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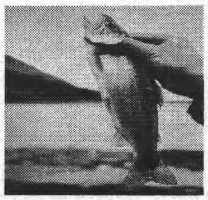
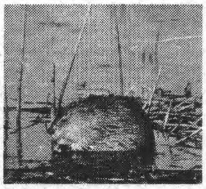
Northern River Basins Study





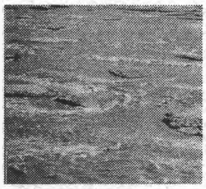





NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 89

ANALYSES FOR CIRCULATING GONADAL SEX STEROIDS AND GONAD MORPHOLOGY IN FISH

PEACE, ATHABASCA AND SLAVE RIVER BASINS, SEPTEMBER TO DECEMBER, 1994






Prepared for the
Northern River Basins Study
under Project 3144-D3

by

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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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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.

F. J. Wrona
(Dr. Fred J. Wrona, Science Director)

14 Feb 96
(Date)

Whereas it is an explicit term of reference of the Science Advisory Committee "to review, for scientific content, material for publication by the Board",
IT IS HERE ADVISED BY THE SCIENCE ADVISORY COMMITTEE THAT;
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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P.A. Larkin
(Dr. P. A. Larkin, Ph.D., Chair)

14 Feb 1996
(Date)

Whereas the Study Board is satisfied that this publication has been reviewed for scientific content and for immediate health implications,
IT IS HERE APPROVED BY THE BOARD OF DIRECTORS THAT;
this publication be released to the public, and that this publication be designated for: **[ ] STANDARD AVAILABILITY [ ] EXPANDED AVAILABILITY**

Lucille Partington
(Lucille Partington, Co-chair)

14/02/96
(Date)

Robert McLeod.
(Robert McLeod, Co-chair)

14/02/96
(Date)

ANALYSES FOR CIRCULATING GONADAL SEX STEROIDS
AND GONAD MORPHOLOGY IN FISH
PEACE, ATHABASCA AND SLAVE RIVERS,
SEPTEMBER TO DECEMBER, 1994

STUDY PERSPECTIVE

The aquatic fauna of the northern rivers in Alberta are exposed to pulp mill effluent, and other types of industrial and municipal effluents. Several recent studies in Canada have reported reproductive problems in fish exposed to bleached kraft pulp mill effluent. In 1992, female longnose suckers collected from the upper Athabasca River showed depressed levels of gonadal sex steroid hormones, but the results were inconclusive. Fish displaying below normal levels of sex steroids typically show changes in reproductive development and/or performance. Given the results of the 1992 project, a follow-up study was necessary to verify the effect on gonadal sex steroids, and to determine the extent of the effect in fish collected from the Peace, Athabasca and Slave rivers. These same fish would also be analyzed for contaminants, liver enzymes, vitamin stores and metal protein synthesis.

The objective of this project was to examine reproductive indices in fish collected from the Peace, Athabasca and Slave rivers and their major tributaries. The fish species targeted for collection and analyses were burbot (primary target species), northern pike, longnose sucker and flathead chub. Biochemical and histological approaches included analyzing levels of gonadal sex steroid hormones, measuring gonad morphology, and estimating fecundity.

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?*

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?*

13b) *What are the cumulative effects of man-made discharges on the water and aquatic environment?*

Fish were collected from sites located on the Peace, Smoky, Little Smoky, Wapiti, Wabasca, Athabasca, McLeod, Pembina, Lesser Slave, Clearwater and Slave Rivers. Results from a total of 211 burbot, 86 longnose sucker, 42 northern pike and 24 flathead chub are incorporated in a portion or all of these analyses. Results from the collection sites were organized into reference (upstream locations and tributaries receiving no inputs form pulp mills), near-field (<100 km downstream of a pulp mill source) and far-field (>100 km downstream of a pulp mill source). Both male and female burbot and longnose suckers showed depressed steroid hormone levels and near-field sites. At most locations there was no evidence of changes in gonad tissue, suggesting that lower levels of reproductive steroids have not impacted gonad growth and development in these species. For both burbot and longnose suckers, higher proportions of non-maturing adult fish were collected downstream of pulp mill effluents. In addition, a very high proportion of immature burbot were collected from the Wabasca River as compared to other reference sites. Due to sample size constraints, similar site specific comparisons were not developed for northern pike and lake chub.

The results from this project are consistent with observations of fish downstream of pulp mills in other parts of Canada and the world. This project is one component of a study representing a large-scale effort to simultaneously evaluate contaminants levels, reproductive parameters and possible physiological effects of potential contaminant exposure. Data from these fish will also provide comparative information in relation to previous contaminant and biochemical analyses conducted on these species by NRBS and other agencies. Results from this study will form important linkages with research on contaminant fate and food chain modelling, ecosystem health, cumulative effects assessment and human health consumption advisory assessments.

REPORT SUMMARY

The objective of this study was to examine reproductive indices in fish collected at a broad spectrum of sites located throughout the Peace and Athabasca river basins. The study represents an pioneering effort of unprecidented scope and scale which simultaneously evaluated contaminants, reproductive parameters and possible biochemical effects of potential contaminant exposure. The collection and sampling protocols for the project included contaminant, biochemical and histological analyses to be performed on the fish. Because of its wide-ranging distribution and relatively sedentary behavior, burbot were targeted for collection and analyses. However, incidental collections of longnose sucker, flathead chub and northern pike were also taken for a broad suite of analyses. The study design was predetermined by the Northern River Basins Study Science Directors and the Contaminants Component Leader and is based on a reference, near-field and far-field location of the collection sites with respect to potential inputs from pulp mills. Reference sites are upstream locations and tributaries receiving no input from pulp mills. Near-field sites are located < 100 km from a pulp mill source. Far-field sites represent downstrean locations > 100 km from a pulp mill source.

There was apparent depressed steroid hormone levels in burbot and longnose suckers of both sexes at near-field sites. This observation was consistent with findingd in longnose suckers and other species downstream of pulp mill effluent in other regions (McMaster et al. 1991; Munkittrick et al. 1991, 1992a,b; Van der Kraak et al. 1992; Brown et al. 1993). Although prolonged reductions in circulating levels of reproductive hormones could adversely affect gonadal development, at most locations there was no evidence of change in morphological measures of gonadal development (GSI, oocyte size measurements, etc.) in burbot or longnose suckers. Therefore, the apparent lower levels of reproductive steroids have not generally impacted gonadal growth and development in fish from the region.

Significantly, higher proportions of non-maturing adult fish were collected downstream of pulp mill inputs (near- and far-field locations). This observation is of sufficient importance to warrant verification and follow-up investigation and the possibility that pulp mill inputs are producing adverse effects on fish in the Peace and Athabasca drainages can not be excluded. More fundamental information about burbot and longnose sucker reproduction and ecology in the Peace and Athabasca Drainages is required to completely understand the implications of apparent depressed reproductive hormones and the distribution of immature fish.

It is recommended that subsequent sampling programs be as timely as possible such that significant developmental changes among sites are minimized. Because of the biological variability, efforts to clearly match samples between reference and exposed areas should be considered. A repeat visit to selected sites would also allow more accurate assessment of data. Based on our findings, an analysis to determine optimal samples sizes should be undertaken prior to performing future surveys and subsequent studies should consider fewer sites, assessed more comphrensively. To allow accurate characterization of gonadal development, future fish collections should continue to include gonad tissues for histological analysis.

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1.0 INTRODUCTION

Several recent studies indicate possible reproductive problems in fish exposed to bleached kraft pulp mill effluent (Brown et al.1993; McMaster et al. 1991; Munkittrick et al. 1991, 1992a,b; Van der Kraak et al. 1992). Reductions in circulating levels of sex steroids have been shown to reliably indicate exposure to compounds known to impact the reproductive system. Recent studies which have examined steroids at an appropriate stage of gonadal development have demonstrated reduced levels of plasma sex steroids (testosterone, 11-ketotestosterone, 17ß-estradiol and 17α20ß-dihydroxy-4-pregnene-3-one) in fish exposed to bleach kraft mill effluent relative to fish at reference sites. Fish displaying lower circulating levels of sex steroids typically show changes in reproductive development and/or performance. These include delayed sexual maturity, reduced gonad growth, reduced fecundity with age, reduced egg size and reduced secondary sexual characteristics.

The aquatic fauna in the Northern River Basins Study Area are also exposed to pulp mill, domestic and other industrial effluents from various sites located throughout the region. In the spring and fall of 1992 the Northern River Basins Study collected four fish species from six sites upstream, near and downstream from the bleached kraft mill located at Hinton on the Athabasca River (Barton et al. 1993a&b). These fish were analyzed for MFO induction, circulating sex steroid levels and gonad morphology. The results of these analyses were somewhat inconclusive. Mountain whitefish showed small increases in liver microsomal enzyme activity relative to fish collected from upstream sites (Lockhart et al. 1996). Depressed levels of gonadal steroid hormones were also noted in female longnose suckers and possibly in mountain whitefish collected downstream of the Hinton Mill in the fall (Brown et al. 1993).

The objective of this study was to examine reproductive indices in fish collected at a broad spectrum of sites located throughout the Peace and Athabasca river basins. In September and October 1994 the Northern River Basins Study Science Directors initiated a basin-wide fish collection to examine potential effects of pulp mill and other effluents on fish populations. The collection and sampling protocols for the project were designed to include contaminant, biochemical and histological analyses to be performed on the fish. Because of its wide-ranging distribution and relatively sedentary behavior, burbot were targeted for collection and analyses. However, provisions were also made for the collection of longnose sucker, flathead chub and northern pike for a broad suite of analyses.

In accordance with the terms of reference for Project 3144-D3, fish samples from the 1994 Fall Basin-Wide Fish Collection in the Peace, Athabasca and Slave river drainages (EnviResource Consulting 1995) were analyzed for steroid hormones found in females (17ß-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 gonad changes likely to be found over time in contaminated environments can be detected. The design for statistical analysis of the data was predetermined by the Northern River Basins Study Science Directors and Contaminants Component Leader and is based on collection sites within reference, near-field and far-field locations chosen with respect to potential inputs from pulp mills. Reference sites were upstream locations and tributaries receiving no input from pulp mills. Near-field sites were located < 100 km from a pulp mill source and far-field sites were located > 100 km from a pulp mill source. Field locations and sites are summarized in Table 1.

2.0 METHODS

2.1 Fish Samples and Collection Sites

Plasma and gonad samples of Burbot (*Lota lota*), longnose sucker (*Catostomus catostomus*), northern pike (*Esox lucius*) and flathead chub (*Platygobio gracilis*) were obtained from preselected sites in the Peace, Athabasca and Slave River basins by EnviResource Consulting Ltd., Calgary, Alberta. Frozen (dry-ice) plasma samples and preserved gonad tissues were subsequently sent to the Freshwater Institute for analyses of steroid hormones (females, 17ß-estradiol & testosterone; males, testosterone & 11-ketotestosterone) and histological assessments of sexual maturity and female fecundity. Details of sampling protocols and tissue preservation techniques are outlined in the EnviResource (1995) report. Plasma samples were stored and transported at -60°C, and gonad tissue samples were fixed in Davidson's solution and in 5% formalin. Collection sites were preselected by the Northern River Basins Study Science Directors and Contaminants Component Leader and complete descriptions are also detailed by EnviResource (1995). Designated sites are presented in Figure 1. Northern River Basins Study sample numbers have been used in tables listing data.

2.2 Fish Characteristics

Fish characteristics (weight, length, gonad weight, age, capture day) were obtained from the Fall and Winter Fish Collections From the Peace, Athabasca and Slave River Drainages Report (EnviResource Consulting, 1995). We calculated Fulton's condition factor as:

$$CF = [100*(\text{TOTAL FISH WEIGHT - GONAD WEIGHT})/\text{LENGTH}^3].$$

Increased age to maturity and lower fecundity with age are reproductive indices which may be sensitive to the presence of pulp mill effluent (McMaster et al. 1991; Munkittrick et al. 1991, 1992a,b). The collections reported here did not have comprehensive age distributions within the site groupings, so these parameters could not be evaluated.

Table 1. Fish collection sites for the 1994 basin-wide fish collections in the Northern River Basins Study area (modified from EnviResource 1995). Field and NRBS Group show the groupings used for analysis and presentation of burbot data. Possible discharge locations and approximate distances are indicated.

Drainage /Delta	River	Site Code	Date Sampled	Field	NRBS Group	General Location	Potential Effluent Exposure
Athabasca	Athabasca	A1a	11/09 to 13/09	NEAR	A1	Near Highway 947 crossing	D/S (approx. 95 km) of pulp mill-Hinton
		A1b	13/09 to 15/09	NEAR	A1	Near Berland River	D/S (approx. 80 km) of pulp mill-Hinton
		A2	21/09 to 24/09	REF	A2	U/S (approx. 10 km) of Hinton	D/S of town of Jasper
		A3	27/09	NEAR	A3	Near Fort Assiniboine	D/S (approx. 60 km) of pulp mills-Whitecourt
		A4	08/10 to 09/10	NEAR	A4	Near Calling River	D/S (approx. 25 km) of pulp mill-ALPAC
		A5	14/10 to 15/10	FAR	A5	Near Fort Mackay	D/S (approx. 20 km) of SUNCOR, D/S (approx. 310 km) ALPAC
	McLeod	MR1	16/09 to 19/09	REF	MR	Near town of Whitecourt	D/S town of Edson
		MR2	15/12	REF	MR	U/S town of Edson	Tributary Reference
	Pembina	P	29/09 to 01/10	REF	P	Near town of Jarvie	D/S town of Barrhead
	Lesser Slave	LSV	03/10 to 04/10	NEAR	LSV	Near town of Slave Lake	D/S (approx. 10 km) Slave Lake Pulp
	Clearwater	CW	11/10 to 13/10	REF	CW	U/S of Fort McMurray	Tributary Reference
Peace	Peace	PR1	28/09 to 01/10	FAR	PR1	Near Many Islands Prov. Park	D/S (approx. 150 km) of pulp mills in BC
		PR2	03/10 to 05/10	NEAR	PR2	D/S Diashowa Near the Notikewan River	D/S (approx. 95 km) of pulp mill ans town of Peace River
		PR3	07/10 to 09/10	FAR	PR3	Near Fort Vermilion	Further D/S (approx. 230 km) of pulp mill -Peace River
	Wapati	WR1	22/09 to 26/09	REF	WR	Near Pipestone Creek Prov. Park	U/S (approx. 20 km) pulp mill-Grande Prairie
		WR2	19/10 to 20/10	REF	WR	Near O'Brian Prov. Park	U/S (approx. 5 km) pulp mill-Grande Prairie
	Smoky	SR1	13/09 to 19/09	NEAR	SR1	Near Highway 49 Near Watino	D/S (approx. 90 km) pulp mill -Grande Prairie
		SR2	16/10	REF	SR2	Near Grande Cache	U/S Reference
		SR3	21/12	REF	SR3	U/S confluence of Wapiti near Canfor bridge	U/S Reference
	Little Smoky	LSR1	18/09 to 22/09	REF	LSR	Near Highway 744 crossing	Tributary Reference
		LSR2	18/12	REF	LSR	D/S (3 km) LSR1	
	Wabasca	WB	10/10 to 12/10		WB	Near highway 67 crossing	Tributary Reference
Peace-Athabasca	Delta	JV1	19/10		JV	Near Jackfish Lake Village	
		JV2	20/10		JV	Near Big Eddy	
Slave	Delta	SRD1	15/10	FAR	SD	U/S of Nagle Channel	D/S town of Fort Smith
		SRD2	15/10 to 17/10	FAR	SD	At mouth of Nagle Channel	D/S town of Fort Smith

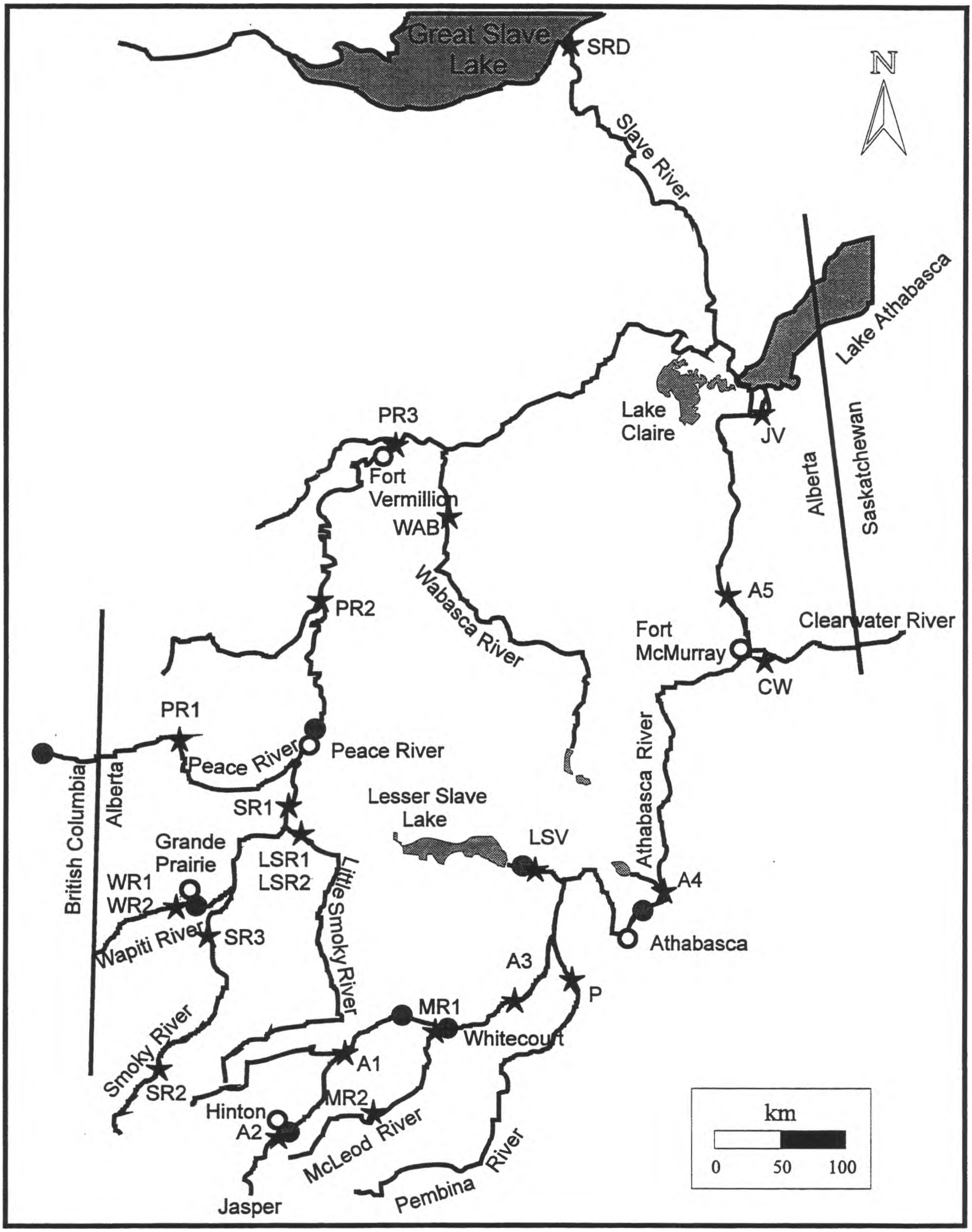

Figure 1. Sampling sites (stars) for the 1994 Basin-Wide Burbot Collection. Towns (open circles and pulp mill (filled circles) locations are indicated.

2.3 Steroid Hormone Assays

Prior to assay, duplicate plasma samples (250 mL) were extracted in 2.5 mL of ethyl acetate:hexane (3:2, v/v). The dried extracts were redissolved in assay buffer (250 mL). After appropriate dilution, aliquots of this redissolved extract were then used for either 17ß-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 17ß-estradiol, testosterone & 11-ketotestosterone) to every sample and counting an aliquot (25 mL) of the redissolved extract by liquid scintillation counting. We had previously demonstrated that each hormone is extracted with nearly identical efficiency. Extraction efficiencies were 76.8±1.9% (mean±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.

2.3.1 Plasma 17ß-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 (17ß-estradiol, 17a-estradiol, estrone, estriol, progesterone, 17α,20ß-dihydroxy-4-pregnen-3-one, testosterone and cortisol). Steroids giving greater than 0.1 CR% with the estradiol antibody were: 17ß-estradiol (100), estrone (1.7), 17α,20ß-dihydroxy-4-pregnen-3-one (0.3) and testosterone (0.1). Intraassay coefficient of variation (CV), from 10 duplicate analyses 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 burbot or longnose sucker plasma was 98.3±4.5 % (mean±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 9 assays. Serial dilution of plasma extracts were parallel to the standard curves and gave estimates of hormone concentrations within 9%.

2.3.2 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, 11ß-hydroxytestosterone, androstenedione, cortisol, progesterone, 17α,20ß-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 11ß-hydroxytestosterone (1.2). Intraassay coefficient of variation (CV), from 10 duplicate analysis of the same sample was 8.8%. Interassay CV of duplicate analyses from 10 assays was 10.9%. Recoveries of testosterone (0.63-2.5 ng/mL) added to fish plasma ranged from 88.9 to 101.3%. 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 14 assays. Serial dilutions of plasma extracts were parallel to the standard curves and gave estimates of hormone concentrations within 8%.

2.3.3 Plasma 11-ketotestosterone

A radioimmunoassay (RIA) was used to assess plasma 11-ketotestosterone. RIA antibody was obtained from Helix Biotech and ^{3}H-labelled 11-ketotestosterone was synthesized in-house from ^{3}H-cortisol. 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, 11ß-hydroxytestosterone, androstenedione, cortisol, progesterone, 17α,20ß-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%), 11ß-hydroxytestosterone (4.8%) and androstenedione (4.6%). Intraassay coefficient of variation (CV), from 10 duplicate analyses 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 90.7 to 104.5 %. The minimum level of sensitivity, defined as that dose level 2 standard deviations away from the 0 dose measurement, averaged 0.5 ng/mL over 5 assays. Serial dilutions of plasma extracts were parallel to the standard curves and gave estimates of hormone concentrations within 7.5%.

2.4 Histology

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

GSI = 100*GONAD WEIGHT/[TOTAL FISH WEIGHT - GONAD WEIGHT].

2.4.1 Female Fish

The ovaries were categorized into one of five Groups (7-11) which are also found under the 'MATURITY INDEX' column in Appendix B, Tables 2-5. The groups are described below:

Index 7 - Those ovaries containing only pre-vitellogenic oocytes. The largest having reached the yolk vesicle stage. In our histograms the frequency mode from 0-150 mm for burbot, 0-700 mm for longnose sucker, and 0-300 mm for northern pike and flathead chub oocyte diameter corresponds to pre-vitellogenic oocytes (e.g. A3BURB5, PR1LNSC7, MRNRPK4, WR1FLCH1).

Index 8 - Those ovaries with only pre-vitellogenic oocytes. The largest are at the yolk vesicle stage, plus a remarkable number of large resorbing oocytes (e.g MCR2BURB5).

Index 9 - Those ovaries with a distinct vitellogenic clutch of developing oocytes plus a core of pre-vitellogenic resting oocytes (e.g. SR1BURB2).

Index 10 - Those ovaries with a distinct vitellogenic clutch of mature oocytes plus a core of pre-vitellogenic resting oocytes (e.g. WR2BURB5).

Index 11 - Ovulated fish. Ovaries comprised almost exclusively of loose clutch oocytes; therefore clutch proportions are skewed (note: none were of this stage).

Fecundity estimates. One hundred 5 % formaldehyde-fixed vitellogenic oocytes were teased out of the ovary tissue of each female, lightly blotted and weighed. The associated connective tissue and pre-vitellogenic oocytes were also weighed to estimate their contribution to overall gonad weight. For longnose sucker and northern pike the proportion of the gonad represented by vitellogenic oocytes was 89% (same as in fall of 1992 samples) and 81%, respectively. This component in burbot and flathead chub ovaries was too small to weigh accurately and so was considered negligible. In these species the vitellogenic oocytes were assumed to be 100% of gonad weight. Absolute fecundity (number of oocytes per fish) was estimated as:

ABSOLUTE FECUNDITY = [GONAD WEIGHT * PROPORTION OF GONAD REPRESENTED BY VITELLOGENIC OOCYTES]/AVERAGE OOCYTE WEIGHT.

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

RELATIVE FECUNDITY = ABSOLUTE FECUNDITY/[TOTAL FISH WEIGHT - GONAD WEIGHT].

Oocyte diameters. The microscopic image of each Davidson's fixed 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 oocytes were measured for each fish. Frequency distribution of oocyte diameters were prepared on histograms (e.g. Mayer et al. 1990) and plotted (see Appendices C-F). From this data the percent of oocytes representing the clutch was calculated. For some samples, in which there were large oocytes (>0.3 mm), 50 Vernier caliper measurements of the formaldehyde fixed oocytes were done under a stereo microscope. The mean diameters for the clutch oocytes were calculated from these measurements and are presented in the Tables.

The mean diameter of the clutch oocytes (on histograms) was 70% (longnose sucker and flathead chub), 75% (burbot), and 80% (northern pike) the diameter of fixed (Davidson's or 5% formalin) but unprocessed oocytes measured with 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 oocytes (approx. 2.0 mm in diameter) measured in the field.

This translates into the histogram clutch oocyte diameters being an estimated 66% (longnose sucker and flathead chub), 71% (burbot), and 76% (northern pike) of actual values (if fixation shrinkage is similar). For example our histogram calculated clutch oocytes for fall burbot ranged from 237-626 mm. The caliper measured clutch oocyte diameters for these fish ranged from 292-775 mm. This would translate into actual diameters of 310-825 mm.

2.4.2 Male Fish

Each testis is classified as to stage of maturity in Appendix B, Tables 2-5 under the 'MATURITY INDEX' column. The histological stages we used are based on those for herring (*Clupea harengus* L.) as outlined by Bowers and Holliday (1961).

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

Stage 1-There are numerous large, spherical, primary germ-cells lying singly or in small groups. Solitary germ cells are about 15 mm in diameter and the germ cells in groups are smaller. Fibrous connective tissue is organizing around the germ cells to form lobules.

Stage 2-The tunica is clearly defined and lobule formation is complete. The groups of primary germ cells become progressively less common. Primary and secondary cysts are comprised of spermatogonia occurring in large numbers. Cysts containing spermatocytes, spermatids and spermatozoa may be present.

Stage 3- All cell types described above are present. The relative numbers differ from 2, there are more cysts containing spermatocytes, spermatids and spermatozoa present. The lobules are wider than stage 2.

Stage 4- Within sperm cysts spermatocytes mostly replaced by spermatids and spermatozoa.

Stage 5- The lobules are tightly packed with spermatozoa, no cysts spermatocytes or spermatids present.

Stage 6- The 'ripe' or 'running' testis. Sperm absent from some lobules, walls thickened.

Stage 7- Fibrous connective tissue thickened by contraction and the tunica is thick and folded. Lobules are distorted and collapsed with relic sperm and cell debris.

2.5 Statistics

The data analysis represents the basin-wide overview and combines like data from both the Athabasca and Peace Drainages. The design was predetermined by the Northern River Basins Study Science Directors and the Contaminants Component Leader and is based on a reference, near-field and far-field location of the collection sites with respect to potential inputs from pulp mills. Reference sites are upstream locations and tributaries receiving no input from pulp mills. Near-field sites are located < 100 km from a pulp mill source. Far-field sites are located > 100 km from a pulp mill source. Site and field locations are summarized in Table 1. Dependant variables used in the reference, near-field and far-field categories in the ANOVA were the mean parameter value calculated for each site. Differences between groups of fish collected at each site for any given parameter were tested by one-way or two-way analysis of variance (ANOVA) computed using the Systat statistical package (Wilkinson et al. 1992). Bartlett's test was applied to test for homogeneity of variance and, where necessary, data were log transformed. Comparisons between absolute fecundity were tested using analysis of covariance, with total body weight adjusted for gonad weight (total weight - gonad weight) as the covariate. Site specific comparisons in body weight adjusted for gonad weight were also tested using analysis of covariance, with age as the covariate. Gutted weight was not used because it was recorded less frequently by the field sampling crew. Frequency analysis using contingency tables were used to test the distribution of immature fish and sex ratio as a function of site category.

Due to the spatial scale covered by the basin-wide survey and the associated constraints in obtaining a simultaneous samples from the various locations (Fig. 1), the variation in the sampling times and associated developmental changes in some of the physiological and morphological measurements confounded site specific comparisons. Most of the sites were sampled once during a 37 day period. The McLeod River (MR2), the Little Smoky River (LSR2) and Smoky River (SR3) sites were sampled some 90 days after the first site was visited. Because these burbot had undergone significant gonadal development relative to those collected at the earlier times, data collected on fish from these sampling sites were not readily comparable to data collected at earlier times. Some burbot measures are affected as well as some parameters measured in longnose suckers. Because the sites in each river were generally visited once some time over the entire sampling period, time and site are confounded. Site specific differences in measures could be due to normal developmental progress or to differences in location. To facilitate site and field specific comparisons, some of the parameters were adjusted based on the time relationships found within each river drainage. The linear relationship of each parameter with sampling day in each river was examined. If this relationship was highly significant ($P<0.01$) then the values were adjusted using the regression equation to a time midpoint in the sampling regimen (Julian day 273). Using this approach we time-adjusted *GSI, oocyte size, oocyte weight, absolute fecundity, relative fecundity and 11-ketotestosterone* measures in burbot and *testosterone, GSI, oocyte size and oocyte weight* in female longnose suckers. Use of adjusted means for a parameter has been directly indicated on the respective figure and in the caption. We were unable to time adjust values from the few fish collected at MR2, LSR2 and SR3 sites in December. Burbot spawn mid-winter beginning early January (Scott and Crossman, 1983). Therefore, the December sampling time was too far removed from the other sites and too close to spawning to adjust values by simple linear regression (Fig. 2). There was insufficient information to define the curvilinear response. Therefore comparisons between locations were computed without values from the MR2, LSR2 and SR3 sites.

