from the corresponding value for fish from site A (Dunnett, $P < 0.05$). GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

Mountain Whitefish Females -- Spring Sample										
Site	N	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (μ m)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (eggs/g)	Maturity Index
A	7	1.00	0.225	0.138 ^{ab}	0.49	666	33.03	8607	16.96	9
		0.03	0.063	0.048	0.05	35	5.12	2071	1.20	
B	11	1.34	0.151	0.058 ^a	0.89	821	28.90	7090	12.54	9
		0.12	0.038	0.022	0.05	27	1.40	958	1.10	
C	8	1.30	0.320	0.273 ^b	0.72	772	35.78	10149	14.64	9
		0.15	0.084	0.048	0.11	54	3.00	1343	1.36	
D	8	1.34	0.182	0.179 ^{ab}	0.76	760	27.75	8574	14.31	9
		0.20	0.032	0.034	0.06	38	1.88	1028	1.77	
E	6	1.07	0.163	0.290 ^b	0.48	664	27.50	12911	15.79	9
		0.24	0.057	0.106	0.05	43	3.21	3238	2.44	
F	6	1.14	0.111	0.355 ^b	0.36	628	15.65	9069	19.74	9
		0.39	0.040	0.058	0.02	15	2.43	1284	4.23	

Although plasma testosterone levels were lowest at site B, values were not different from those at site A. Testosterone concentrations at sites C, D, E and F were significantly higher than at site B. No site related differences were apparent in plasma estrogen levels.

When compared to site A fish egg weight was higher in fish from sites B, C and D. Also, egg size was increased in fish from site B while percent clutch eggs was reduced at sites E and F. The proportion of the ovary comprised of pre-vitellogenic oocytes and connective tissue varied between 50 and 90% of gonad weight in these samples. Therefore to estimate fecundity a correction factor was applied on an individual basis. Compared to fall samples relative fecundity estimates in spring fish were about 50% greater. However, no site related differences were found in the fecundity estimates.

1.6. Northern Pike -- Spring Sample (Table 11 & 12; Appendix 6)

Analytical results for each female northern pike collected in the spring (N=14) are outlined in Table 11. The spring sample contained one immature fish (A-II-5) lacking clutch eggs. The rest were mature but many had ovulated and had begun or nearly completed spawning. There was no gonadal tissue for sample A-II-2. Sample F-II-9 had no vitellogenic eggs in the sample, presumably they were all spawned out. Vitellogenic oocytes were loose in fish A-II-3, D-II-1, E-II-6, E-II-10 and F-II-8 and most were presumably spawned.

Table 11. Physical characteristics (length, weight & gonad weight), plasma steroid hormones (E2 & Test) and reproductive indices (GSI, clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of each female northern pike collected during the fall 1992 Special Fish Collection. GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

Northern Pike Females -- Spring Sample													
Sample	Site	Length (mm)	Weight (g)	Gonad (g)	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (μ m)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (eggs/g)	Maturity Index
A-II-3	A	659	1792	27	1.53	0.452	0.050	3.30	1229	**	**	**	11
A-II-5	A	440	632	3	0.48	0.147	0.047	*	*	*	*	*	7
A-II-6	A	483	910	32	3.64	0.361	0.055	3.90	1285	**	**	**	11
B-II-1	B	477	781	18	2.36	0.752	0.077	3.08	1113	**	**	**	11
D-II-1	D	698	2596	31	1.21	0.076	0.090	10.00	1708	**	**	**	11
E-II-1	E	699	2152	287	15.39	3.475	1.409	5.55	1481	10.1	40025	21.5	10
E-II-4	E	835	5409	532	10.91	0.490	1.043	7.63	1555	18.1	53967	11.1	10
E-II-5	E	665	2413	174	7.77	1.970	1.233	5.45	1474	17.0	24711	11.0	10
E-II-6	E	667	2212	13	0.59	0.272	0.780	4.67	1446	**	**	**	11
E-II-10	E	631	720	15	2.13	0.346	0.187	9.60	1762	**	**	**	11
F-II-1	F	455	632	21	3.44	0.692	0.035	3.10	1169	**	**	**	11
F-II-8	F	792	3735	38	1.03	0.062	0.007	7.09	1747	**	**	**	11
F-II-9	F	521	1045	5	0.48	0.079	0.021	**	**	**	**	**	11
F-II-10	F	637	2045	219	11.99	12.063	3.446	6.90	1588	27.9	24566	13.5	10
* - Immature, no clutch oocytes.													
** - Because these fish had ovulated and contained loose eggs, accurate estimates of % clutch and fecundity cannot be obtained.													

Table 12. GSI, plasma steroid hormones (E2 & Test) and reproductive indices (clutch egg weight, clutch egg diameter, absolute fecundity, relative fecundity & maturity index) of female northern pike collected during the spring 1992 Special Fish Collection. Values represent mean and SE of mature fish from each site. GSI=gonadosomatic index, E2=17 β -estradiol, Test=testosterone.

Northern Pike Females -- Spring Sample										
Site	N	GSI (%)	Plasma E2 (ng/mL)	Plasma Test (ng/mL)	Clutch Egg Wt (mg)	Clutch Egg Diam (μ m)	Amount Clutch (%)	Absolute Fecundity (# eggs)	Relative Fecundity (eggs/g)	Maturity Index *
A	2	2.59	0.407	0.053	3.60	1257				11
		1.06	0.046	0.003	0.30	40				
B	1	2.36	0.752	0.077	3.08	1113				11
D	1	1.21	0.076	0.090	10.00	1708				11
E	2	1.36	0.309	0.484	7.14	1604				11
		0.77	0.037	0.297	2.47	158				
E	3	11.36	1.978	1.228	6.21	1503	15.1	39568	14.5	10
		2.21	0.862	0.106	0.71	26	2.5	8448	3.5	
F	3	1.65	0.278	0.021	5.10	1458				11
		0.91	0.207	0.008	2.00	289				
F	1	11.99	12.063	3.446	6.90	1588	27.9	24566	13.5	10
* - Immature fish not included.										

A diameter for these eggs was measured by caliper (60 eggs) and reduced to 72.7% to be comparable with eggs measured by digitizer and microscope. All fecundity values and % clutch for ovulated fish are not reliable because possible spawned eggs or those lost due to handling are unaccountable. Site specific means and standard errors are summarized in Table 12. The low numbers collected at each site and the presence of differing development states preclude detailed statistical evaluation.

As previously stated we are unaware of published information regarding plasma steroid hormone levels in northern pike from North America. The limited study on European stains (Simontacchi et al. 1983) shows generally similar values but comprehensive information is lacking. Similar to mountain whitefish (see Table 4), plasma steroid levels were much lower in ovulated and spent fish.

The pre-vitellogenic oocytes were generally less than 300 μm in diameter. Clutch egg sizes measured by digitizer and microscope are about 70 % actual diameter. When corrected for this difference egg sizes in northern pike from the Athabaska River are still near the low end of the spawning size (2.5-3.0) reported by Scott and Crossman (1973) and others (Treasurer, 1990; Lebeau 1991). The reported relative fecundity estimate is approximately 20 eggs/g fish and absolute fecundity averaged 32,000 eggs per fish (Scott and Crossman 1973). Spring northern pike from the Athabaska River had similar absolute fecundity but the relative fecundity appeared lower in the 4 fish where it could be determined. Also relative fecundity appeared lower than in fall fish. However, due to the small numbers, no firm conclusion can be considered.

2. Male Fish

2.1. Longnose Suckers -- Fall Sample (Table 13 & 14)

Results obtained for each male longnose sucker collected (N=26) are outlined in Table 13. The sample contained 6 male fish with immature gonads (< stage 5) in the sample. Site specific means and standard errors are summarized in Table 14. Comparison between male fish taken at site A and those from downstream sites cannot be made because fish were not in the same developmental state. Sufficient numbers of fish at the same stage were collected only from sites B, C & D.

Plasma levels of testosterone, 11-ketotestosterone and GSI were similar to values reported for male longnose sucker collected during September in Lake Superior (Munkittrick et al. 1992a).

Table 13. Physical characteristics (length, weight & gonad weight), GSI, plasma steroid hormones (Test & Ktest) and maturity stage) of each male longnose sucker collected during the fall 1992 Special Fish Collection. GSI=gonadosomatic index, Test=testosterone, Ktest=11-ketotestosterone.

Longnose Sucker Males -- Fall Sample								
Sample	Site	Length (mm)	Weight (g)	Gonad (g)	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage
G-IV-3	A	362	570	2.5	0.44	0.044	0.35	3
G-IV-4	A	375	470	2.8	0.60	0.025	0.35	2
G-IV-7	A	378	670	7.1	1.07	0.150	1.86	4
H-IV-2	B	393	760	29.2	4.00	0.746	20.68	5
H-IV-3	B	411	820	31.5	3.99	0.999	24.33	5
H-IV-4	B	374	670	3.5	0.53	0.199	4.46	2
H-IV-6	B	400	800	33.2	4.33	1.550	17.97	5
H-IV-9	B	402	850	31.2	3.81	1.434	15.22	5
H-IV-10	B	386	700	25.4	3.77	1.479	13.44	5
J-IV-3	C	352	580	27.8	5.03	0.620	17.72	5
J-IV-4	C	361	650	*	*	2.717	32.53	5
J-IV-5	C	381	780	34.4	4.61	2.353	37.14	5
J-IV-7	C	398	870	47.4	5.76	1.515	18.91	5
J-IV-8	C	397	715	28.5	4.15	2.517	32.49	5
J-IV-9	C	397	720	39.2	5.76	3.676	48.43	5
K-IV-1	D	377	600	32.2	5.67	0.519	10.33	5
K-IV-2	D	348	560	20.8	3.86	2.606	19.66	5
K-IV-3	D	411	825	34.8	4.40	2.363	18.91	5
K-IV-4	D	356	555	22.2	4.17	0.953	6.94	5
K-IV-5	D	384	660	35.3	5.65	2.242	13.14	5
K-IV-6	D	357	590	19.3	3.38	2.999	17.63	5
K-IV-9	D	376	670	20.3	3.12	2.436	24.44	5
K-IV-10	D	356	590	2.5	0.43	0.097	0.81	2
L-IV-5	E	358	540	17.5	3.35	1.787	17.55	5
L-IV-6	E	392	765	31.4	4.28	2.368	27.05	5
M-IV-2	F	383	690	4.7	0.69	0.029	0.79	2
* - No gonad weight given in Fish Collection Report.								

Based on plasma testosterone, plasma 11-ketotestosterone and GSI lower values were evident between fish with stage 5 gonads and those at more immature stages (<5). In stage 5 fish, plasma testosterone or GSI did not differ between sites. Although, plasma 11-ketotestosterone levels were lowest in fish from site D no firm conclusions can be derived.

Table 14. GSI, plasma steroid hormones (Test & Ktest) and maturity stage of male longnose sucker collected during the fall 1992 Special Fish Collection. Values represent mean and SE of fish from each site. GSI=gonadosomatic index, Test=testosterone, Ktest=11-ketotestosterone.

Longnose Sucker Males -- Fall Sample					
Site	N	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage
A	3	0.70	0.073	0.85	< 5
		0.19	0.039	0.87	
B	5	3.98	1.241	18.33	5 *
		0.10	0.157	1.94	
C	6	5.06	2.233	31.20	5
		0.32	0.429	4.72	
D	7	4.32	2.017	15.86	5 *
		0.38	0.346	2.28	
E	2	3.82	2.078	22.30	5
		0.46	0.291	4.75	
F	1	0.69	0.029	0.79	< 5
* -- Immature fish were not included.					

2.2. Mountain Whitefish -- Fall Sample (Table 15 & 16)

Analytical results for each male mountain whitefish collected (N=17) are detailed in Table 15. All fish were either at stage 5 or 6, however, the low numbers and the distribution between stages complicates site specific comparisons. Site specific means and standard errors are summarized in Table 16. Sufficient numbers of fish are available to compare differences only between site A and F or site A and various combinations of the others. The comparison is also complicated by the 2 differing developmental stages. When stages are compared (stages 5 vs 6), plasma 11-ketotestosterone was higher in stage 6 (ripe and running) fish while plasma testosterone and GSI tended to be lower. These differences in stage and the low numbers of fish collected preclude the development of conclusions regarding site specific differences.

Table 15. Physical characteristics (length, weight & gonad weight), GSI, plasma steroid hormones (Test & Ktest) and maturity stage) of each male mountain whitefish collected during the fall 1992 Special Fish Collection. GSI=gonadosomatic index, Test=testosterone, Ktest=11-ketotestosterone.

Mountain Whitefish Males -- Fall Sample								
Sample	Site	Length (mm)	Weight (mm)	Gonad (g)	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage
G-I-1	A	323	380	11.9	3.23	26.16	13.33	6
G-I-2	A	322	380	16.4	4.51	7.48	4.60	5
G-I-4	A	350	435	20.8	5.02	14.72	6.13	5
G-I-7	A	326	390	20.3	5.49	32.08	13.75	5
G-I-10	A	370	575	36.7	6.82	15.66	5.59	5
H-I-9	B	341	485	18.3	3.92	11.84	30.24	6
H-I-11	B	334	420	6.8	1.65	8.79	35.71	5
J-I-1	C	316	385	23.2	6.41	6.01	9.09	5
J-I-3	C	306	345	20.7	6.38	5.91	12.76	5
K-I-8	D	337	425	18.9	4.65	3.53	16.96	6
K-I-10	D	332	345	25.5	7.98	10.24	23.60	6
L-I-1	E	332	390	12.9	3.42	16.49	21.20	6
M-I-1	F	431	960	40.1	4.36	4.49	10.58	6
M-I-3	F	359	435	13.7	3.25	8.87	39.18	6
M-I-5	F	363	585	21.3	3.78	9.39	15.68	6
M-I-6	F	389	720	38.2	5.60	20.22	35.42	6
M-I-10	F	347	510	20.4	4.17	1.18	14.35	6

Table 16. GSI, plasma steroid hormones (Test & Ktest) and maturity stage of male mountain whitefish collected during the fall 1992 Special Fish Collection. Values represent mean and SE of fish from each site. GSI=gonadosomatic index, Test=testosterone, Ktest=11-ketotestosterone.

Mountain Whitefish Males -- Fall Sample					
Site	N	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage
A	5	5.02	19.22	8.68	5 *
		0.59	4.38	2.00	
B	2	2.78	10.31	32.97	5 *
		1.14	1.53	2.74	
C	2	6.40	5.96	10.93	5
		0.02	0.05	1.84	
D	2	6.32	6.88	20.28	6
		1.66	3.35	3.32	
E	1	3.42	16.49	21.20	6
F	5	4.23	8.83	23.04	6
		0.39	3.22	5.91	

* - Includes one fish at stage 6.

2.3. White Sucker -- Fall Sample (Table 17 & 18)

Analytical results for each male white sucker collected (N=6) are given in Table 17. There was no gonad for one fish (H-III-1) and judging from the low steroid hormone levels this fish was immature. All other fish were at stage 5, however, the very low numbers preclude site specific comparisons. Site specific means and standard errors are summarized in Table 18.

Plasma levels of testosterone, 11-ketotestosterone and GSI were similar to values reported for male white sucker collected during September in Lake Superior (Munkittrick et al. 1992b) and in small headwater lakes at ELA (Brown and Evans, unpublished).

Table 17. Physical characteristics (length, weight & gonad weight), GSI, plasma steroid hormones (Test & Ktest) and maturity stage) of each male white sucker collected during the fall 1992 Special Fish Collection. GSI=gonadosomatic index, Test=testosterone, Ktest=11-ketotestosterone.

White Sucker Males -- Fall Sample								
Sample	Site	Length (mm)	Weight (g)	Gonad (g)	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage
H-III-1	B	370	730	*	*	0.003	0.35	*
L-III-1	E	352	636	37.4	6.25	0.340	5.76	5
L-III-5	E	360	570	35.4	6.62	0.127	1.77	5
L-III-7	E	377	720	38.3	5.62	0.172	7.23	5
M-III-6	F	350	570	25.9	4.76	0.214	1.80	5
M-III-7	F	389	750	46.1	6.55	0.143	2.19	5
* - No gonad collected, classified as immature in Collection Report.								

Table 18. GSI, plasma steroid hormones (Test & Ktest) and maturity stage of male white sucker collected during the fall 1992 Special Fish Collection. Values represent mean and SE of fish from each site. GSI=gonadosomatic index, Test=testosterone, Ktest=11-ketotestosterone.

Not for circulation.

White Sucker Males -- Fall Sample					
Site	N	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage
B	1	*	0.003	0.35	*
E	3	6.16	0.213	4.92	5
		0.90	0.065	1.63	
F	2	5.66	0.179	2.00	5
		0.90	0.500	0.08	

* -- Immature fish.

2.4. Northern Pike -- Fall Sample (Table 19 & 20)

Analytical results for each northern pike collected (N=11) are detailed in Table 19. Two fish were judged to differ from the others. One (L-II-2) contained only relic sperm and no developing new sperm cysts. Due to the presence of relic sperm this fish had likely spawned previously and it had the appearance of a stage 7 gonad. This lack of new development was atypical relative to other fish in the sample. The second (M-II-9) was a virgin male at maturity stage 3. All other fish were at stage 4-5, however, the very low numbers preclude detailed site specific comparisons. Site specific means and standard errors are summarized in Table 18.

Table 19. Physical characteristics (length, weight & gonad weight), GSI, plasma steroid hormones (Test & Ktest) and maturity stage) of each male northern pike collected during the fall 1992 Special Fish Collection. GSI=gonadosomatic stage, Test=testosterone, Ktest=11-ketotestosterone.

Northern Pike Males -- Fall Sample								
Sample	Site	Length (mm)	Weight (g)	Gonad (g)	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage
K-II-1	D	491	925	8.5	0.93	0.570	4.10	5
L-II-2	E	441	600	6.9	1.16	0.045	0.40	7
L-II-4	E	538	1050	10.8	1.04	0.912	7.61	4
L-II-5	E	643	2150	42.0	1.99	0.956	10.32	5
L-II-6	E	499	930	11.6	1.26	0.565	8.77	4
L-II-9	E	573	1340	15.3	1.15	2.084	18.28	4
L-II-10	E	549	1450	14.5	1.01	0.344	6.69	5
M-II-3	F	471	765	11.3	1.50	1.237	14.34	4
M-II-5	F	677	2540	64.1	2.59	4.163	9.79	4
M-II-9	F	667	2340	54.6	2.39	0.097	2.00	3
M-II-10	F	770	3715	65.8	1.80	0.400	10.20	4

Table 20. GSI, plasma steroid hormones (Test & Ktest) and maturity stage of male northern pike collected during the fall 1992 Special Fish Collection. Values represent mean and SE of fish from each site. GSI=gonadosomatic index, Test=testosterone, Ktest=11-ketotestosterone.

Northern Pike Males -- Fall Sample					
Site	N	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage *
D	1	0.93	2.017	15.86	5
E	5	1.27	0.972	10.33	4-5
		0.15	0.300	2.08	
F	3	1.96	1.933	11.44	4
		0.32	1.141	1.45	
* -- Stage 3 and 7 fish not included.					

For the most part, plasma levels of testosterone and 11-ketotestosterone were fairly high in northern pike and similar to levels found in salmonids at the same stage of development (Scott et al. 1980).

2.5. Mountain Whitefish -- Spring Sample (Table 21 & 22)

Results for each male mountain whitefish collected in the spring (N=16) are outlined in Table 21. Fish were in early maturation stages (<3) and most fish were in stage 1. With the exception of a single stage 4 fish from site C, GSI values were <1%. Site specific means and standard errors are summarized in Table 22. Generally, Low levels of plasma testosterone and 11-ketotestosterone were found. The low hormone levels found collected in May were similar to unpublished data from the Procter and Gamble study on the Smokey/Wapiti system (Klopper-Sams and Benton 1992). When data from sites A and B are combined plasma testosterone levels are lower than those found further downstream. However, the low numbers collected at each site (≤4) do not allow detailed comparisons.

Table 21. Physical characteristics (length, weight & gonad weight), GSI, plasma steroid hormones (Test & Ktest) and maturity stage) of each male mountain whitefish collected during the spring 1992 Special Fish Collection. GSI=gonadosomatic index, Test=testosterone, Ktest=11-ketotestosterone.

Mountain Whitefish Males -- Spring Sample								
Sample	Site	Length (mm)	Weight (g)	Gonad (g)	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage
A-I-2	A	330	381	3.0	0.79	0.086	0.35	1
A-I-7	A	363	492	3.0	0.61	0.109	0.79	2
A-I-9	A	333	409	2.0	0.49	0.116	0.98	1
B-I-8	B	314	357	2.0	0.56	0.154	0.35	3
C-I-4	C	393	742	11.0	1.50	0.459	0.61	4
C-I-9	C	406	692	2.0	0.29	1.135	0.73	2
D-I-1	D	373	631	2.0	0.32	0.107	0.38	1
D-I-4	D	326	422	2.0	0.48	0.420	0.67	2
E-I-1	E	335	412	2.0	0.49	0.208	0.35	1
E-I-6	E	369	648	1.0	0.15	0.338	0.57	1
E-I-7	E	389	748	2.0	0.27	1.246	1.04	2
E-I-10	E	370	611	2.0	0.33	0.169	0.35	*
F-I-1	F	418	891	3.0	0.34	0.407	0.58	1
F-I-3	F	343	502	1.0	0.20	0.219	0.35	1
F-I-4	F	327	395	1.0	0.25	0.375	0.71	1
F-I-9	F	414	914	3.0	0.33	0.424	1.80	1
* - No gonad tissue in capsule.								

Table 22. GSI, plasma steroid hormones (Test & Ktest) and maturity stage of male mountain whitefish collected during the spring 1992 Special Fish Collection. Values represent mean and SE of fish from each site. GSI=gonadosomatic index, Test=testosterone, Ktest=11-ketotestosterone.

Mountain Whitefish Males -- Spring Sample					
Site	N	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage
A	3	0.63	0.103	0.71	< 5
		0.09	0.009	0.19	
B	1	0.56	0.154	0.35	< 5
C	2	0.90	0.797	0.67	< 5
		0.61	0.338	0.06	
D	2	0.40	0.264	0.53	< 5
		0.08	0.157	0.15	
E	4	0.31	0.490	0.58	< 5
		0.07	0.254	0.16	
F	4	0.28	0.357	0.86	< 5
		0.03	0.047	0.32	

2.6. Northern Pike -- Spring Sample (Table 23 & 24)

The values obtained for each male northern pike collected (N=26) are outlined in Table 23. The sample contained 14 male fish all with mature gonads (\geq stage 5). Most fish were ripe or nearly spent. Male pike taken at site A and B appeared to have spawned sometime earlier than fish at sites E and F because lobular lumens in the testes contained only relic sperm. Site specific means and standard errors are summarized in Table 24. Comparison between male fish taken at upstream sites (A & B) and those from downstream sites are difficult due to the differing developmental stages and low numbers.

Plasma levels of testosterone and 11-ketotestosterone found in northern pike were generally somewhat lower than values reported for male mountain whitefish also at full maturation (see Table 20). However, the levels of steroid hormones northern pike donot seem to decline as rapidly in nearly spent fish. Spent fish with only relic sperm (Stage 7) had very low hormone levels (see Table 24).

Table 23. Physical characteristics (length, weight & gonad weight), GSI, plasma steroid hormones (Test & Ktest) and maturity stage) of each male northern pike collected during the spring 1992 Special Fish Collection. GSI=gonadosomatic index, Test=testosterone, Ktest=11-ketotestosterone.

Northern Pike -- Spring Sample								
Sample	Site	Length (mm)	Weight (g)	Gonad (g)	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage
A-II-1	A	470	785	4	0.51	0.049	0.56	7
A-II-4	A	590	1399	3	0.21	0.009	0.35	7
B-II-2	B	395	415	1	0.24	0.046	0.39	7
E-II-2	E	405	543	2	0.37	0.344	1.46	6
E-II-3	E	612	1693	22	1.32	0.583	4.19	6
E-II-7	E	650	1680	21	1.27	0.382	3.07	6
E-II-8	E	548	1110	11	1.00	0.620	4.80	6
E-II-9	E	507	1093	9	0.83	0.749	4.07	6
F-II-2	F	465	674	9	1.35	0.154	2.02	6
F-II-3	F	540	1129	13	1.16	0.660	3.49	6
F-II-4	F	537	1100	10	0.92	0.274	3.30	6
F-II-5	F	538	1084	4	0.37	0.118	1.76	6
F-II-6	F	530	970	22	2.32	0.364	3.68	6
F-II-7	F	546	1118	8	0.72	0.358	3.27	6

Table 24. GSI, plasma steroid hormones (Test & Ktest) and maturity stage of male northern pike collected during the Spring 1992 Special Fish Collection. Values represent mean and SE of fish from each site. GSI=gonadosomatic index, Test=testosterone, Ktest=11-ketotestosterone.

Northern Pike Males -- Spring Sample					
Site	N	GSI (%)	Plasma Test (ng/mL)	Plasma Ktest (ng/mL)	Maturity Stage
A	2	0.36	0.029	0.46	7
		0.15	0.020	0.11	
B	1	0.24	0.046	0.39	7
E	5	0.96	0.536	3.52	6
		0.17	0.076	0.59	
F	6	1.14	0.321	2.92	6
		0.27	0.079	0.33	

RECOMMENDATIONS

Technical Quality of Samples. Blood and tissues appear to have been collected in a timely and suitable fashion. Sampling stress appears to have been kept to a minimum and most plasma samples were removed within 15 min. Plasma volumes were adequate but in several cases more could have been taken without affecting other sampling. This simply allows greater scope for analyses. Some pieces of gonad tissue preserved in Davidson's fluid were very small. It is recommended that samples fill the tissue capsules to at least one quarter capacity. Also, many independent eggs were found at the bottom of the sample bottles. We presume they fell through the holes in the 38 mm tissue capsules. Using smaller sized tissue capsules (29 mm) where necessary would eliminate this problem. Given the large number of samples to process, it would also help considerably if the actual samples contained in each one liter bottle were listed on an attached label. Taking more than one tissue capsule per fish was a good idea because sometimes a capsule pops open in the fixation bottle. If sufficient tissue was not present in one capsule, it could generally be found in another.

Scientific Quality of Collections. Future collections need to avoid collection of fish near transition stages. The presence of ovulated and spent mountain whitefish in the fall collection and northern pike in the spring collection affects interpretation of that data with regard to location. Is the presence of less mature fish at upstream sites a result of temporal differences in sampling, location of suitable spawning sites or an actual effect of proximity to the mill. Also, altering the sampling time to avoid ovulated fish would eliminate egg losses and compromised fecundity estimates. Mountain whitefish should be sampled in mid to late August to avoid transition between mature and ovulated fish. Because significant gonad development occurs in most spring spawners by this time (e.g. longnose sucker, white sucker or northern pike), evaluation of plasma concentrations of reproductive hormones and fecundity estimates will still be possible for the species collected in this study.

Because mature male longnose sucker were not obtained at site A in the fall, conclusions regarding exposure could not be derived. Values of plasma testosterone were lower with proximity to the mill (site B versus C,D & E). *If at all possible, fish collections must obtain fish of appropriate developmental stages from the purported reference area.*

The importance of obtaining samples from a remote reference site can not be over-emphasized. This would have made present data much more interpretable. Fish can range widely within systems and baseline variability in parameters can be more credibly assessed in a system not impacted in any way by pulp mill effluent. For example, the data obtained for female mountain

whitefish in the spring at sites A and B are quite variable. There are fish with very low 17 β -estradiol (<1 ng/mL) values and fish with higher levels (>1 ng/mL). Does this indicate the presence of different groups/exposure levels moving between sites? Without data from a remote reference, what constitutes normal variability cannot be ascertained. We view this as extremely important due to the paucity of baseline information on mountain whitefish and longnose sucker as far as reproductive biomarkers are concerned.

The small site-specific sample sizes often prohibited site specific statistical analyses. Suitable sample sizes are essential for comprehensive data analyses. If data from different sites were combined the sample sizes would not be as great a problem. Therefore, we believe that future collections should place less emphasis on sampling the Upper Athabaska River itself. Due to the high potential for movement by species like mountain whitefish. Sampling the upstream site A and only two downstream sites (B/C & D/E) would seem sufficient. Between 10 and 15 individuals of each sex should be obtained for each area. *Generally, greater emphasis needs to be placed on the collection of appropriate control data.*

CONCLUSIONS

Any conclusions regarding the general state of the free-swimming fish populations are compromised to a certain extent by the lack of suitable reference data from completely unaffected sites. Also, in many cases site specific sample sizes were too small. Due to the close proximity of the upstream reference location (site A) and sites with effluent exposure (e.g. site B or C), it is highly conceivable that fish may readily move between locations. This would add to overall variability making it more difficult to detect potential changes.

Female Longnose Sucker. Plasma 17 β -estradiol was significantly reduced in female longnose sucker downstream of site A. Under the control of pituitary gonadotropins, 17 β -estradiol is produced in the ovary and is carried by the circulatory system to the liver where it stimulates production of yolk proteins for incorporation into developing clutch oocytes. Thus, prolonged reductions in its circulating level could adversely affect ovary development. There were no site specific differences in measures of ovarian development (GSI, egg size measurements or fecundity estimates) so despite lower 17 β -estradiol levels gonadal growth appears unaffected. However, it is striking that fecundity estimates were near the low end of reported ranges.

Female Mountain Whitefish. Due to proximity to spawning, fall data for mountain whitefish females are more difficult to interpret than that for longnose sucker. Peak pre-spawning levels of plasma steroid hormones rapidly decline in salmonids as

spawning begins (Scott et al. 1980). The fish which had ovulated were easily discernable by hormone levels and from the oocyte size-frequency histograms and were not included in the statistical analyses. The decline in plasma estrogen levels with site could simply indicate that individuals were progressing nearer ovulation. Moreover, the temporal sequence of sampling is such that E & F follow sites A and B by 4 to 5 days. To support this hypothesis egg weight was greater at sites D, E and F than at site A. Sampling in late August or early September would reduce the potential for temporal effects to act as a source of variability. We note that the pattern of lower plasma 17 β -estradiol in maturity stage 10 females at locations downstream of site A was **very similar** to the changes observed in longnose sucker. Again, fecundity estimates appeared unaffected by the low plasma 17 β -estradiol and were fairly close to literature values. In the spring data, hormone levels are much lower and changes are not as clear but there is a definite trend for lower testosterone levels (precursor for 17 β -estradiol in female fish) with proximity to site A. Generally, fecundity estimates were higher than found in fall fish. The lower relative fecundity in fish from site B was not significant. Without more information regarding other parameters (e.g. temperature, diet) few conclusions regarding the larger egg size and weight at sites B, C and D in spring fish can be made.

Female White Sucker and Northern Pike. White sucker and pike were mostly obtained from the two downstream sites (E & F). Thus, no firm conclusions regarding site differences within the river can be made. Hormone levels, GSI, egg size and fecundity of white sucker fell within ranges cited in the literature.

No conclusions regarding site differences are derived for northern pike, because similar to white suckers fish were mostly obtained at the two sites furthest downstream (E & F). To effectively facilitate comparisons, other reference data are essential. The spring sample is further complicated by the fact that females were either ovulated or spent. As previously described for the fall mountain whitefish, estimates of fecundity cannot be obtained from spent females and they have differing hormone profiles from gravid fish. There is very little literature data available on hormones for northern pike. The northern pike from the Athabaska River had similar absolute fecundity but the relative fecundity appeared lower than most values reported in the literature. However, due to the small numbers, it is difficult to determine whether this difference is real.

The values reported here do establish hormone levels, fecundity, and egg size for white suckers and northern pike captured in 1992 from the upper Athabaska River.

Male Longnose Sucker, Mountain Whitefish, White Sucker and Northern Pike. Generally, too few male fish were collected and their state of maturity tended to vary between sites. These two factors precluded the derivation of many conclusions regarding their status. We observed no histopathological aberrations in male gonad tissue. Values for male longnose and white suckers generally fell near ranges reported elsewhere (Hodson et al. 1992; Munkittrick et al. 1992a; Van der Kraak et al. 1992). The paucity of control data presents a problem in actually determining whether the values observed in male fish fall within normal ranges.

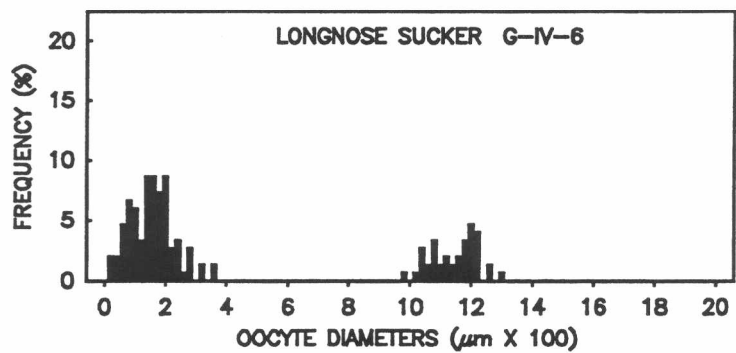
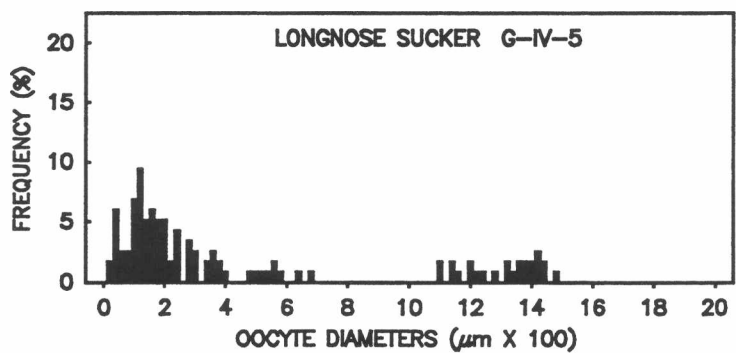
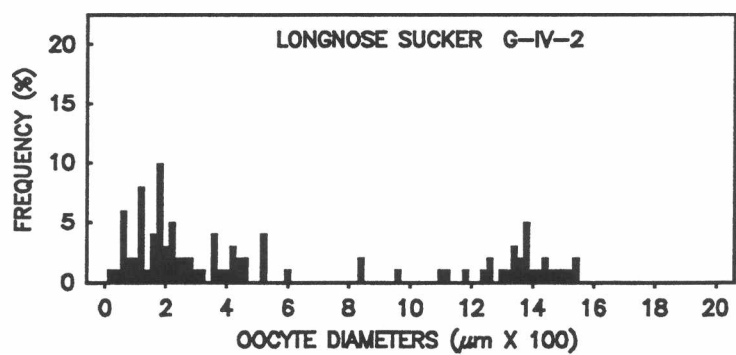
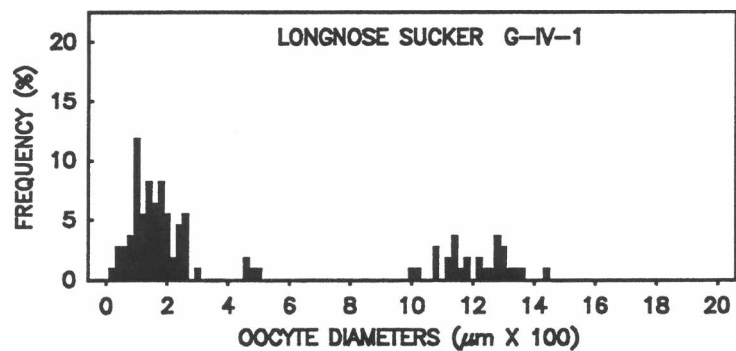
Overall, depressed circulating levels of gonadal steroid hormones occurred in female longnose suckers and possibly also in mountain whitefish fish collected downstream of the Hinton mill. These findings were consistent with observations in longnose suckers and other species downstream of pulp mill effluent (McMaster et al. 1991; Munkittrick et al. 1991, 1992a,b; Van der Kraak et al. 1992). However, corresponding site related differences in egg size or fecundity estimates were not readily apparent. Further study to verify the present findings and to determine the consequences of low plasma 17 β -estradiol levels are required. Other aspects of reproduction (e.g. time and synchronization of spawning, gamete viability and embryo survival) have not been investigated and their examination is required to ensure reproductive competence. Age to maturity and lower fecundity with age are also reproductive indices which may be sensitive to the presence of pulp mill effluent (McMaster et al. 1991; Munkittrick et al. 1991, 1992a,b). *Aging structures have been collected by EMA but require assessment and integration with the present data before these can be determined.* Additionally, the present data needs to be consolidated with other data collected on the same individuals (chemical residues, liver enzyme induction, condition, etc.) to assess possible interactions.

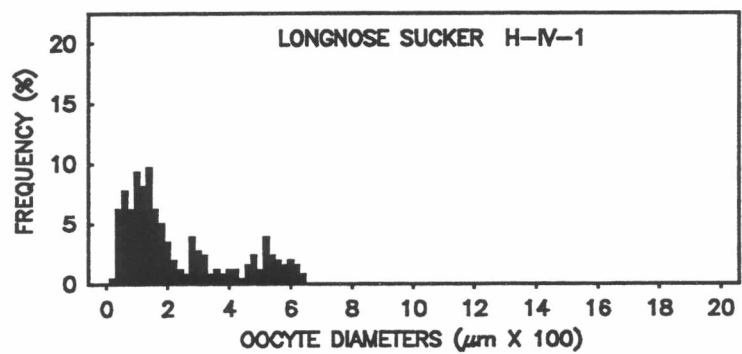
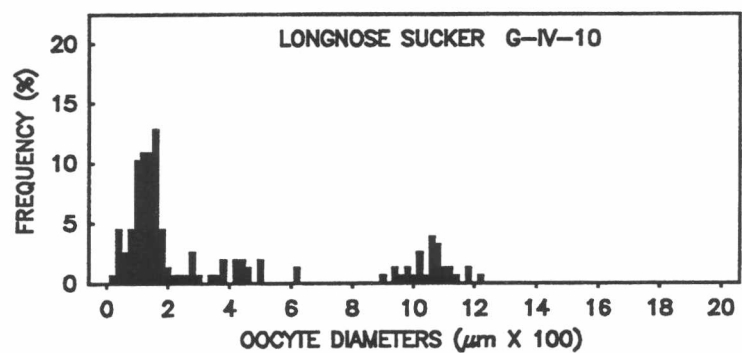
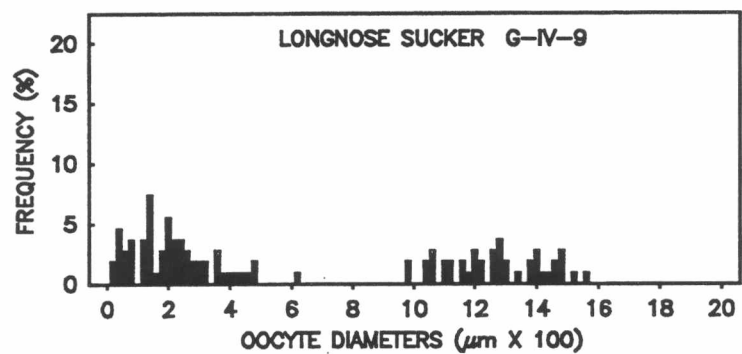
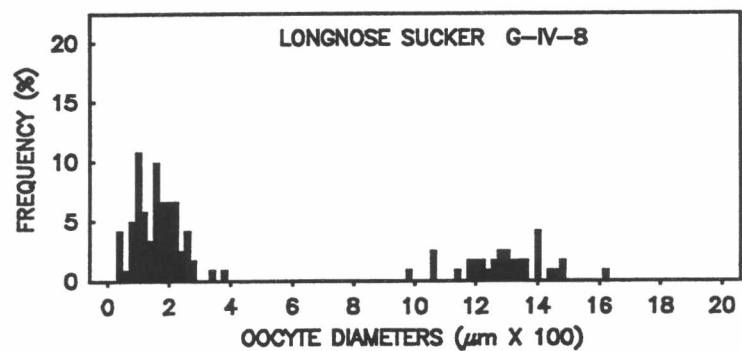
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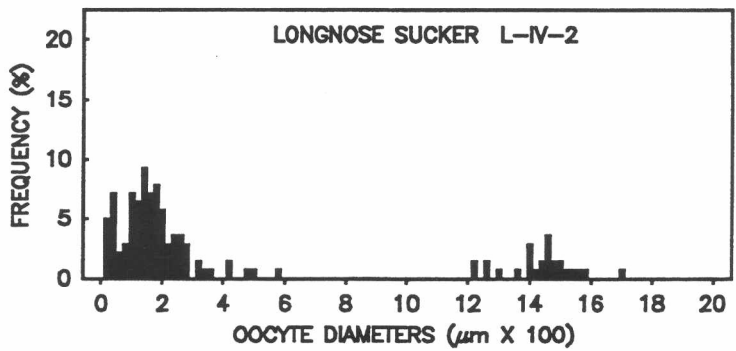
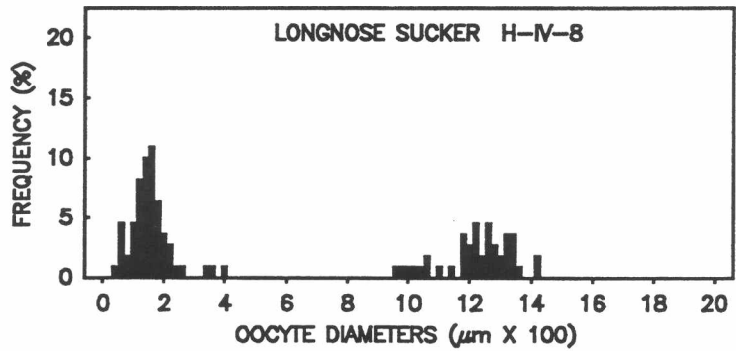
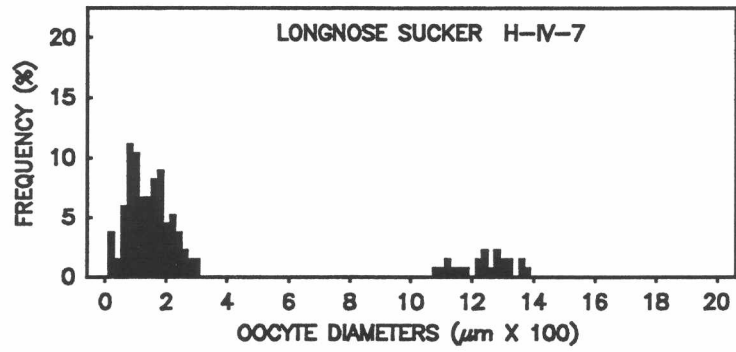
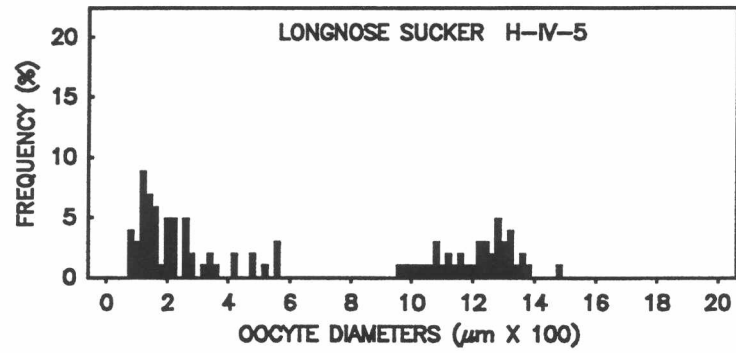
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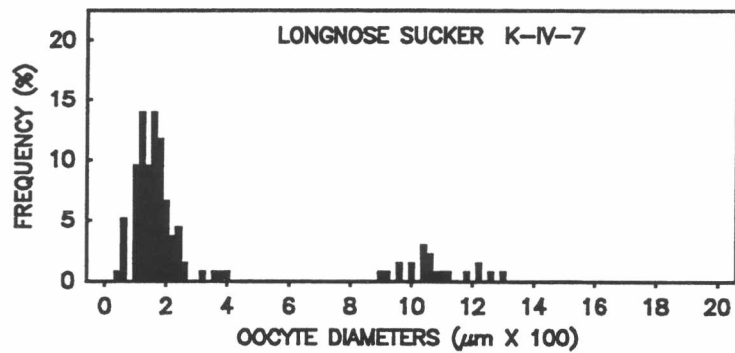
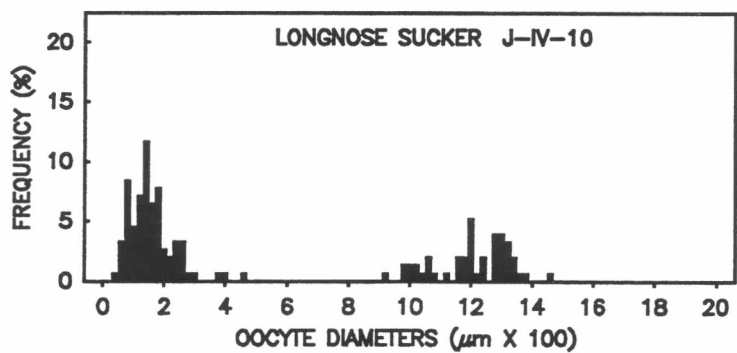
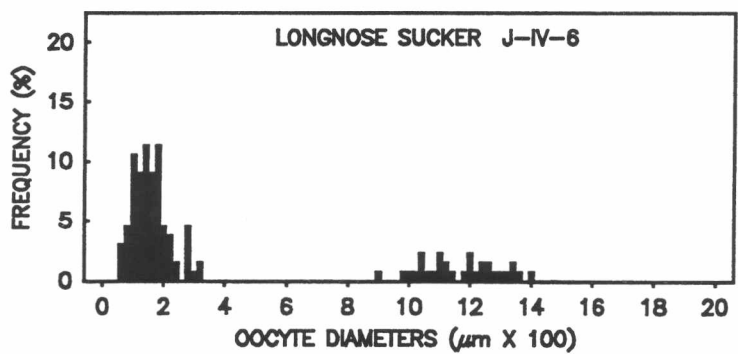
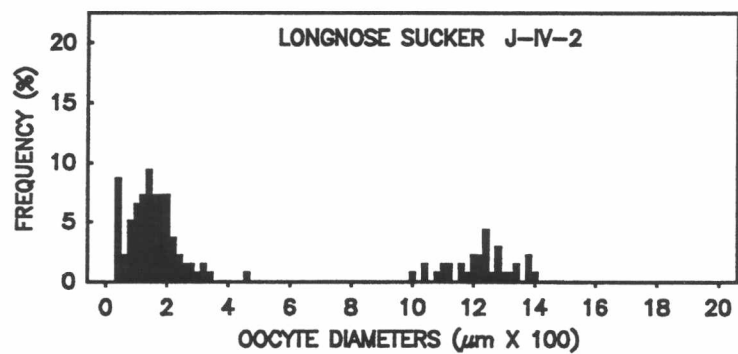
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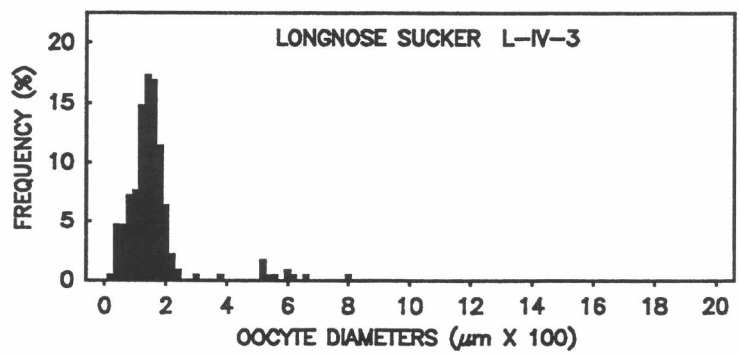
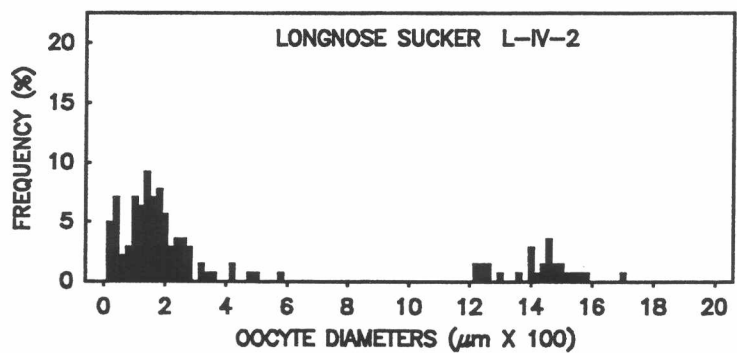
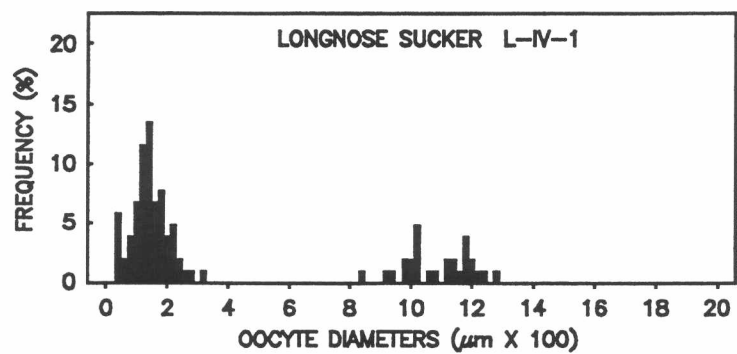
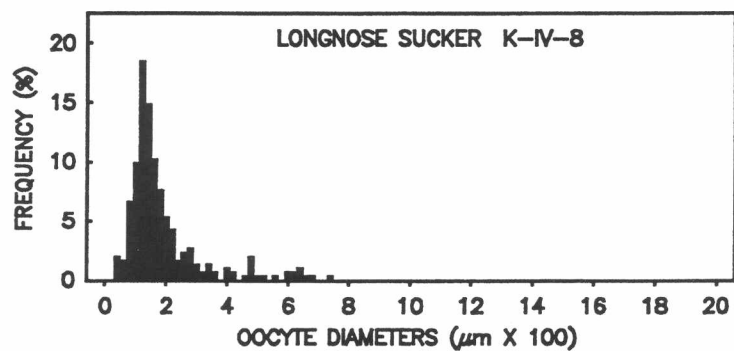
APPENDIX 1
Oocyte Diameter
Frequency Distributions
Fall Longnose Sucker