The application of this adjustment for time x site interaction is based on several biological assumptions: 1) We assume that the response profile can be described by a simple linear function. While over the long-term gonadal development parameters follow curvilinear response patterns (Scott et al. 1980), based on our findings linear regression fairly closely approximates short-term changes in the curvilinear response profiles (Fig. 1B). 2) We assume that rate of growth or change is similar among all sites in a river. 3) The procedure assumes that impacted sites were sampled in a random fashion. The latter two assumptions also require comment. Rates of gonadal development are possibly impaired at sites impacted by discharges like pulp mill effluent. Additionally, the rivers were more or less sampled from upper to lower reaches so it is possible that impacted sites are not randomly distributed. *Generally, we believe the applied approach was conservative because far fewer site-specific differences were found.* By assuming similar developmental rates throughout, potential differences in response parameters from both the fastest and slowest developing groups are dampened.

Pairwise comparisons were conducted by applying the LSD or Dunnett's test to the least squared means produced by the ANOVAs. Arithmetic means and standard error are given in the histograms. For variables requiring temporal correction, adjusted means with the standard error are presented.

Burbot. Two sets of site comparisons are given in the figures. Data are summarized according to the reference, near-field and far-field overview. Additionally, site specific means and standard deviations are presented. The Wabasca site (WB) was separated from the other possible reference sites becauses male and immature fish show a mild MFO induction response, 2-4 fold increase over background levels (Lockhart and Metner 1996).

Longnose Sucker. Data are organized as to reference, near-field and far-field overview. Because the locations where longnose suckers were captured are mostly in the Peace, data were analyzed according to capture site outlined in Table 1. Wapiti River (WR) , upper Smoky River (SR2), Little Smoky River (LSR2), Smoky River (SR1) and Peace River one (PR1), two (PR2) and three (PR3). Athabasca fish are few and data are indicated for comparative purposes.

Northern Pike. To simplify presentation, data for northern pike collected on the Peace drainage were grouped according to capture site: Wapiti River (WR1 & WR2), Smoky River (SR1) and Peace River (PR1, PR2 & PR3). Mostly single fish were collected on the Athabasca drainage and site-specific values are provided for comparison. The site categorizations are listed in Appendix B, Table 3 with the data for each fish. Due to the small numbers no statistical analyses was undertaken.

Flathead Chub. Only 24 flathead chub were collected and data are presented according to river or tributary. For example the Peace River sites (PR1 & PR2) are grouped as site PR. The site categorizations are listed in Appendix B, Table 4 with the data for each fish.

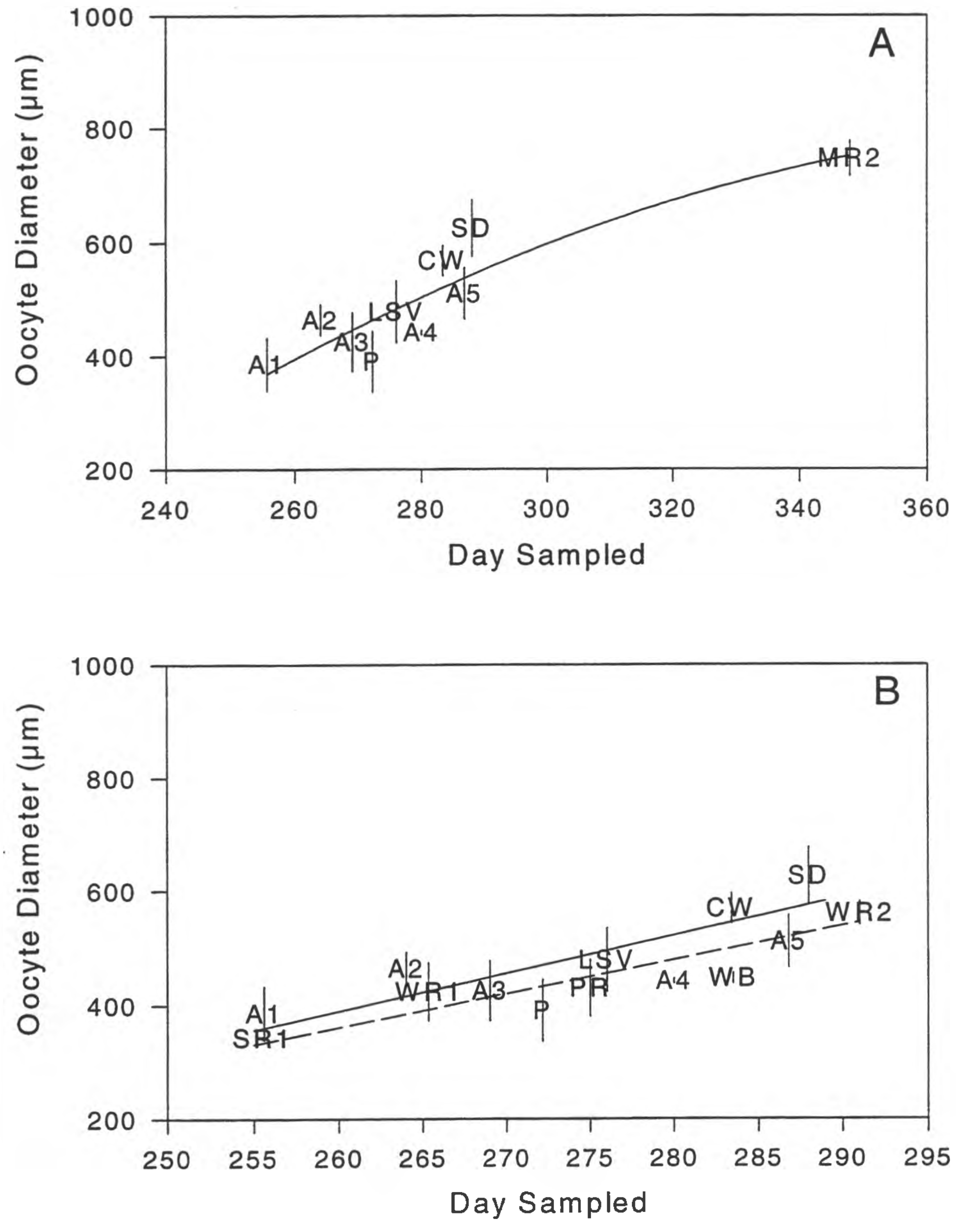

Figure 2. Relationship and fitted regressions between mean oocyte diameter and day sampled for female burbot (A) collected at the various sites in the Athabasca and Slave drainages and (B) for the Athabasca sites (solid line) and the Peace sites (broken line). Vertical bars represent the 95 % confidence intervals.

2.6 Technical Quality of Samples

Generally, plasma samples were good with very little hemolysis. Blood was to be removed immediately after fish capture but average time for its removal was not recorded in the sampling report (EnviResource 1995). The times between fish capture and tissue sampling for gonad histology were, in some cases, long and some fish died before processing (see Appendix B, EnviResource, 1995). Despite this there seemed little identifiable impact on the histological samples for gonad morphology. In several cases measurements of gonad weight, particularly for small fish, appeared erroneous, probably because sensitivity of field balances was inadequate. Consistent recording of all the associated measures is important to maximize available information about each fish. The field crews reported incorrect sex in 4 % of the fish. This mostly involved categorizations of immature males and females. The field collection crew also inaccurately represented gonad maturity in nearly 40 % of female fish. This mostly involved categorizing immature females as mature. Therefore it was important to collect gonad tissues for histological analysis. Capture of longnose sucker on the Athabasca River would have provided valuable comparative data to the previous work conducted on the upper Athabasca (Barton et al. 1993b; Brown et al. 1993).

3.0 RESULTS AND DISCUSSION

3.1 Burbot

Overall, the ratio of mature female to male burbot collected was near unity. However, when the data were parsed as to reference, near-field and far-field locations the sex ratios became skewed. The female to male ratios were 72/28 (N=39), 40/60 (N=42) and 34/66 (N=56) in the reference, near-field and far-field locations, respectively. Based on frequency analyses the near-field (Pearson chi-square = 13.19, $P < 0.001$) and far-field (Pearson chi-square = 8.03, $P < 0.01$) locations differed significantly from the reference category. Sex ratios did not differ between near-field and far-field locations. There are a variety of possible explanations for the observed pattern. Further information about burbot ecology is necessary but the observation could be related to differing movement patterns between sexes.

3.1.1 Maturing Female Fish

Results obtained for each female burbot analyzed are summarized in Appendix B, Table 1. For maturing females (N=64) location and site specific means and standard errors are summarized in Figures 3-8. Based on their age-length relationship female burbot in the Athabasca/Peace basins mature about age 5. All but two maturing females in this collection from Peace and Athabasca drainages were age 5 and older. Depending on latitude female burbot can mature between 2 and 7 years (Chen 1969; Bailey 1972; Scott and Crossman 1973).

There were no differences in the mean size, condition and age of maturing females collected at the reference, near-field and far-field locations (Fig. 3). The largest burbot were captured at sites A2 and PR2 while the smallest fish were collected at sites A1 and WB (Fig 4). Post hoc analysis indicated that the condition of the burbot collected at the Slave River delta site was superior to fish collected elsewhere while fish from sites P and A1 had the lowest condition factor. Condition was similar to that reported previously for burbot from the lower Mackenzie River (Lockhart et al. 1989). Burbot collected from sites A2, PR2, PR1 and SD tended to be the oldest while the burbot collected at sites CW, A1 and WB tended to be youngest. Relative to the REF locations, plasma estrogen levels (Fig. 5) were lower in fish from the near-field locations. Two of the five reference locations had higher estradiol levels than all near-field locations except LSV (Fig. 6). Plasma testosterone levels did not differ between field locations (Fig. 6). On a site specific basis, testosterone levels were variable with site SD highest but there was no clear trend. To our knowledge with the exception of Giles et al. (1996) there is little comparable information regarding steroid hormone levels in burbot. Steroid hormone concentrations in female burbot from reference locations were comparable to those found in maturing burbot held in the laboratory (Giles et al. 1996). The gonadosomatic index indicated that relative gonad size was similar between the field location and sites (Figs. 5 & 6). The clutch oocyte diameters estimated from the histograms (Appendix C) are 75.6% (63 - 91%) of those derived from caliper measurements presented in Appendix B, Table 2. The gonadal oocyte measures (Fig. 7) did not differ between the reference, near-field or far field locations. Post hoc analysis showed that mean oocyte size and weight was higher in fish at the SD site but significantly lower in fish collected from the Pembina River. Females collected at the Slave River delta sites tended to have larger oocytes while fish collected from the Pembina and Wabasca rivers had smaller oocytes (Fig. 8) than those found in fish from the reference sites. The adjusted absolute fecundity was similar across all sites (Fig. 8). The relative fecundity tended to be higher in burbot collected from sites WB and P and lowest in burbot from the SD site. Generally, fecundity (appoximately 500 oocytes/g female) was near values reported in northern studies (Chen 1969; Lawler 1963; Scott and Crossman 1973). Higher fecundity was observed in fish from Lake Superior (Bailey 1972).

3.1.2 Maturing Male Fish

Results obtained for each male burbot analyzed are summarized in Appendix B, Table 1. For maturing males (N=73) locations and site specific means and standard errors are summarized in Figures 9 - 12. Based on their age-length relationship male burbot in the Athabasca/Peace basins mature about age 4. All but one maturing male in the Peace and Athabasca drainages were age 4+. Depending on latitude male burbot can mature between 2 and 6 years (Chen 1969: Bailey 1972).

The size, condition and age of maturing male fish were comparable across the reference, near-field and far-field locations (Fig 9). Fish collected at the PR2 site were larger than those collected at most other sites (Fig. 10). Compared to the REF locations, plasma levels of 11-

ketotestosterone tended to be lower (*P*=0.086) in fish from the near-field location (Fig. 11). Post hoc testing indicated plasma 11-ketotestosterone was highest at the WR site in the reference category. Plasma testosterone concentrations were similar between the field locations (Fig, 11). On a site specific basis, plasma testosterone levels (Fig. 12) were variable with site WR and SD the highest; values from the A3 site were lowest. As stated for female burbot, there is little comparable information regarding steroid hormone levels in burbot. The general plasma steroid concentrations in male burbot from reference areas were comparable to those found in maturing burbot held in the laboratory (Giles et al. 1996). On a site specific basis, male GSIs were variable without any clear trend and did not differ significantly from the reference locations when examined from the basin-wide perspective.

3.1.3 Immature Fish

Results obtained for each immature burbot analyzed (N=60) are summarized in Appendix B, Table 1. The age of maturity for female and male burbot in the Athabasca and Peace drainages were age 5+ and 4+, respectively. Therefore, we designated younger fish with immature gonads as juveniles (N=7) and excuded them from further analyses. There were 53 burbot of the appropriate age and size to have maturing gonads but for some reason did not. The size, condition and age of these immature fish were almost identical to that of the maturing burbot collected at the various sites throughout the Peace and Athabasca drainages (Fig 13). Immature burbot were near age of first maturity at sites WB, A3 and A5 (Fig 14). Steroid hormone measures were very low and consistent with immature fish (Appendix B, Table 1).

The distribution of immature fish at the various locations is summarized in Figure 15. Significantly higher percentages of immature fish were collected from the near-field (Pearson chi-square = 18.41, $P < 0.001$) and the far-field (Pearson chi-square = 9.47, $P < 0.005$) locations. Immature fish were collected only at the A2 site in the reference locations (Fig. 15). In the near- and far-field categories immature fish were collected at all sites except A1 and SD. The overall averages for the Peace and Athabasca drainages were 40.6 and 26.5 %, respectively. For the Peace River mainstem sites (PR1, PR2, PR3), immature burbot comprised 63.0 % of the total collection (N=27).

Similar to the overall proportion of immature fish found in this study for the Athabasca drainage, Chen (1969) found that 25 % of a small number (N=34) of adult burbot in the Tanana River, Alaska were unripe near spawning. Thus, it is probable that not all fish spawn each year at these latitudes. Ecological explanations are also possible, perhaps the immature fish congregate more downstream on the mainstem where food supplies might be better. In contrast, other investigations document that a low percentage of adult burbot fail to mature. Previous studies carried out in Lake Superior (Bailey 1972) and in Scandinavian reference areas (Pulliainen et al. 1992) show that < 10-15 % of adult burbot have immature gonads. While the overall rate of immature adults (26.5 %) in the Athabasca drainage may seem unremarkable, we find their distribution is exceptional. Most immature burbot were collected from the A4 and LSV sites which are located less than 50 km from pulp mill input. The Peace and Smoky River sites downstream of the reference sites on the Wapiti and Little Smoky Rivers all show high rates of immature adult fish. Mills are located at Grande Praire near the Wapiti/Smoky confluence, on the upper Peace in British Columbia and near the town of Peace River. High incidences of immature burbot in areas impacted by pulp mill and metal mining discharges have been previously reported on the northern coast of the Bothnian Bay and in Lake Kemijävie, Finland (Pulliainen et al. 1992; Pulliainen and Korhonen 1993). As observed in the Finnish burbot (Pulliainen and Korhonen 1990), comparable condition, size and age between mature and immature fish from both the Peace and Athabasca rivers argue against a nutritional deficit as an underlying cause for the high proportion of non-mature fish.

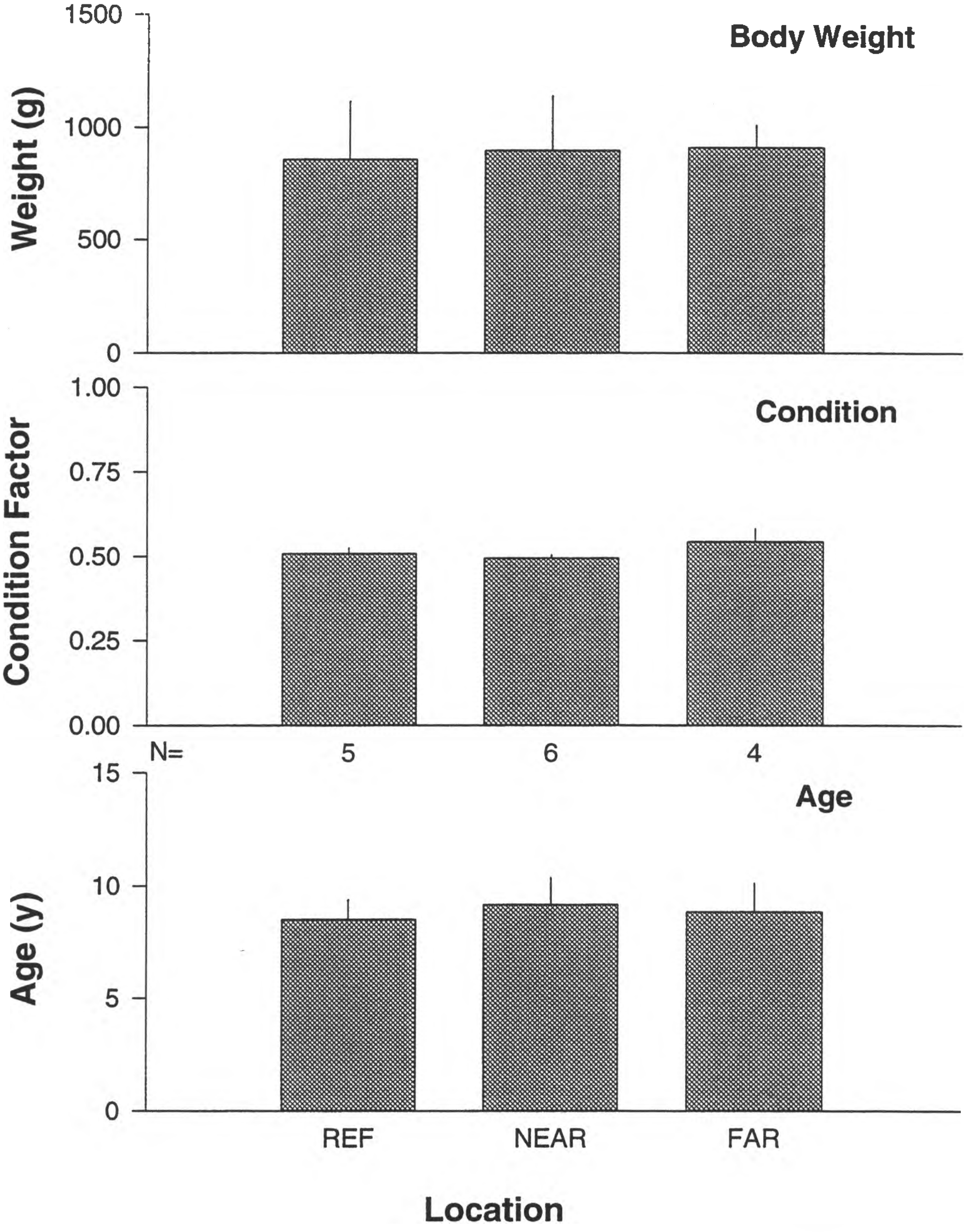

Figure 3. Mean values weight, condition and age for female burbot from reference (REF), near-field (NEAR) and far-field (FAR) locations. Histogram bars represent mean and standard errors. The number of sites used in the analysis are indicated after N=.

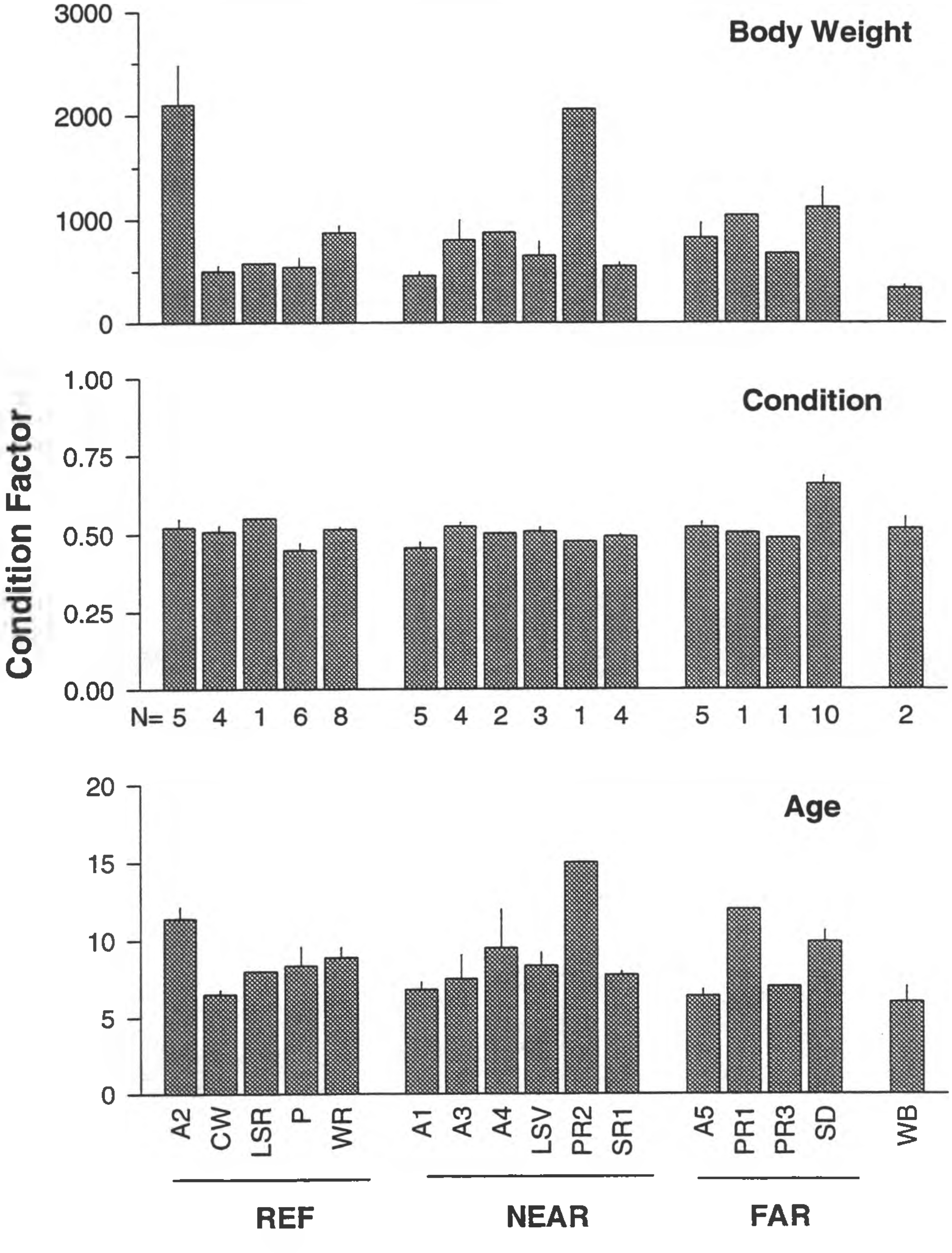

Figure 4. Weight, condition and age for female burbot collected at the various sites. Histogram bars represent mean and standard errors. Sample sizes are indicated after N=.

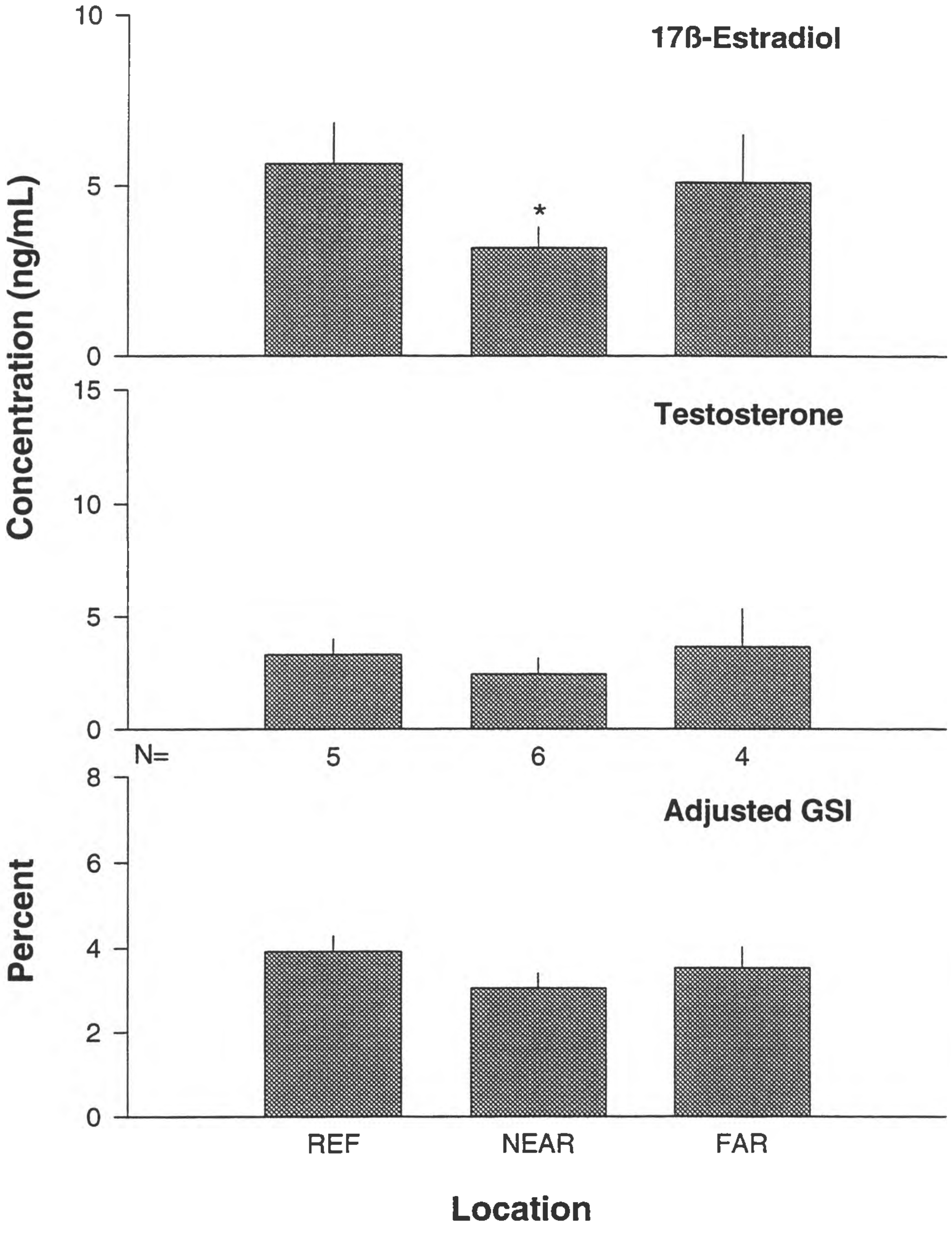

Figure 5. Mean values for steroid hormones (17ß-estradiol & testosterone) and adjusted GSI for female burbot from reference (REF), near-field (NEAR) and far-field (FAR) locations. Histogram bars represent mean and standard errors. Asterisk indicates means significantly different from (REF) by Dunnett's test ($P<0.05$). The number of sites used in the analysis are indicated after N=.

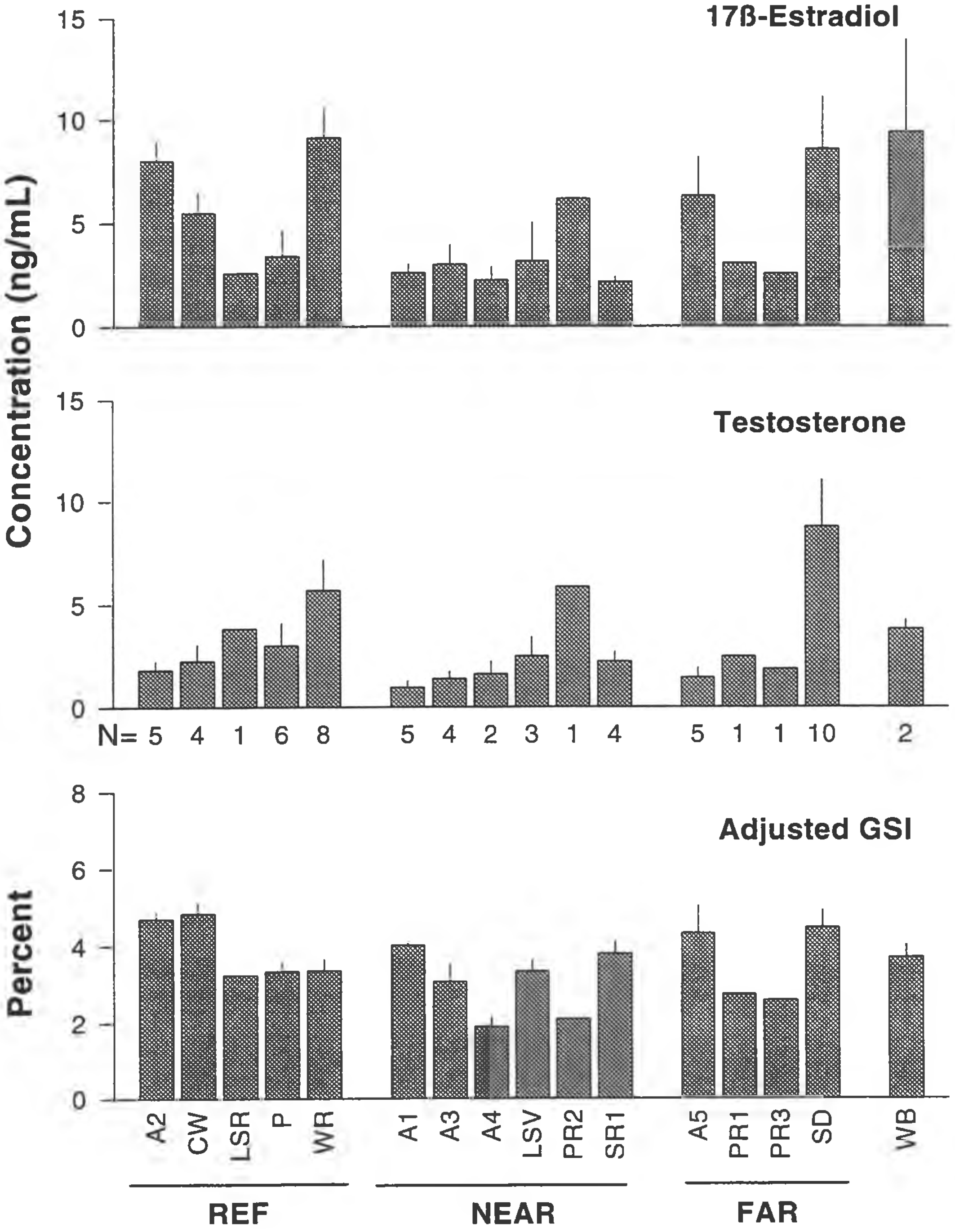

Figure 6. Steroid hormones (17ß-estradiol & testosterone) and adjusted GSI for female burbot collected at the various sites. Histogram bars represent mean and standard error. Sample sizes are indicated after N=.