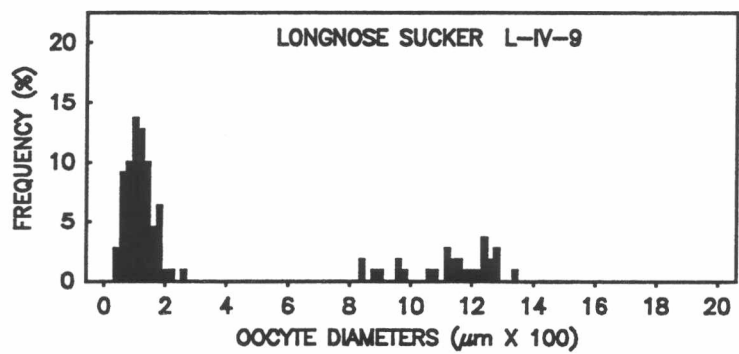
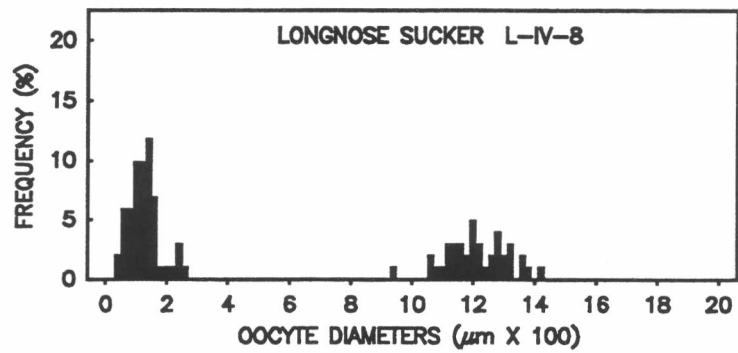
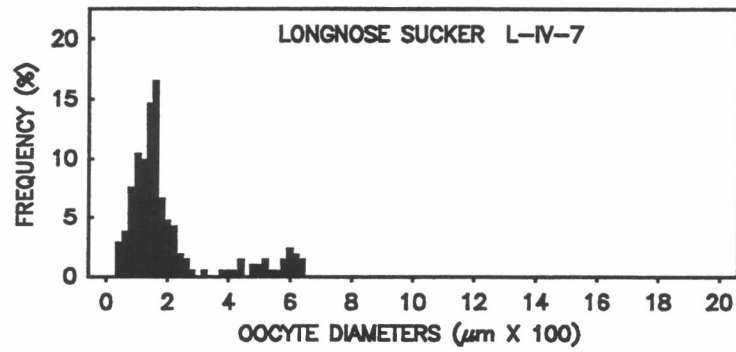
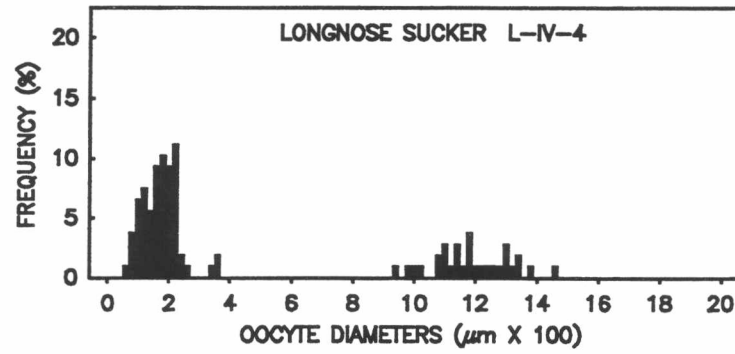


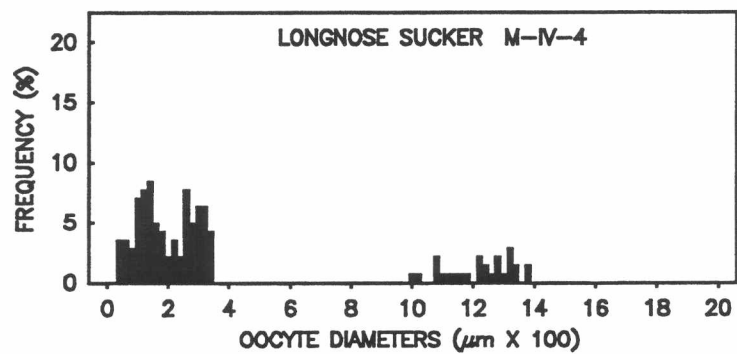
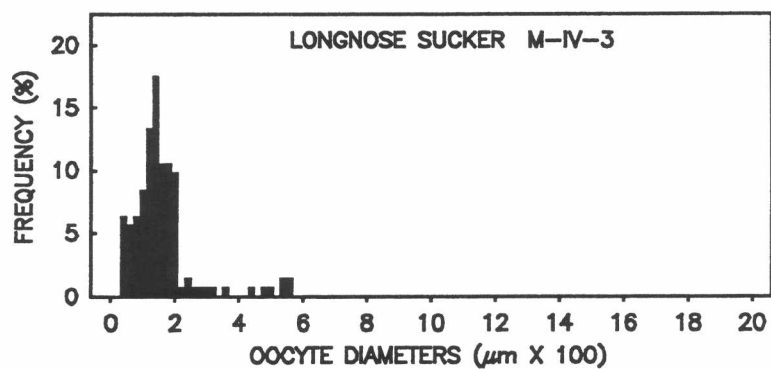
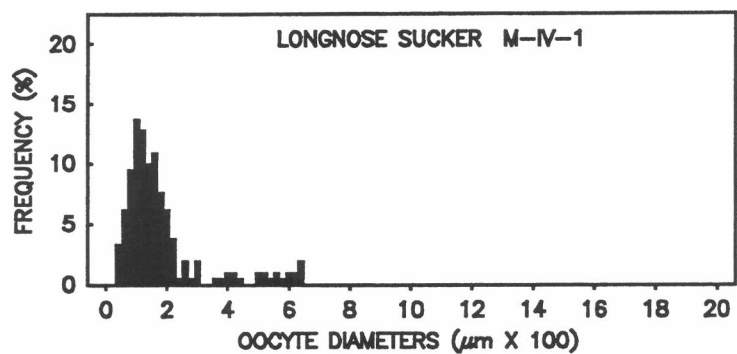
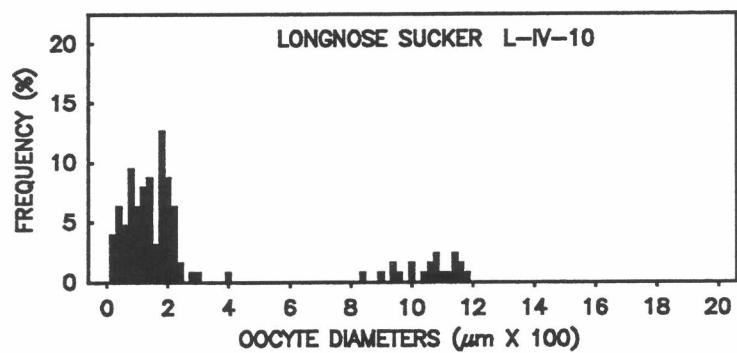


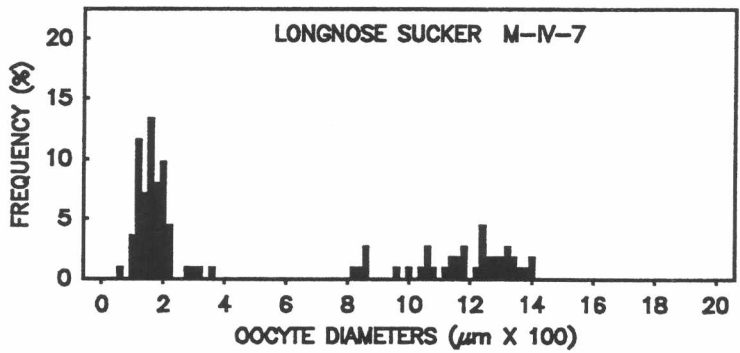
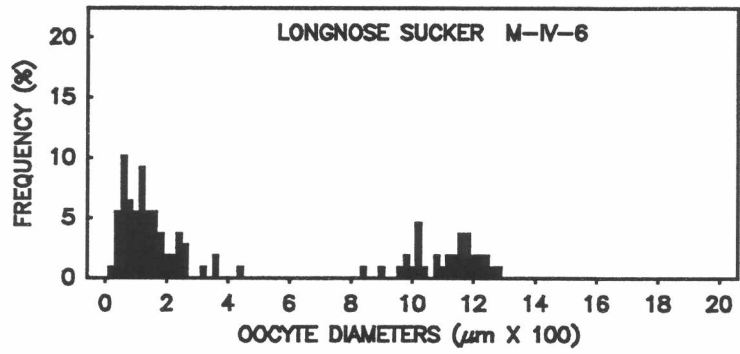
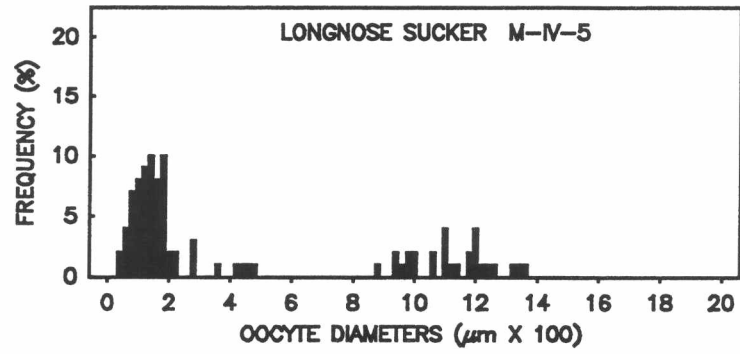




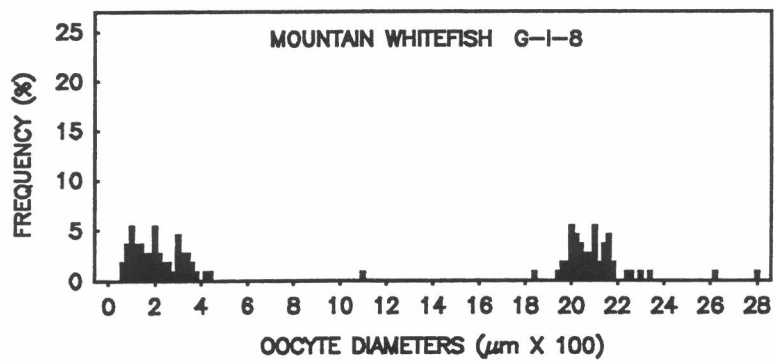
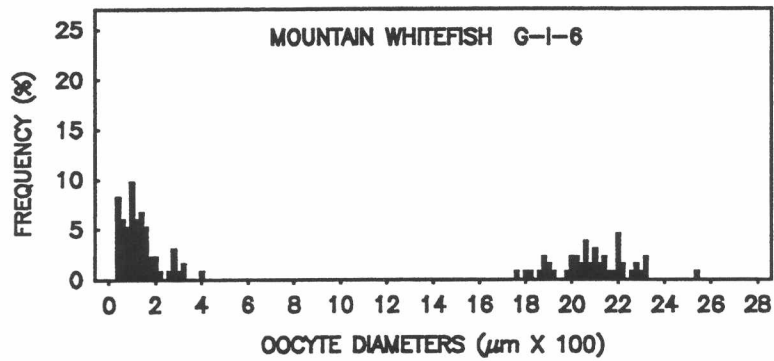
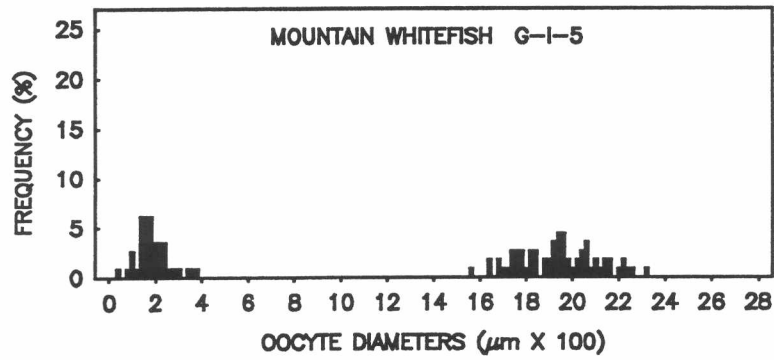
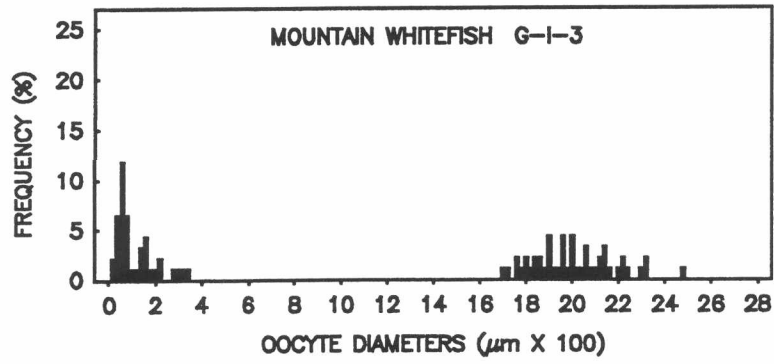


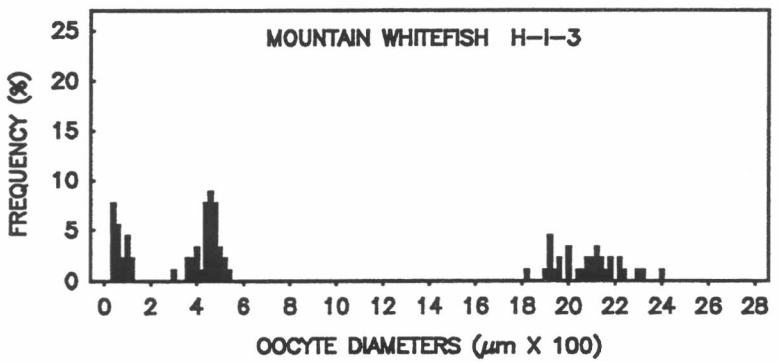
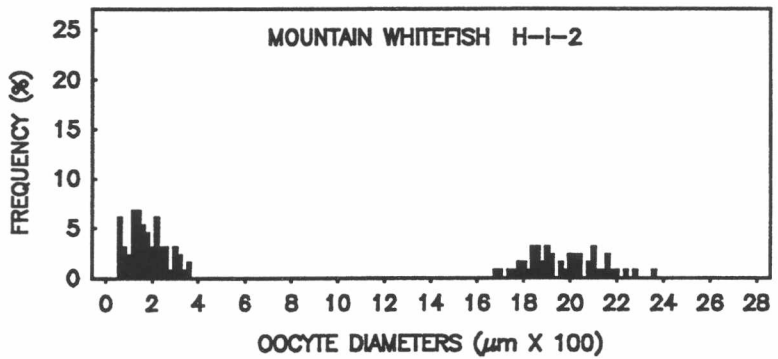
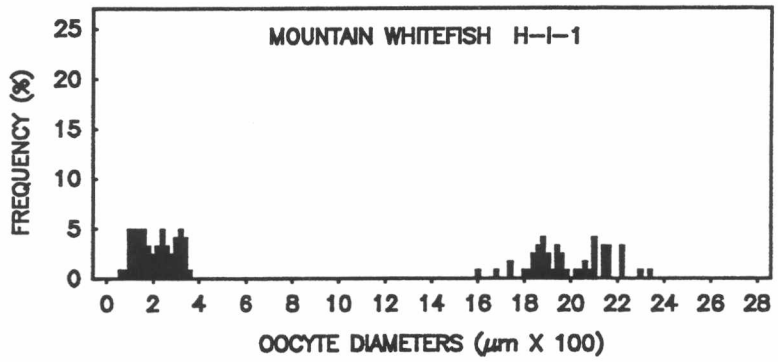
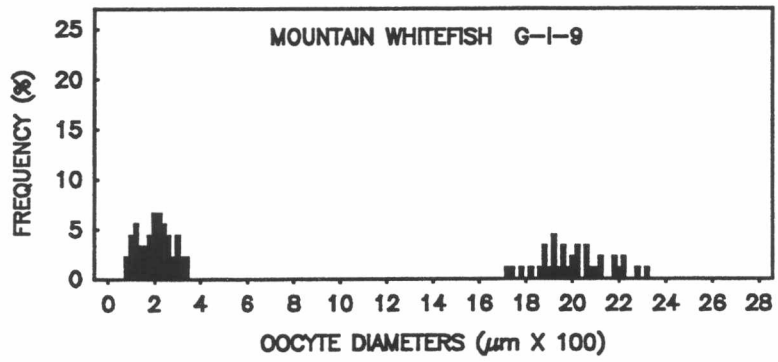


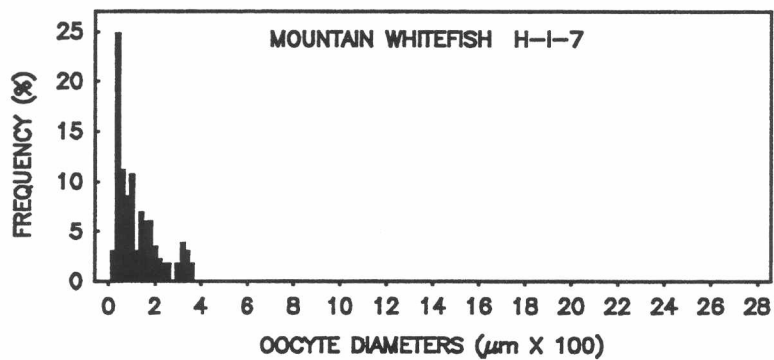
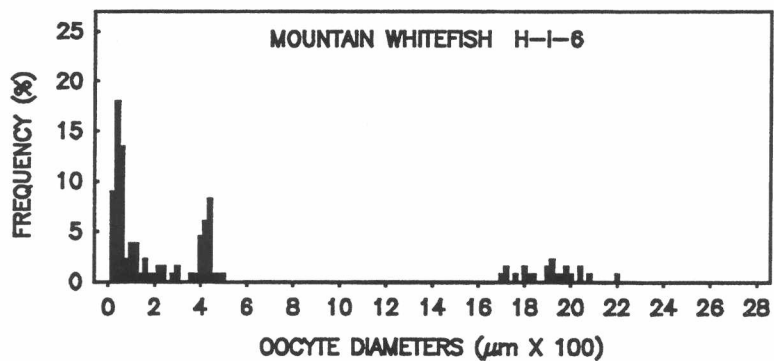
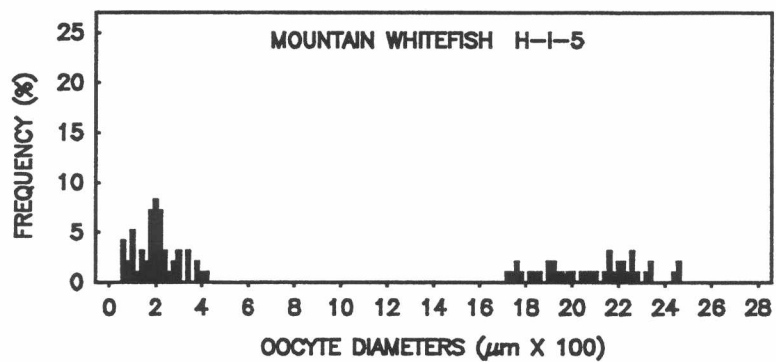
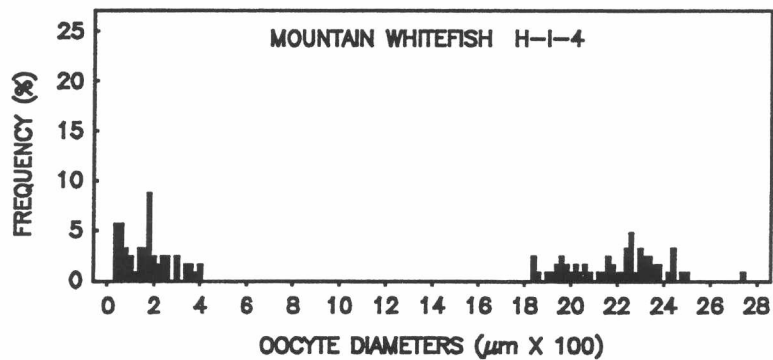


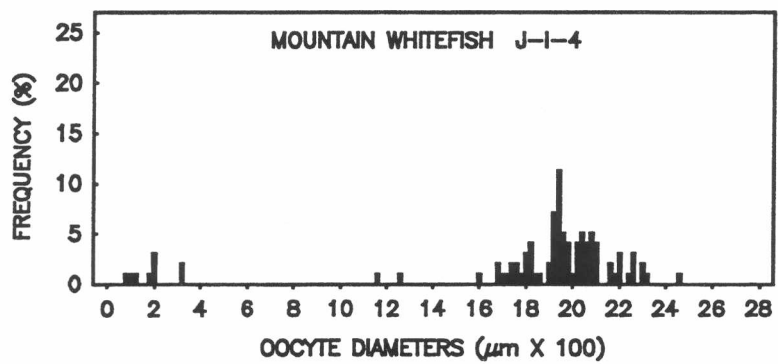
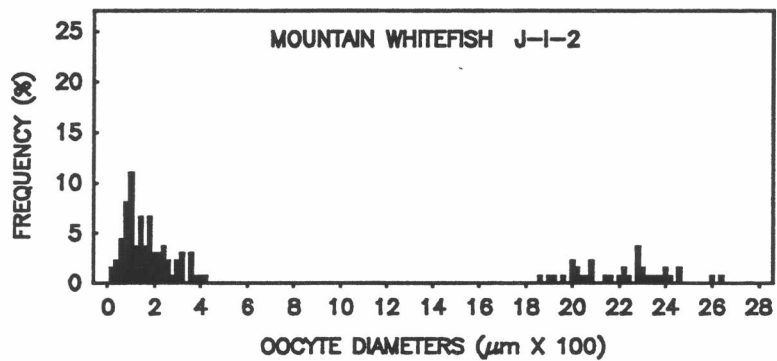
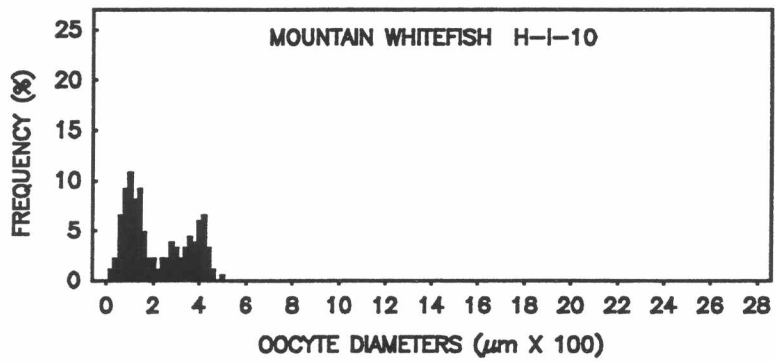
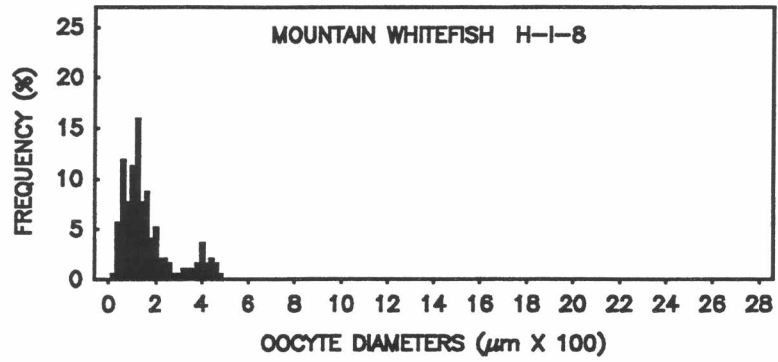


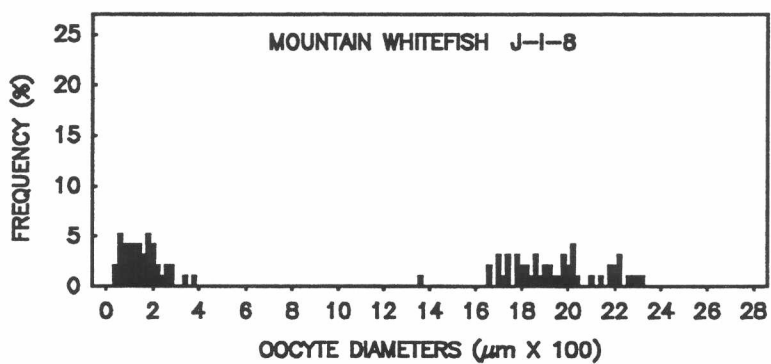
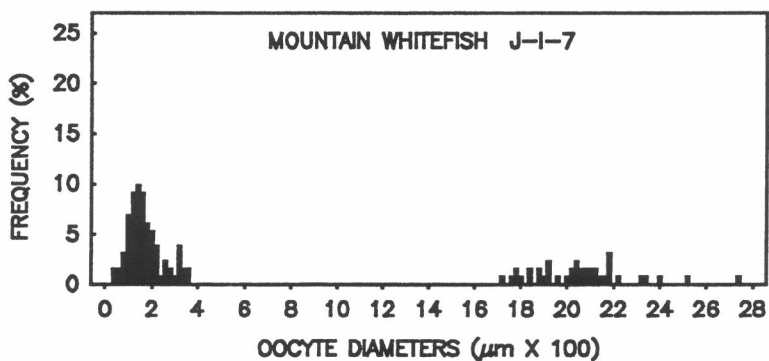
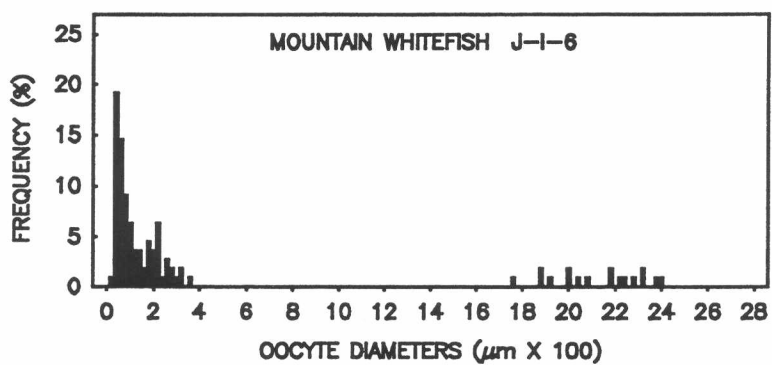
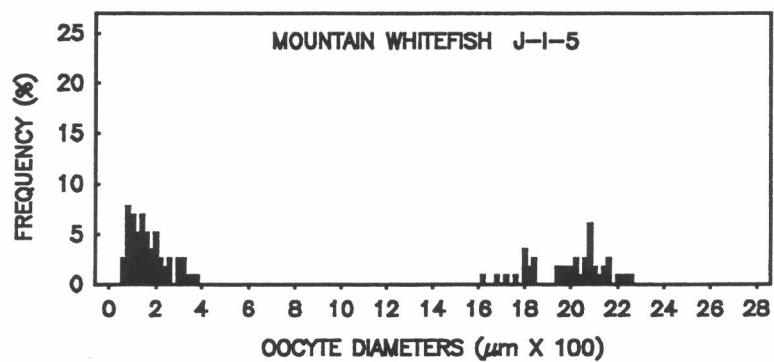
APPENDIX 2
Oocyte Diameter
Frequency Distributions
Fall Mountain Whitefish

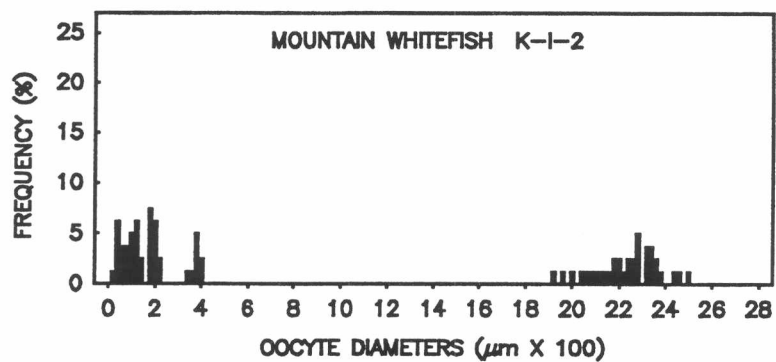
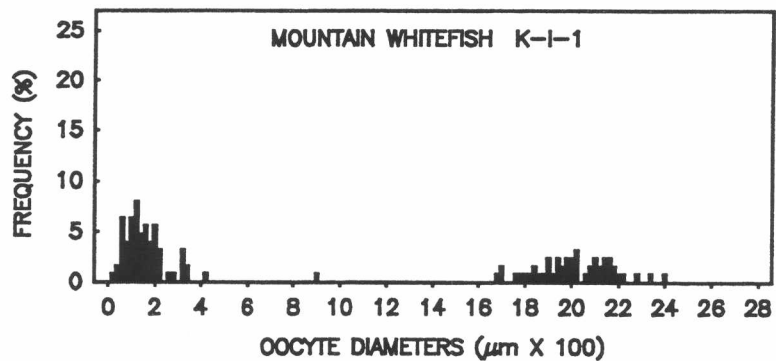
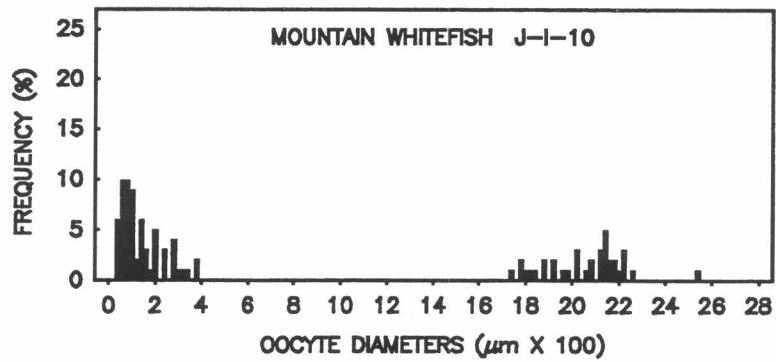
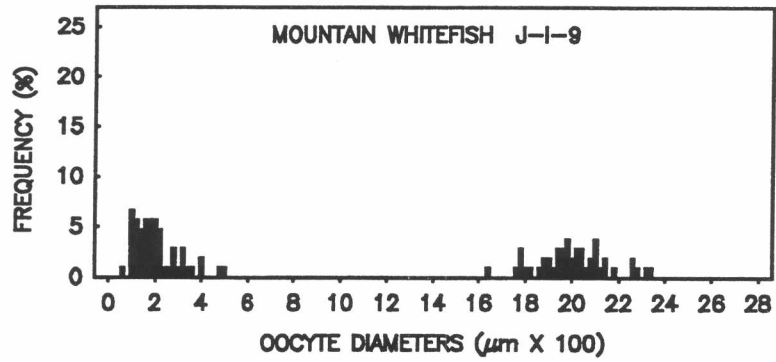


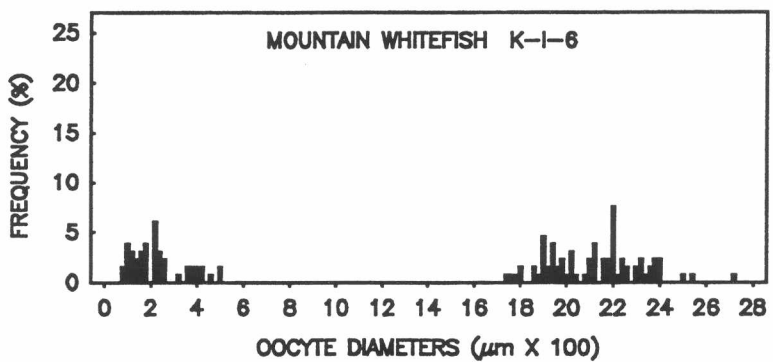
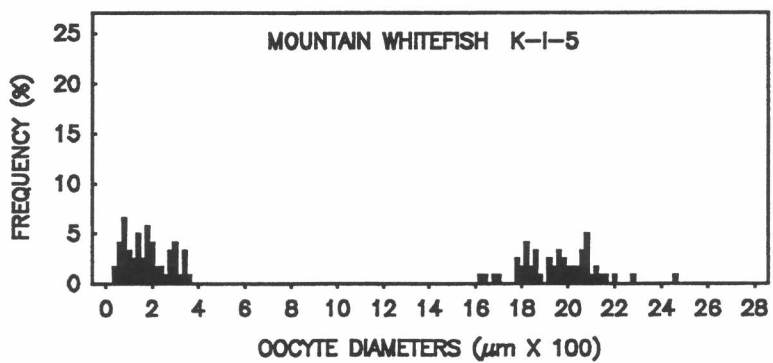
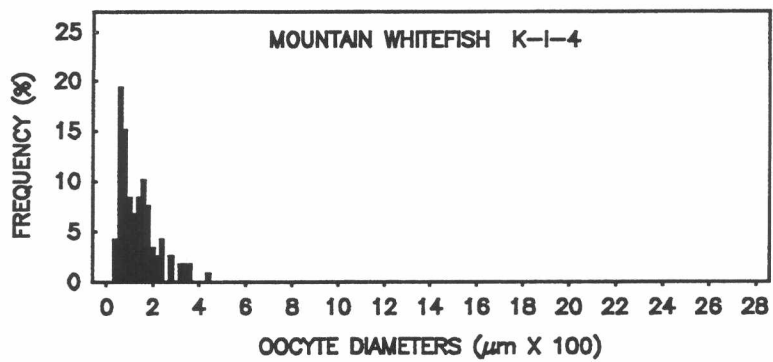
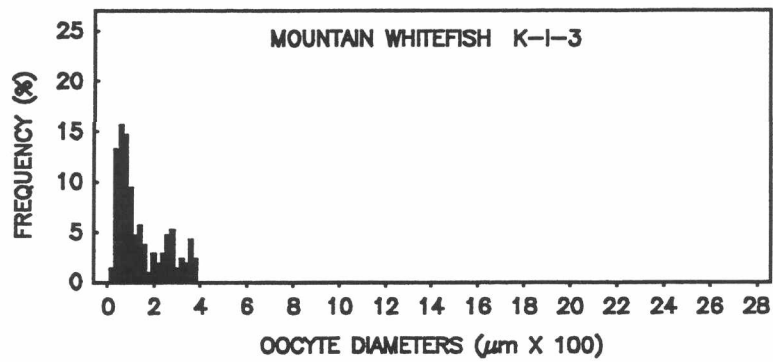


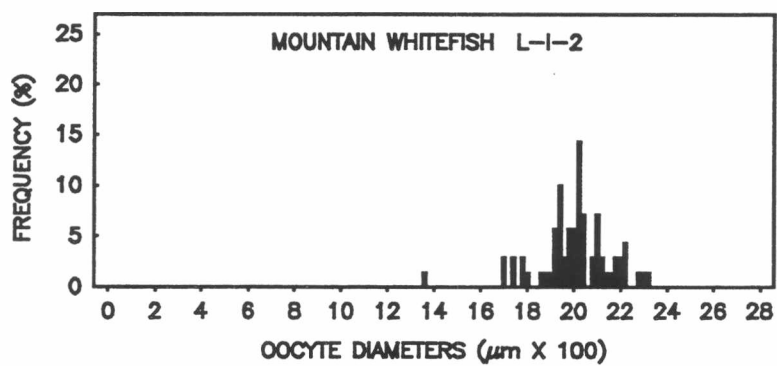
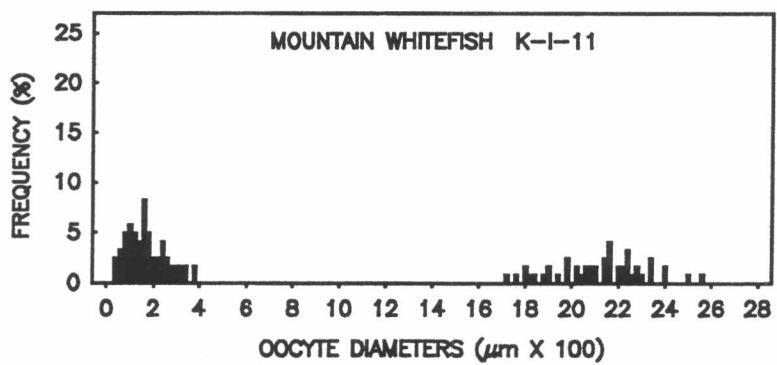
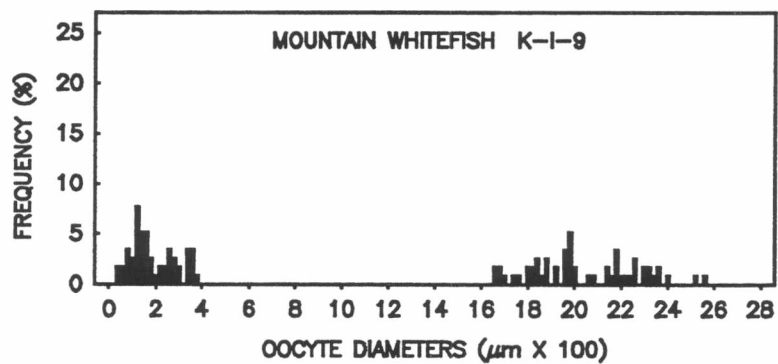
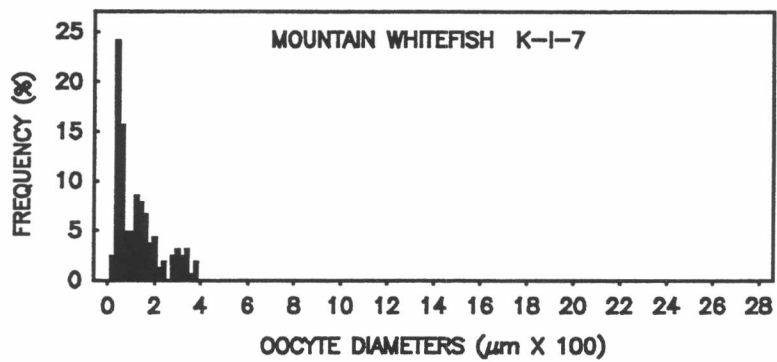