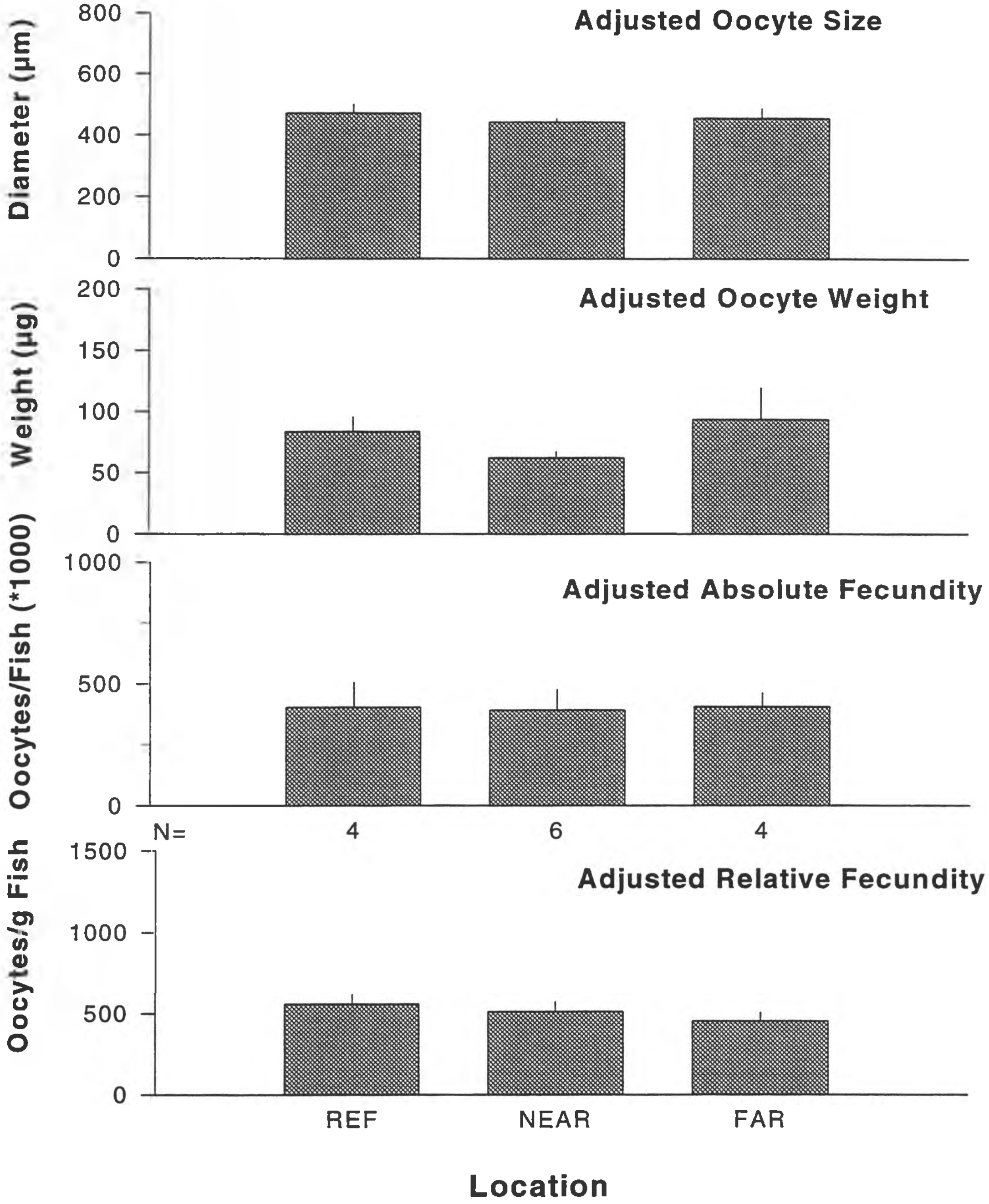

Figure 7. Mean values for oocyte size (diameter & weight) and fecundity (absolute & relative) for female burbot from reference (REF), near-field (NEAR) and far-field (FAR) locations. Histogram bars represent mean and standard errors. The number of sites used in the analysis are indicated after N=.

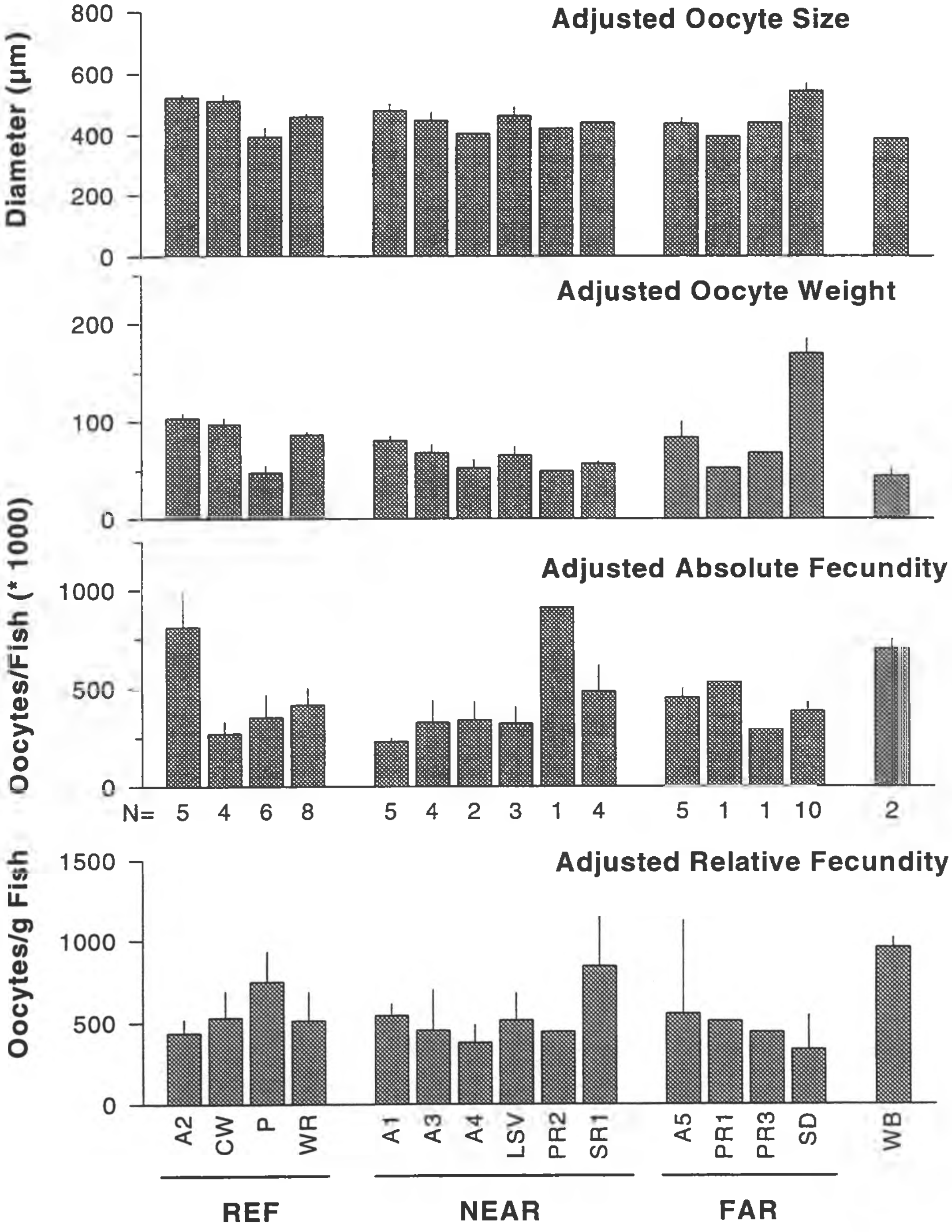

Figure 8. Oocyte size (diameter & weight) and fecundity (absolute & relative) for female burbot collected at the various sites. Histogram bars represent mean and standard error. Sample sizes are indicated after N=.

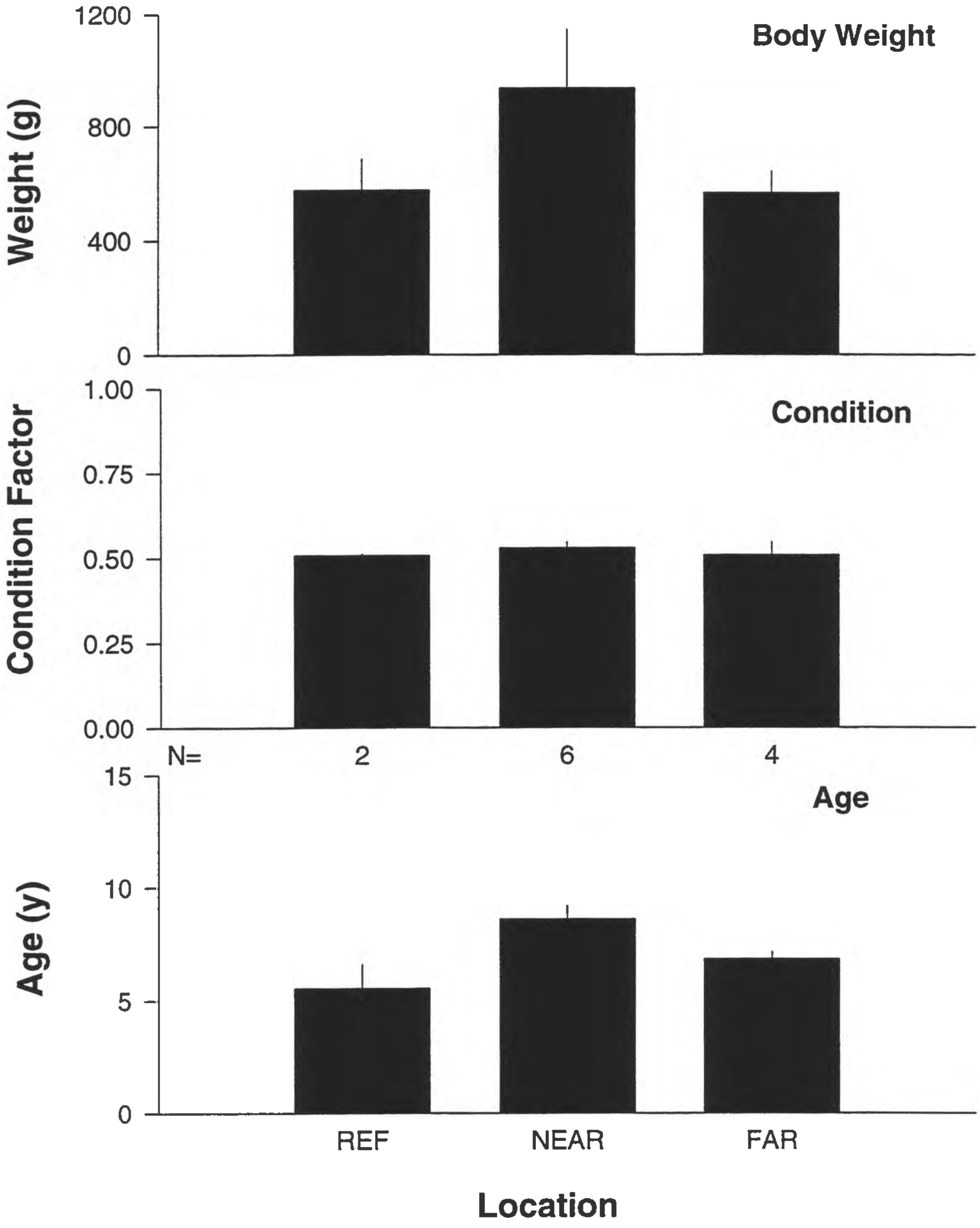

Figure 9. Mean values weight, condition and age for male burbot from reference (REF), near-field (NEAR) and far-field (FAR) locations. Histogram bars represent mean and standard errors. The number of sites used in the analysis are indicated after N=.

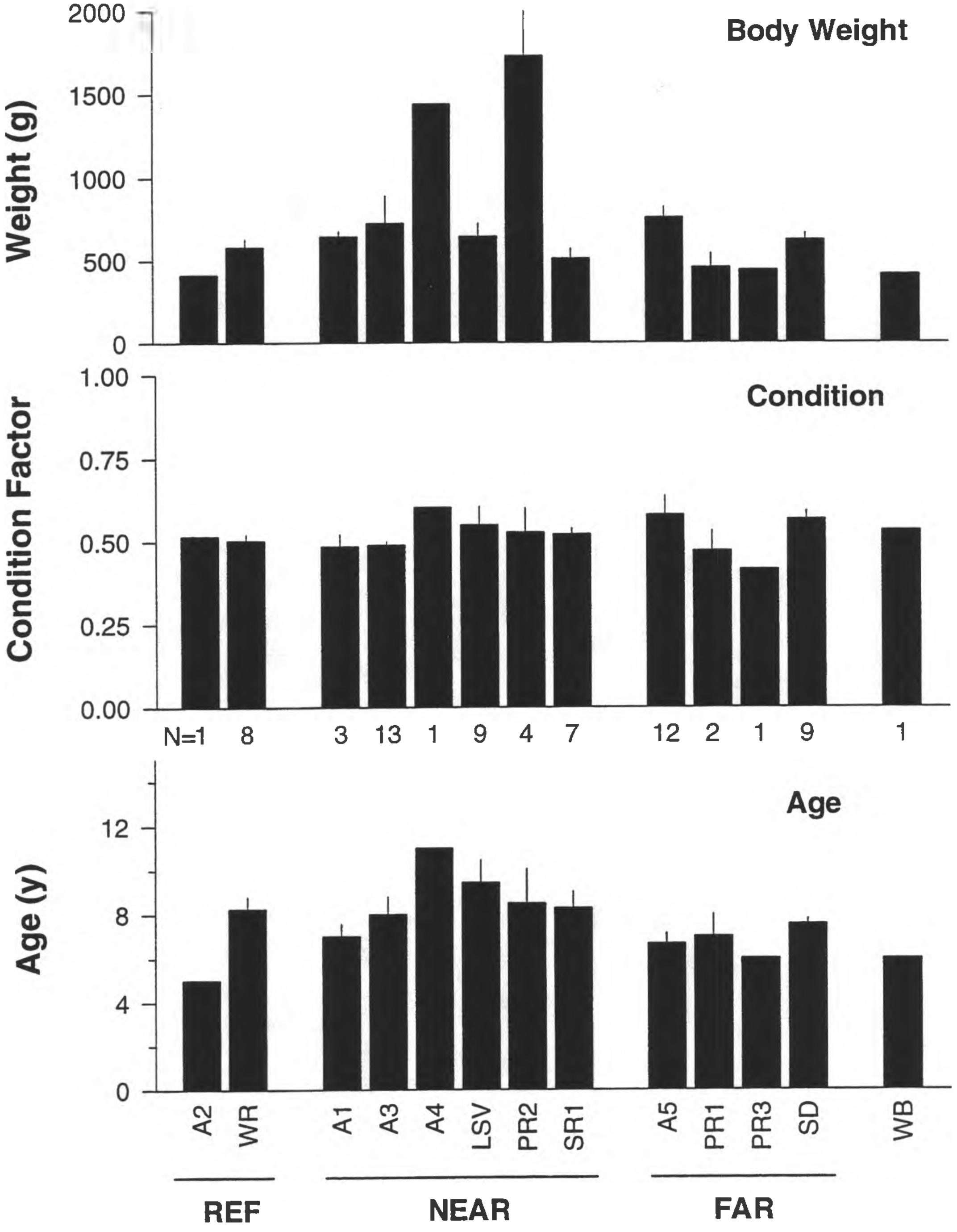

Figure 10. Weight, condition and age for male burbot collected at the various sites. Histogram bars represent mean and standard error. Sample sizes are indicated after N=.

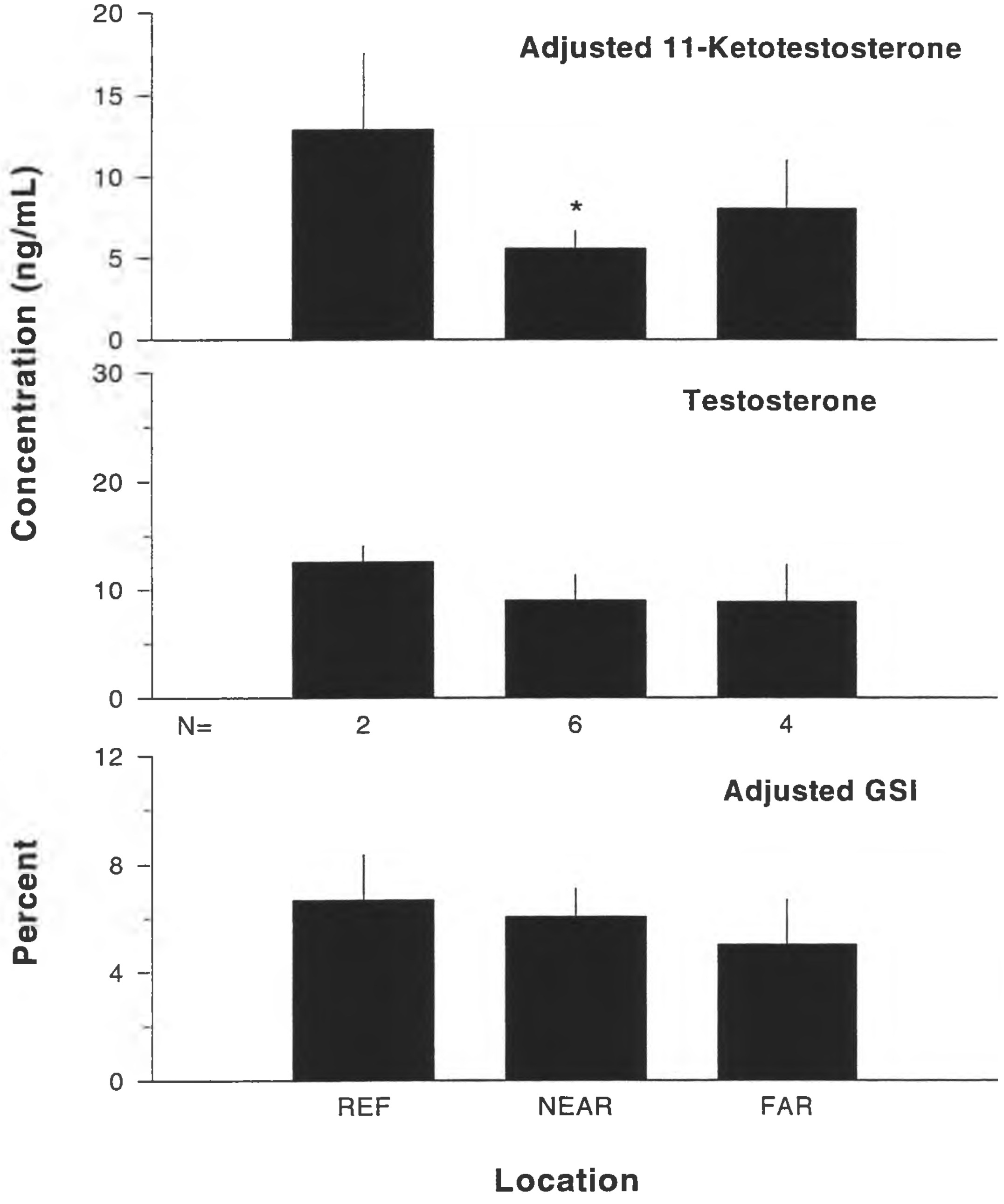

Figure 11. Mean values for steroid hormones (11-ketotestosterone & testosterone) and adjusted GSI for male burbot from reference (REF), near-field (NEAR) and far-field (FAR) locations. Histogram bars represent mean and standard errors. Asterisk indicates means significantly different from (REF) by Dunnett's test (P<0.05). The number of sites used in the analysis are indicated after N=.

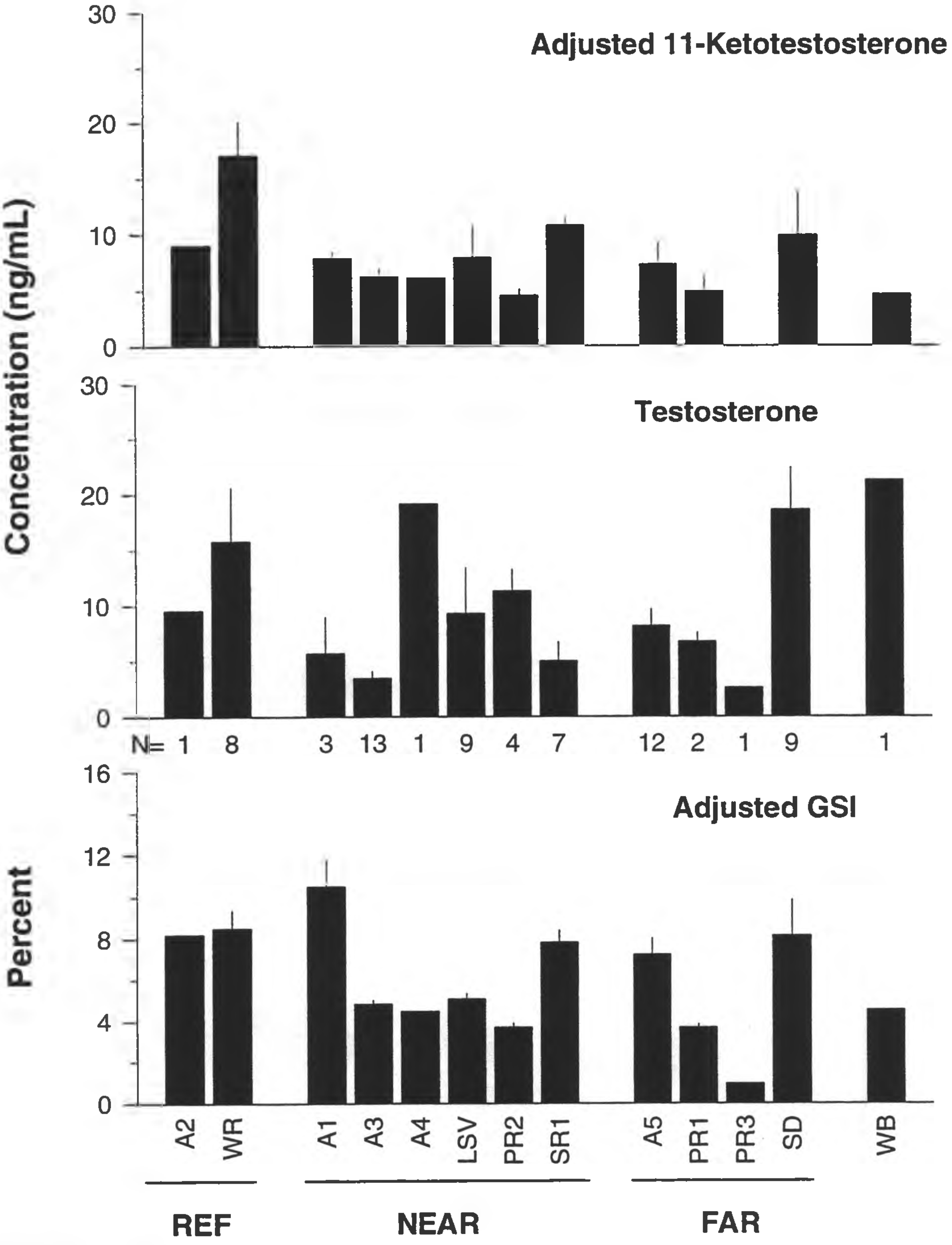

Figure 12. Steroid hormones (11-ketotestosterone & testosterone) and adjusted GSI for male burbot collected at the various sites. Histogram bars represent mean and standard error. Sample sizes are indicated after N=.

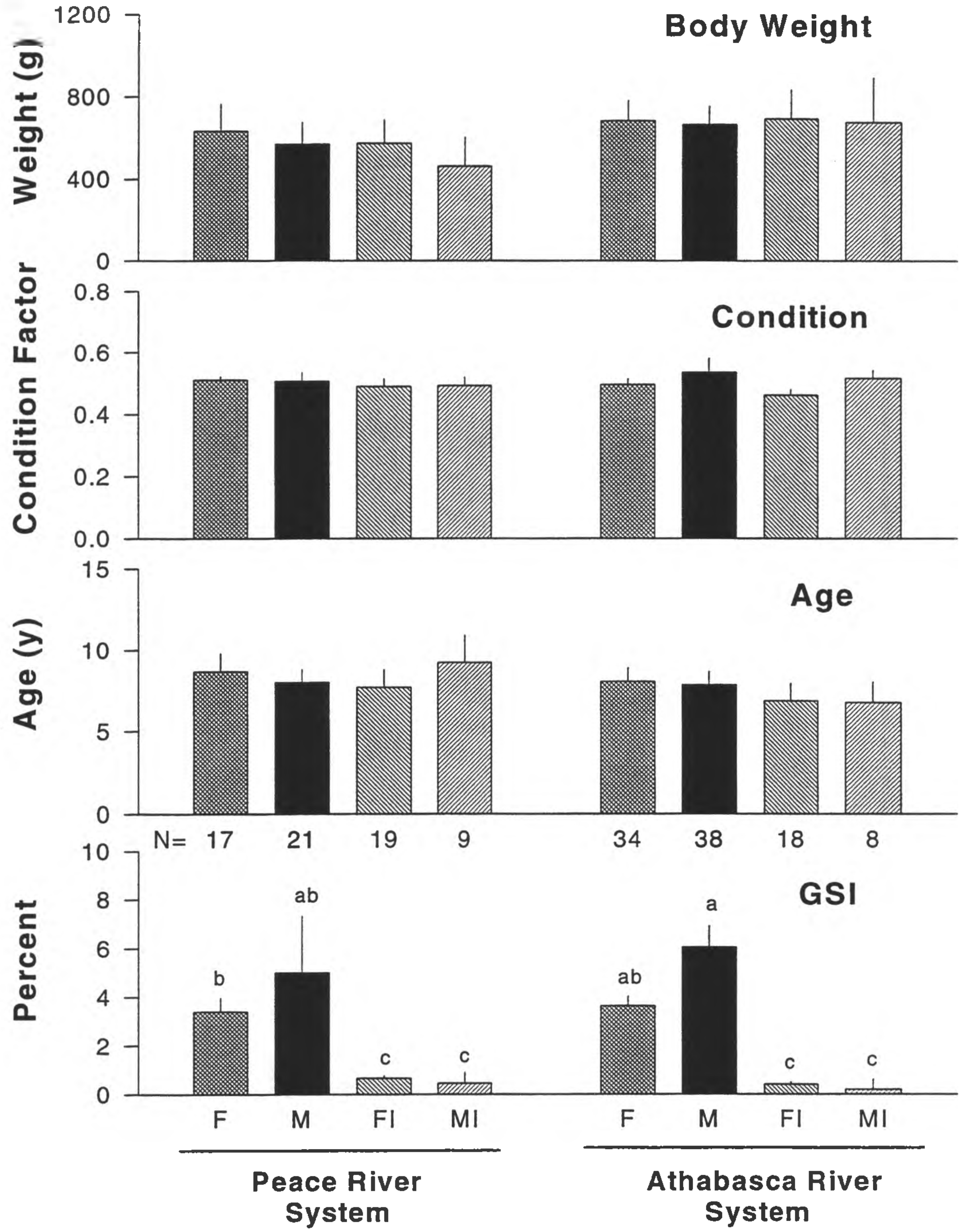

Figure 13. Comparison of weight, condition, age and GSI between mature and immature burbot from the Peace and Athabasca drainages. The same letters above the bars indicate similar means (P<0.05). Histogram bars represent mean and 95 % confidence intervals, Sample sizes are indicated after N=.

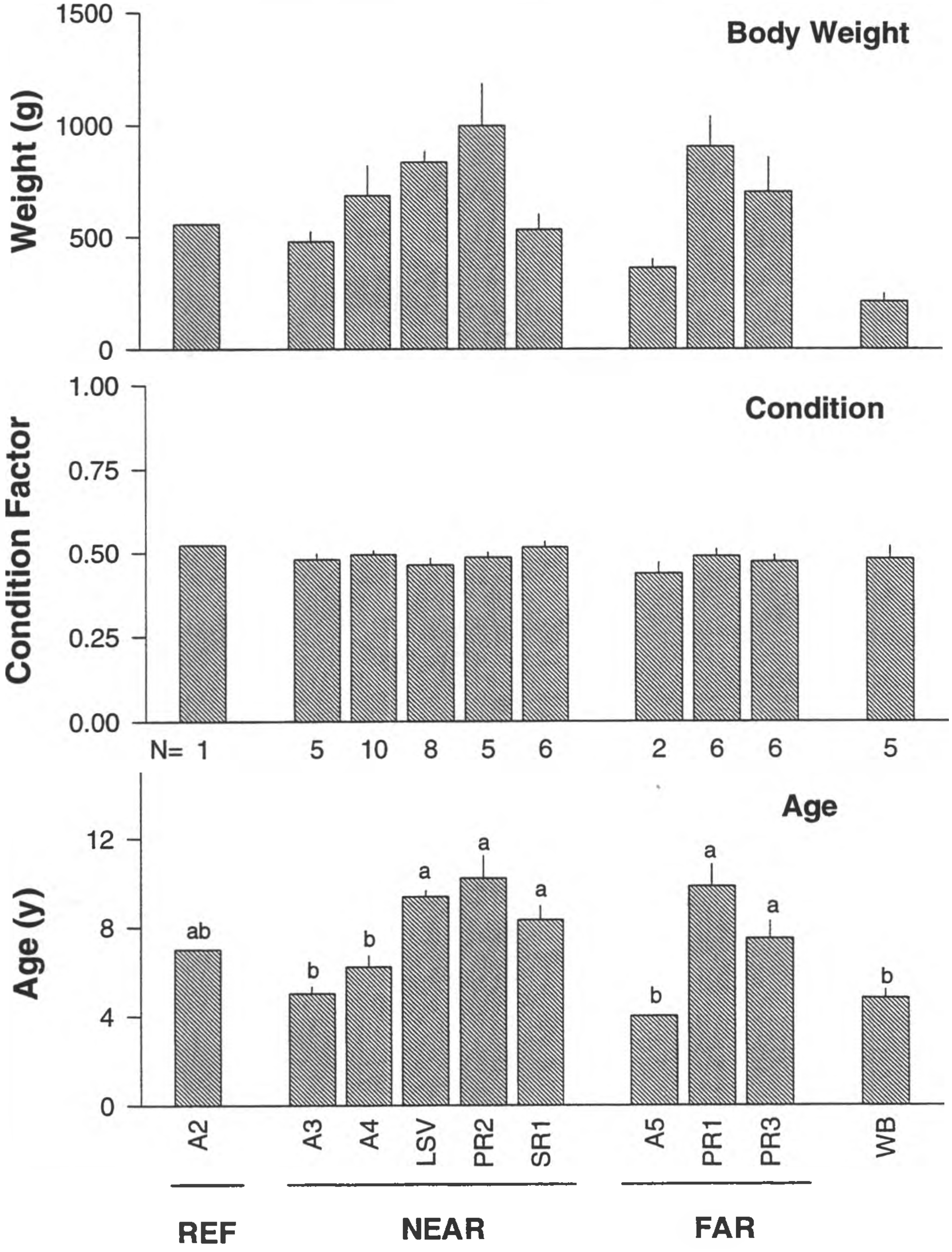

Figure 14. Weight, condition and age for immature burbot collected at the various sites. Histogram bars represent mean and standard error. The same letters above the bars indicate similar means ($P<0.05$). Sample sizes are indicated after N=.