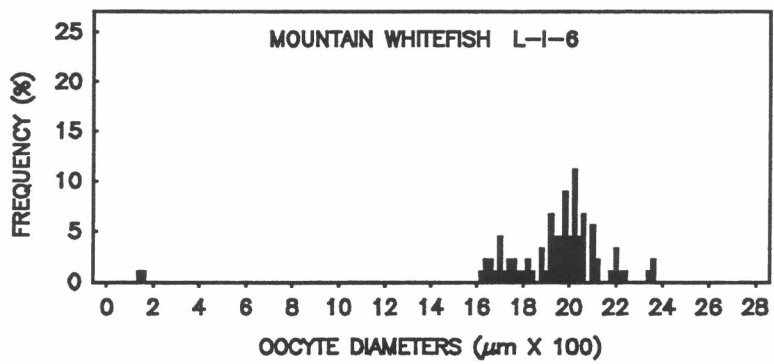
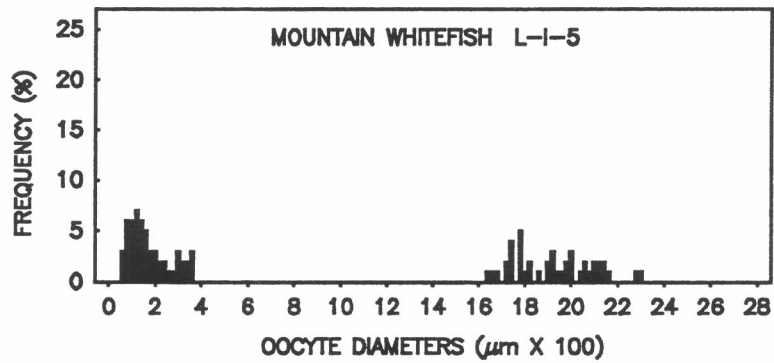
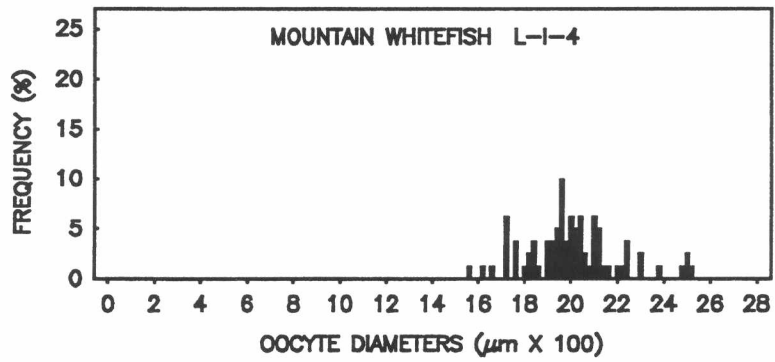
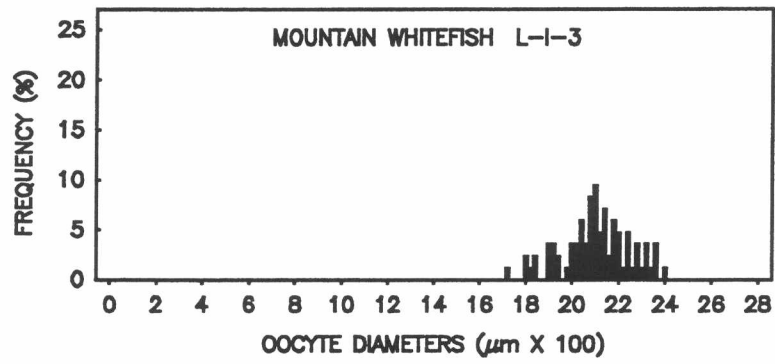


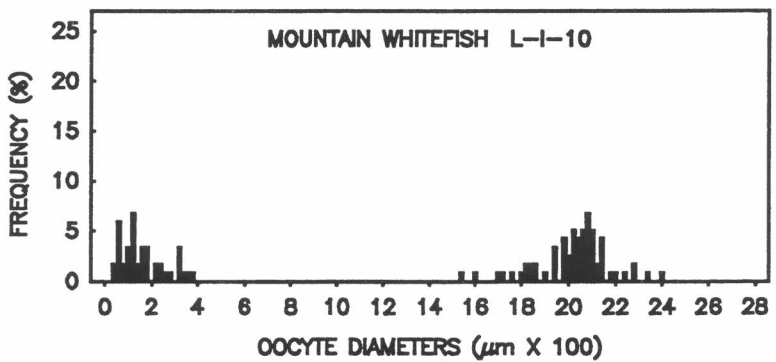
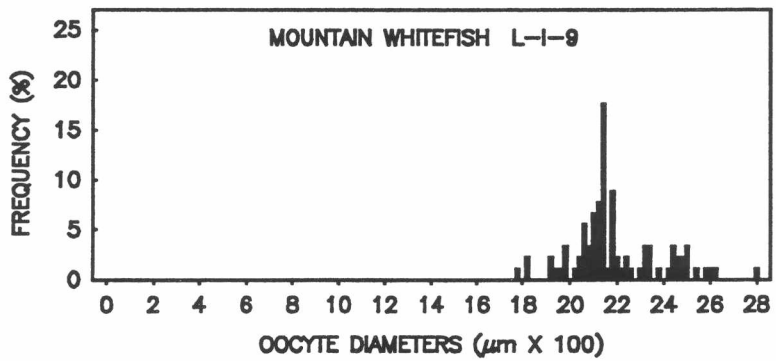
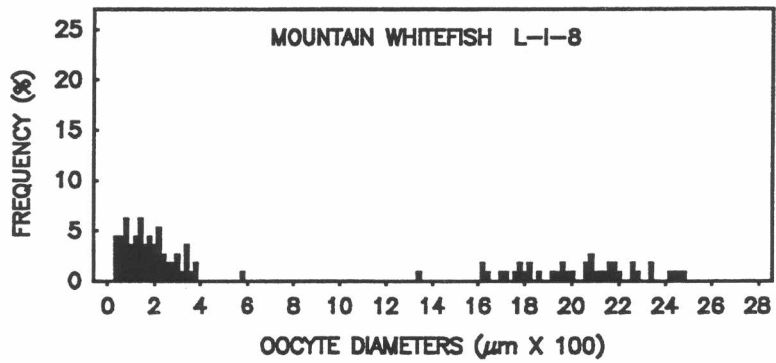
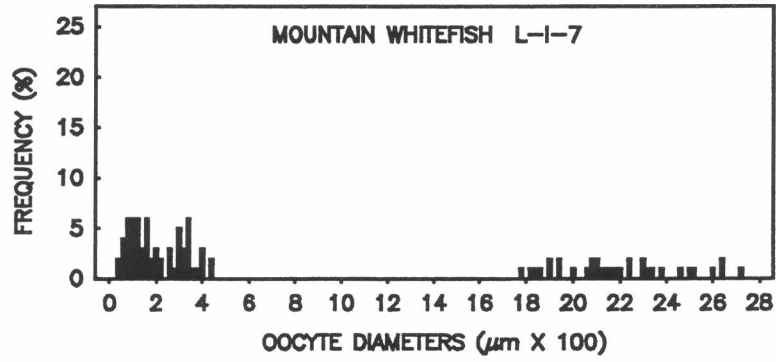


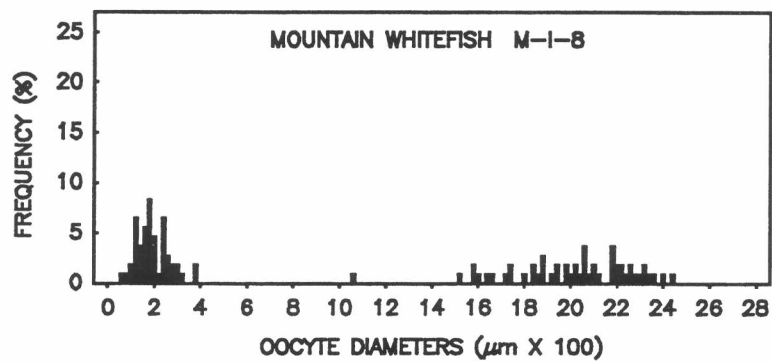
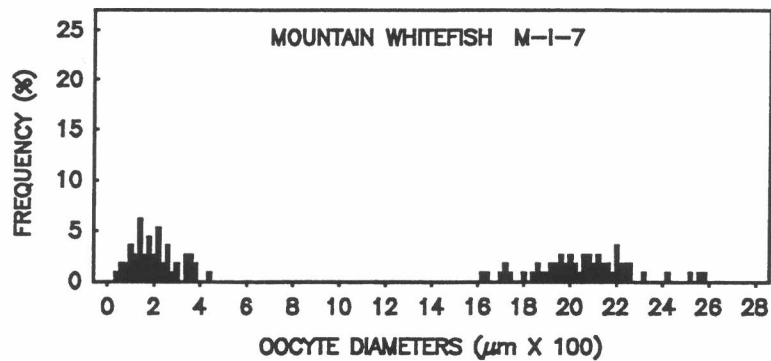
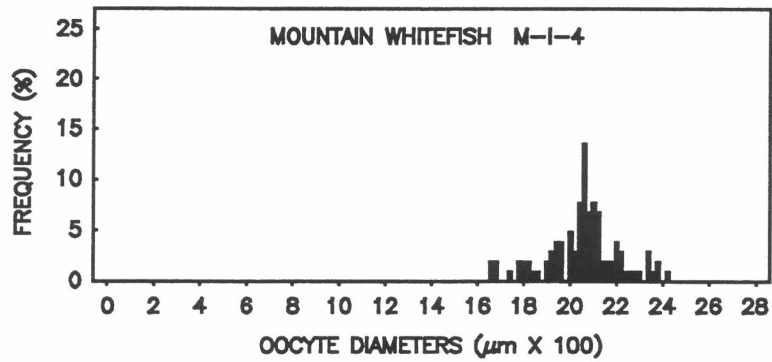
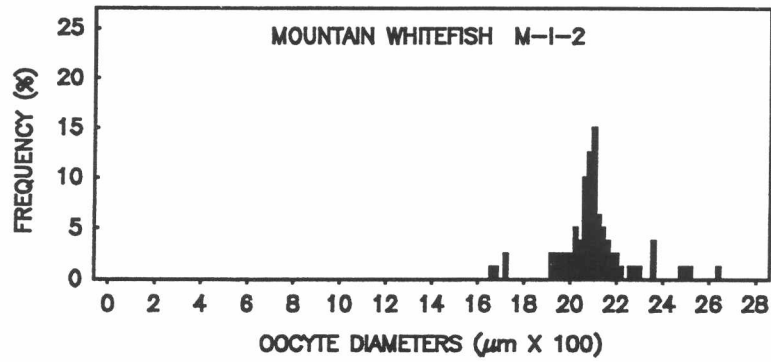


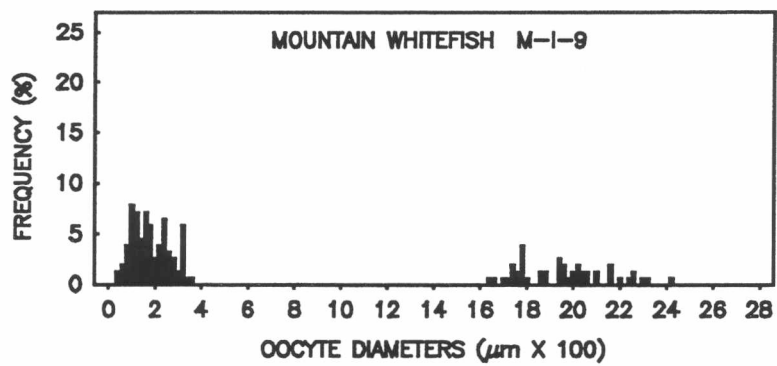




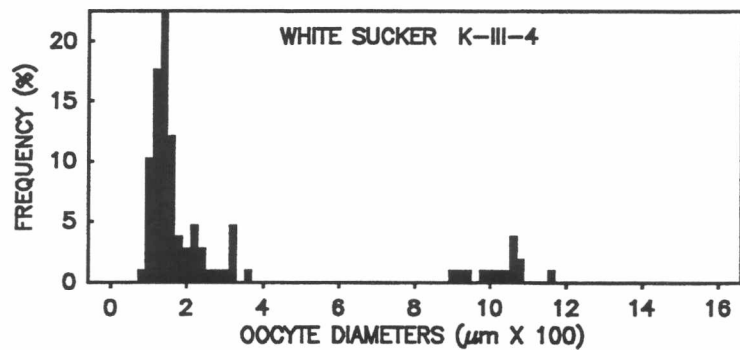
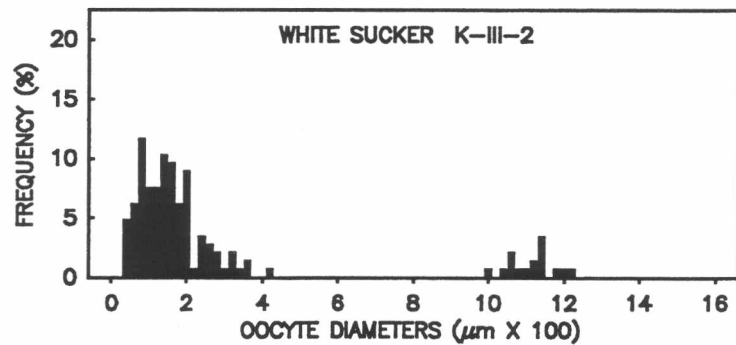
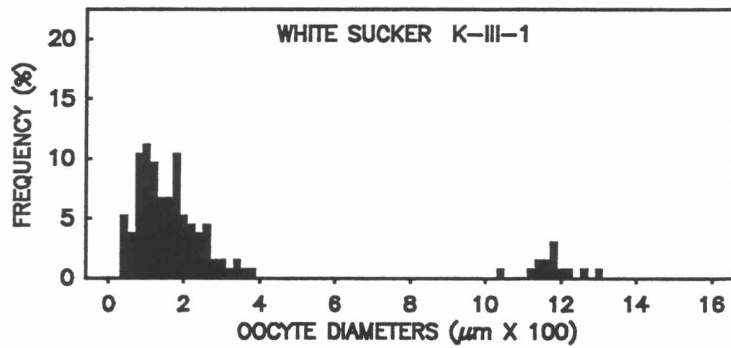
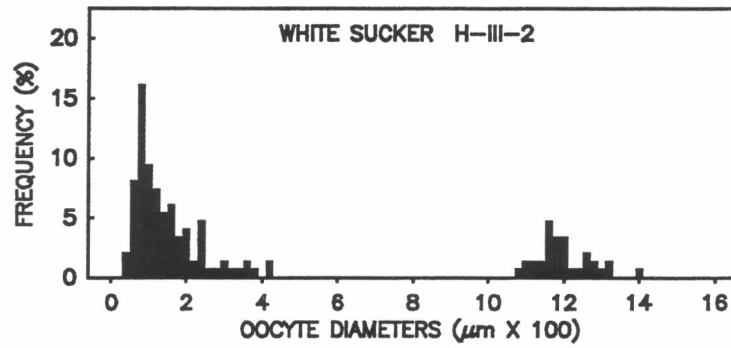


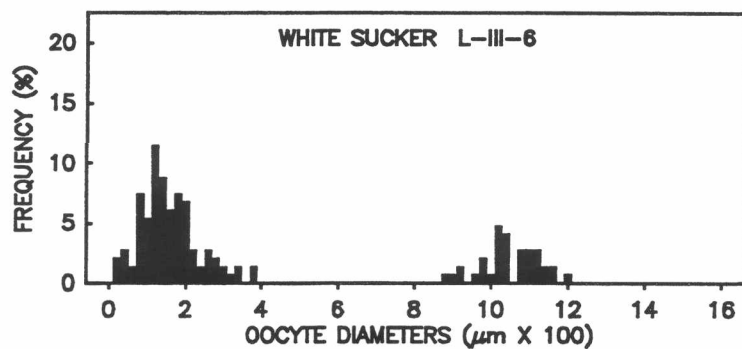
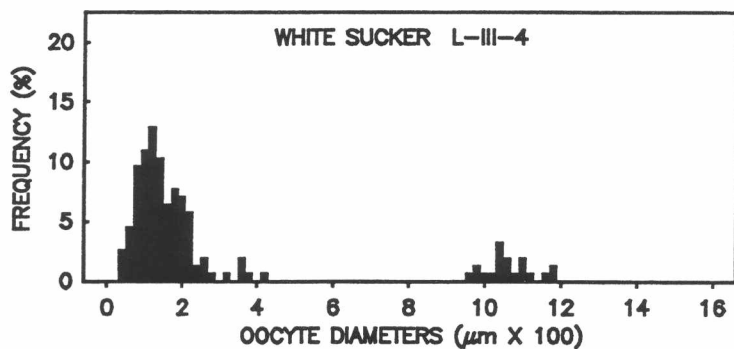
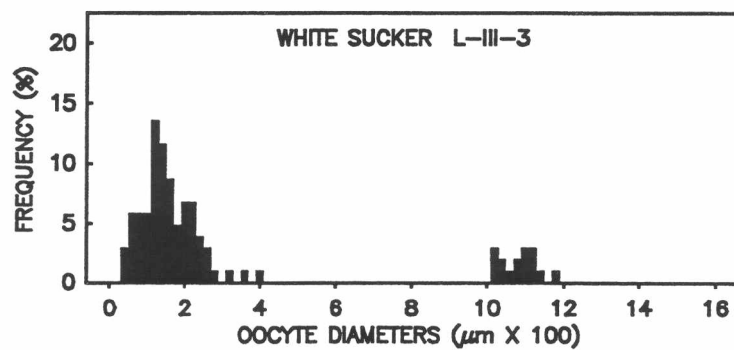
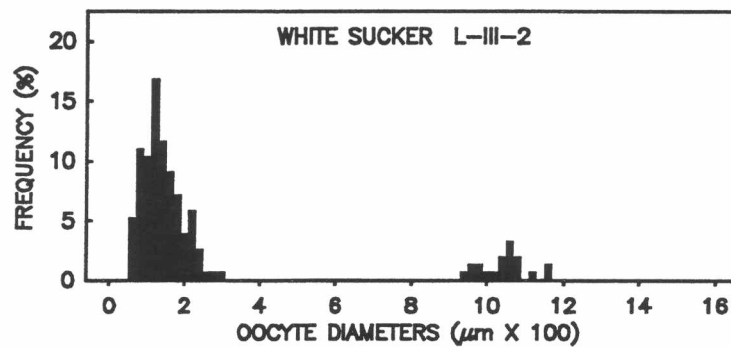


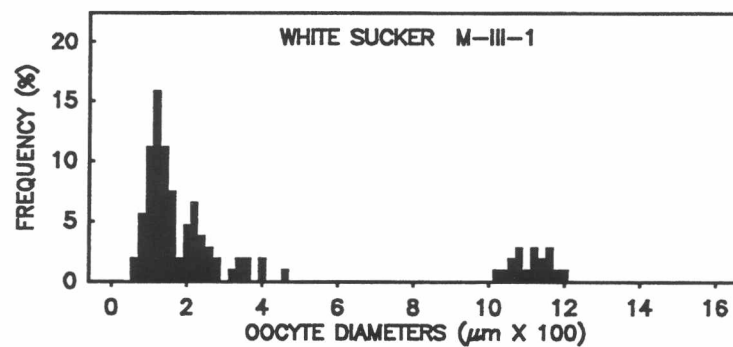
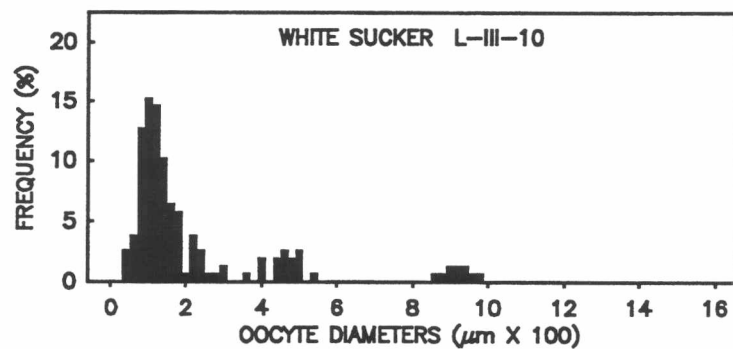
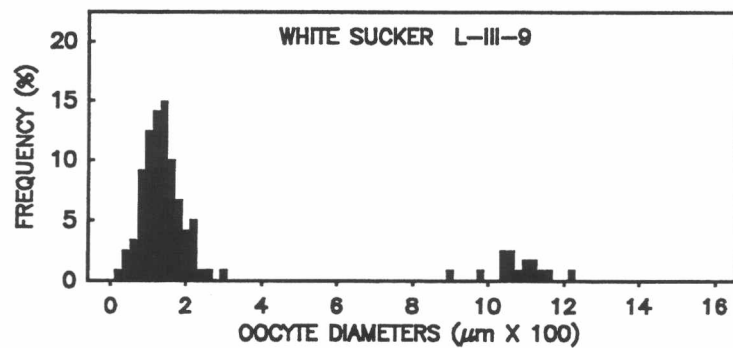
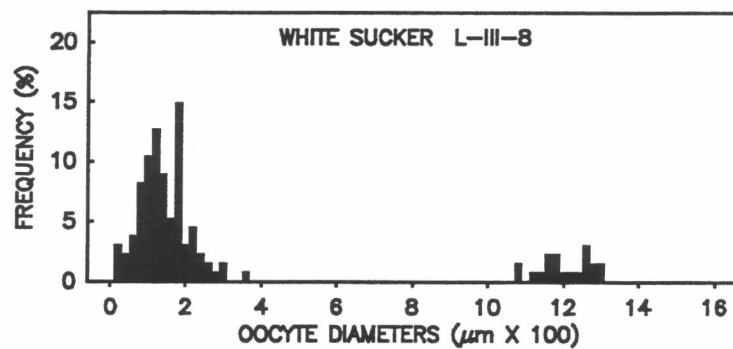