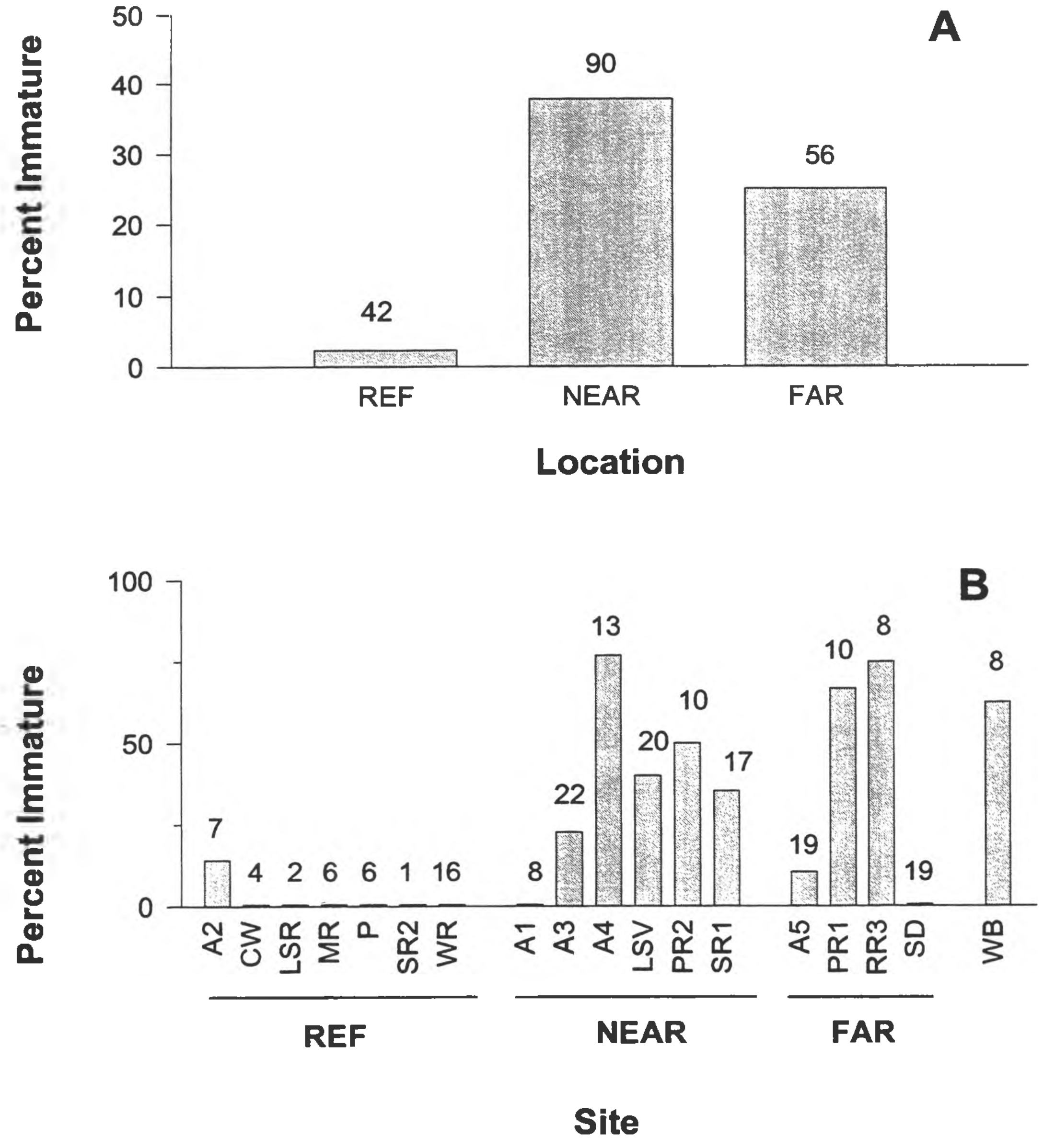

Figure 15. A -Percent immature burbot from reference (REF), near-field (NEAR) and far-field (FAR) locations. B - Percent immature burbot found at each site. Numbers over the bars indicate the total numbers of burbot collected for each location or site.

3.2 Longnose Sucker

3.2.1 Maturing Female Fish

Results obtained for each female longnose sucker analyzed (N=60) are summarized in Appendix B, Table 3. For maturing females (N=34) site specific means and standard errors are summarized in Figures 16 - 18. Based on their age-length relationship female longnose in the Peace basin mature about age 8 - 9. All maturing females in this collection were longer than 370 mm. This observation is consistent with those in Great Slave Lake where no spawners younger than 9 years were seen (Harris 1962). Too few fish were collected in the Athabasca drainage to develop conclusions regarding site specific differences.

The fish collected at the Peace River sites tended to be larger than those collected at the other sites (Fig. 16). Mean condition and ages were similar between locations. Because ages for fish collected at the LSR and SR1 sites were missing in the EnviResource (1995) Report, theses sites could not be included in the age and body weight comparisons. Plasma estrogen levels were variable but were signifcantly lower in fish collected from the PR2 site (Fig. 17). However, neither plasma testosterone nor GSI differed between locations. 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 steroids (estradiol & testosterone) and GSI values were similar to values we previously measured in longnose suckers collected on the Athabasca River in the fall of1992 (Brown et al. 1993). Plasma testosterone levels are somewhat higher than those reported by Munkittrick et al. (1992a) for longnose sucker collected in late September from Lake Superior. As expected plasma steroid hormone levels in longnose suckers were between the pre- and post- ovulatory levels reported for spring spawning fish in the Wapiti/Smoky River (Swanson et al. 1993).

The pre-vitellogenic oocytes including yolk vesicle stage oocytes were usually <700 mm in diameter (Appendix D). These oocytes and connective tissue portion of the ovary comprised 11% of total gonad weight and therefore a single correction factor (89%) was applied for fecundity estimates. The clutch weight ranged from 81 to 95% of total gonad weight. Clutch oocyte diameters and weights were similar between locations (Fig. 18). Oocyte size parameters were comparable to those previously reported in fish collected in the fall from the Athabasca River (Brown et al. 1993). Although absolute and relative fecundity were lower in fish from the PR2 and PR3 sites than those found at the SR1 abnd PR1 sites, fish from the PR2 and PR3 sites did not differ from reference fish. Similar to previous findings in longnose from the Athabasca River (Brown et al. 1993) and the Wapiti River (Swanson et al. 1993) , absolute fecundity estimates averaged 21,104±1031 (mean±SE) eggs per female (Fig. 18). This falls near the low end of the range (17,000 to 60,000 eggs per female) reported by Scott and Crossman (1973).

3.2.2 Maturing Male Fish

Results obtained for each male longnose sucker analyzed (N=22) are summarized in Appendix B, Table 2. For maturing males (N=16) site specific means and 95 % confidence intervals are summarized in Figures 19 & 20. Based on their age-length relationship male longnose in the Peace basin mature about age 7-8. Most maturing males in this collection from Peace and Athabasca drainages were longer than 370 mm. Too few mature male longnose suckers were collected to develop site specific comparisons. Plasma levels of 11-ketotestosterone, testosterone and GSI were similar to values reported for male longnose sucker from fall collections in Lake Superior (Munkittrick et al. 1992a) and in the Athabasca River (Brown et al. 1993). No hormone information was reported for male longnose suckers in the Wapiti/Smoky River by Swanson et al. (1993).

3.2.3 Immature Fish

Results obtained for each immature longnose sucker analyzed (N=32) are summarized in Appendix B, Table 3. The age of maturity for female and male longnose sucker in the Athabasca and Peace drainages were age 7+ and 8+, respectively. We designated younger fish with immature gonads as juveniles (N=7) and excuded them from further analyses. There were 19 longnose sucker of the appropriate size to have maturing gonads but for some reason did not. The size, condition and age of these immature fish were almost identical to that of the maturing longnose sucker collected at the various sites throughout the Peace drainage (Fig. 21). Steroid hormone measures were very low and consistent with immature fish (Appendix B, Table 3)

The distribution of immature fish in the field locations is outlined in Fig. 22. The highest precentages of immature fish were collected from the mainstem near-field and far field sites. There were no immature fish collected from the Reference locations, however, the sample size was small (N=12) and may not be completely representative. Swanson et al. (1993) does not provide any information regarding the proportion of mature and immature longnose suckers in their previous study on fish from the Wapiti/Smoky River. Despite reporting that a higher proportion of immature longnose sucker were collected on the Athabasca mainstem downstream of the town of Athabasca, Shelast et al. (1994) provide insuffcent detail regarding age distribution and maturity to allow comparison with the present findings. Not all longnose suckers spawn in successive years (Scott and Crossman 1973), however few details regarding rates of maturity were supplied.

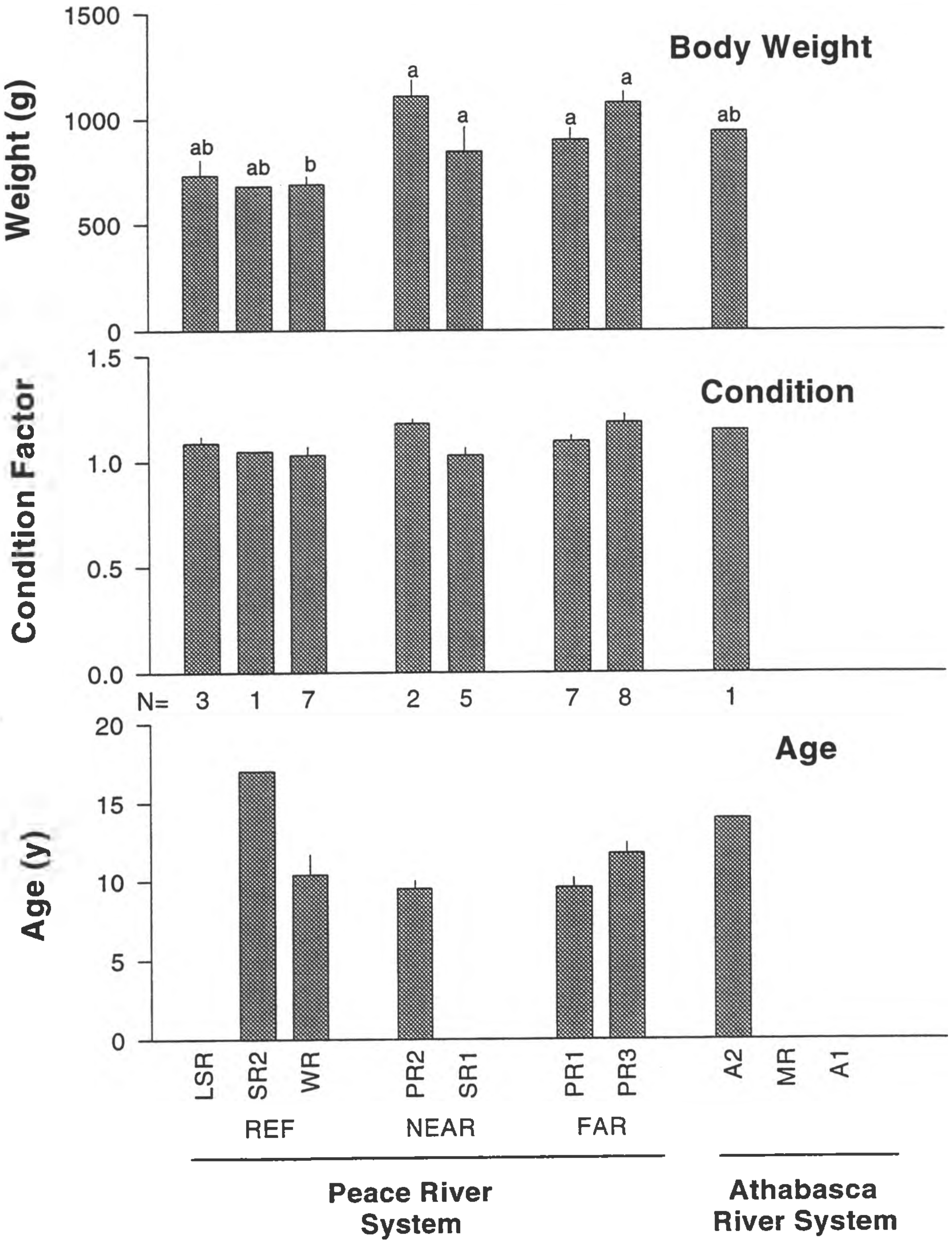

Figure 16. Weight, condition and age for female longnose sucker. Histogram bars represent mean and standard errors. The same letters above the bars indicate similar means (P<0.05). Sample sizes are indicated after N=.

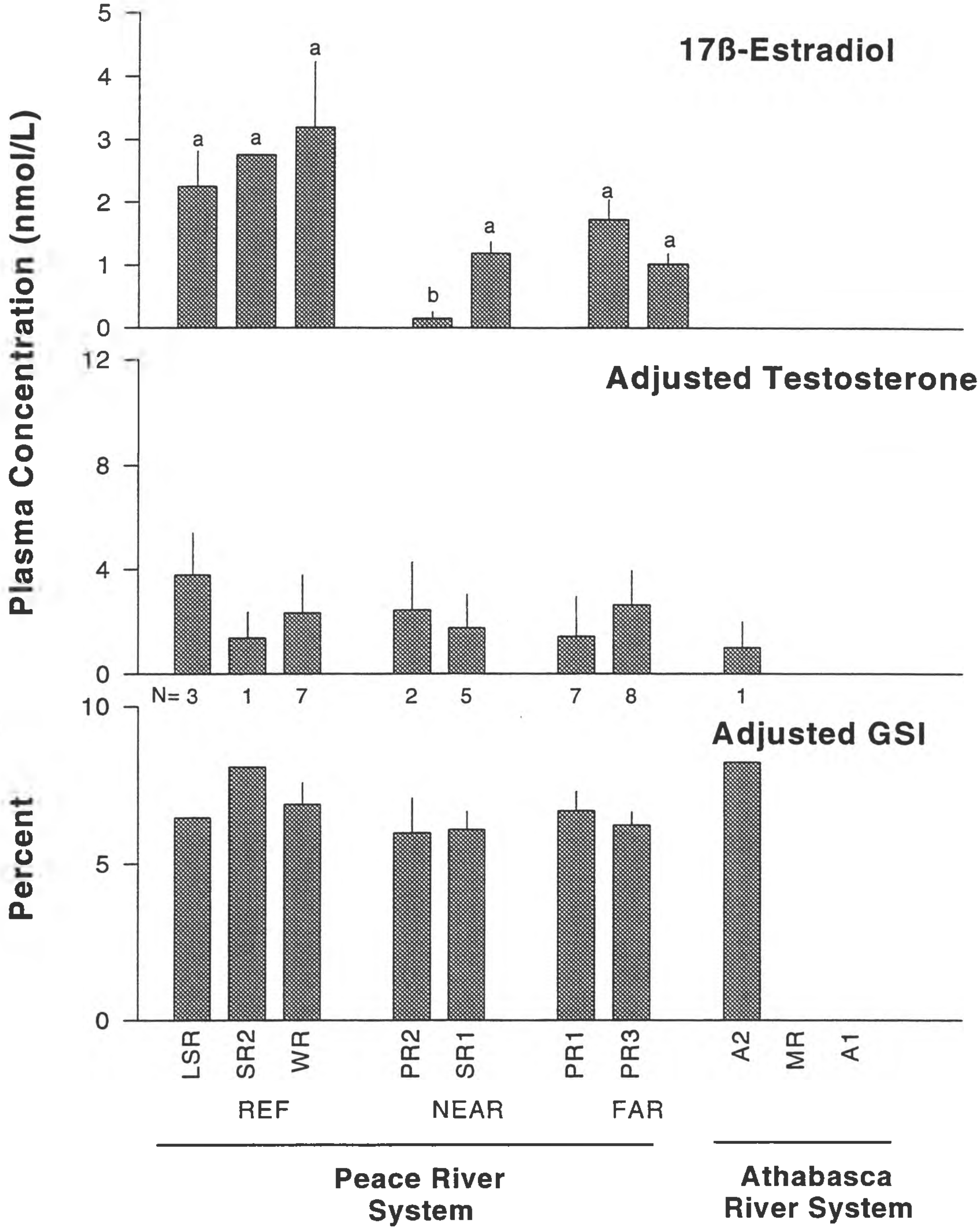

Figure 17. Steroid hormones (17ß-estradiol & testosterone) and adjusted GSI for female longnose sucker. Histogram bars represent mean and standard errors. The same letters above the bars indicate similar means (P<0.05). Sample sizes are indicated after N=.

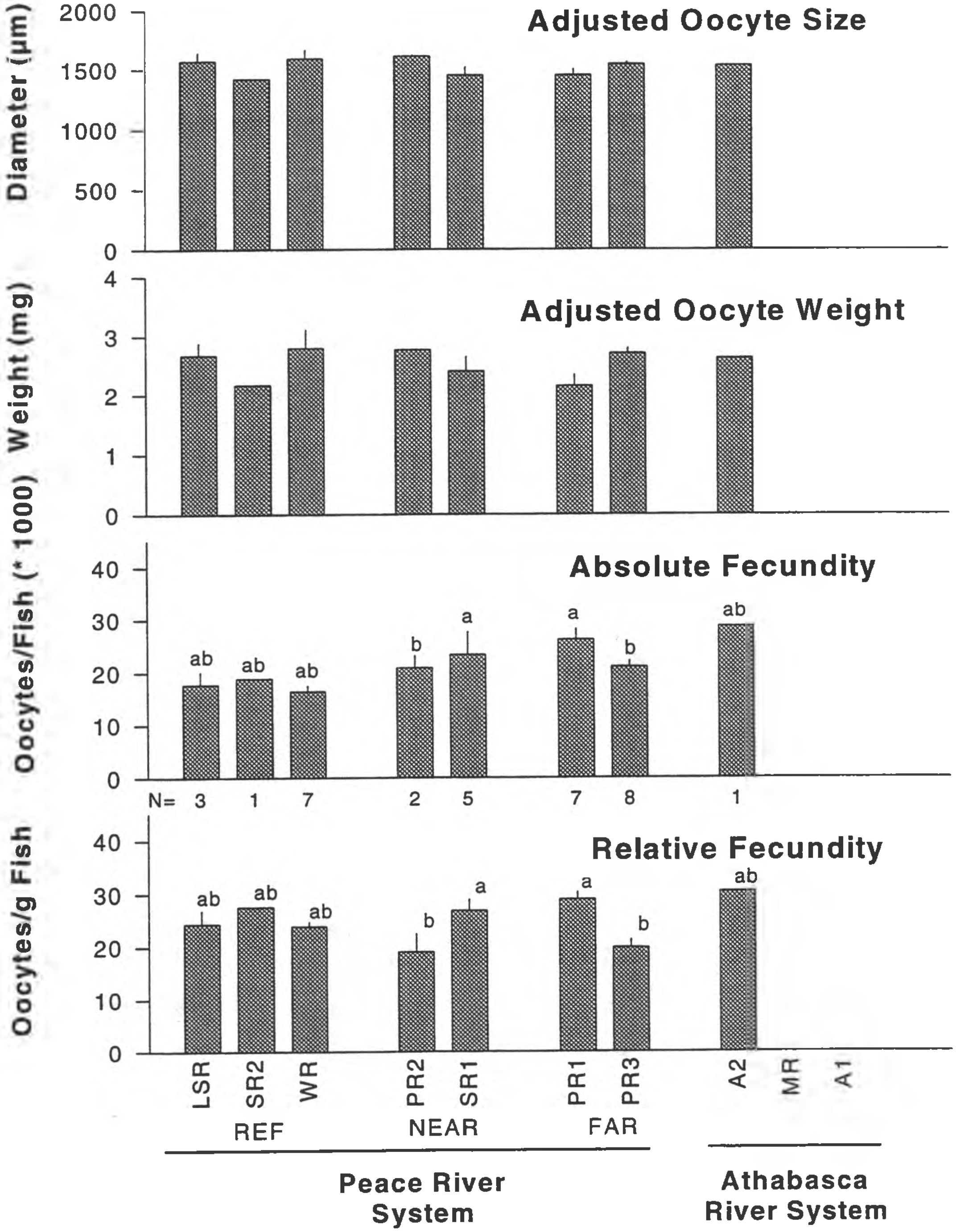

Figure 18. Oocyte size (diameter & weight) and fecundity (absolute & relative) for female longnose sucker. Histogram bars represent mean and standard errors. The same letters above the bars indicate similar means (P<0.05). Sample sizes are indicated after N=.

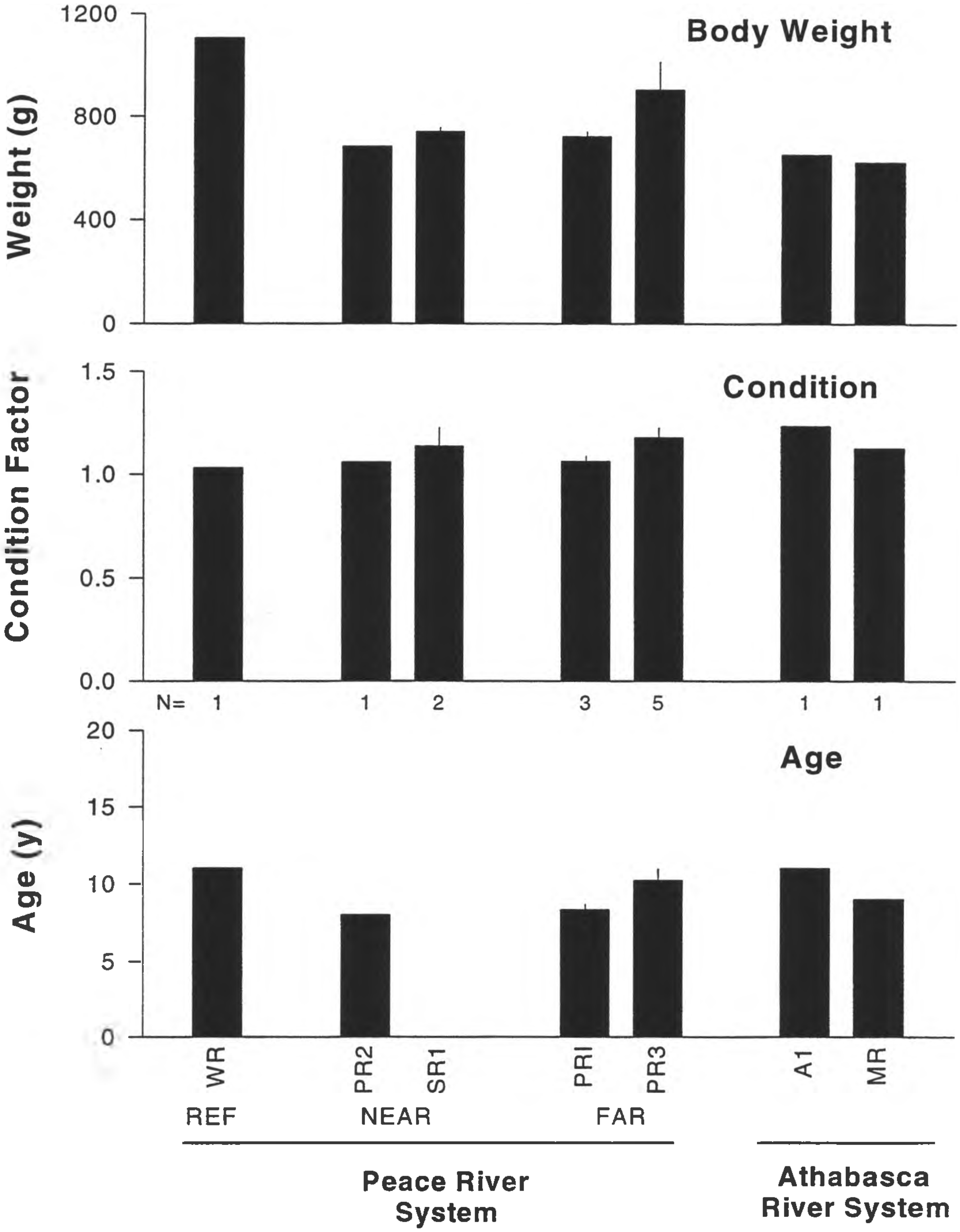

Figure 19. Weight, condition and age for male longnose sucker. Histogram bars represent mean and standard errors. Sample sizes are indicated after N=.

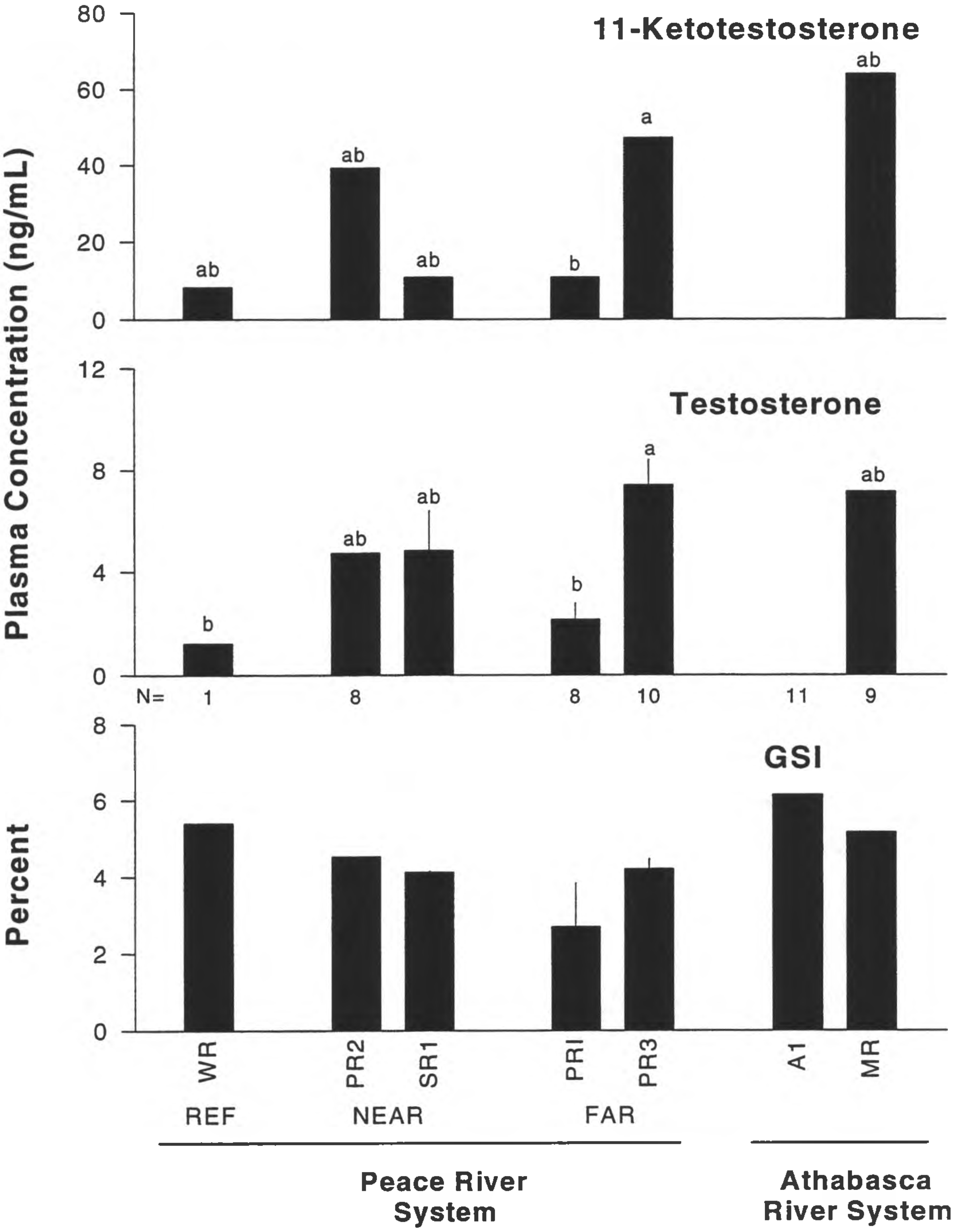

Figure 20. Steroid hormones (11-ketotestosterone & testosterone) and adjusted GSI for male longnose sucker. Histogram bars represent mean and standard errors. The same letters above the bars indicate similar means (P<0.05). Sample sizes are indicated after N=.

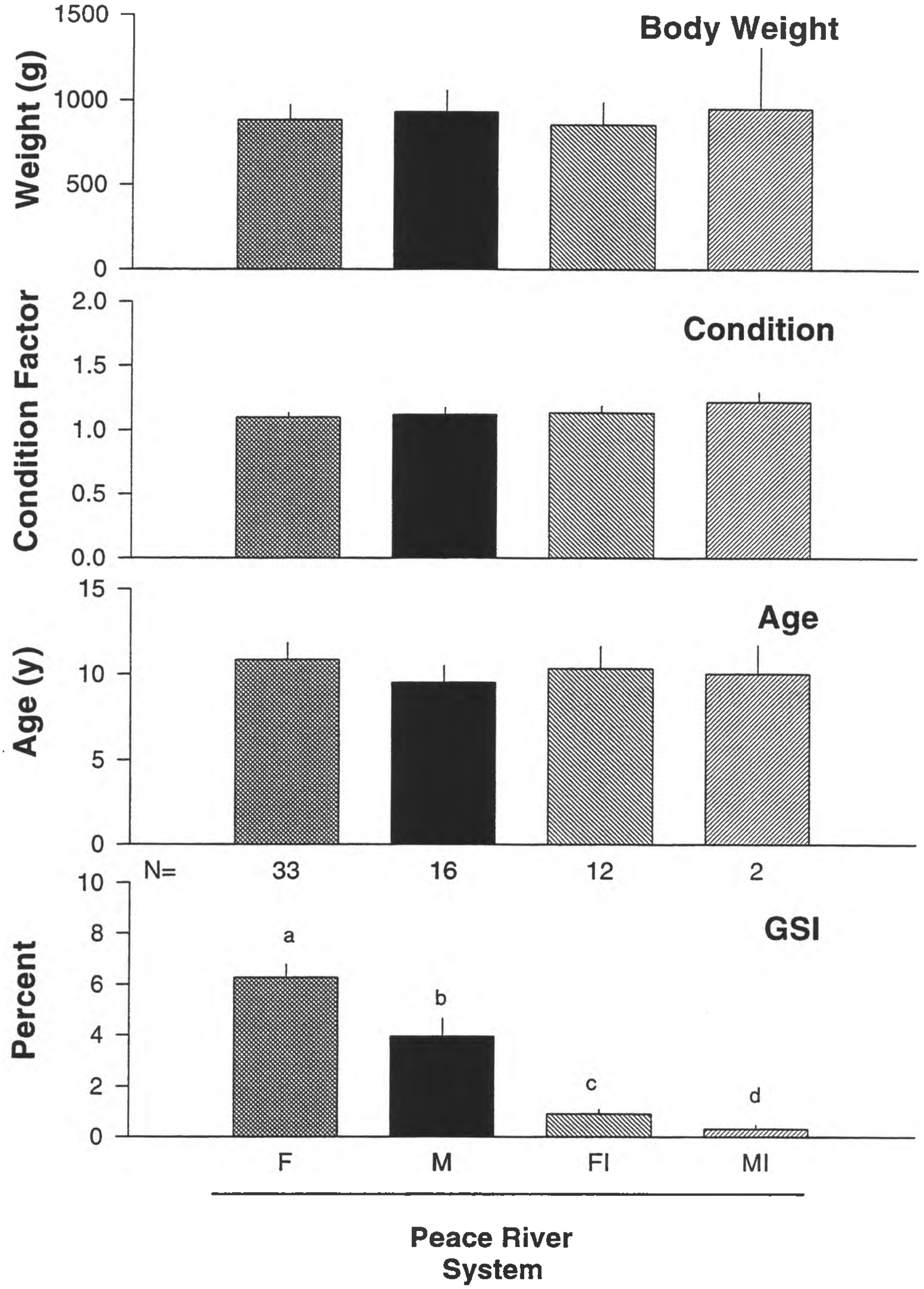

Figure 21. Comparison of weight, condition, age and GSI between mature and immature longnose sucker from the Peace drainages. Histogram bars represent mean and 95 % confidence intervals. The same letters above the bars indicate similar means (P<0.05). Sample sizes are indicated after N=.

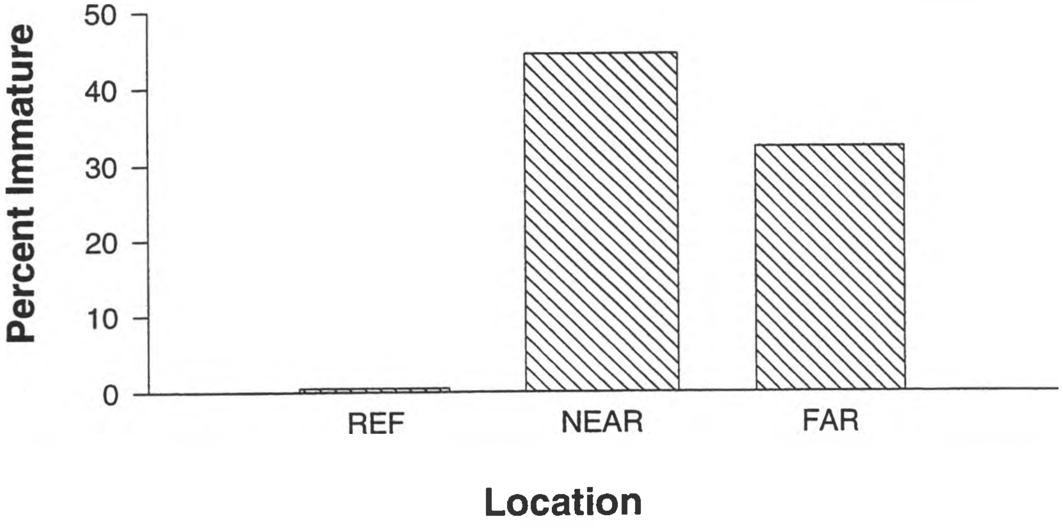

Figure 22. Distribution of mature and immature longnose suckers >370 mm in length between location groupings.

3.3 Northern Pike

3.3.1 Maturing Female Fish

Results obtained for each female northern pike analyzed (N=23) are summarized in Appendix I, Table 3. For maturing females (N=14) site specific means and 95 % confidence intervals are summarized in Figures 23-25. Based on their age-length relationship female northern pike in the Peace and Athabasca drainages mature about age 3-4. This was similar to the age range reported for maturity of female pike in the southern areas of Canada (Scott and Crossman 1973). Too few fish were collected to develop conclusions regarding site specific differences. All maturing females in this collection from Peace and Athabasca drainages were longer than 450 mm.

The pre-vitellogenic oocytes were generally less than 300 μm in diameter (Appendix E). The vitellogenic oocyte component of maturing ovaries was estimated to be 80.3% (range, 64-91%) of gonad weight and this value was applied to all fish when calculating fecundity estimates. For the samples collected on the Athabasca River in 1992 (Brown et al. 1993) this value was 77.4%. The histogram estimates of clutch diameters was 80.8% (64-91%) of caliper measurements. Other than the previous work on the Athabasca River (Brown et al. 1993), we are unaware of published information regarding plasma steroid hormone levels in northern pike from North America. The limited study on European strains (Simontacchi et al. 1983) generally shows values similar to our observations (Brown et al. 1993) but comprehensive information is lacking. The reported relative fecundity estimate is approximately 20 eggs/g fish and absolute fecundity averaged 32,000 eggs per fish (Scott and Crossman 1973). Fall collected northern pike from the Peace and Athabasca had similar absolute and relative fecundities.

3.3.2 Maturing Male Fish

Results obtained for each male northern pike analyzed (N=19) are summarized in Appendix B, Table 4. For maturing males (N=18) site specific means and 95 % confidence intervals are summarized in Figures 26 & 27. Based on their age-length relationship male northern pike in the Athabasca and Peace basins mature about age 3. Males mature at 2 - 3 years in southern Canada and at age 5 in the North (Scott and Crossman 1973). Most maturing males in this collection from Peace and Athabasca drainages were longer than 450 mm. Too few mature male northern pike were collected to develop site specific comparisons. For the most part, plasma levels of testosterone and 11-ketotestosterone were fairly high in northern pike (Brown et al. 1993) and similar to levels found in salmonids near the same stage of gonadal development (Scott et al. 1980) .

3.3.3. Immature Fish

Results obtained for each immature northern pike analyzed (N=10) are summarized in Appendix B, Table 4. The age of maturity for female and male northern pike in the Athabasca and Peace drainages were age 3+. All immature fish except one were ages 2-3. Too few immature northern pike were collected to draw specific conclusions.

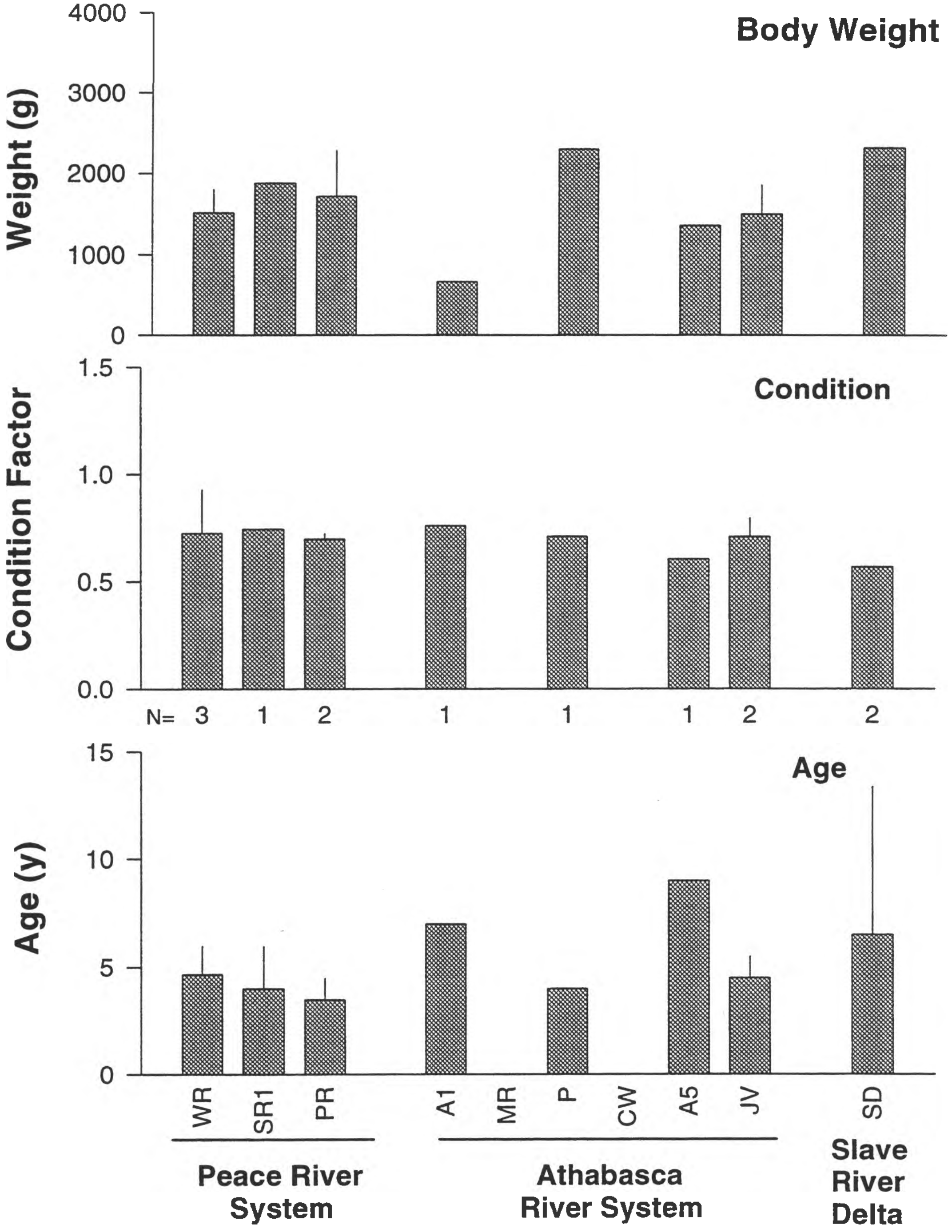

Figure 23. Weight, condition and age for female northern pike. Histogram bars represent mean and 95 % confidence intervals. Sample sizes are indicated after N=.

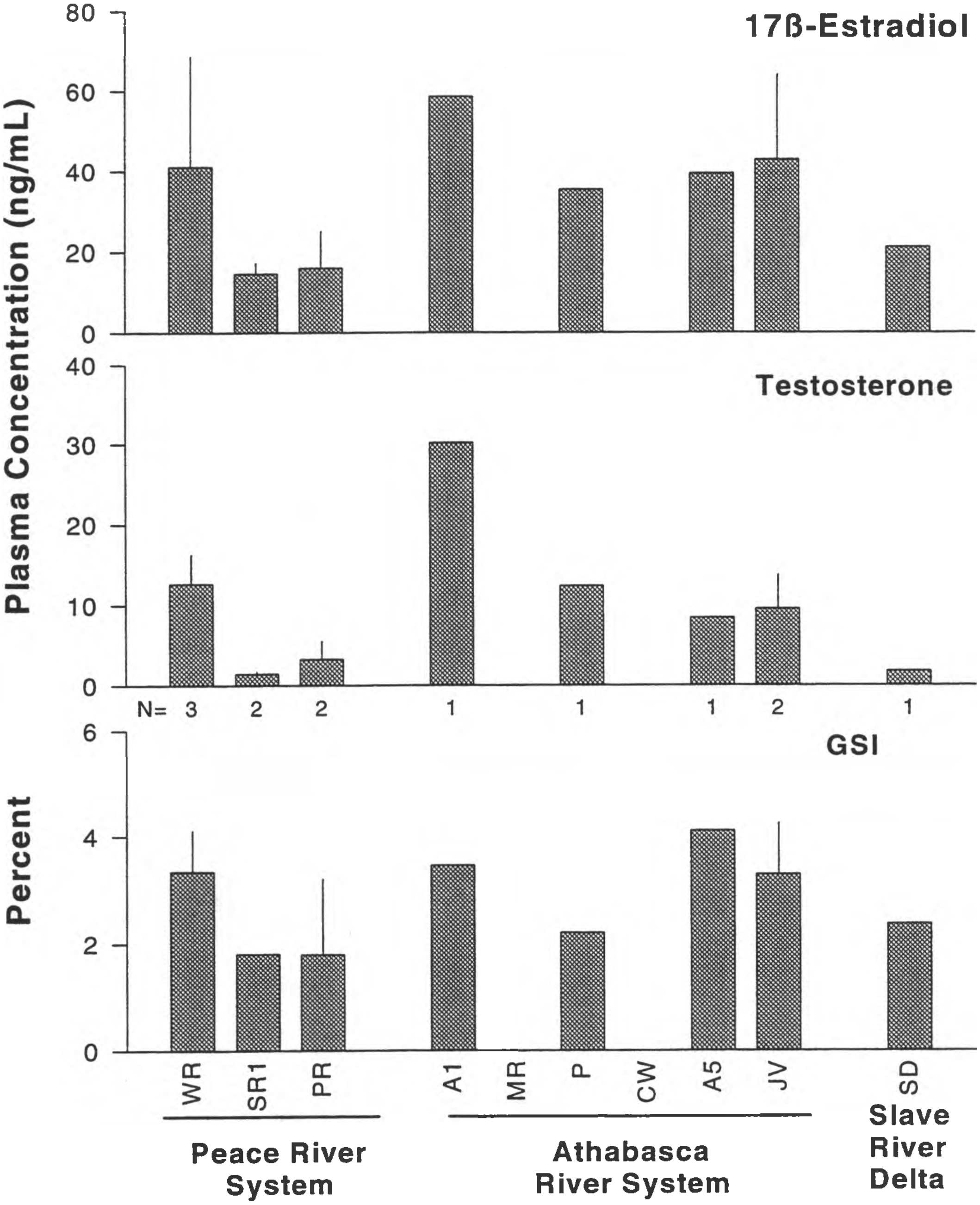

Figure 24. Steroid hormones (17ß-estradiol & testosterone) and GSI for female northern pike. Histogram bars represent mean and 95 % confidence intervals. Sample sizes are indicated after N=.

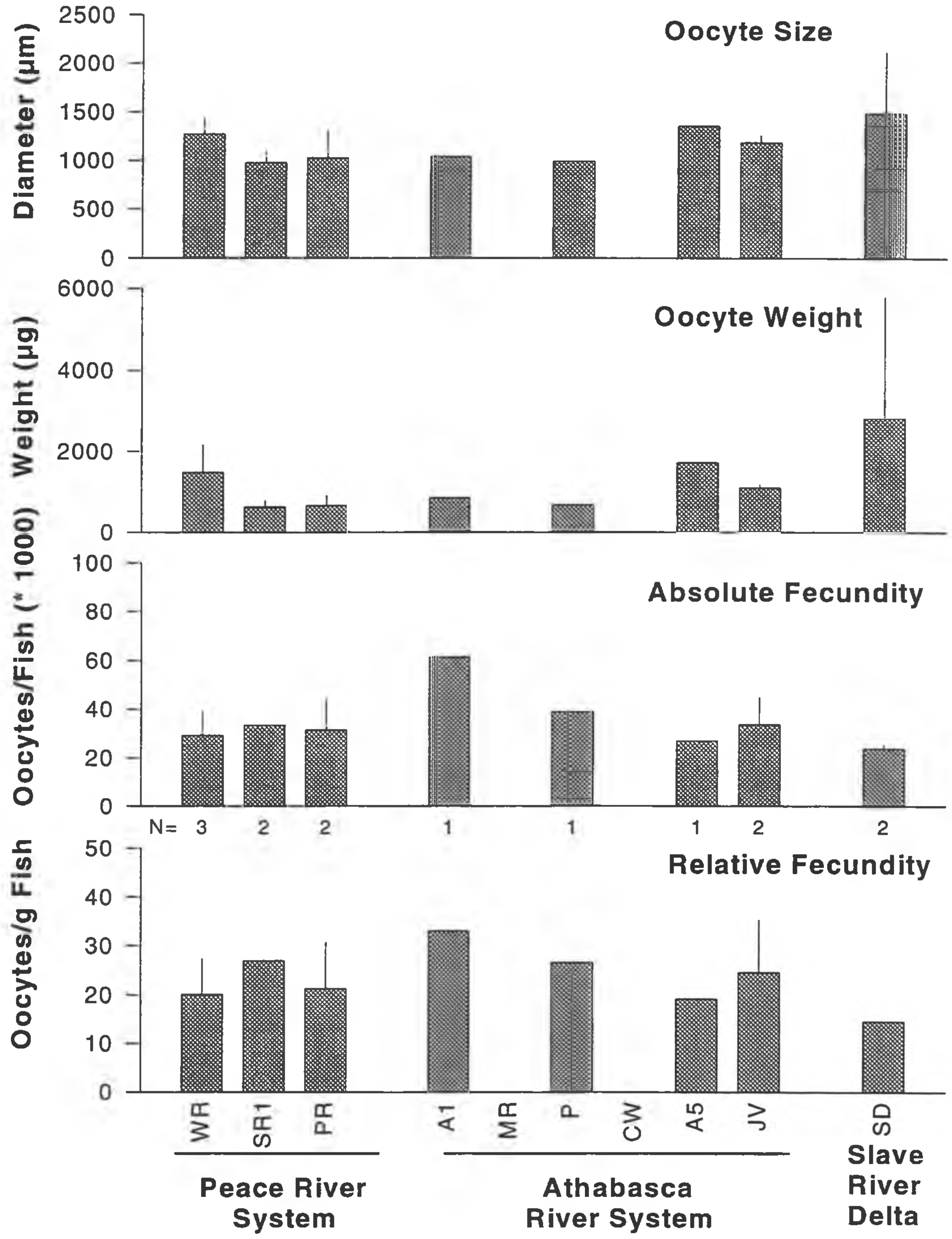

Figure 25. Oocyte size (diameter & weight) and fecundity (absolute & relative) for female northern pike. Histogram bars represent mean and 95 % confidence intervals. Sample sizes are indicated after N=.

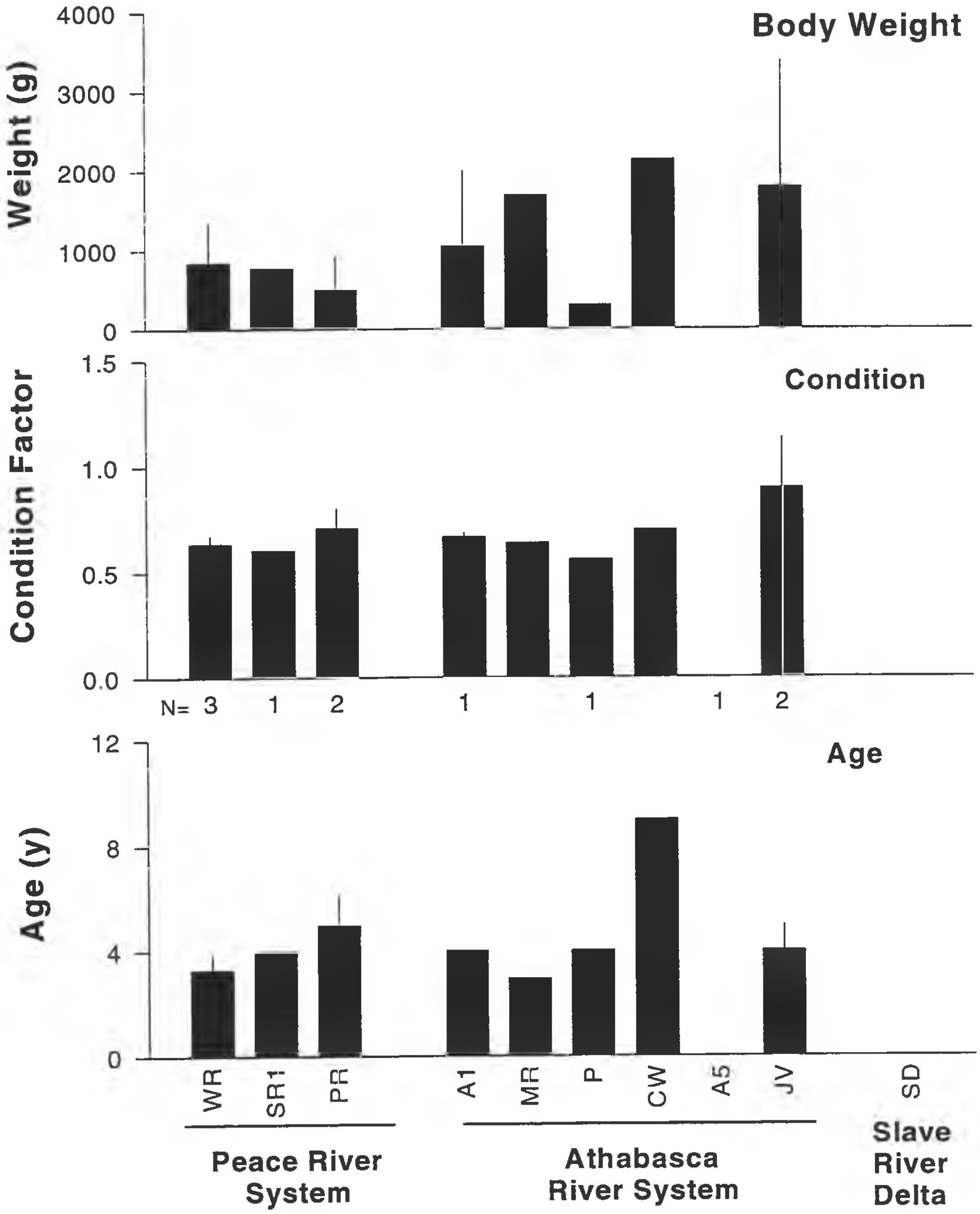

Figure 26. Weight, condition and age for male northern pike. Histogram bars represent mean and 95 % confidence intervals. Sample sizes are indicated after N=.

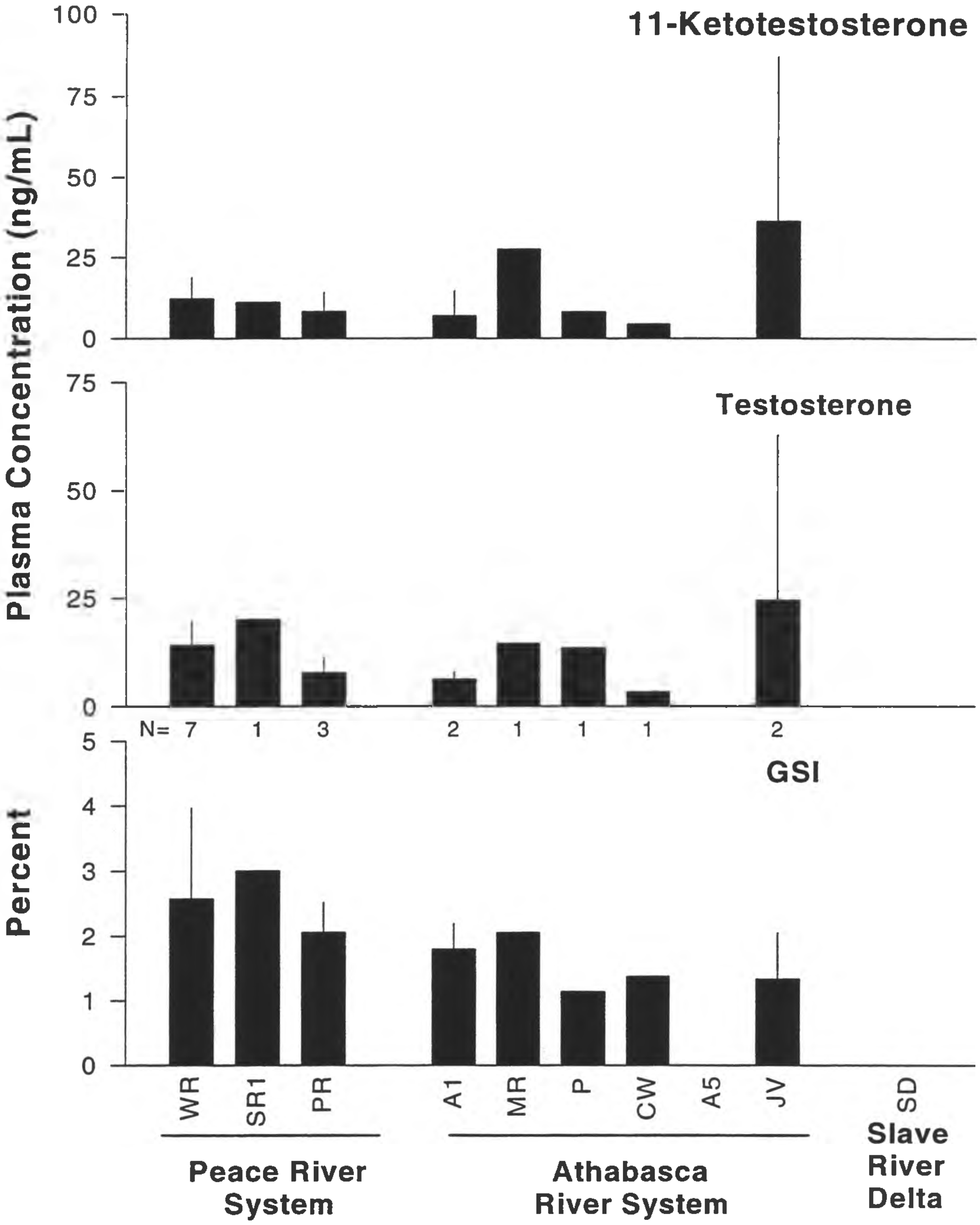

Figure 27. Steroid hormones (11-ketotestosterone & testosterone) and GSI for male northern pike. Histogram bars represent mean and 95 % confidence intervals. Sample sizes are indicated after N=.

3.4 Flathead Chub

3.4.1 Maturing Female Fish

Results obtained for each female flathead chub analyzed (N=16) are summarized in Appendix B, Table 5. For maturing females (N=11) site specific means and 95 % confidence intervals are summarized in Figures 28 - 30. Based on their age-length relationship female flathead chub in the Peace and Athabasca drainages mature about age 4. This age of maturity is consistent with previous findings on the Athabasca River (Bond and Berry 1980a & b). Too few fish were collected to develop conclusions regarding site specific differences. All maturing females in this collection from Peace and Athabasca drainage were longer than 200 mm.

The flathead chub pre-vitellogenic oocytes, including early yolk vesicle stage oocytes were considered to be <300 mm in diameter. The percent clutch and clutch oocyte diameters were estimated from the histograms using 450 mm (Appendix E) as a minimum size for inclusion. The oocyte sizes spanned the range from smallest to largest without an obvious size separation between the clutch and resting stages. Therefore flathead chub oocyte development appears to be continuous. Histologically, the vitellogenic oocytes looked similar over their range. Adapting a minimum size for inclusion in the clutch was justifiable in that it worked out to be 71 % (66-78 %) of the caliper measurements (presented in Appendix B, Table 5) for these fish. Further, when performing caliper measurements to estimated clutch diameter only those oocytes showing a yellow coloration were included. From this one group of samples we cannot determine definitively that flathead chub are asynchronous spawners.

3.4.2 Maturing Male Fish

Results obtained for each male flathead chub analyzed (N=6) are summarized in Appendix B, Table 5. For maturing males (N=4) site specific means and 95 % confidence intervals are summarized in Figures 28 & 29. Based on their age-length relationship male flathead chub in the Peace basin mature about age 3+. Bond and Berry (1980a & b) found the earliest age of maturity at 3 years for flathead chub on the lower Athabasca River. Male gonads were at Stage 2 and cysts contained mostly spermatocytes with few spermatids and spermatozoa. No stage 3 and 4 gonads were collected so it is likely that these males would be first time spawners the following year. Most maturing males in this collection from Peace drainages were longer than 150 mm. Too few mature male flathead chub were collected to develop site specific comparisons.

3.4.3 Immature Fish

Results obtained for each immature flathead chub analyzed (N=9) are summarized in Appendix B, Table 5. The age of the immature fish collected was 2 - 5 years Because many gonad weights were recorded simply as <1g in the field report (EnviResource 1995), measurements are too inaccurate to provide GSI estimates for immature flathead chub. Too few immature flathead chub were collected to develop site specific conclusions.