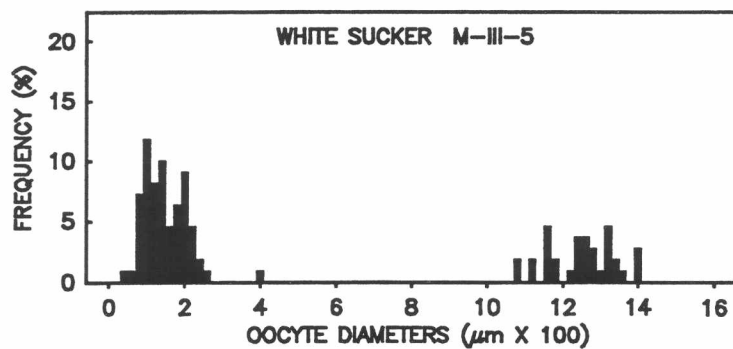
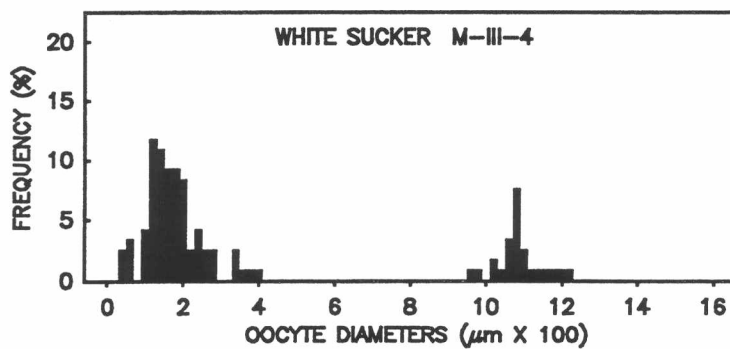
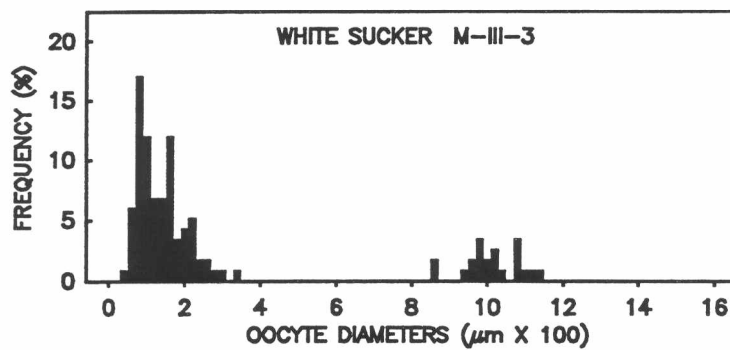
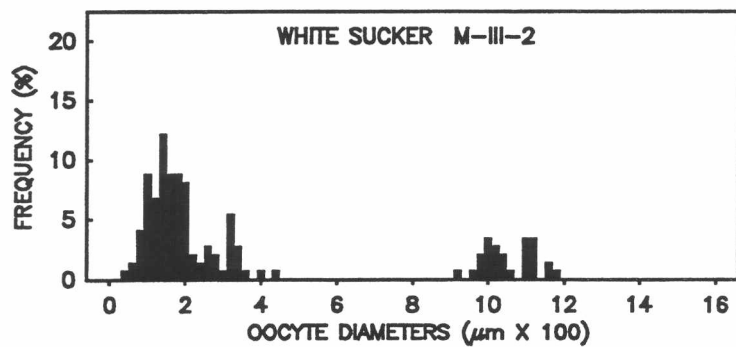


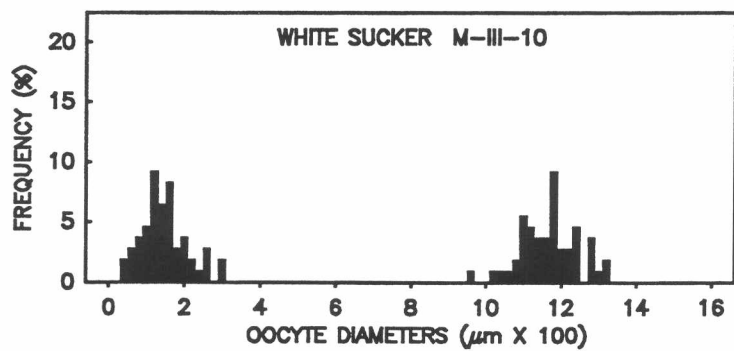
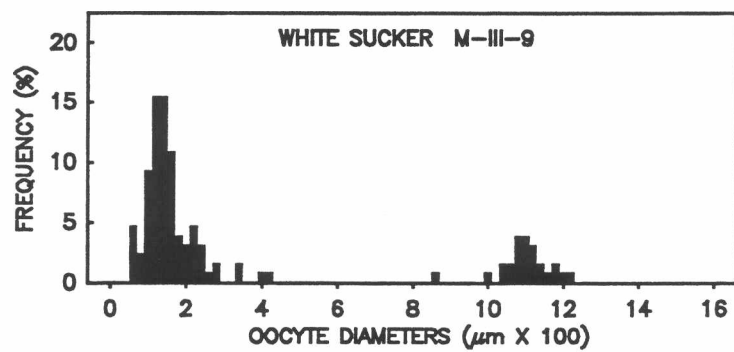
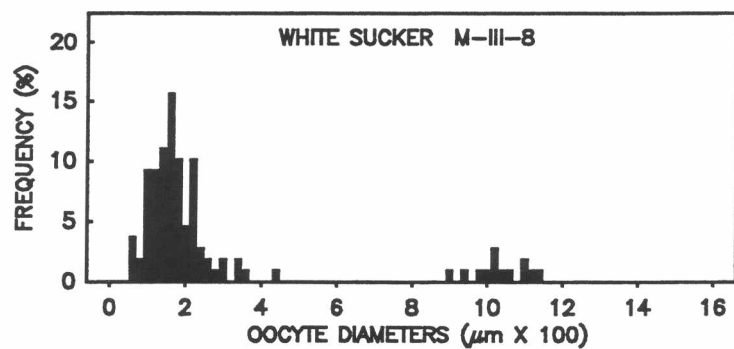
APPENDIX 3
Oocyte Diameter
Frequency Distributions
Fall White Sucker



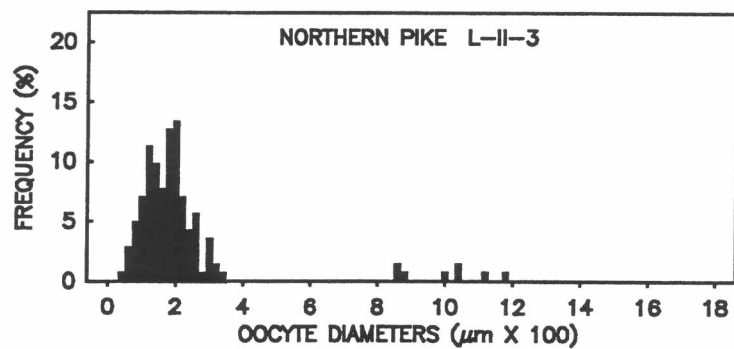
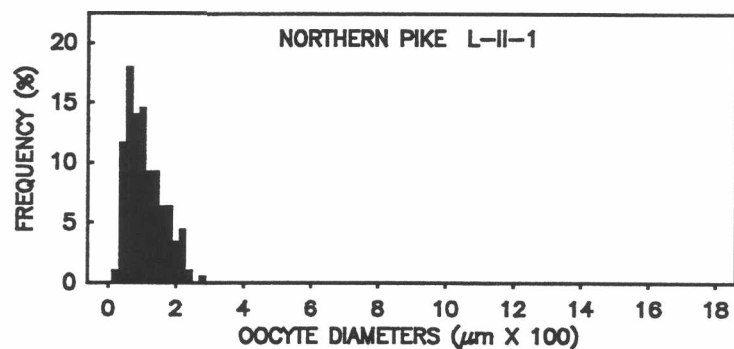
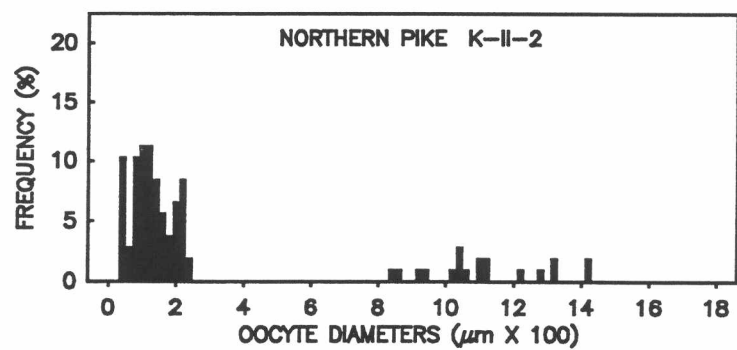
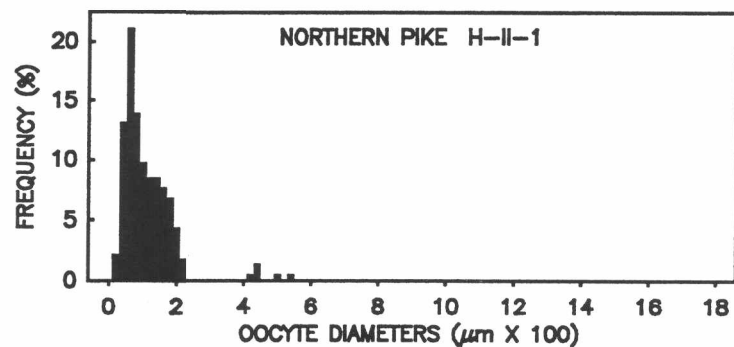


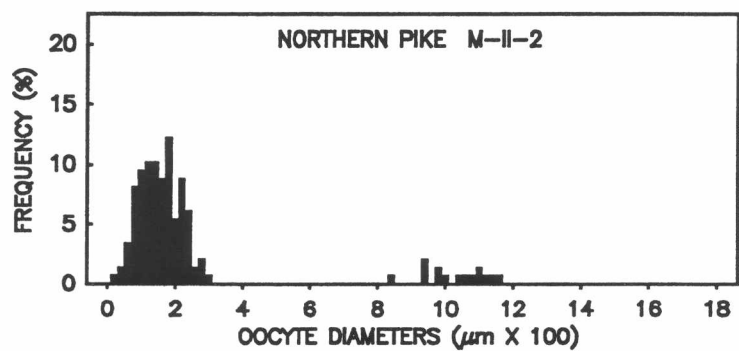
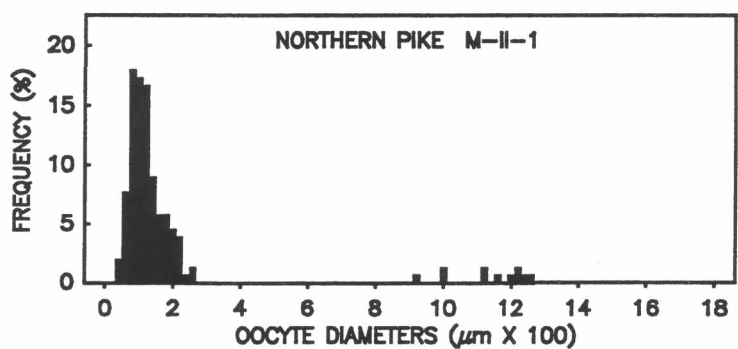
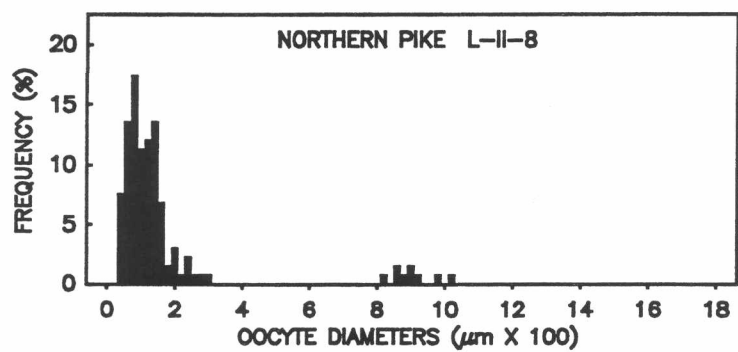
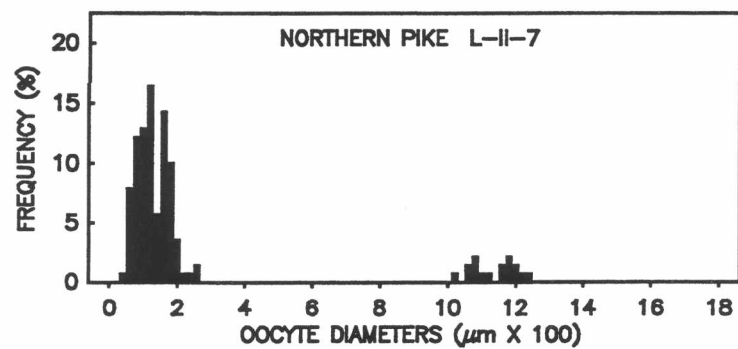


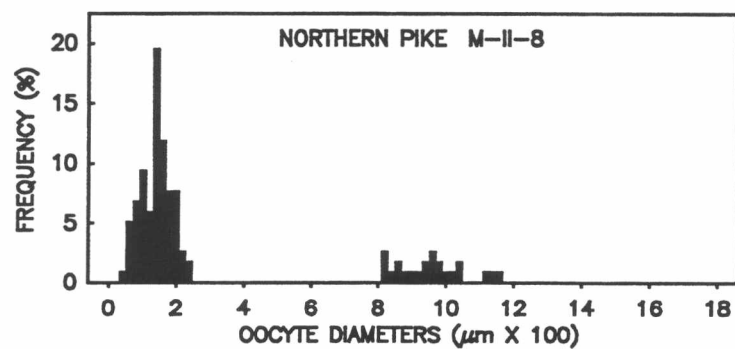
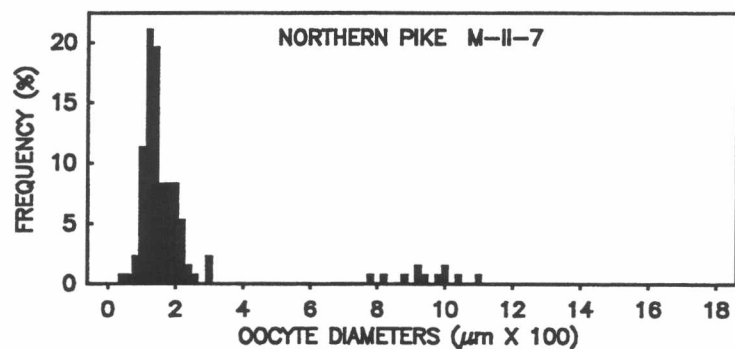
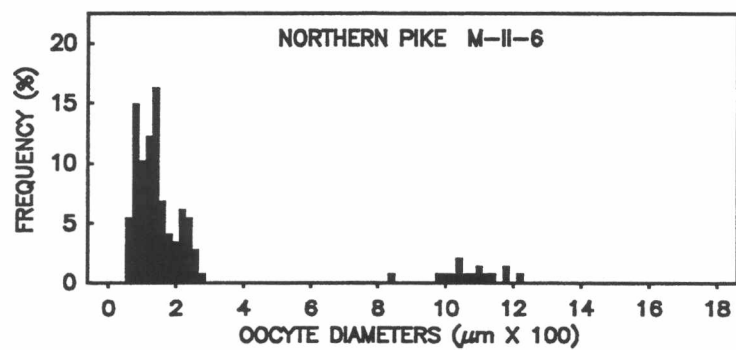
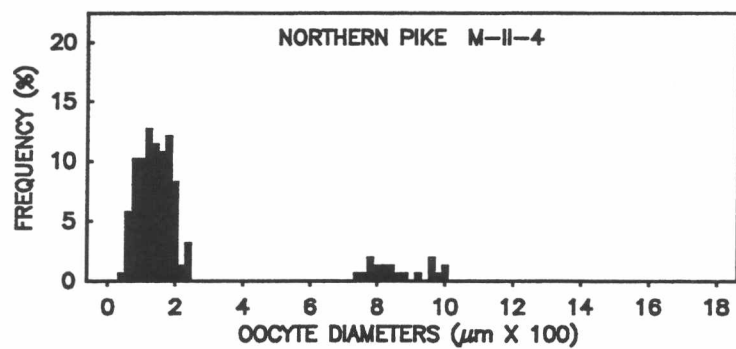




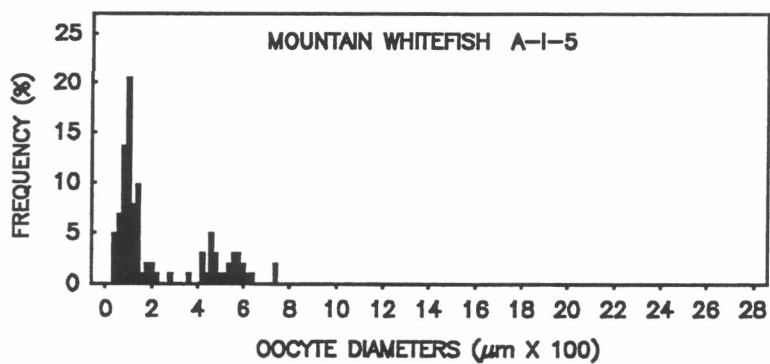
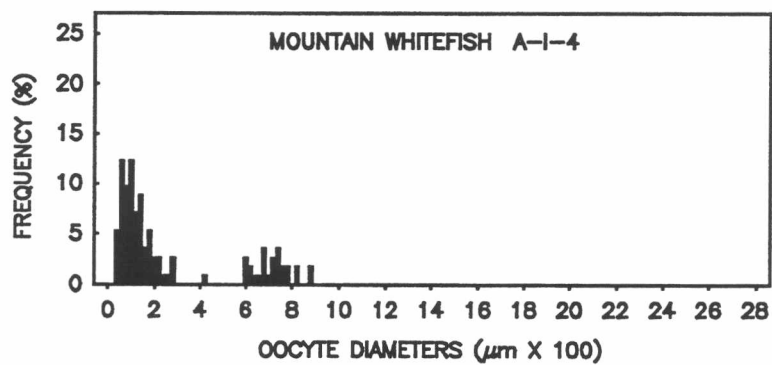
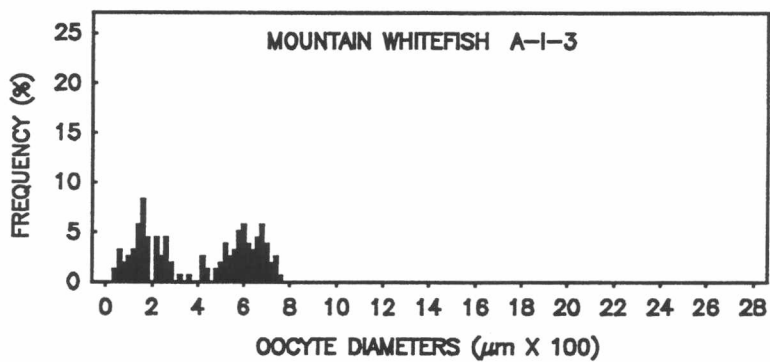
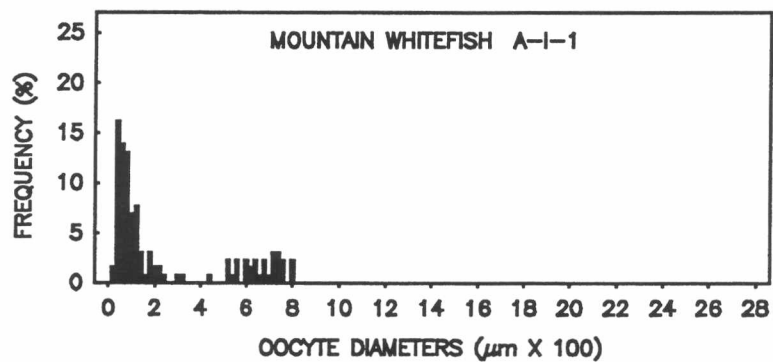
APPENDIX 4
Oocyte Diameter
Frequency Distributions
Fall Northern Pike

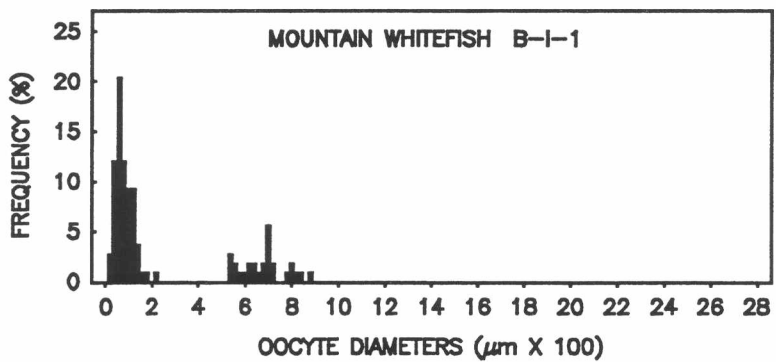
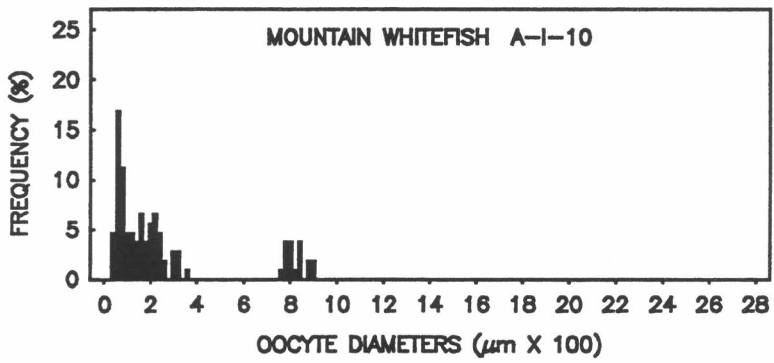
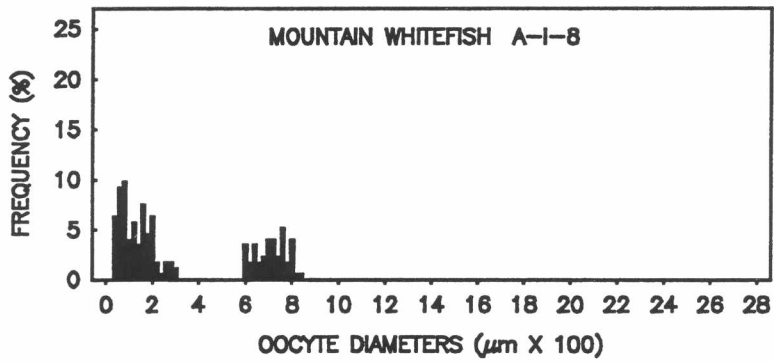
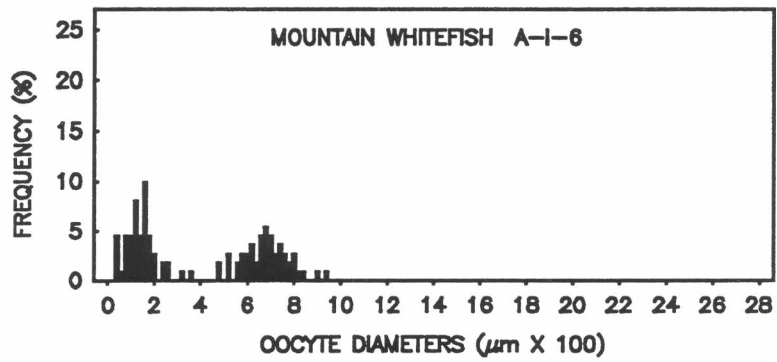


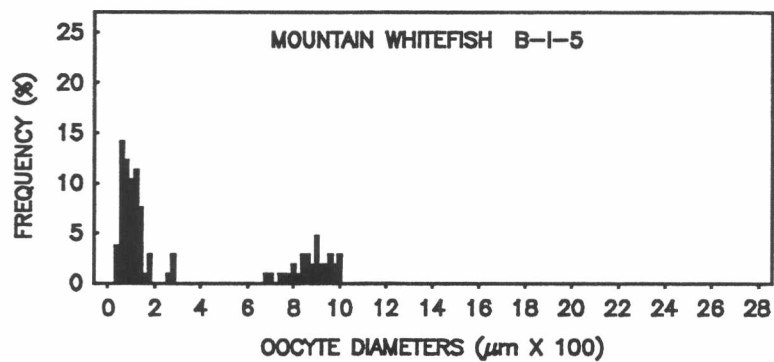
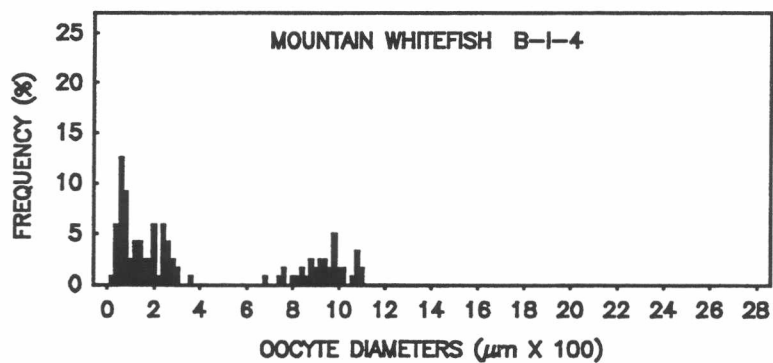
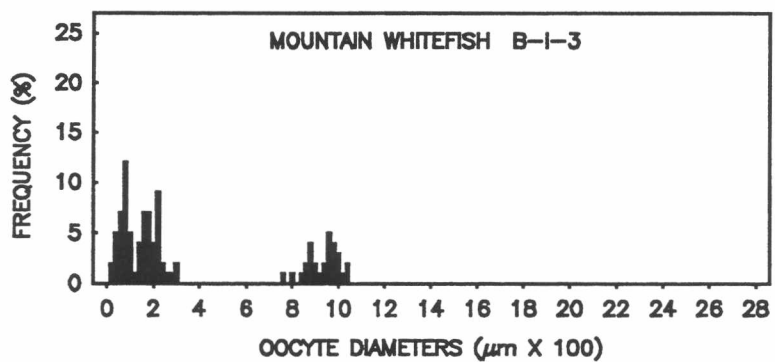
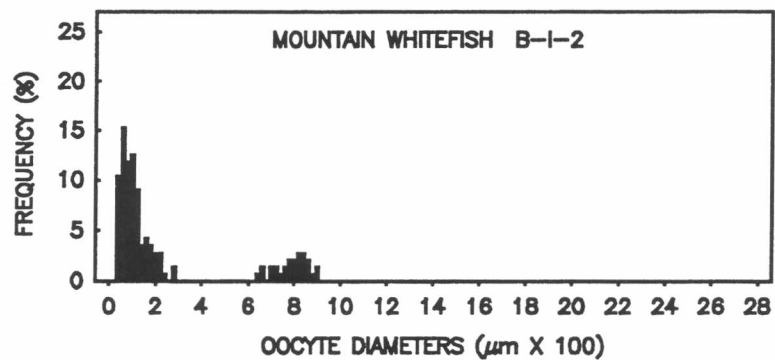


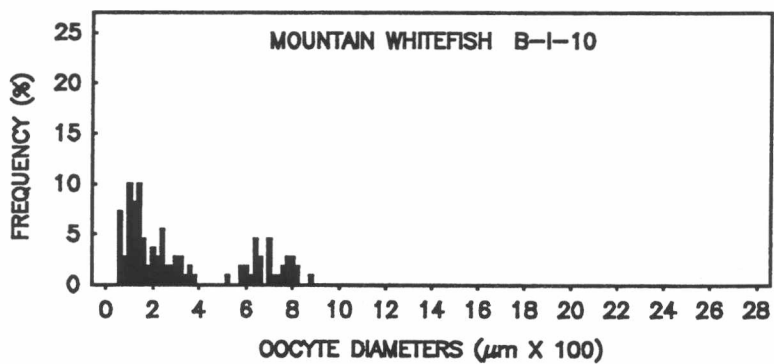
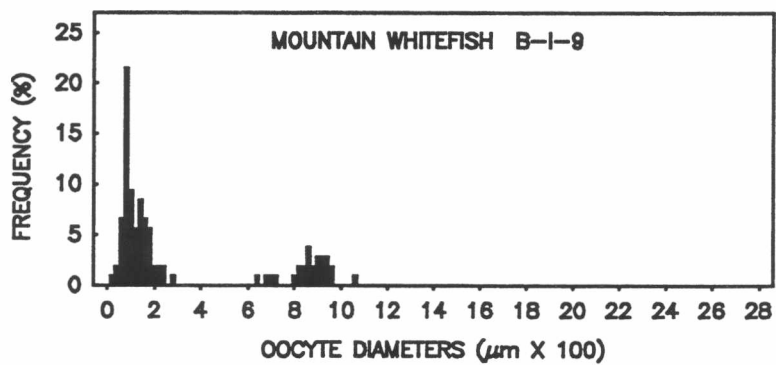
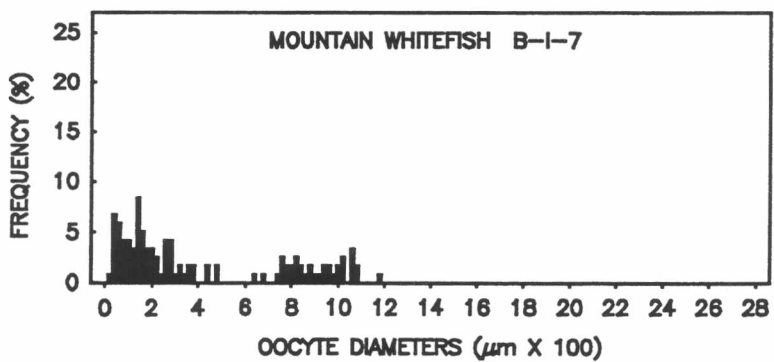
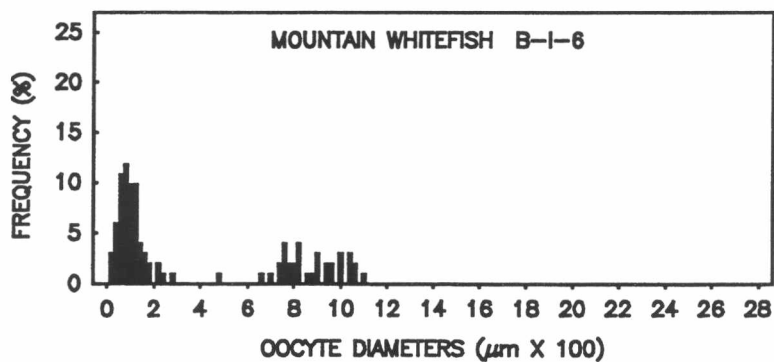


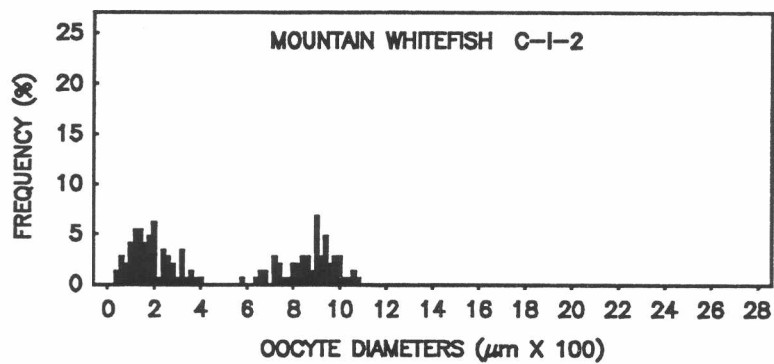
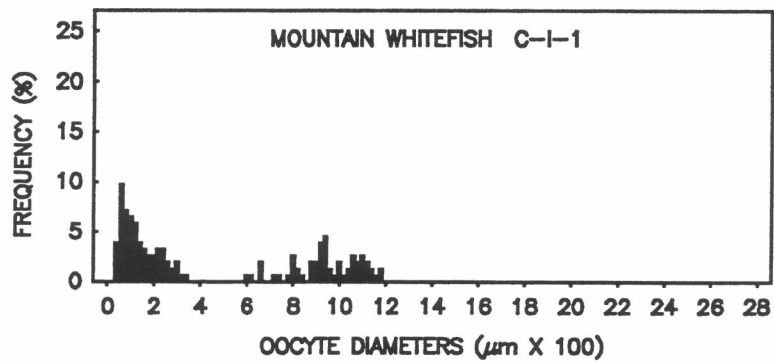
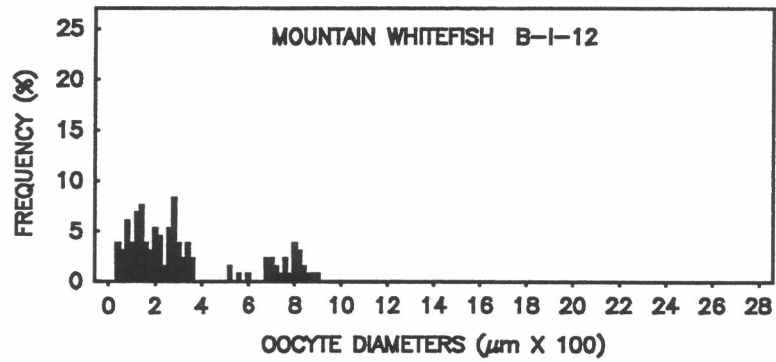
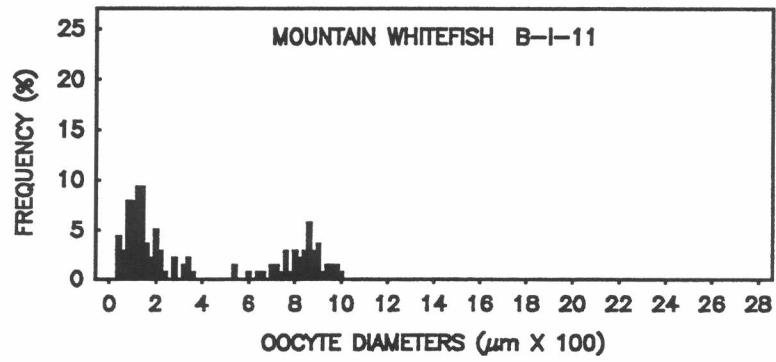
APPENDIX 5
Oocyte Diameter
Frequency Distributions
Spring Mountain Whitefish

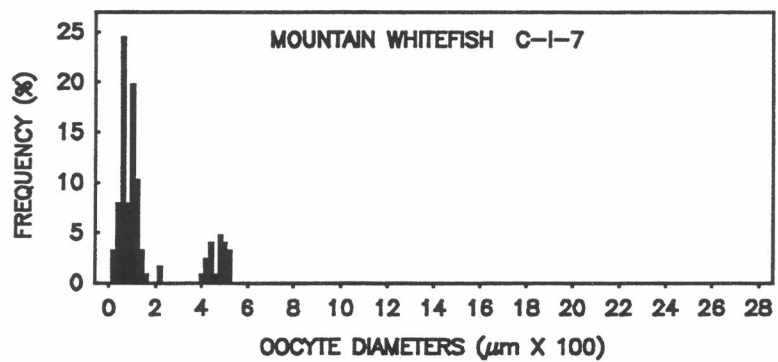
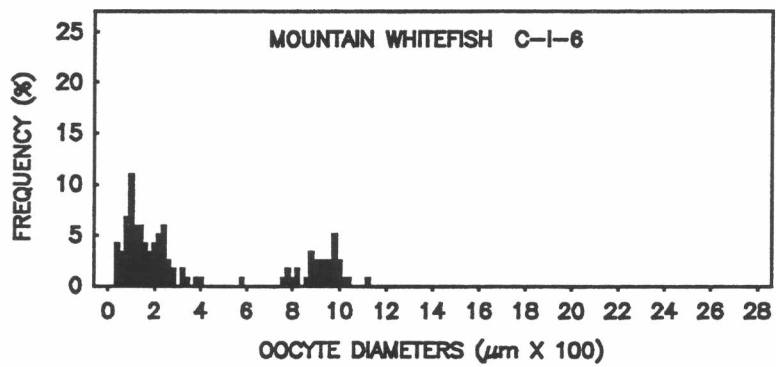
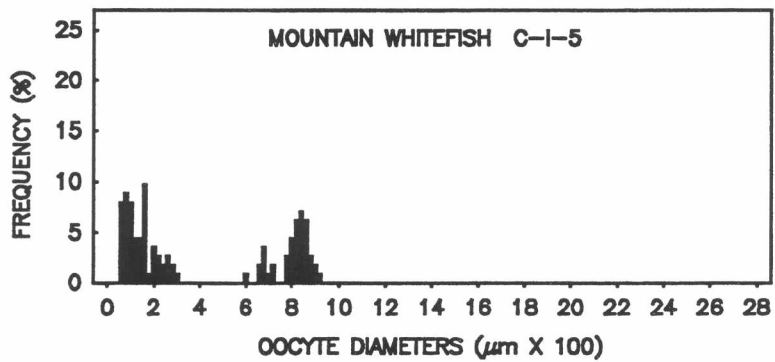
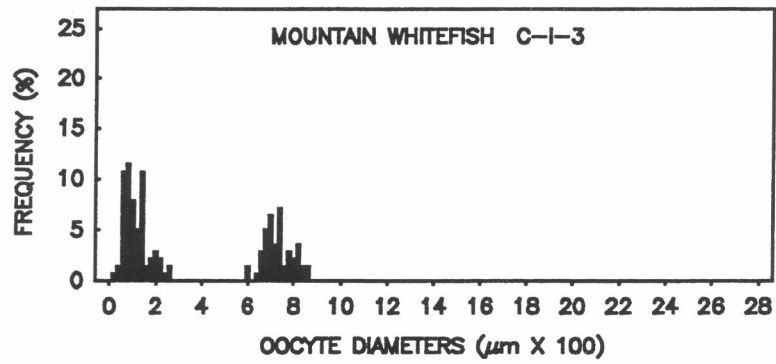


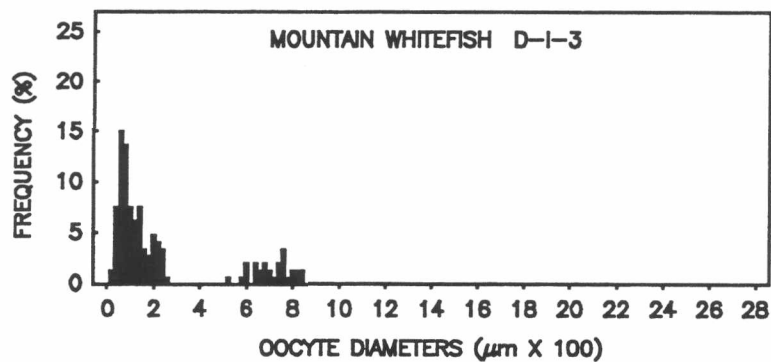
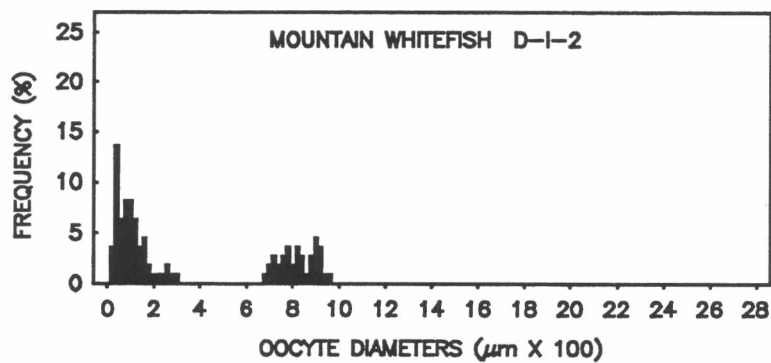
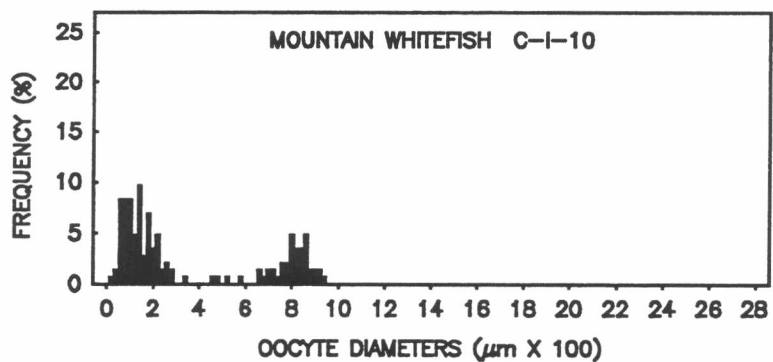
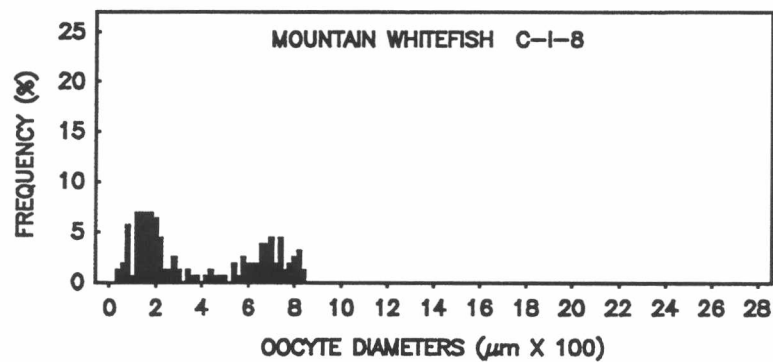


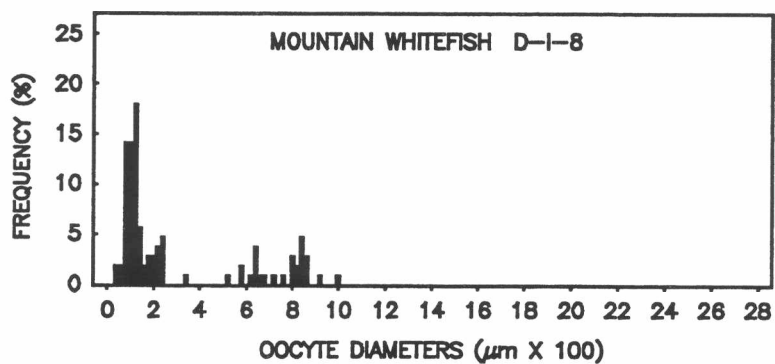
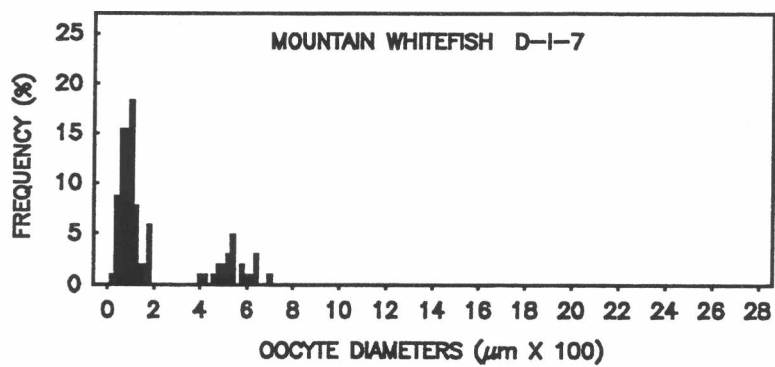
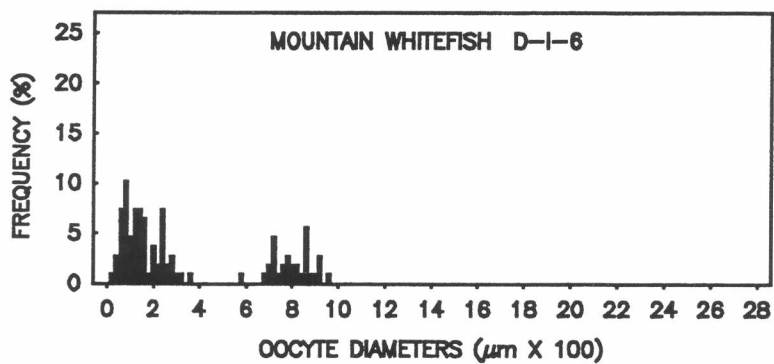
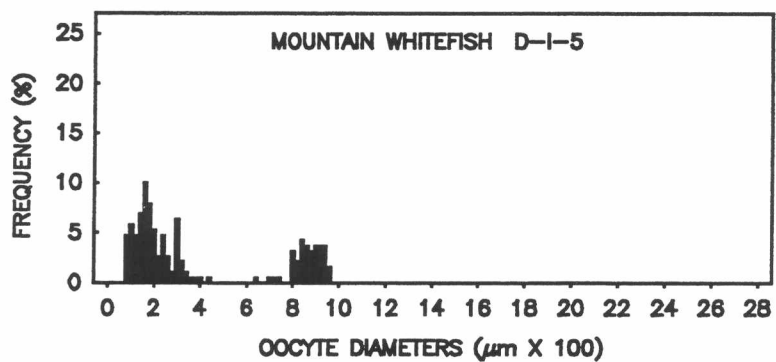