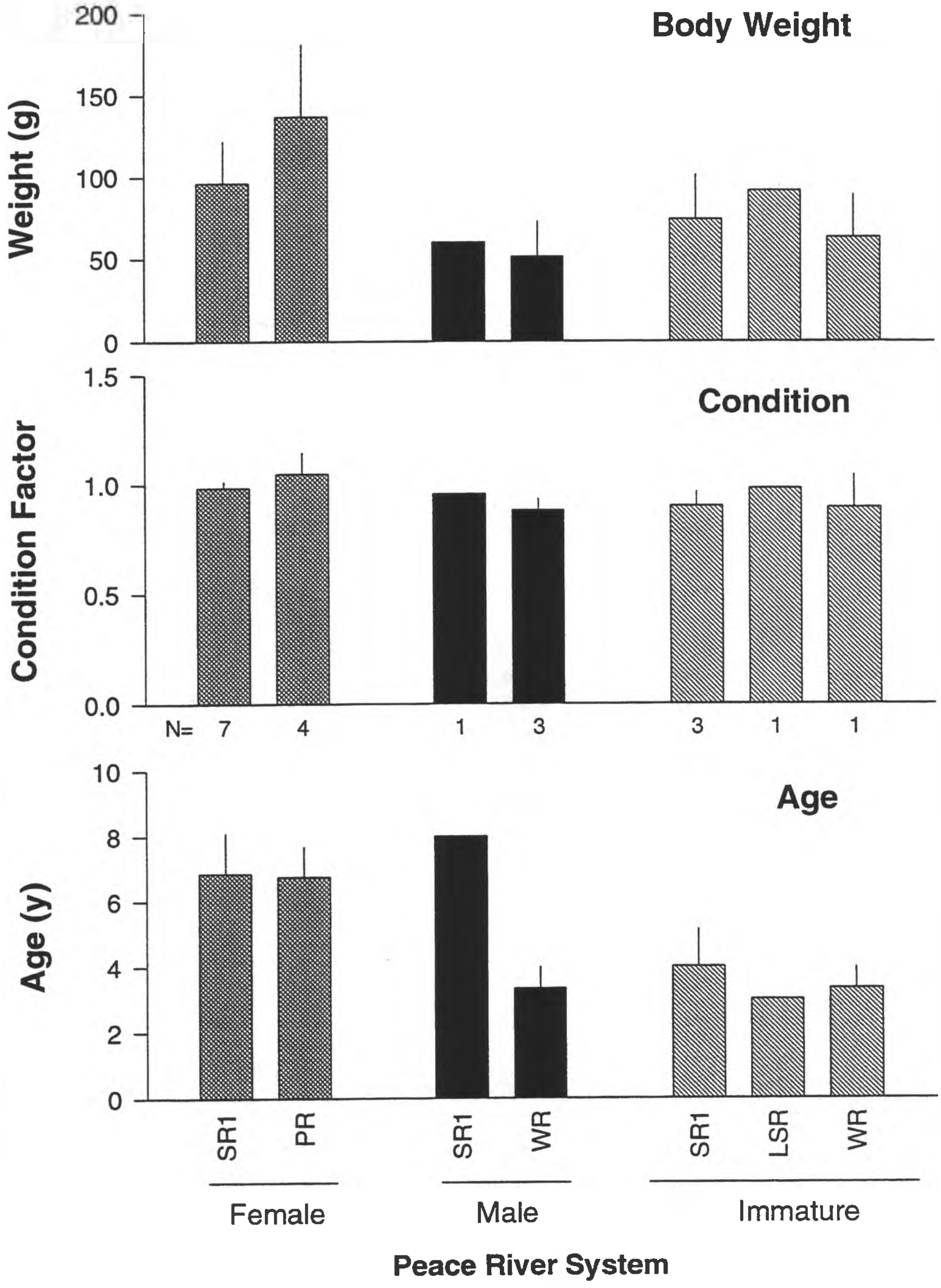

Figure 28. Weight, condition and age for flathead chub. Histogram bars represent mean and 95 % confidence intervals. Sample sizes are indicated after N=.

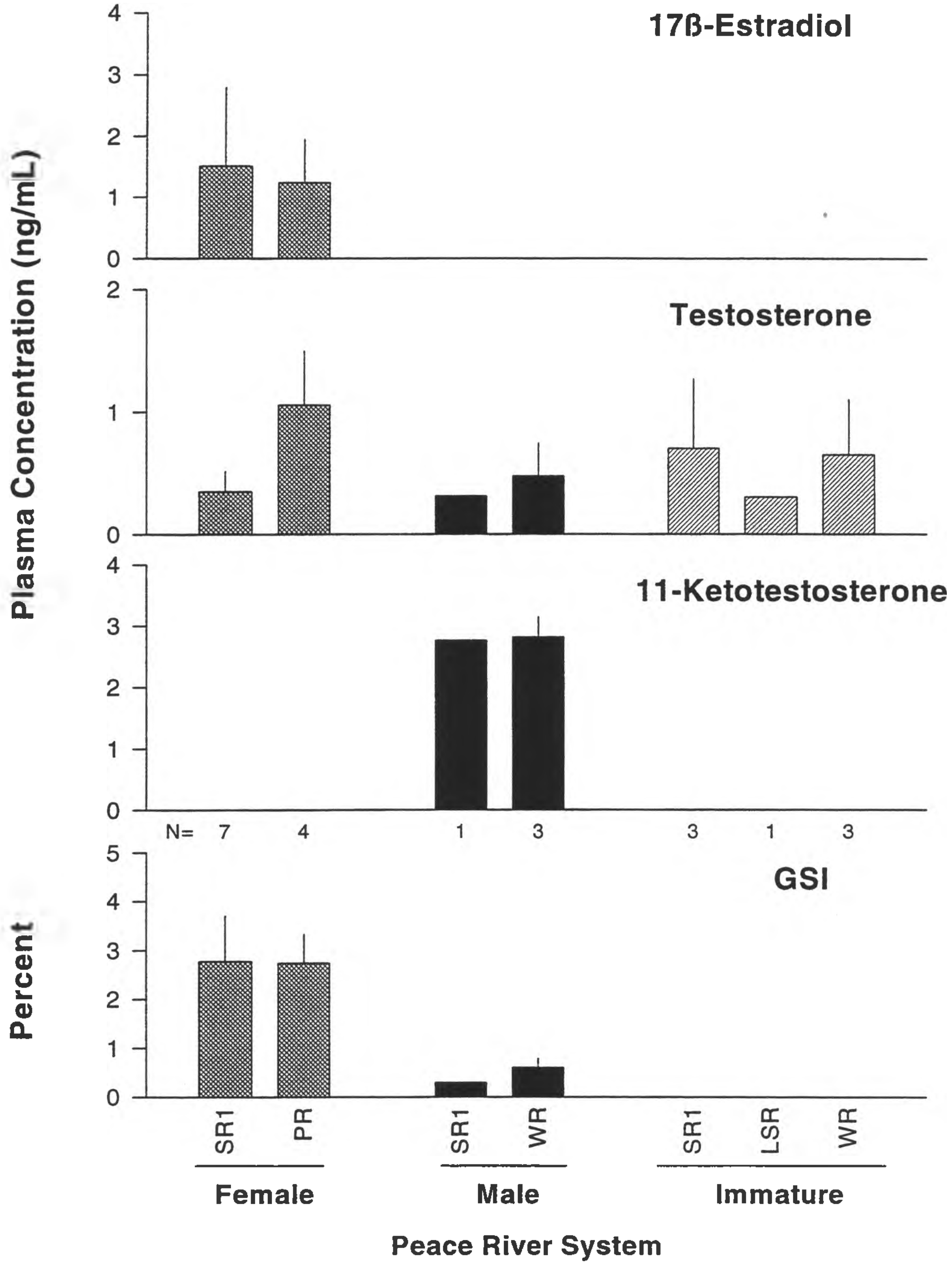

Figure 29. Steroid hormones (17ß-estradiol, testosterone & 11-ketotestosterone) and GSI for flathead chub. Histogram bars represent mean and 95 % confidence intervals. Sample sizes are indicated after N=.

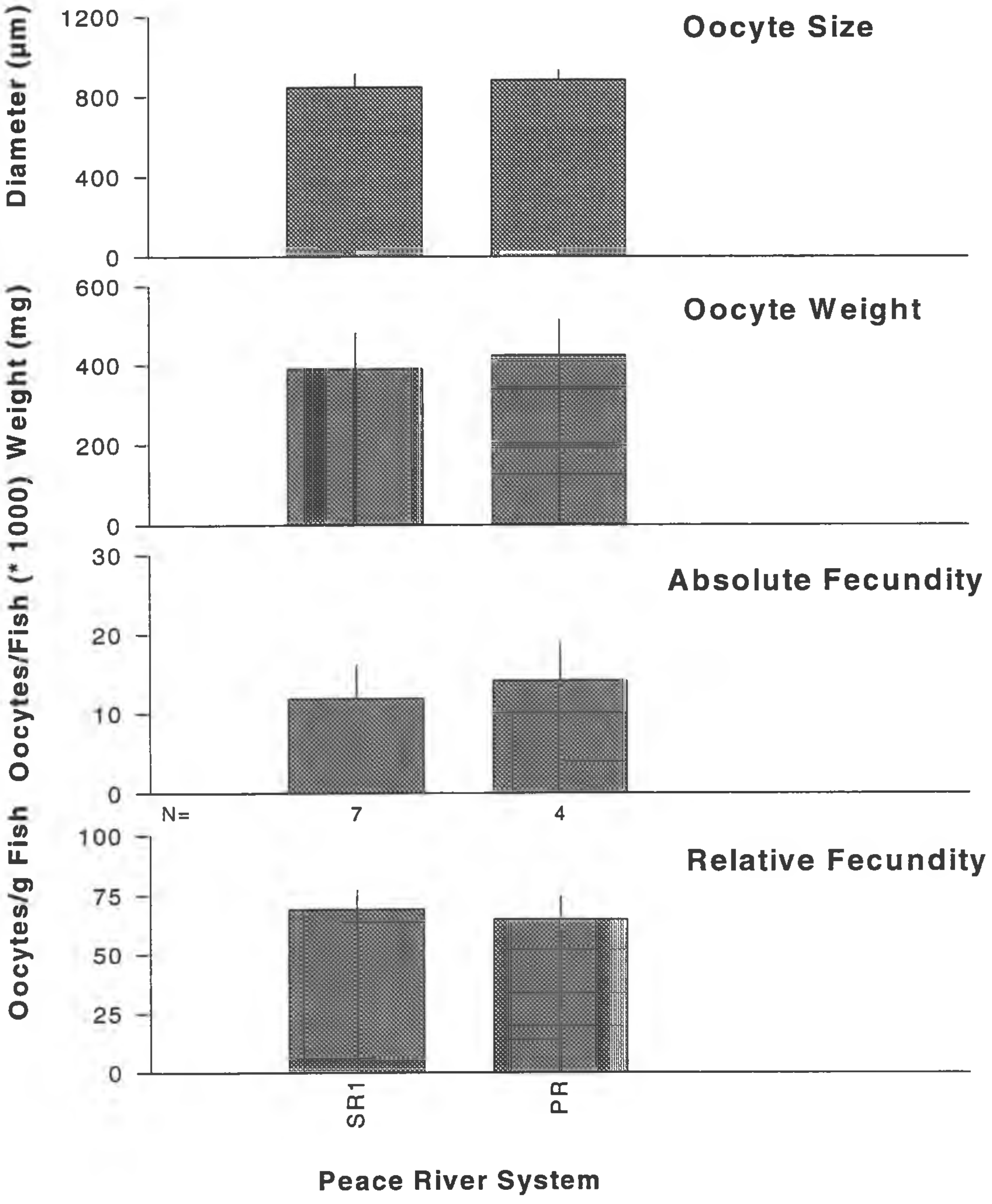

Figure 30. Oocyte size (diameter & weight) and fecundity (absolute & relative) for female flathead chub. Histogram bars represent mean and 95 % confidence intervals. Sample sizes are indicated after N=.

4.0 CONCLUSIONS AND RECOMMENDATIONS

The study represents an pioneering effort of unprecidented scope and scale which attempted to simultaneously evaluate contaminants, reproductive parameters and possible biochemical effects of potential contaminant exposure. Thus, the data provides heretofor undocumented information about feral species captured in the fall from the Northern River Basins Study area. Due to the large spatial scale covered by the basin-wide survey and the associated constraints in obtaining a simultaneous samples from the various locations, the variation in the sampling times and associated developmental changes in some of the physiological and morphological measurements confounded site specific comparisons. To account for this relationship with sampling time for certain parameters, we adjusted those showing a significant relationship by linear regression to a common date (*GSI, oocyte size, oocyte weight, absolute fecundity, relative fecundity and 11-ketotestosterone* in burbot; *testosterone, GSI, oocyte size and oocyte weight* in longnose sucker). After time adjustment only 11-ketotestosterone in male burbot showed a significant difference with respect to field location. Apparent differences in other parameters prior to adjustment were no longer present. Concentrations of 17ß-estradiol and other unadjusted parameters appeared unrelated to to sampling time, therefore no correction was performed. The differences in sampling times between locations may confound conclusions regarding measured reproductive parameters in maturing fish, but it does not alter the observations about the developmental state (mature versus immature). It is recommended that subsequent sampling programs be as timely as possible such that significant developmental changes among sites are minimized. Because of the biological variability, efforts to clearly match samples between reference and exposed areas should be considered. A repeat visit to selected sites would also allow more accurate assessment of data. Based on our findings an analysis to determine optimal samples sizes should be undertaken prior to performing future surveys and subsequent studies should consider fewer sites, assessed more comphrensively. To allow accurate characterization of gonadal development, future fish collections should continue to include gonad tissues for histological analysis.

Generally, any observed differences between fish collected from reference locations and the other regions would be consistent with effects found in fish collected from waters receiving pulp mill inputs in other locations. The higher proportion of females in the reference areas versus regions more downstream and potentially more impacted by effluent discharge raise questions about general burbot movements and residency in the upper reaches and tributaries. Investigations of movement patterns seems essential to develop a complete understanding burbot distribution in the area.

Female Fish. In the near-pulpmill sites from the Peace and Athabasca drainages, plasma 17ß-estradiol appeared lower in female burbot and longnose sucker relative to the reference sites. Levels of 17ß-estradiol in fish from reference locations was similar to concentrations found in maturing burbot held in the laboratory (Giles et al. 1996). Under the control of pituitary gonadotropins, 17ß-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. However, there was no evidence of change in measures of ovarian development (GSI, oocyte size measurements) in burbot so the possible deficit in reproductive steroid levels has not generally impacted gonadal growth and development in fish from the region. Burbot from the Slave River were in the best condition with the largest oocytes but fecundity estimates were lowest. Gonads from the smaller female burbot collected from the Pembina River near Jarvie were under-developed relative to fish collected at other potential reference sites. The cause of this is uncertain and requires further investigation. As previously reported for longnose sucker collected in the upper Athabasca River (Brown et al. 1993), site related differences in oocyte size or fecundity estimates corresponding to low 17ß-estradiol levels were not apparent in fish collected on the Peace River.

Lower steroid hormone levels have been previously reported in longnose suckers (Brown et al. 1993) and other species downstream of pulp mill effluent (McMaster et al. 1991; Munkittrick et al. 1991, 1992a,b; Van der Kraak et al. 1992). There were insufficient samples and information to evaluate potental site-specific differences for female northern pike and flathead chub. Studies to verify the present findings and to determine the consequences of low plasma 17ß-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 complete reproductive competence.

Male Fish. In the Peace and Athabasca Drainages, plasma 11-ketotestosterone appeared marginally depressed in most male burbot collected from the near-pulp mill locations. Levels of 11-ketotestosterone and testosterone in fish from reference locations was similar to concentrations found in maturing burbot held int he laboratory (Giles et al. 1996). The exact role of 11-ketotestosterone in male reproduction has yet to be elucidated, however, its presence is associated with the appearance of sperm in the testes (Schulz and Blum 1990). We observed no histopathological aberrations in male gonad tissue, however investigation of sperm quantity and quality in burbot may represent a worthwhile endeavour. Due to the small numbers, it is impossible to evaluate site groups in male longnose suckers, northern pike and flathead chub. Values for steroid hormones and GSI in male longnose suckers and northern pike generally fell near ranges reported elsewhere (Munkittrick et al. 1992a; Brown et al. 1993). The lowered steroid hormone levels in burbot is similar to observations in white suckers collected downstream of pulp mill input (McMaster et al. 1991; Munkittrick et al. 1991, 1992a,b; Van der Kraak et al. 1992).

Immature Fish. It is not unusual to find a small proportion (10-15%) of adult burbot that were not sexually mature in surveys (Bailey 1972). However, the high proportion (approx 40%) found at the near-pulp mill locations (Fig 13) is concerning. The overall proportion f immature adults (26.5%) in the Athabasca drainage does not seem excessive. Because most immature burbot were collected from the A4 and LSV sites which are located less than 50 km from pulp mill inputs, their distribution is remarkable. The rates of non-maturing adult fish Peace (62.0%) and Smoky River (35.3%) sites downstream of the reference sites on the Wapiti and Little Smoky Rivers seem noteworthy. The observations about have statistical significance and are of sufficient importance to warrant verification and follow-up investigation. While it is possible that differential movement patterns due to spawning activities may account for the distribution of immature fish, similar observations by Finnish researchers are regarded as 'a reproduction disorder' (Pulliainen and Korhonen 1993). In Scandinavia, substantial numbers of immature adult burbot have been located in waters affected by loading from metal industries or pulp mills (Pulliainen and Korhonen 1993). Moreover, the area-specific differences could not be explained by differences in fish size, condition or age. Higher proportions of immature fish were also apparent in longnose sucker collected from the Peace mainstem but the relatively low numbers of fish collected from reference areas may not comprise a completely representative sample. More fundamental information about burbot and longnose sucker reproduction and ecology in the Peace and Athabasca Drainages is required to completely understand the distribution of immature fish.

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APPENDIX A

Terms of Reference

NORTHERN RIVER BASINS STUDY

SCHEDULE A - TERMS OF REFERENCE

Project 3144-D3: 1994 Fall Basin-Wide Burbot Collection - Circulating Gonadal Sex Steroid and Gonad Morphology Analyses

I. BACKGROUND AND OBJECTIVES

Monitoring the effects of industrial wastes in receiving waters includes monitoring the responses of fish. Many biochemical indicators and physiological processes are known to be sensitive to compounds discharged in industrial effluent, including bleach kraft mill effluent (BKME). However, many of the responses reported are of limited use in assessing damage to aquatic ecosystems because they are not linked to effects at the population. For example, the induction of the hepatic mixed function oxygenase (MFO) system has been shown to be a good indicator of exposure to BKME and a variety of other contaminants. The measurement of hepatic MFO enzymes in fish is now required under the Aquatic Environmental Effects Monitoring Requirements for pulp mill effluents (Environment Canada 1991), as an indicator of exposure to pulp mill effluent.

Although MFO induction has been consistently found at bleached kraft mills, the consequences of increased activity are currently unknown. It has been hypothesized that increased MFO activity may be responsible for the impacts of many lipophilic contaminants on reproduction. Several studies have shown that gonadal sex steroid levels become altered when MFOs have been induced. Reductions in circulating levels of sex steroids has also been shown to reliably indicate exposure to compounds known to impact the reproductive system. Recent studies which have examined steroids at an appropriate stage of gonadal development have demonstrated reduced levels of plasma sex steroids (testosterone, 11-ketotestosterone, 17 -estradiol and 17 20 -dihydroxy-4-pregnene-3-one) in fish exposed to bleach kraft mill effluent relative to fish at reference sites. Fish displaying lower circulating levels of sex steroids typically show changes in reproductive development and/or performance. These include delayed sexual maturity, reduced gonad growth, reduced fecundity with age, reduced egg size and reduced secondary sexual characteristics.

MFO induction and measurements of sex steroid levels can be used as a two-tiered system for measuring the effects of BKME (McMaster *et al.* 1993). MFO induction can be employed to indicate exposure to effluent, but in situations where MFO activity levels are induced, sex steroid analyses are required as an estimate of biological response to the effluent.

The aquatic fauna of the northern river basins are exposed to BKME and other types of municipal and industrial effluents. In the spring and fall of 1992 the Northern River Basins Study (NRBS) collected four fish species from six sites upstream, near and downstream from the bleached kraft mill located at Hinton on the Athabasca River (Barton *et al.* 1993a&b). These fish were analyzed for MFO induction, circulating sex steroid levels and gonad morphology. The results of these analyses were somewhat inconclusive. Mountain whitefish showed small increases in liver microsomal enzyme activity relative to fish collected from upstream sites (Lockhart *et al.* 1993). Depressed levels of gonadal steroid hormones were also noted in female longnose suckers and possibly in mountain whitefish collected downstream of the Hinton Mill (Brown *et al.* 1993).

In September and October 1994 the NRBS initiated a basin-wide fish collection to further determine the effects of pulp mill and other effluents on fish populations. The collection and sampling protocols for the project were designed to allow biochemical, contaminant and histological analyses to be performed on the fish. Because of its wide-ranging distribution and relatively sedentary behavior, burbot were targeted for collection and analyses. However, provisions were also made for the collection of longnose sucker, flathead chub and northern pike for a broad suite of analyses.

The purpose of this project is to analyze blood samples of burbot, northern pike, longnose sucker and flathead chub collected in the fall of 1994 for circulating levels of gonadal sex steroids. A histological examination of gonads from these fish is also to be performed to provide maturity and fecundity estimates.

II. GENERAL REQUIREMENTS

1. Various sample sizes of fish species were collected at a number of different sites in the fall of 1994. The contractor is to conduct circulating sex steroid and gonad morphology analyses on all burbot, northern pike, longnose sucker and flathead chub submitted from each collection site. The contractor is to contact Dr. Don Metner regarding the location, disposition and number of the blood plasma and gonad samples for each of the four fish species.

2. Plasma samples, stored and transported at -60°C, and gonad tissue samples fixed in Davidson's solution and in 5% formalin have been supplied to Dr. Don Metner by EnviResource Consulting Limited, Calgary. The contractor is expected to maintain these tissue samples in a suitable condition to allow for analyses of circulating levels of gonadal sex steroids and to make histological measurements.

3. The contractor will record all information supplied with each plasma and gonad sample and code laboratory record numbers with NRBS sample numbers (see Boag in prep.) so that the results of sex steroid and histological analyses can be compared with other data generated on the same fish.

4. The contractor will apply appropriate radio-immunoassays or enzyme-immunoassays to plasma samples to determine circulating levels of gonadal sex steroids in the samples provided. The contractor will also conduct appropriate histological examinations to determine gonadosomatic index, female fecundity, ovarian and testicular maturity index, clutch oocyte size, and percent oocytes representing the clutch.

5. Details of all calculations will be retained by the laboratory, but will be made available to the NRBS upon request.

III. ANALYTICAL REQUIREMENTS

Analyses of circulating levels of gonadal sex steroids and histological measurements carried out under this contract are to conform to the methods outlined in Brown *et al.* (1993). Specifically, the methodology is as follows:

Steroid Hormone Assays

1. Before assays are conducted, duplicate plasma samples (250 µL) are to be extracted in 2.5 mL of ethyl acetate:hexane (3:2, v/v). Dried extracts are then to be redissolved in assay buffer (250 µL). After appropriate dilution, aliquots of this redissolved extract are to be used for analyses of 17 -estradiol and testosterone in female fish and for analyses of testosterone and 11-ketotestosterone in male fish. Enzyme-immunoassays are to be used to assess plasma estradiol and plasma testosterone levels; a radioimmunoassay is to be used to determine 11-ketotestosterone levels.

2. A complete standard curve (6 to 8 concentrations) and quality control samples (not supplied) must be run each time an assay is performed. All samples, blanks and standards are to be analyzed in duplicate or triplicate. For each assay procedure, the contract laboratory is required to assess the following assay performance characteristics:

   a) The recovery of hormone from extracted samples.
   b) The recovery of known amounts of authentic hormone added to representative plasma samples for each species.
   c) Assay precision, both intra- and interassay variability are to be less than 15%.
   d) The parallelism of serial dilutions of representative biological samples.
   e) The antibody specificity for closely related hormones found in fish plasma.
   f) The detection limit for each assay procedure.
   g) The blank values determined following extraction of plasma pools where the endogenous hormones have been removed by absorption with charcoal.

3. The percent recovery of hormones from each extracted sample is to be determined by addition of a mixture of ^{3}H-labelled steroid tracers (1500 cpm each of 17 -estradiol, testosterone and 11-ketotestosterone) to every sample and counting an aliquot (25 µL) of the redissolved extract by liquid scintillation counting. Extraction efficiencies are to be

calculated for the samples processed. For calculating the final hormone concentration the extraction efficiency for each individual sample is to be used to correct for losses.

4. The cross-reactivity between testosterone and 11-ketotestosterone as well as other closely related steroids (e.g. 11 -hydroxytestosterone) must be determined for their respective assays. If significant interference is present, samples must be chromatographically purified before analysis.

Histology - Female Fish

5. The contractor is to dehydrate Davidson's solution fixed tissue in n-butanol and then embed the tissue samples in paraffin. Microscope sections of 8 μm are then be prepared and stained with Harris' hematoxylin and eosin.

6. Ovaries are to be examined and scored into one of the following categories to determine a Maturity Index for each fish.

Index 7	fish with only pre-vitellogenic oocytes, the largest having reached the yolk vesicle stage.
Index 8	fish with only pre-vitellogenic oocytes, the largest at the yolk vesicle stage, plus a remarkable number of large resorbing eggs.
Index 9	fish with a distinct vitellogenic clutch of developing oocytes plus a core of pre-vitellogenic resting oocytes.
Index 10	fish 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.

7. Oocyte diameters are to be determined from microscopic images of each ovary. Two diameter measurements are to be recorded for each oocyte to determine an average diameter. Depending on oocyte size and variety within the ovaries, 75 to 250 eggs are to be measured for each fish. The mean diameters for clutch oocytes from individual fish are to be determined from these measurements. Frequency distribution histograms are also to be prepared from diameter measurements of oocytes from each fish. The percent of oocytes representing the clutch is also to be calculated from these measurements.

8. For comparative purposes, gonadosomatic indexes (GSI) are to be calculated for each fish based on the following formula:

$$GSI = 100 * \text{Gonad Weight}/(\text{Total Fish Weight} - \text{Gonad Weight})$$

9. Fecundity estimates are to be derived from ovary samples fixed in 5% formalin. Between 60 and 100 vitellogenic oocytes are to be teased out of the ovarian tissue, lightly blotted and weighed. The associated connective tissue and pre-vitellogenic oocytes are also to be weighed to estimate their overall contribution to gonad weight.

   Absolute fecundity (number of eggs per fish) is to be estimated as follows:

$$\text{Absolute Fecundity} = \frac{(\text{Gonad Weight} * \text{Proportion of Gonad Represented by Vitellogenic Oocytes})}{\text{Average Egg Weight}}$$

   Relative fecundity (eggs per gram of fish) is to be calculated as follows:

$$\text{Relative Fecundity} = \text{Absolute Fecundity}/(\text{Total Fish Weight} - \text{Gonad Weight})$$

<u>Histology - Male Fish</u>

10. The contractor is to dehydrate Davidson's solution fixed tissue in n-butanol and then embed the tissue samples in paraffin. Microscope sections of 8 μm are then be prepared and stained with Harris' hematoxylin and eosin.

11. Testes are then to be examined with a microscope and scored into one of the following categories to determine a Maturity Index for each fish.

   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

12. For comparative purposes, gonadosomatic indexes (GSI) are to be calculated for each fish based on the following formula:

$$GSI = 100 * \text{Gonad Weight}/(\text{Total Fish Weight} - \text{Gonad Weight})$$

IV. REPORTING REQUIREMENTS

1. Prepare a comprehensive report outlining the results of the gonadal morphology and fecundity analyses carried out under this contract. To the extent possible, the results should also be discussed in relation to the possible effects of industrial and municipal effluents on the health of the fish populations examined. Specifically, the report is to include:

a) a brief description of the actual assay procedure employed and a summary of assay performance characteristics are to be included for each sex steroid investigated.
b) a brief description of how oocyte size measurements and percent clutch eggs were determined.
c) an appendix or tables indicating the mean sex steroid concentrations from replicate assays for each fish.
d) an appendix or tables indicating the gonadosomatic index, relative and absolute fecundity, maturity index, clutch oocyte size, and percent oocytes representing the clutch for each female fish.
e) an appendix or tables indicating the gonadosomatic index and maturity index for each male fish.

The report is to indicate that the details pertaining to the collection of fish analyzed under this contract are outlined in Boag (in prep.). Sample numbers indicated in the report are to conform to those outlined in Boag (in prep.).

2. Ten copies of the draft report along with an electronic disk copy are to be submitted to the Component Coordinator by **March 31, 1995**.

3. Three weeks after the receipt of review comments on the draft report, the Contractor is to provide the Project Liaison Officer with two unbound, camera ready copies and ten cerlox bound copies of the final report along with an electronic version.

4. The Contractor is to provide draft and final reports in the style and format outlined in the NRBS document, "A Guide for the Preparation of Reports," which will be supplied upon execution of the contract.

   The final report is to include the following: an acknowledgement section that indicates any local involvement in the project, Report Summary, Table of Contents, List of Tables, List of Figures and an Appendix with the Terms of Reference for this project.

   Text for the report should be set up in the following format:

   a) Times Roman 12 point (Pro) or Times New Roman (WPWIN60) font.
   b) Margins; are 1" at top and bottom, 7/8" on left and right.
   c) Headings; in the report body are labelled with hierarchical decimal Arabic numbers.
   d) Text; is presented with full justification; that is, the text aligns on both left and right margins.
   e) Page numbers; are Arabic numerals for the body of the report, centred at the bottom of each page and bold.

   - If photographs are to be included in the report text they should be high contrast black and white.
   - All tables and figures in the report should be clearly reproducible by a black and white photocopier.
   - Along with copies of the final report, the Contractor is to supply an electronic version of the report in Word Perfect 5.1 or Word Perfect for Windows Version 6.0 format.
   - Electronic copies of tables, figures and data appendices in the report are also to be submitted to the Project Liaison Officer along with the final report. These should be submitted in a spreadsheet (Quattro Pro preferred, but also Excel or Lotus) or database (dBase IV) format. Where appropriate, data in tables, figures and appendices should be geo-referenced.