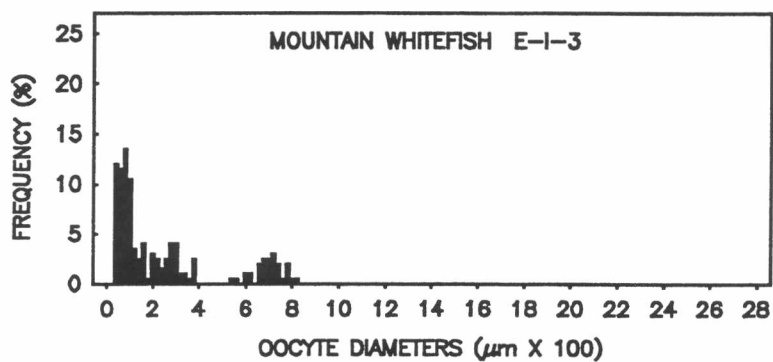
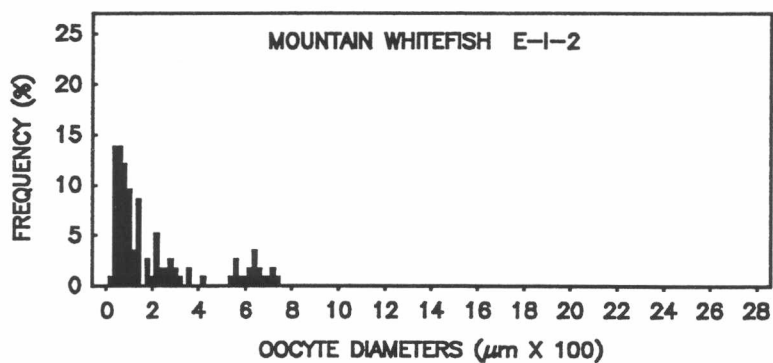
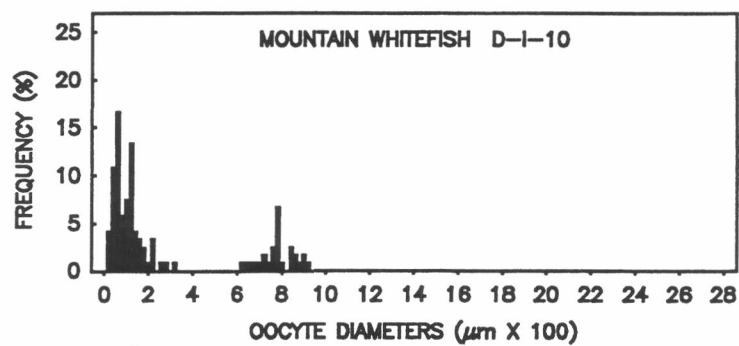
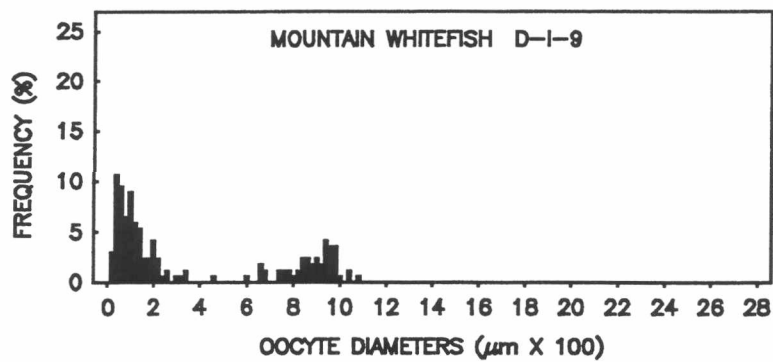


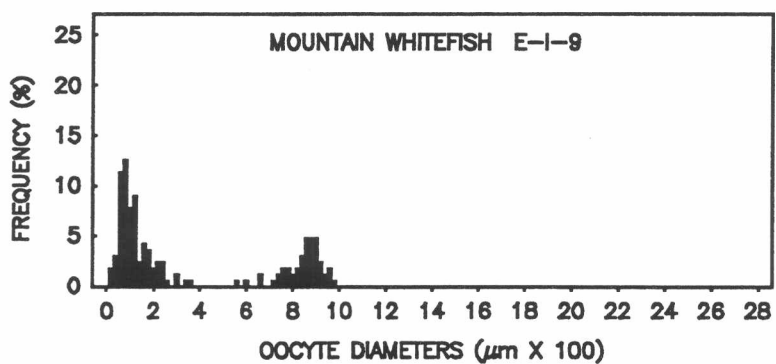
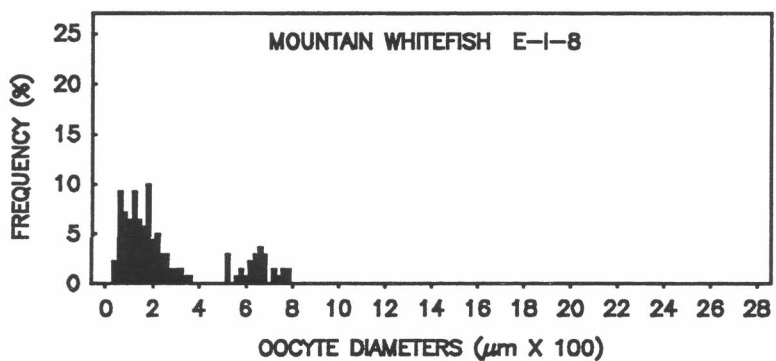
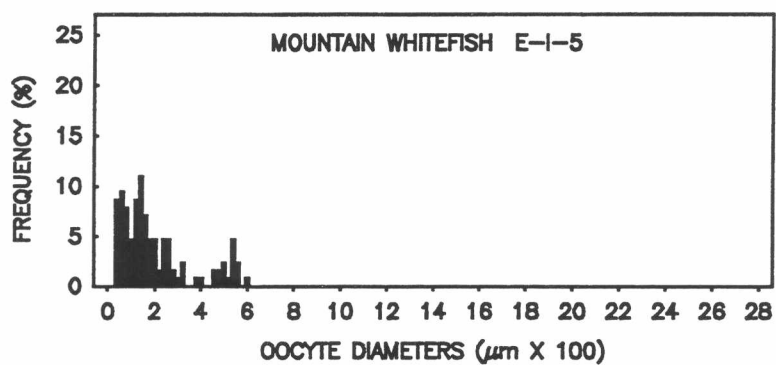
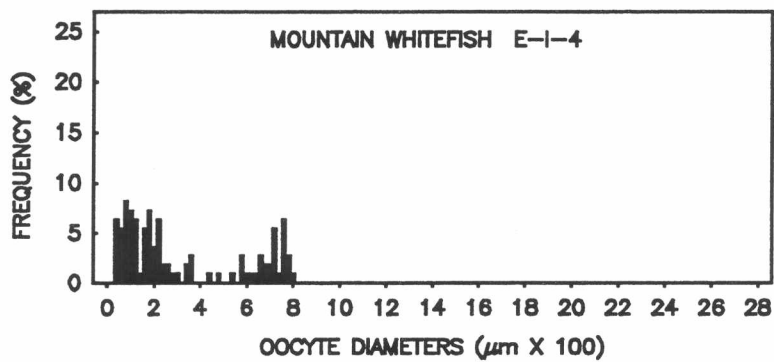


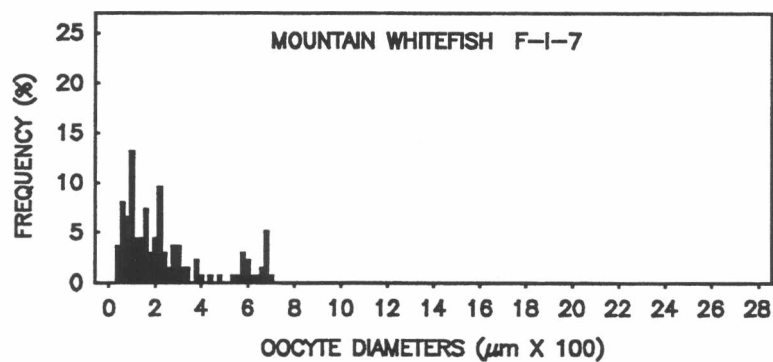
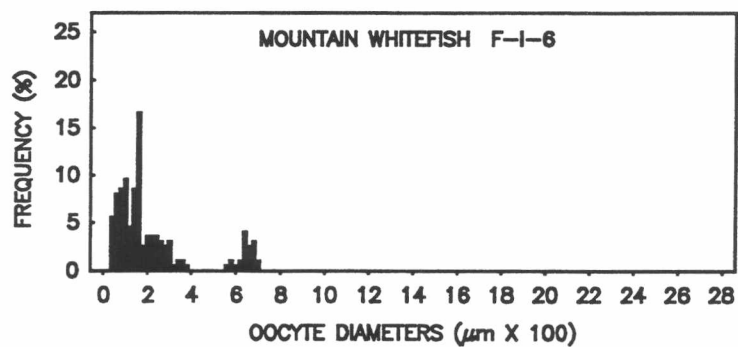
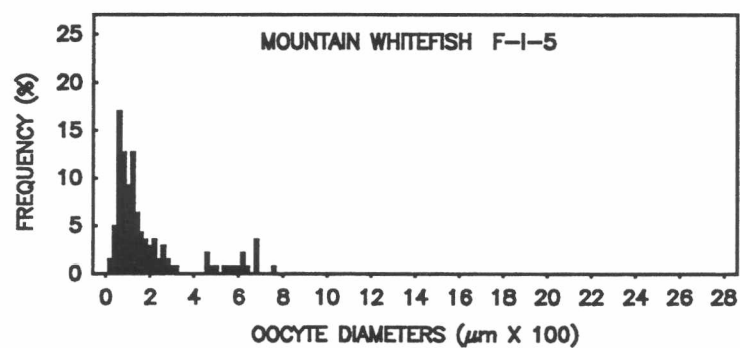
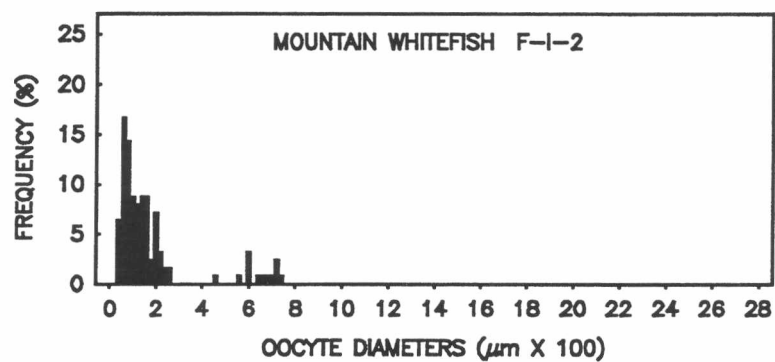


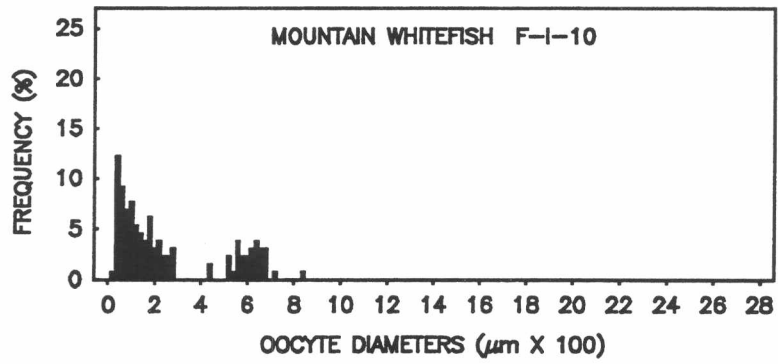
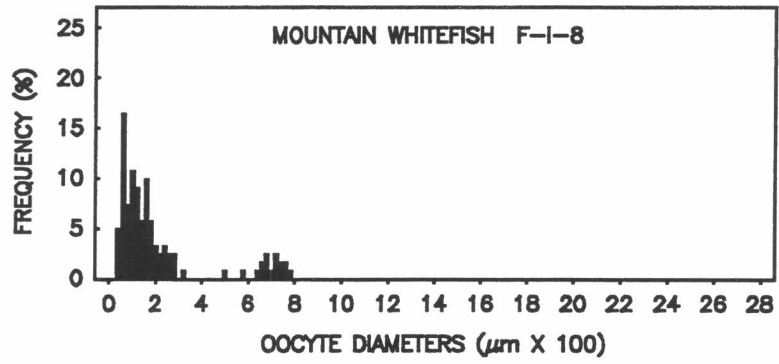




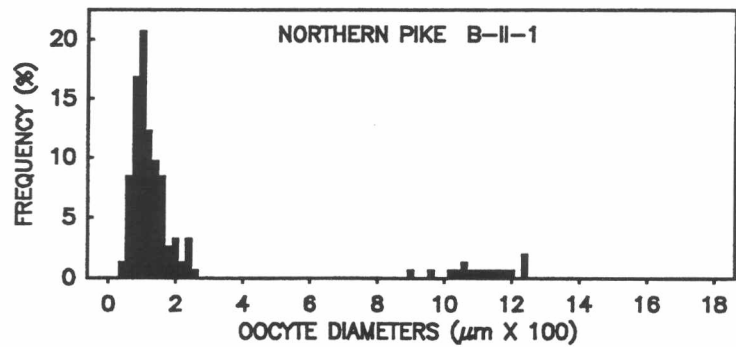
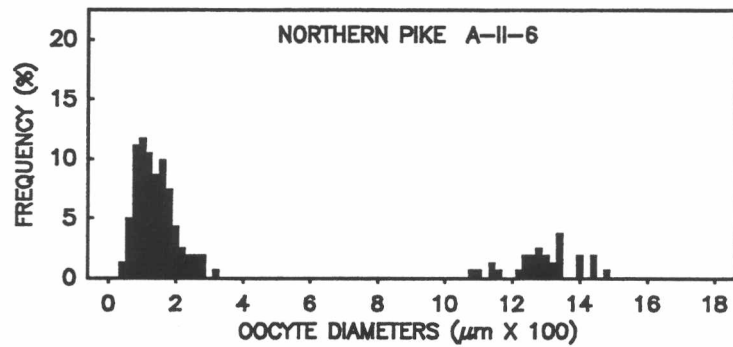
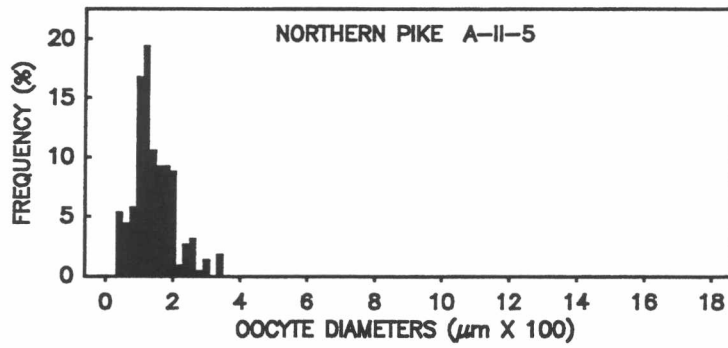
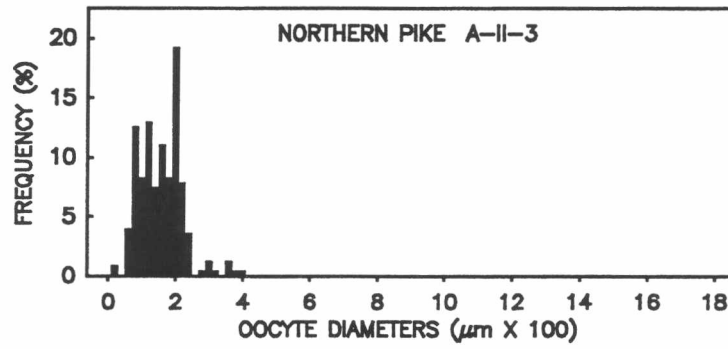


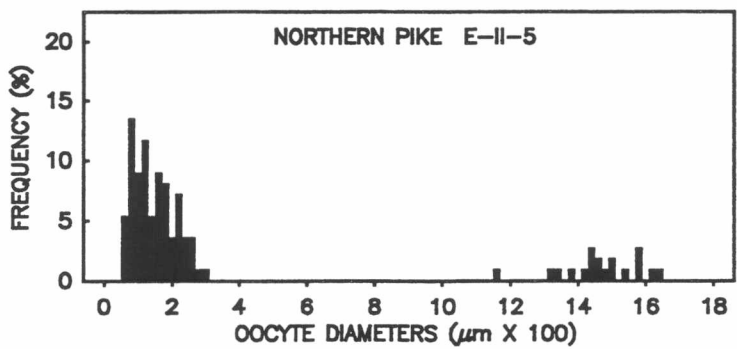
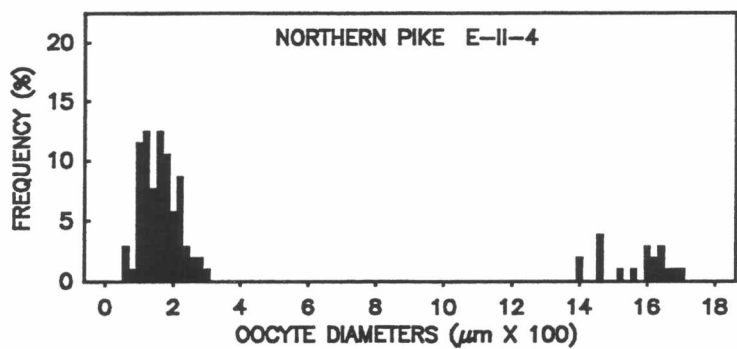
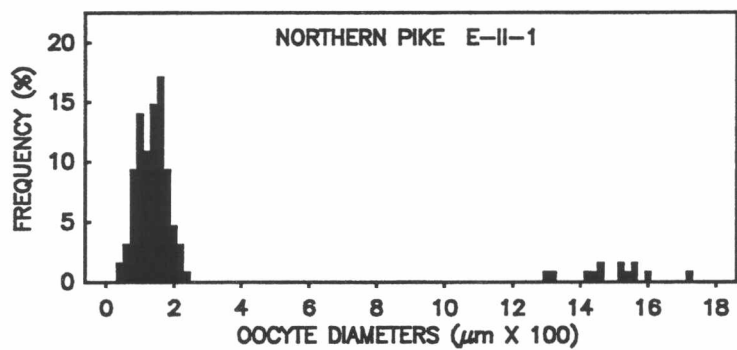
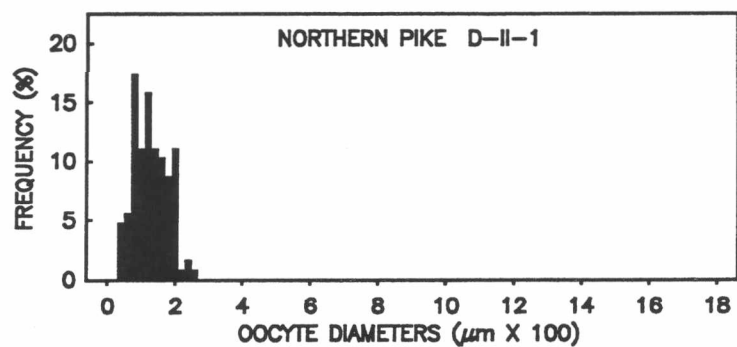


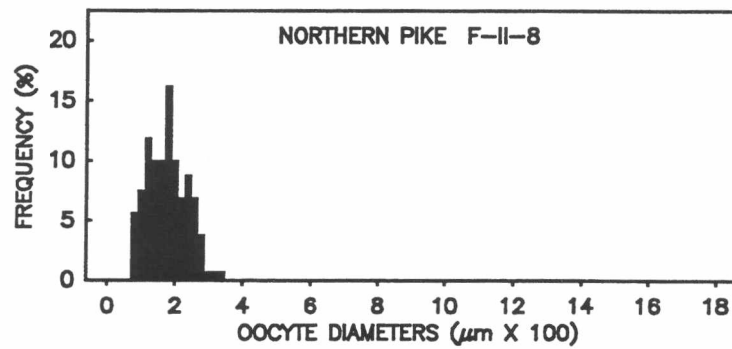
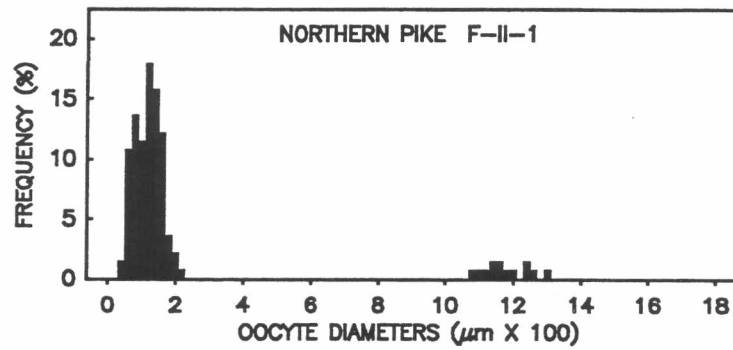
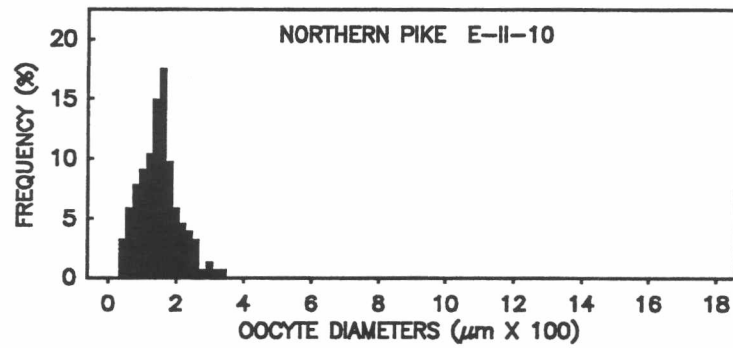
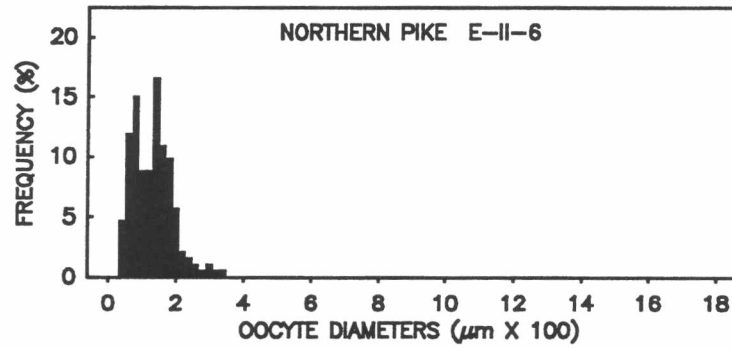




APPENDIX 6
Oocyte Diameter
Frequency Distributions
Spring Northern Pike







3 1510 00135 5719