5. All figures and maps are to be delivered in both hard copy (paper) and digital formats. Acceptable formats include: DXF, uncompressed E , VEC/VEH, Atlas and ISIF. All digital maps must be properly geo-referenced.

6. All sampling locations presented in report and electronic format should be geo-referenced. This is to include decimal latitudes and longitudes (to six decimal places) and UTM coordinates. The first field for decimal latitudes / longitudes should be latitudes (10 spaces wide). The second field should be longitude (11 spaces wide).

7. A presentation package of 35 mm slides to be used at public meetings is to comprise of one original and four duplicates of each slide.

V. DELIVERABLES

1. A data interpretation report, including the methods and results for the circulating gonadal sex steroid and gonad morphology analyses for NRBS fish samples collected in fall 1994.

1. Ten to twenty-five 35 mm slides that can be used at public meetings to summarize the project methods and key findings.

VI. CONTRACT ADMINISTRATION

This contract is being conducted under the Contaminants Component of the NRBS. The Contaminants Component leader is:

Dr. John Carey
National Water Research Institute
Environment Canada
867 Lakeshore Road
P.O. Box 5050
Burlington, Ontario L7R 4A6
phone: (905) 336-4913
fax: (905) 336-4972

The Component Coordinator for this contract is:

Richard Chabaylo
Northern River Basins Study
690 Standard Life Centre
10405 Jasper Avenue
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VII. LITERATURE CITED

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APPENDIX B

Fish Data

Table 2. Sample identification (Unique ID), year day of sample (Day), Northern River Basins Code (NRBS#), fish number (Fish#), sample collection site (Site), physical characteristics (length, weight & gonad weight), condition factor, gonadosomatic index (GSI), sex/maturity (Sex), age, plasma steroid hormones (Ktest, Test & E2) and reproductive indices (clutch oocyte weight, clutch oocyte diameter, amount clutch, absolute fecundity, relative fecundity & maturity index) for each burbot collected during the fall 1994 Basin Wide Fish Collection. Ktest=11-ketotestosterone, E2=17ß-estradiol, Test=testosterone.

Unique ID	Day	NRBS#	Fish#	Site	Total Length (mm)	Weight (g)	Gonad Weight (g)	Condition Factor	GSI (%)	Sex	Age (y)	Plasma Ktest (ng/mL)	Plasma Test (ng/mL)	Plasma E2 (ng/mL)	Clutch Oocyte Wt (µg)	Clutch Oocyte Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# oocytes)	Relative Fecundity (oocytes/g)	Maturity Index
90287	255	A1-BURB-1	1	A1a	550	722	35.8	0.412	5.22	M	6									3
90288	255	A1-BURB-2	2	A1b	498	700	38.7	0.535	5.85	M	8	1.690	2.408							3
90289	255	A1-BURB-6	6	A1b	469	406	13.2	0.381	3.36	F	7		2.168	2.120	37.3	353	33.1	353887	901	9
90290	255	A1-BURB-7	7	A1b	450	438	11.9	0.468	2.79	F	7		0.423	2.162	43.2	379	19.3	275463	646	9
90291	256	A1-BURB-8	8	A1b	452	448	14.4	0.470	3.32	F	7		0.825	2.897	34.6	354	18.5	416185	960	9
90292	256	A1-BURB-9	9	A1b	498	624	19.7	0.489	3.26	F	8		0.404	1.538	62.1	481	34.9	317230	525	9
90293	256	A1-BURB-10	10	A1b	447	435	12.1	0.473	2.86	F	5		0.938	4.072	43.6	363	22.7	277523	656	9
90294	257	A1-BURB-11	11	A1b	485	635	56.5	0.507	9.77	M	7	3.200	8.944							3
90315	263	A2-BURB-1	1	A2	519	613	29.3	0.418	5.02	F			0.442	3.153	56.5	408	24.6	518584	888	9
90316	263	A2-BURB-2	2	A2	599	1082	37.3	0.486	3.57	F	9		1.513	4.268	66.9	466	22.1	557549	534	9
90317	263	A2-BURB-3	3	A2	681	1508	59	0.459	4.07	F	11		1.073	8.839	80.5	440	25.0	732919	506	9
90318	264	A2-BURB-4	4	A2	768	2530	107	0.535	4.42	F	13		2.832	8.829	82.4	484	28.8	1298544	536	9
90319	265	A2-BURB-5	5	A2	431	440	27.1	0.516	6.56	M	5	6.410	9.549							3
90320	265	A2-BURB-6	6	A2	474	590	33.5	0.523	6.02	MI	7									2
90321	265	A2-BURB-7	7	A2	843	3890	170	0.621	4.58	F	13		2.833	9.568	105.2	505	24.8	1617871	435	9
90322	266	A2-BURB-8	8	A2	720	1962	85.9	0.503	4.58	F	11		0.937	8.360	89.1	484	33.6	964085	514	9
90350	269	A3-BURB-1	1	A3	424	378	14.2	0.477	3.90	M	7	2.210	1.044							3
90351	269	A3-BURB-2	2	A3	529	744	26.4	0.485	3.68	M	10	5.950	6.660							3
90352	269	A3-BURB-3	3	A3	440	458	18.4	0.516	4.19	M	5	2.800	3.940							4
90353	269	A3-BURB-4	4	A3	395	342	8.1	0.542	2.43	M	5	8.580	8.629							3
90354	269	A3-BURB-5	5	A3	416	323	1.6	0.446	0.50	FI	4		0.023	1.056						7
90355	269	A3-BURB-6	6	A3	463	519	25.3	0.497	5.12	M	5	5.950	3.985							3
90356	269	A3-BURB-7	7	A3	450	362	9.5	0.387	2.70	M	4	1.540	1.797							3
90357	269	A3-BURB-8	8	A3	515	704	26.5	0.496	3.91	M	10	2.300	2.641							3
90358	269	A3-BURB-9	9	A3	613	1242	49.3	0.518	4.13	F	9		0.653	2.609	75.0	472	29.2	657333	551	9
90359	269	A3-BURB-10	10	A3	536	743	26.8	0.465	3.74	M	11	1.120	0.465							3
90360	269	A3-BURB-11	11	A3	467	508	0.3	0.498	0.06	MI	5	0.500	0.177							1
90361	269	A3-BURB-12	12	A3	462	496	10.9	0.492	2.25	F	6		1.844	4.790	68.0	445	16.8	160294	330	9
90362	269	A3-BURB-13	13	A3	583	1094	24.9	0.540	2.33	F	11		1.637	1.527	57.0	437	35.1	436842	409	9
90363	269	A3-BURB-14	14	A3	590	931	32.3	0.438	3.59	M	11	7.580	1.744							3

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90364	269	A3-BURB-15	15	A3	458	445	2.1	0.461	0.47	FI	5		0.113	0.337						7
90365	269	A3-BURB-16	16	A3	480	594	0.6	0.537	0.10	MI	5	0.500	0.054							1
90366	269	A3-BURB-17	17	A3	487	529	2.6	0.456	0.49	FI	6		0.060	0.416						7
90367	269	A3-BURB-18	18	A3	475	514	24.2	0.457	4.94	M	6	6.120	2.860							3
90368	269	A3-BURB-19	19	A3	455	493	22	0.500	4.67	M		4.310	2.065							3
90369	269	A3-BURB-20	20	A3	514	736	32.2	0.518	4.58	M	7	8.380	3.709							3
90370	269	A3-BURB-21	21	A3	787	2774	108	0.547	4.04	M	13	4.180	4.008							3
90371	269	A3-BURB-22	22	A3	459	432	14.3	0.432	3.42	M	6	5.250	2.879							3
90372	269	A3-BURB-23	23	A3	436	467	12.6	0.548	2.77	F	4				38.0	347	32.7	331579	730	9
90406	280	A4-BURB-1	1	A4	570	982	0.8	0.530	0.08	MI	8	0.500	0.289							1
90405	280	A4-BURB-2	2	A4	558	896	17.5	0.506	1.99	F	12		2.237	2.861	76.0	440	28.9	230263	262	9
90407	280	A4-BURB-3	3	A4	559	896	21.9	0.500	2.51	F	7		0.984	1.580	58.3	445	44.4	375643	430	9
90408	280	A4-BURB-4	4	A4	523	615	0.4	0.430	0.07	MI	4	0.500	0.249							1
90409	280	A4-BURB-5	5	A4	575	1059	3.1	0.555	0.29	FI	8		0.172	2.205						7
90410	280	A4-BURB-6	6	A4	620	1523	85	0.603	5.91	M	11	8.360	19.086							3
90411	280	A4-BURB-7	7	A4	593	1050	5	0.501	0.48	FI	7		0.012	0.300						7
90412	280	A4-BURB-8	8	A4	603	1128	4.6	0.512	0.41	FI	7		0.132	0.401						7
90413	280	A4-BURB-9	9	A4	391	274				MI	4	0.500	0.001							1
90414	280	A4-BURB-10	10	A4	374	245	0.8	0.467	0.33	FI	5		0.030	0.263						7
90415	280	A4-BURB-11	11	A4	425	408	0.2	0.531		MI	5									2
90416	280	A4-BURB-12	12	A4	392	294	0.3	0.488	0.10	MI	6									1
90417	280	A4-BURB-13	13	A4	310	134	0.2	0.449	0.15	FI	4									7
90418	281	A4-BURB-14	14	A4	425	356	1.6	0.462	0.45	FI	4		0.085	0.364						7
90419	281	A4-BURB-15	15	A4	645	1213	5	0.450	0.41	FI	8		0.067	0.339						7
90442	286	A5-BURB-1	1	A5	571	1103	95.8	0.541	9.51	M	7	25.860	12.451							3
90443	286	A5-BURB-2	2	A5	457	468	30.5	0.458	6.97	F	5		0.494	9.915	74.6	494	33.0	408847	935	10
90444	286	A5-BURB-3	3	A5	377	260	0.1	0.485		MI	3	0.500	0.164							2
90445	286	A5-BURB-4	4	A5	567	805	49.7	0.414	6.58	M	7	6.330	9.747							3
90446	287	A5-BURB-5	5	A5	435	912	60.9	1.034	7.16	M	9	10.930	13.385							3
90447	287	A5-BURB-6	6	A5	410	326	1.6	0.471	0.49	FI	4		0.380	1.484						7
90448	287	A5-BURB-7	7	A5	514	726	70.4	0.483	10.74	M	7	12.650	9.074							3
90449	287	A5-BURB-8	8	A5	513	813	64.2	0.555	8.57	M	5	10.630	10.091							3
90450	287	A5-BURB-9	9	A5	462	399	0.5	0.404	0.13	FI	4		0.050	0.714						7
90451	287	A5-BURB-10	10	A5	463	530	0.6	0.533	0.11	MI	3	0.500	0.223							2
90452	287	A5-BURB-11	11	A5	443	920	82.2	0.964	9.81	M	6	12.690	14.298							3
90453	287	A5-BURB-12	12	A5	560	945	82.4	0.491	9.55	M	7	11.160	5.822							3
90454	287	A5-BURB-13	13	A5	451	477	56	0.459	13.30	M	5	5.950	4.293							3

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90455	287	A5-BURB-14	14	A5	513	758	20.6	0.546	2.79	F	6		1.789	3.632	80.9	429	29.6	254635	345	9
90456	287	A5-BURB-15	15	A5	599	1162	81.7	0.503	7.56	M	10	15.090	0.586							3
90457	287	A5-BURB-16	16	A5	403	373	51.4	0.491	15.98	M	5	1.960	1.278							3
90458	287	A5-BURB-17	17	A5	613	1358	72.6	0.558	5.65	F	7		2.034	5.286	156.6	563	30.1	463602	361	10
90459	287	A5-BURB-18	18	A5	569	1002	41.6	0.521	4.33	F	7				141.1	530	29.6	294826	307	10
90460	287	A5-BURB-19	19	A5	505	701	55.1	0.502	8.53	M	5									3
90461	287	A5-BURB-20	20	A5	553	1004	116	0.525	13.11	M	7									3
90462	287	A5-BURB-21	21	A5	510	730	37.1	0.522	5.35	F	7				108.7	540	23.7	341306	493	10
90429	283	CW-BURB-1	1	CW	520	688	33.5	0.465	5.12	F	7		0.278	8.330	120.9	564	23.5	277089	423	10
90430	283	CW-BURB-2	2	CW	374	317	15.5	0.576	5.14	F	3		2.586	8.209	120.2	571	21.7	128952	428	10
90431	283	CW-BURB-3	3	CW	423	432	22.6	0.541	5.52	F	6		2.899	5.118	129.1	599	21.4	175058	428	10
90432	283	CW-BURB-4	4	CW	427	404	18.3	0.495	4.74	F	6		1.883	3.764	126.4	590	28.6	144778	375	10
90433	285	CW-BURB-5	5	CW	469	586	33.6	0.535	6.08	F	7		3.997	4.600	100.7	521	29.3	333664	604	10
90072	262	LSR2-BURB-1	1	LSR1	471	590	14.5	0.551	2.52	F	8		3.833	2.548			38.1			9
90511	351	LSR3-BURB-1	1	LSR2	450	508	52.9	0.499	11.62	F	6		14.027	68.377	0.309	720	22.2	171031	376	10
90383	275	LSV-BURB-1	1	LSV	460	555	34.8	0.534	6.69	M	9	2.600	1.696							3
90384	275	LSV-BURB-2	2	LSV	549	715	3.1	0.430	0.44	FI	9		0.082	0.130						7
90385	275	LSV-BURB-3	3	LSV	465	566	32.8	0.530	6.15	M	8	6.090	4.695							3
90386	275	LSV-BURB-4	4	LSV	470	538	23.7	0.495	4.61	M	7	8.310	8.228							3
90387	275	LSV-BURB-5	5	LSV	552	802	5.8	0.473	0.73	FI	10		0.105	0.326						7
90388	275	LSV-BURB-6	6	LSV	607	1042	4.2	0.464	0.40	FI	10									7
90389	276	LSV-BURB-7	7	LSV	446	405	21.7	0.432	5.66	M	6	4.260	1.537							3
90390	276	LSV-BURB-8	8	LSV	515	718	36	0.499	5.28	M	8	9.130	1.714							3
90391	276	LSV-BURB-9	9	LSV	621	926	4.3	0.385	0.47	FI	9		0.034	0.193						7
90392	276	LSV-BURB-10	10	LSV	520	631	0.9	0.448	0.14	MI	10	0.500	0.162							1
90393	276	LSV-BURB-11	11	LSV	540	800	23.9	0.493	3.08	F	8		1.578	5.000	55.2	432	45.1	432971	558	9
90394	276	LSV-BURB-12	12	LSV	565	793	3.7	0.438	0.47	FI	9		0.109	0.437						7
90395	276	LSV-BURB-13	13	LSV	629	1226	58.4	0.469	5.00	M	15	2.780	4.005							3
90396	276	LSV-BURB-14	14	LSV	583	970	6.4	0.486	0.66	FI	10		0.105	0.483						7
90397	276	LSV-BURB-15	15	LSV	423	392	15.2	0.498	4.03	F	7		3.408	1.247	78.4	475	24.1	193878	515	9
90398	276	LSV-BURB-16	16	LSV	387	593	27.2	0.976	4.81	M	14	10.110	17.212							3
90399	276	LSV-BURB-17	17	LSV	312	169	0.5	0.555	0.30	FI	3		0.338	1.749						7
90400	276	LSV-BURB-18	18	LSV	525	835	1.5	0.576	0.18	MI	8	0.500	0.709							2
90401	276	LSV-BURB-19	19	LSV	547	805	46.7	0.463	6.16	M	9	27.030	34.985							3
90402	276	LSV-BURB-20	20	LSV	529	819	26.8	0.535	3.38	F	10				82.0	530	40.7	326829	413	9
90403	276	LSV-BURB-21	21	LSV	495	706	42	0.547	6.33	M	9									3
90308	259	MR-BURB-1	1	MR1	475	463	3.5	0.429	0.76	FI			0.394	0.074						7

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90504	348	MCR2-BURB-1	1	MR2	490	702	59.8	0.546	9.31	F	8		51.112	20.810	312.5	773	33.7	191360	298	10
90505	348	MCR2-BURB-2	2	MR2	452	514	78	0.472	17.89	M	9	1.690	47.853							4
90506	348	MCR2-BURB-3	3	MR2	481	530	48.9	0.432	10.16	F	7		62.416	0.499	346.6	775	45.5	141085	293	10
90507	348	MCR2-BURB-4	4	MR2	430	386	34.7	0.442	9.88	F	5		110.581	21.977	251.1	715	46.4	138192	393	10
90509	348	MCR2-BURB-6	6	MR2	513	579	67.7	0.379	13.24	F	7		141.953	12.912	274.1	725	30.4	246990	483	10
90510	349	MCR2-BURB-7	7	MR2	463	563	91.5	0.475	19.41	M	8	65.910	27.537							4
90373	271	P-BURB-1	1	P	380	225	8.7	0.394	4.02	F	5		1.565	0.765	26.0	323	26.7	334615	1547	9
90374	271	P-BURB-2	2	P	517	671	15.9	0.474	2.43	F	11		6.112	6.589	40.0	446	31.3	397500	607	9
90375	272	P-BURB-3	3	P	500	490	17	0.378	3.59	F	10		2.848	2.253	58.9	397	33.9	288625	610	9
90376	272	P-BURB-4	4	P	538	806	24	0.502	3.07	M	4	10.180	13.533							3
90377	273	P-BURB-5	5	P	394	290	8.7	0.460	3.09	F	5		1.514	3.862	77.0	432	28.6	112987	402	9
90378	273	P-BURB-6	6	P	511	632	20.5	0.458	3.35	F	7				29.0	292	36.3	706897	1156	9
90381	273	P-BURB-7	7	P	570	1014	32.6	0.530	3.32	F	12				46.0	453	39.5	708696	722	9
90144	270	PR1-BURB-1	1	PR1	462	505				M		3.390	4.212							
90145	271	PR1-BURB-2	2	PR1	590	1063	27	0.504	2.61	F	12		2.481	3.037	48.1	385	18.9	561331	542	9
90148	272	PR1-BURB-3	3	PR1	645	1468	8.1	0.544	0.55	FI	8		0.001	0.413						7
90158	272	PR1-BURB-4	4	PR1	517	642	5.2	0.461	0.82	FI	7		0.202	0.497						7
90157	272	PR1-BURB-5	5	PR1	411	382	13	0.531	3.52	M	6	5.750	5.881							3
90161	273	PR1-BURB-6	6	PR1	541	734	5	0.460	0.69	FI	12		0.079	0.151						7
90162	273	PR1-BURB-7	7	PR1	582	1108	9.1	0.557	0.83	FI	8		0.003	0.133						7
90163	273	PR1-BURB-8	8	PR1	508	557	19	0.410	3.53	M	8	3.670	7.527							3
90164	273	PR1-BURB-9	9	PR1	518	596	6.2	0.424	1.05	FI	12									7
90165	273	PR1-BURB-10	10	PR1	570	911	3	0.490	0.33	MI	12									1
90171	275	PR2-BURB-1	1	PR2	618	1137	7	0.479	0.62	FI	11		0.001	0.610						7
90176	275	PR2-BURB-2	2	PR2	474	507	1	0.475	0.20	MI	7	0.500	0.012							1
90179	275	PR2-BURB-3	3	PR2	503	667				M	6	6.040	16.695							3
90173	275	PR2-BURB-4	4	PR2	513	604	2	0.446	0.33	MI	13	0.500	0.001							1
90175	275	PR2-BURB-5	5	PR2	804	3672	167	0.674	4.76	M	13	6.420	8.041							3
90172	275	PR2-BURB-6	6	PR2	756	2108	46	0.477	2.23	F	15		5.826	6.137	53.4	432	21.2	861423	418	9
90183	276	PR2-BURB-7	7	PR2	643	1452	9	0.543	0.62	FI	11		0.009	0.145						7
90181	276	PR2-BURB-8	8	PR2	559	827	36	0.453	4.55	M	8	4.260	9.310							3
90182	276	PR2-BURB-9	9	PR2	580	931	40	0.457	4.49	M	7	6.130	11.135							3
90194	277	PR2-BURB-10	10	PR2	645	1312	6	0.487	0.46	FI	9		0.008	0.100						7
90212	279	PR3-BURB-1	1	PR3	464	423	1	0.422	0.24	MI	11	0.500	0.133							2
90208	279	PR3-BURB-2	2	PR3	635	1242	5	0.483	0.40	FI	7		0.111	0.835						7
90211	279	PR3-BURB-3	3	PR3	516	691	20	0.488	2.98	F	7		1.862	2.519	80.6	474	21.2	248139	370	9
90214	279	PR3-BURB-4	4	PR3	442	454	2	0.523	0.44	FI	6		0.042	0.420						7

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90213	279	PR3-BURB-5	5	PR3	569	859	6	0.463	0.70	FI	8		0.019	0.071						7
90209	279	PR3-BURB-6	6	PR3	473	452	13	0.415	2.96	M	6	1.770	2.579							3
90231	281	PR3-BURB-8	8	PR3	398	268	1	0.424	0.37	FI	6		0.092	0.103						7
90230	281	PR3-BURB-9	9	PR3	570	972	1	0.524	0.10	MI	7	0.500	0.024							2
90007	255	SR1-BURB-1	1	SR1	444	411	2	0.467	0.49	MI	9	0.500	0.019							1
90008	255	SR1-BURB-2	2	SR1	495	612	17	0.491	2.86	F	8		2.064	2.765	17.1	341	40.8	994152	1671	9
90009	255	SR1-BURB-3	3	SR1	460	412				F	7		2.668	2.201						
90001	256	SR1-BURB-4	4	SR1	391	364	5.8	0.599	1.62	M	8	4.890	5.628							3
90012	256	SR1-BURB-5	5	SR1	502	675	5.7	0.529	0.85	MI	9	4.300	4.494							2
90011	256	SR1-BURB-6	6	SR1	480	577	17	0.506	3.04	F	8		0.957	1.919	17.5	341	31.8	971429	1735	9
90002	256	SR1-BURB-7	7	SR1	402	306	1.8	0.468	0.59	FI	7		0.102	0.216						7
90003	256	SR1-BURB-8	8	SR1	412	352	2.2	0.500	0.63	M	6	1.050	1.242							3
90005	256	SR1-BURB-9	9	SR1	441	483	5.1	0.557	1.07	FI	6		0.190	0.167						7
90013	256	SR1-BURB-10	10	SR1	475	597	18	0.540	3.11	MI	10	0.500	0.187							2
90014	256	SR1-BURB-11	11	SR1	518	756	4	0.541	0.53	FI	9		1.248	0.100						7
90006	256	SR1-BURB-12	12	SR1	545	749	16.6	0.452	2.27	M	11	1.560	2.783							3
90017	256	SR1-BURB-13	13	SR1	486	628	29	0.522	4.84	M	11	2.440	2.279							3
90016	256	SR1-BURB-14	14	SR1	449	479	6.2	0.522	1.31	M	7	0.870	0.565							3
90018	256	SR1-BURB-15	15	SR1	465	495	10	0.482	2.06	F	8		3.208	1.637	20.9	345	27.2	478469	987	9
90020	257	SR1-BURB-16	16	SR1	352	244	4	0.550	1.67	M	4	4.400	3.726							3
90021	257	SR1-BURB-17	17	SR1	375	252	1	0.476	0.40	MI	4	0.500	0.149							1
90019	257	SR1-BURB-18	18	SR1	369	284	1	0.563	0.35	MI	4	0.500	0.105							1
90031	259	SR1-BURB-19	19	SR1	466	553	16	0.531	2.98	M	7	5.900	10.604							3
90024	259	SR1-BURB-20	20	SR1	410	400				M	8	5.130	11.696							
90512	354	SR3-BURB-1	1	SR3	456	581	76.4	0.532	15.14	F	7				0.342	774	30.0	223131	442	10
90479	287	SR-BURB-1	1	SD1	536	840	54.9	0.510	6.99	M	8	6.050	8.728							3
90478	287	SR-BURB-2	2	SD1	575	1170	39.3	0.595	3.48	F	8		0.807	3.837	92.3	447	31.0	425785	377	9
90477	287	SR-BURB-3	3	SD1	642		118			F	13		14.373	25.135	218.8	630	19.8	541133		10
90476	287	SR-BURB-4	4	SD1	454	465	76.6	0.415	19.72	M	8	6.220	6.544							4
90483	288	SR-BURB-6	6	SD2	454	646	66	0.620	11.38	M	8	10.490	14.919							3
90481	288	SR-BURB-7	7	SD2	470	642	45.4	0.575	7.61	M	6	7.860	12.776							4
90493	288	SR-BURB-8	8	SD2	463	630	42.5	0.592	7.23	M	8	9.960	24.273							3
90486	288	SR-BURB-9	9	SD2	540	1120	64.5	0.670	6.11	F	8		10.833	16.781	174.5	583	17.8	369628	350	10
90484	288	SR-BURB-10	10	SD2	598	1740	60.5	0.785	3.60	F	11		4.432	3.130	214.6	695	18.3	281920	168	10
90485	288	SR-BURB-11	11	SD2	495	760	56.1	0.580	7.97	M	7	11.100	22.543							3
90494	288	SR-BURB-12	12	SD2	472	650	107	0.516	19.68	M	7	12.540	32.101							3
90482	288	SR-BURB-13	13	SD2	465	724	28.8	0.691	4.14	F	10		6.433	3.320	190.3	644	17.0	151340	218	10

Unique ID	Day	NRBS#	Fish#	Site	Total Length (mm)	Weight (g)	Gonad Weight (g)	Condition Factor	GSI (%)	Sex	Age (y)	Plasma Ktest (ng/mL)	Plasma Test (ng/mL)	Plasma E2 (ng/mL)	Clutch Oocyte Wt (µg)	Clutch Oocyte Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# oocytes)	Relative Fecundity (oocytes/g)	Maturity Index
90487	288	SR-BURB-14	14	SD2	455	675	69.2	0.643	11.42	M	8	40.820	6.617							4
90497	288	SR-BURB-15	15	SD2	490	699	46.3	0.555	7.09	F	9		4.380	8.273	215.2	610	26.1	215149	330	10
90498	288	SR-BURB-16	16	SD2	495	842	65.3	0.640	8.41	M	8	26.720	38.313							3
90495	288	SR-BURB-17	17	SD2	482	710	43	0.596	6.45	F	7		4.523	3.233	171.9	563	29.8	250145	375	10
90496	288	SR-BURB-18	18	SD2	490	886	36.9	0.722	4.35	F	9		9.238	4.092	240.3	680	17.9	153558	181	10
90490	289	SR-BURB-20	20	SD2	695	2500	112	0.711	4.67	F	14		23.621	9.092	259.6	730	19.8	429507	180	10
90489	289	SR-BURB-21	21	SD2	525	936	62.6	0.604	7.17	F	10				233.2	672	16.4	268439	307	10
90235	282	WAB1-BURB-1	1	WB1	381	211	1	0.380	0.48	FI	6		0.018	0.136						
90236	283	WAB1-BURB-2	2	WB1	340	232	2	0.585	0.87	FI	4		0.082	0.333						7
90239	283	WAB1-BURB-3	3	WB1	314	135	2	0.430	1.50	FI	4		0.036	0.391						7
90237	283	WAB1-BURB-4	4	WB1	395	334	4	0.535	1.21	MI	5	0.500	0.077							1
90241	283	WAB1-BURB-5	5	WB1	320	159	1	0.482	0.63	FI	5									7
90238	283	WAB1-BURB-6	6	WB1	423	381	18	0.480	4.96	F	7		3.398	4.828	72.2	442	44.4	249307	687	9
90240	283	WAB1-BURB-7	7	WB1	386	305	27	0.483	9.71	M	4									3
90243	284	WAB1-BURB-8	8	WB1	426	447	34	0.534	8.23	M	6	4.650	21.183							3
90244	284	WAB1-BURB-9	9	WB1	380	317	13	0.554	4.28	F	5		4.265	13.950	60.3	452	48.7	215589	709	9
90093	266	WR1-BURB-1	1	WR1	509	712	15.7	0.528	2.25	F	12		1.075	2.296	84.4	450	23.0	186019	267	9
90092	266	WR1-BURB-2	2	WR1	560	824	55	0.438	7.15	M	11	7.010	1.021							3
90106	268	WR1-BURB-3	3	WR1	446	468	27.7	0.496	6.29	M	7	12.180	20.389							3
90107	268	WR1-BURB-4	4	WR1	628	1277	38.3	0.500	3.09	F	10		13.258	7.021	59.5	397	32.5	643697	520	9
90278	291	WR2-BURB-1	1	WR2	472	756	126	0.599	20.00	M	9	23.840	0.589							3
90280	291	WR2-BURB-2	2	WR2	576	1044	44	0.523	4.40	F	6		8.595	7.655	119.5	568	25.0	368201	368	10
90279	291	WR2-BURB-3	3	WR2	510	758	85	0.507	12.63	M	7	40.130	11.708							3
90275	291	WR2-BURB-4	4	WR2	322	192	24	0.503	14.29	M	2	15.890	3.335							3
90281	291	WR2-BURB-5	5	WR2	505	676	26	0.505	4.00	F	10		0.308	7.160	124.9	513	22.8	208167	320	10
90284	291	WR2-BURB-6	6	WR2	434	491	59	0.528	13.66	M	7	26.850	18.273							3
90274	291	WR2-BURB-7	7	WR2	549	831	30	0.484	3.75	F	8		5.768	12.822	140.2	555	26.5	213980	267	10
90276	291	WR2-BURB-8	8	WR2	527	821	33	0.538	4.19	F	7		7.019	8.559	128.4	586	19.0	257009	326	10
90277	291	WR2-BURB-9	9	WR2	465	491	55	0.434	12.61	M	7	33.400	5.218							3
90282	291	WR2-BURB-10	10	WR2	472	601	78	0.497	14.91	M	8	26.770	36.215							3
90285	291	WR2-BURB-11	11	WR2	619	1262	74	0.501	6.23	F	9		5.855	12.061	126.3	581	27.7	585907	493	10
90273	291	WR2-BURB-12	12	WR2	518	825	94	0.526	12.86	M	10	11.760	32.517							3
90283	291	WR2-BURB-13	13	WR2	483	641	32	0.540	5.25	F	9		3.452	15.222	137.7	561	51.4	232389	382	10

Table 3. Sample identification (Unique ID), year day of sample (Day), Northern River Basins Code (NRBS#), fish number (Fish#), sample collection site (Site), physical characteristics (length, weight & gonad weight), condition factor, gonadosomatic index (GSI), sex/maturity (Sex), age, plasma steroid hormones (Ktest, Test & E2) and reproductive indices (clutch oocyte weight, clutch oocyte diameter, amount clutch, absolute fecundity, relative fecundity & maturity index) for each longnose sucker collected during the fall 1994 Basin Wide Fish Collection. Ktest=11-ketotestosterone, E2=17ß-estradiol, Test=testosterone.

Unique ID	Day	NRBS#	Fish#	Site	Total Length (mm)	Weight (g)	Gonad Weight (g)	Condition Factor	GSI (%)	Sex	Age (y)	Plasma Ktest (ng/mL)	Plasma Test (ng/mL)	Plasma E2 (ng/mL)	Clutch Oocyte Wt (mg)	Clutch Oocyte Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# oocytes)	Relative Fecundity (oocytes/g)	Maturity Index
90025	259	SR1-LNSC-2	2	SR1	393	704	7	1.15	1.00	FI	8		0.03	0.13			7.8			7
90027	259	SR1-LNSC-1	1	SR1	381	664	35	1.14	5.56	F			0.48	1.14	2.44	1437	23.0	12787	20.3	10
90029	259	SR1-LNSC-3	3	SR1	296	245	1	0.94	0.41	FI			0.03	0.07						7
90032	260	SR1-LNSC-5	5	SR1	395	787	31	1.23	4.10	M		3.6	3.28							5
90033	260	SR1-LNSC-7	7	SR1	470	1115	66	1.01	6.29	F			0.76	1.59	1.72	1318	23.7	34211	32.6	10
90035	261	SR1-LNSC-6	6	SR1	410	752	30	1.05	4.16	M		17.9	6.40							4
90036	261	SR1-LNSC-4	4	SR1	425	762	10	0.98	1.33	FI			0.07	0.02			13.8			7
90037	261	SR1-LNSC-8	8	SR1	487	1200	36	1.01	3.09	F			0.29	1.59	1.03	1051	15.6	31198	26.8	9
90041	261	SR1-LNSC-10	10	SR1	445	866	46	0.93	5.61	F			0.48	0.95	1.68	1317	30.4	24340	29.7	10
90042	261	SR1-LNSC-11	11	SR1	381	638	4	1.15	0.63	FI			0.12	0.54			0.9			7
90043	261	SR1-LNSC-9	9	SR1	415	771	6	1.07	0.78	FI			0.14	7.66						7
90048	261	SR1-LNSC-12	12	SR1	376	592	24.6	1.07	4.34	F			0.19	0.65	1.56	1283	30.1	14017	24.7	10
90071	262	LSR2-LNSC-1	1	LSR2	405	807	42	1.15	5.49	F			2.41	3.07	1.69	1290	33.3	22158	29.0	10
90074	263	LSR2-LNSC-2	2	LSR2	432	885	47	1.04	5.61	F			1.14	2.52	2.48	1529	21.1	16887	20.2	10
90075	263	LSR2-LNSC-3	3	LSR2	380	623	33	1.08	5.59	F			0.52	1.16	2.09	1477	27.5	14086	23.9	10
90077	263	LSR2-LNSC-5	5	LSR2	252	171	2	1.06	1.18	FI			0.02	0.00						7
90078	263	LSR2-LNSC-4	4	LSR2	268	210	1.8	1.08	0.86	FI			0.01	0.10						7
90079	263	LSR2-LNSC-6	6	LSR2	196	85	0.8	1.12	0.95	FI			0.02	0.00						7
90083	265	WR1-LNSC-5	5	WR1	352	452	3.3	1.03	0.74	FI			0.03	0.00						7
90084	265	WR1-LNSC-7	7	WR1	418	865	59	1.10	7.32	F			1.44	1.03	2.40	1521	48.7	21888	27.2	10
90085	265	WR1-LNSC-3	3	WR1	378	648	37	1.13	6.06	F			0.67	4.68	2.37	1440	21.7	13883	22.7	10
90086	265	WR1-LNSC-4	4	WR1	363	490	6	1.01	1.24	FI			0.09	0.17			9.6			7
90087	265	WR1-LNSC-6	6	WR1	412	808	53.5	1.08	7.09	F	8		0.56	1.59	2.74	1605	17.4	17365	23.0	10
90088	265	WR1-LNSC-2	2	WR1	341	515	21.3	1.25	4.31	M	7	26.7	3.63							5
90089	265	WR1-LNSC-1	1	WR1	420	884	74.6	1.09	9.22	F	11		2.68	5.58	3.96	1840	30.6	16775	20.7	10

Unique ID	Day	NRBS#	Fish#	Site	Total Length (mm)	Weight (g)	Gonad Weight (g)	Condition Factor	GSI (%)	Sex	Age (y)	Plasma Ktest (ng/mL)	Plasma Test (ng/mL)	Plasma E2 (ng/mL)	Clutch Oocyte Wt (mg)	Clutch Oocyte Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# oocytes)	Relative Fecundity (oocytes/g)	Maturity Index
90091	265	WR1-LNSC-8	8	WR1	392	653	30.3	1.03	4.87	F	10		3.44	7.60	1.62	1330	17.3	16605	26.7	10
90094	266	WR1-LNSC-10	10	WR1	430	702	28.8	0.85	4.28	F	15		0.32	0.63	1.68	1337	20.0	15266	22.7	10
90095	266	WR1-LNSC-9	9	WR1	390	580	26	0.93	4.69	F	8		0.42	1.25	1.74	1340	17.6	13299	24.0	10
90096	266	WR1-LNSC-11	11	WR1	363	469	25.3	0.93	5.70	M	7	19.8	2.15							4
90097	266	WR1-LNSC-12	12	WR1	312	292	0.5	0.96	0.17		5	1.7	0.15	0.00						
90103	267	WR1-LNSC-13	13	WR1	475	1165	59.7	1.03	5.40	M	11	8.3	1.24							4
90129	270	PR1-LNSC-14	14	PR1	437	916	68.2	1.02	8.04	F	9		0.77	2.82	2.19	1523	45.5	27779	32.8	10
90130	270	PR1-LNSC-3	3	PR1	370	563	3	1.11	0.54	FI	7		3.05	0.60						7
90131	270	PR1-LNSC-8	8	PR1	455	1002	39	1.02	4.05	F	9		4.48	0.52	1.33	1207	33.6	26117	27.1	10
90132	270	PR1-LNSC-9	9	PR1	402	738	18	1.11	2.50	M	8	10.8	1.77							4
90133	270	PR1-LNSC-4	4	PR1	405	755	11	1.12	1.48	FI	8		0.17	0.27	0.50	838	9.4	19541	26.3	7
90134	270	PR1-LNSC-11	11	PR1	414	801						0.5	1.01	0.00						
90135	270	PR1-LNSC-2	2	PR1	397	759	52.5	1.13	7.43	F	7		1.00	1.13	2.44	1451	36.2	19189	27.2	10
90136	270	PR1-LNSC-7	7	PR1	363	590	6.1	1.22	1.04	FI	7		0.08	0.00			5.9			7
90137	270	PR1-LNSC-12	12	PR1	408	724	33	1.02	4.78	M	8	20.0	3.37							4
90138	270	PR1-LNSC-5	5	PR1	422	934	67.5	1.15	7.79	F	10		0.79	1.94	2.05	1494	23.7	29276	33.8	10
90139	270	PR1-LNSC-1	1	PR1	481	1217	52	1.05	4.46	F	11		0.23	1.74	1.29	1273	27.3	35848	30.8	10
90141	270	PR1-LNSC-10	10	PR1	387	726	8	1.24	1.11	FI	8		0.15	0.00			3.5			7
90142	270	PR1-LNSC-6	6	PR1	335	458	0.4	1.22	0.09	MI	5	4.8	0.36							2
90143	270	PR1-LNSC-13	13	PR1	414	760	5.9	1.06	0.78	M	9	1.5	1.33							5
90146	272	PR1-LNSC-17	17	PR1	415	905	53.6	1.19	6.30	F	9		5.49	2.69	2.05	1377	50.0	23317	27.4	10
90147	272	PR1-LNSC-15	15	PR1	431	973	66	1.13	7.28	F	12		0.47	1.24	2.74	1580	16.0	21415	23.6	10
90156	272	PR1-LNSC-16	16	PR1	407	739	1.2	1.09	0.16	FI	9		0.07	0.04						7
90166	275	PR2-LNSC-4	4	PR2	393	634	3	1.04	0.48	FI	8		0.10	0.23						7
90167	275	PR2-LNSC-2	2	PR2	462	1246	60	1.20	5.06	F	10		1.69	0.04	2.89	1645	40.3	18497	15.6	10
90168	275	PR2-LNSC-3	3	PR2	446	1107	75	1.16	7.27	F	9		5.67	0.25	2.88	1627	23.6	23185	22.5	10
90174	275	PR2-LNSC-1	1	PR2	476	1304	17	1.19	1.32	FI	10						9.1			7
90177	275	PR2-LNSC-5	5	PR2	474	1202	14	1.12	1.18	FI	13		0.08	0.00						7
90191	277	PR2-LNSC-7	7	PR2	401	714	31	1.06	4.54	M	8	39.2	4.74							4
90192	277	PR2-LNSC-8	8	PR2	399	697	3	1.09	0.43	FI	8		0.04	0.00						7

Unique ID	Day	NRBS#	Fish#	Site	Total Length (mm)	Weight (g)	Gonad Weight (g)	Condition Factor	GSI (%)	Sex	Age (y)	Plasma Ktest (ng/mL)	Plasma Test (ng/mL)	Plasma E2 (ng/mL)	Clutch Oocyte Wt (mg)	Clutch Oocyte Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# oocytes)	Relative Fecundity (oocytes/g)	Maturity Index
90198	279	PR3-LNSC-5	5	PR3	468	1131	71	1.03	6.70	F	15		7.24	1.43	3.31	1602	44.3	19096	18.0	10
90199	279	PR3-LNSC-8	8	PR3	463	1430	85	1.36	6.32	F	10		4.02	1.04	3.33	1705	46.3	22704	16.9	10
90200	279	PR3-LNSC-1	1	PR3	458	1221	55	1.21	4.72	M	10	45.1	6.95							4
90201	279	PR3-LNSC-3	3	PR3	445	1067	72	1.13	7.24	F	10		3.40	0.83	3.02	1629	26.9	21212	21.3	10
90202	279	PR3-LNSC-4	4	PR3	416	954	39	1.27	4.26	M	13	63.4	9.15							5
90203	279	PR3-LNSC-10	10	PR3	446	1124	10	1.26	0.90	FI	13		0.02	0.00						7
90204	279	PR3-LNSC-11	11	PR3	378	650	30	1.15	4.84	M	10	20.5	5.50							3
90205	279	PR3-LNSC-2	2	PR3	453	1106	45	1.14	4.24	F	11		4.93	0.35	2.80	1552	24.3	14309	13.5	10
90206	279	PR3-LNSC-6	6	PR3	485	1252	11	1.09	0.89	FI	15		0.05	0.03			8.4			7
90207	279	PR3-LNSC-9	9	PR3	411	943	70	1.26	8.02	F	10		1.51	1.35	2.67	1600	28.5	23307	26.7	10
90210	279	PR3-LNSC-7	7	PR3	362	587	1	1.24	0.17	MI	7	0.8	0.07							1
90217	280	PR3-LNSC-13	13	PR3	458	1304	79	1.28	6.45	F	14		6.79	0.62	3.25	1654	34.2	21634	17.7	10
90218	280	PR3-LNSC-12	12	PR3	411	725	26	1.01	3.72	M	9	44.0	5.18							5
90219	280	PR3-LNSC-16	16	PR3	410	871	2	1.26	0.23	MI	10	1.2	0.09							1
90220	280	PR3-LNSC-19	19	PR3	446	1154	39	1.26	3.50	M	9	62.7	10.27							5
90221	280	PR3-LNSC-18	18	PR3	500	1433	16	1.13	1.13	FI	12		1.85	0.00			9.4			7
90222	280	PR3-LNSC-20	20	PR3	435	1198	11	1.44	0.93	FI	10		1.97	0.00			10.3			7
90223	280	PR3-LNSC-17	17	PR3	441	1016	4	1.18	0.40	MI	10	2.5	0.72							1
90224	280	PR3-LNSC-14	14	PR3	450	915	8	1.00	0.88	FI	12		0.03	0.00						7
90225	280	PR3-LNSC-15	15	PR3	452	1066	76	1.07	7.68	F	12		7.54	0.78	3.23	1630	22.4	20928	21.1	10
90226	280	PR3-LNSC-23	23	PR3	340	431	1	1.09	0.23		6		0.18	0.00						
90227	280	PR3-LNSC-21	21	PR3	442	1155	83	1.24	7.74	F	12		25.76	1.75	2.95	1643	27.3	25015	23.3	10
90228	280	PR3-LNSC-22	22	PR3	332	426							0.07	0.00						
90245	288	SR2-LNSC-1	1	SR2	402	746	64	1.05	9.38	F	17		7.99	2.76	3.02	1615	21.1	18842	27.6	10
90299	254	A1-LNSC-1	1	A1b	375	691	40	1.23	6.14	M	11									4
90314	258	MR-LNSC-1	1	MR1	381	655	32.2	1.13	5.17	M	9	64.0	7.16							5
90323	263	A2-LNSC-1	1	A2	291	290	1.2	1.17	0.42	FI	6		0.12	0.00						7
90324	264	A2-LNSC-2	2	A2	311	332	0.3	1.10	0.09	MI	6									1
90325	266	A2-LNSC-3	3	A2	355	503	5.7	1.11	1.15	FI	8						9.7			7
90326	266	A2-LNSC-4	4	A2	434	1013	71.6	1.15	7.61	F	14				2.22	1437	20.9	28653	30.4	10

Unique ID	Day	NRBS#	Fish#	Site	Total Length (mm)	Weight (g)	Gonad Weight (g)	Condition Factor	GSI (%)	Sex	Age (y)	Plasma Ktest (ng/mL)	Plasma Test (ng/mL)	Plasma E2 (ng/mL)	Clutch Oocyte Wt (mg)	Clutch Oocyte Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# oocytes)	Relative Fecundity (oocytes/g)	Maturity Index
90327	266	A2-LNSC-5	5	A2	266	200	0.7	1.06	0.35	MI	6									1

Table 4. Sample identification (Unique ID), year day of sample (Day), Northern River Basins Code (NRBS#), fish number (Fish#), sample collection site (Site), data group, physical characteristics (length, weight & gonad weight), condition factor, gonadosomatic index (GSI), sex/maturity (Sex), age, plasma steroid hormones (Ktest, Test & E2) and reproductive indices (clutch oocyte weight, clutch oocyte diameter, amount clutch, absolute fecundity, relative fecundity & maturity index) for each northern pike collected during the fall 1994 Basin Wide Fish Collection. Ktest=11-ketotestosterone, E2=17ß-estradiol, Test=testosterone.

Unique ID	Day	NRBS#	Fish#	Site	Data Group	Total Length (mm)	Weight (g)	Gonad Weight (g)	Condition Factor	GSI (%)	Sex	Age (y)	Plasma Ktest (ng/mL)	Plasma Test (ng/mL)	Plasma E2 (ng/mL)	Clutch Oocyte Wt (mg)	Clutch Oocyte Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# oocytes)	Relative Fecundity (oocytes/g)	Maturity Index
90098	267	WR1-NRPK-4	4	WR1	WR	710	3431	130.6	0.92	3.96	F	6		15.6	24.6	1.601	1338	21.1	65912	20.0	10
90105	268	WR1-NRPK-9	9	WR1	WR	586	1187	30.1	0.57	2.60	F	4		13.1	29.5	0.790	1103	14.9	30786	26.6	10
90286	292	WR2-NRPK-1	1	WR2	WR	512	933	31.0	0.67	3.44	F	4		9.2	69.0	2.014	1372	11.6	12440	13.8	10
90010	255	SR1-NRPK-1	1	SR1	SR1	610		25.0			F	5		1.6	17.6	0.698	1036	12.5	28923		9
90034	261	SR1-NRPK-3	3	SR1	SR1	460	733	13.0	0.74	1.81	F	3		1.2	11.5	0.542	915	16.6	19369	26.9	9
90380	272	P-NRPK-2	2	P	P	606	1606	34.6	0.71	2.20	F	4		12.4	35.3	0.670	996	21.7	41727	26.6	10
90297	257	A1-NRPK-3	3	A1b	A1	695	2628	87.7	0.76	3.45	F	7		30.1	58.5	0.846	1041	26.9	83761	33.0	10
90473	287	A5-NRPK-1	1	A5	A5	608	1411	55.6	0.60	4.10	F	9		8.4	39.3	1.725	1360	21.3	26043	19.2	10
90500	291	JV1-NRPK-2	2	JV1	JV1	625	1660	45.0	0.66	2.79	F	5		7.4	53.6	1.177	1233	16.9	30887	19.1	10
90503	291	JV1-NRPK-5	5	JV1	JV1	535	1190	43.2	0.75	3.77	F	4		11.6	32.0	1.012	1144	26.4	34478	30.1	10
90491	289	SR-NRPK-2	2	SRD2	SD	540	913	21.1	0.57	2.37	F	3		1.8	21.1	1.313	1171	14.8	12987	14.6	10
90492	289	SR-NRPK-3	3	SRD2	SD	870		49.5			F	10				4.338	1816	22.1	9220		10
90128	270	PR1-NRPK-2	2	PR1	PR	446	611	6.5	0.68	1.08	F	3		4.4	11.3	0.526	883	11.5	9983	16.5	9
90169	275	PR2-NRPK-1	1	PR2	PR	565	1307	32.0	0.71	2.51	F	4		2.1	20.6	0.778	1172	14.7	33234	26.1	10
90080	264	WR1-NRPK-1	1	WR1	WR	345	282	3.6	0.68	1.29	M	3	15.9	19.6							4
90081	264	WR1-NRPK-2	2	WR1	WR	542	1095	68.0	0.65	6.62	M	4	26.9	22.6							4
90090	265	WR1-NRPK-3	3	WR1	WR	475	720	22.0	0.65	3.15	M	4	17.0	20.4							4
90100	267	WR1-NRPK-5	5	WR1	WR	356	283	4.5	0.62	1.62	M	3	3.0	8.2							4
90099	267	WR1-NRPK-6	6	WR1	WR	464	681	12.0	0.67	1.79	M	3	9.8	16.7							4
90104	268	WR1-NRPK-10	10	WR1	WR	543	1085	18.6	0.67	1.74	M	4	12.1	9.1							4
90113	268	WR1-NRPK-12	12	WR1	WR	290	128	2.3	0.52	1.83	M	2	1.0	2.1							3
90004	256	SR1-NRPK-2	2	SR1	SR1	490	740	21.6	0.61	3.01	M	4	11.1	20.1							4
90310	260	MR-NRPK-2	2	MR1	MR	494	785	15.8	0.64	2.05	M	3	27.6	14.4							4
90379	271	P-NRPK-1	1	P	P	365	275	3.1	0.56	1.14	M	4	8.2	13.4							4
90434	283	CW-NRPK-1	1	CW	CW	672	2168	29.5	0.70	1.38	M	9	4.4	3.3							4
90295	255	A1-NRPK-1	1	A1b	A1	489	773	12.1	0.65	1.59	M	4	3.1	5.3							4
90298	257	A1-NRPK-4	4	A1b	A1	568	1273	24.9	0.68	2.00	M	4	10.8	7.0							4
90499	291	JV1-NRPK-1	1	JV1	JV1	562	1820	17.5	1.02	0.97	M	4	10.3	4.8							4

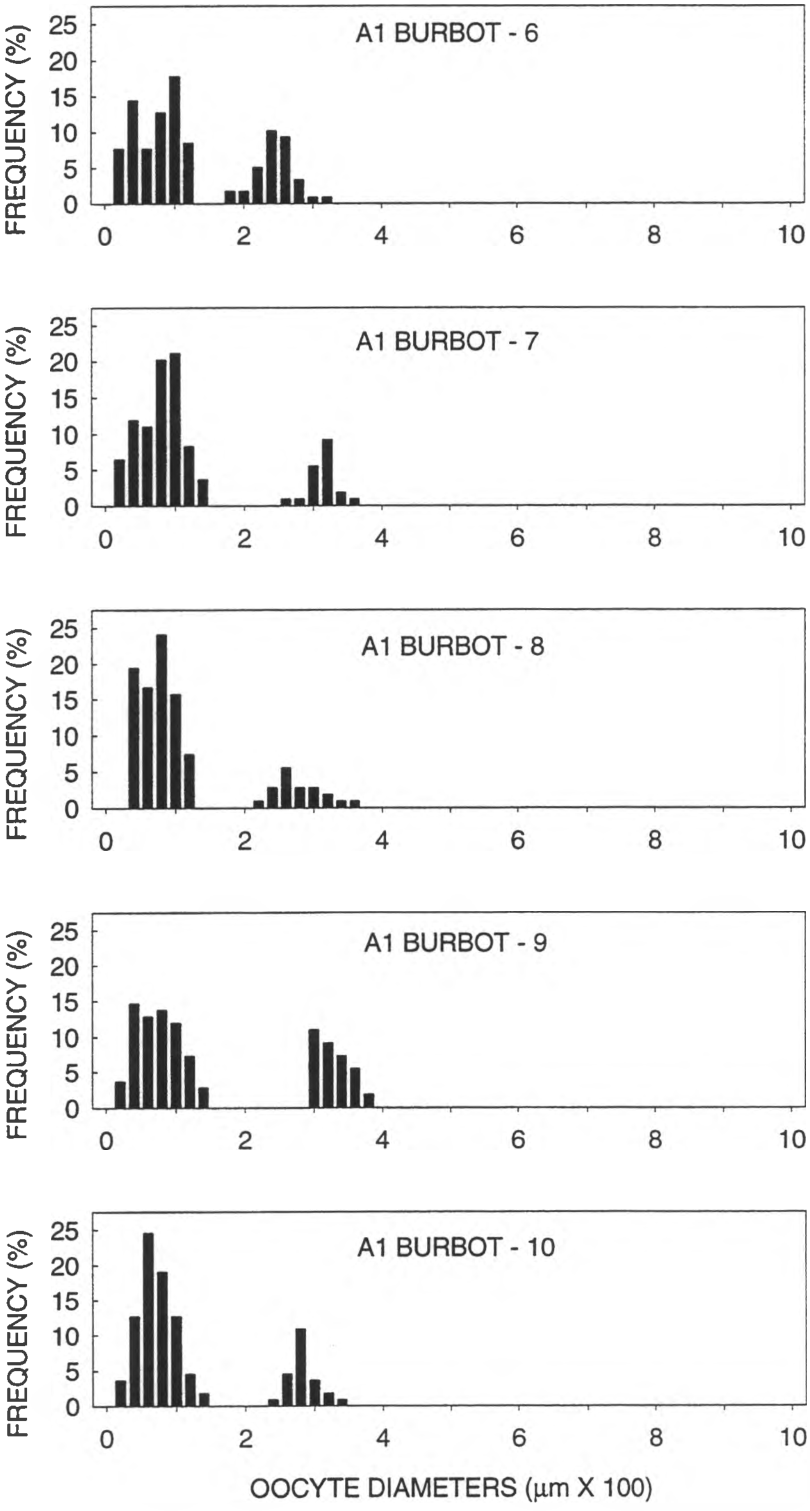
Unique ID	Day	NRBS#	Fish#	Site	Data Group	Total Length (mm)	Weight (g)	Gonad Weight (g)	Condition Factor	GSI (%)	Sex	Age (y)	Plasma Ktest (ng/mL)	Plasma Test (ng/mL)	Plasma E2 (ng/mL)	Clutch Oocyte Wt (mg)	Clutch Oocyte Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# oocytes)	Relative Fecundity (oocytes/g)	Maturity Index
90502	291	JV1-NRPK-4	4	JV1	JV1	580	1536	25.7	0.77	1.70	M	4	62.2	44.1							4
90127	270	PR1-NRPK-1	1	PR1	PR	504	923	18.0	0.71	1.99	M	6	5.8	10.8							4
90140	270	PR1-NRPK-3	3	PR1	PR	590	1678	40.9	0.80	2.50	M	5	14.1	7.6							4
90170	275	PR2-NRPK-2	2	PR2	PR	441	543	9.0	0.62	1.69	M	4	4.8	4.6							4
90102	267	WR1-NRPK-7	7	WR1	WR	341	272	2.4	0.68	0.89	FI	2		1.6	1.8						7
90101	267	WR1-NRPK-8	8	WR1	WR	353	299	2.0	0.68	0.67	FI	2		0.8	1.4						7
90112	268	WR1-NRPK-11	11	WR1	WR	357	262	1.6	0.57	0.61	FI	2		0.3	0.5						7
90309	260	MR-NRPK-1	1	MR1	MR	370	322	1.1	0.63	0.34	FI	2		1.4	4.3						7
90311	261	MR-NRPK-3	3	MR1	MR	393	350	1.3	0.57	0.37	FI	2		0.3	0.7						7
90312	261	MR-NRPK-4	4	MR1	MR	429	474	2.4	0.60	0.51	FI	3		1.2	3.3						7
90296	255	A1-NRPK-2	2	A1b	A1	498	830	9.2	0.66	1.12	FI	3		1.2	10.6	0.279	776	15.5	26672	32.5	7
90501	291	JV1-NRPK-3	3	JV1	JV1	705	1975	19.3	0.56	0.99	FI	8		0.6	1.1	0.641	792	13.5	24328	12.4	7
90159	272	PR1-NRPK-4	4	PR1	PR	264	154	1.0	0.83	0.65	MI	2	0.5	4.9							
90178	275	PR2-NRPK-3	3	PR2	PR	264	112	1.0	0.60	0.9	FI	2		0.1	0.8						

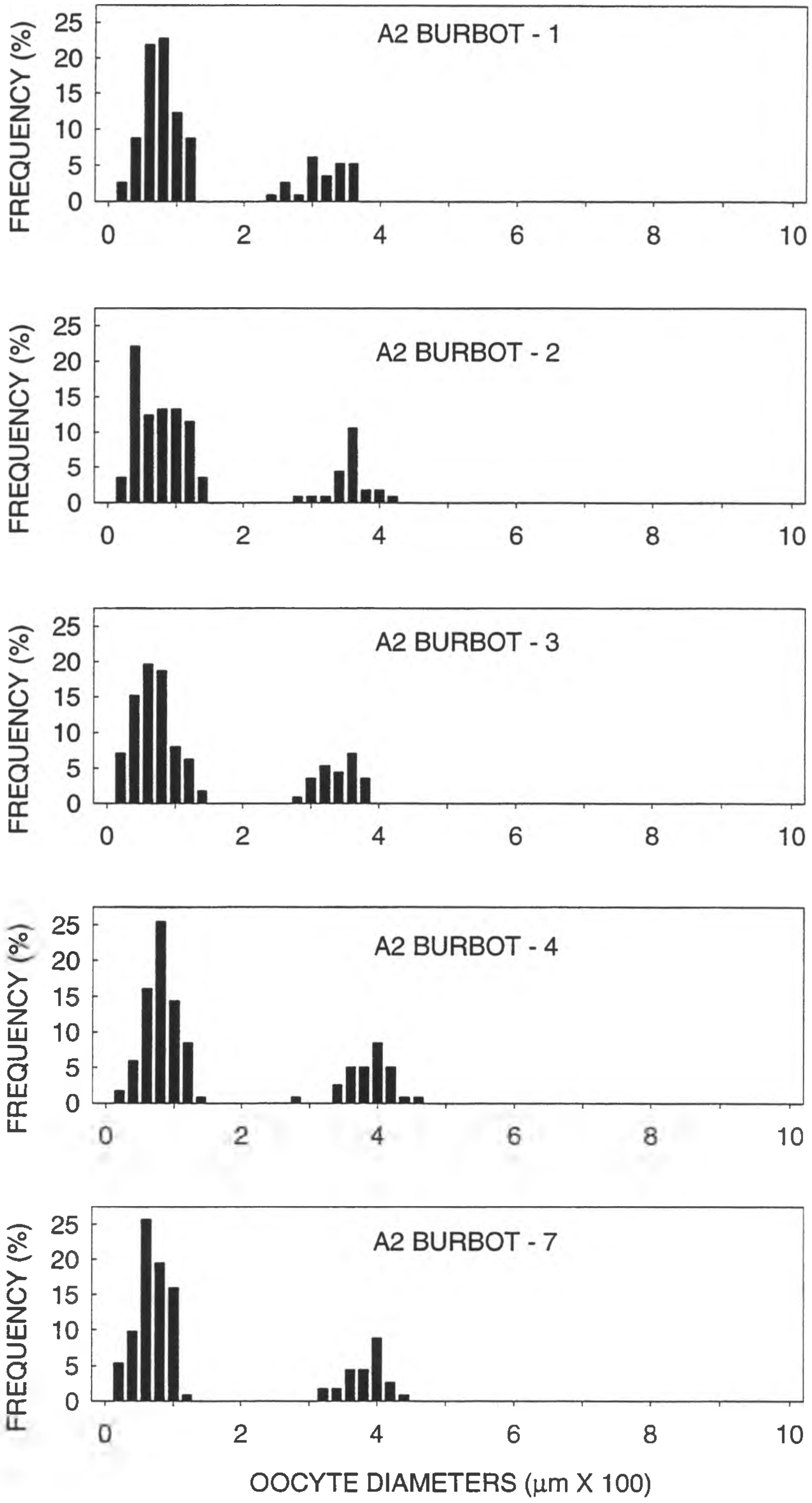
Table 5. Sample identification (Unique ID), year day of sample (Day), Northern River Basins Code (NRBS#), fish number (Fish#), sample collection site (Site), data group, physical characteristics (length, weight & gonad weight), condition factor, gonadosomatic index (GSI), sex/maturity (Sex), age, plasma steroid hormones (Ktest, Test & E2) and reproductive indices (clutch oocyte weight, clutch oocyte diameter, amount clutch, absolute fecundity, relative fecundity & maturity index) for each flathead chub collected during the fall 1994 Basin Wide Fish Collection. Ktest=11-ketotestosterone, E2=17ß-estradiol, Test=testosterone.

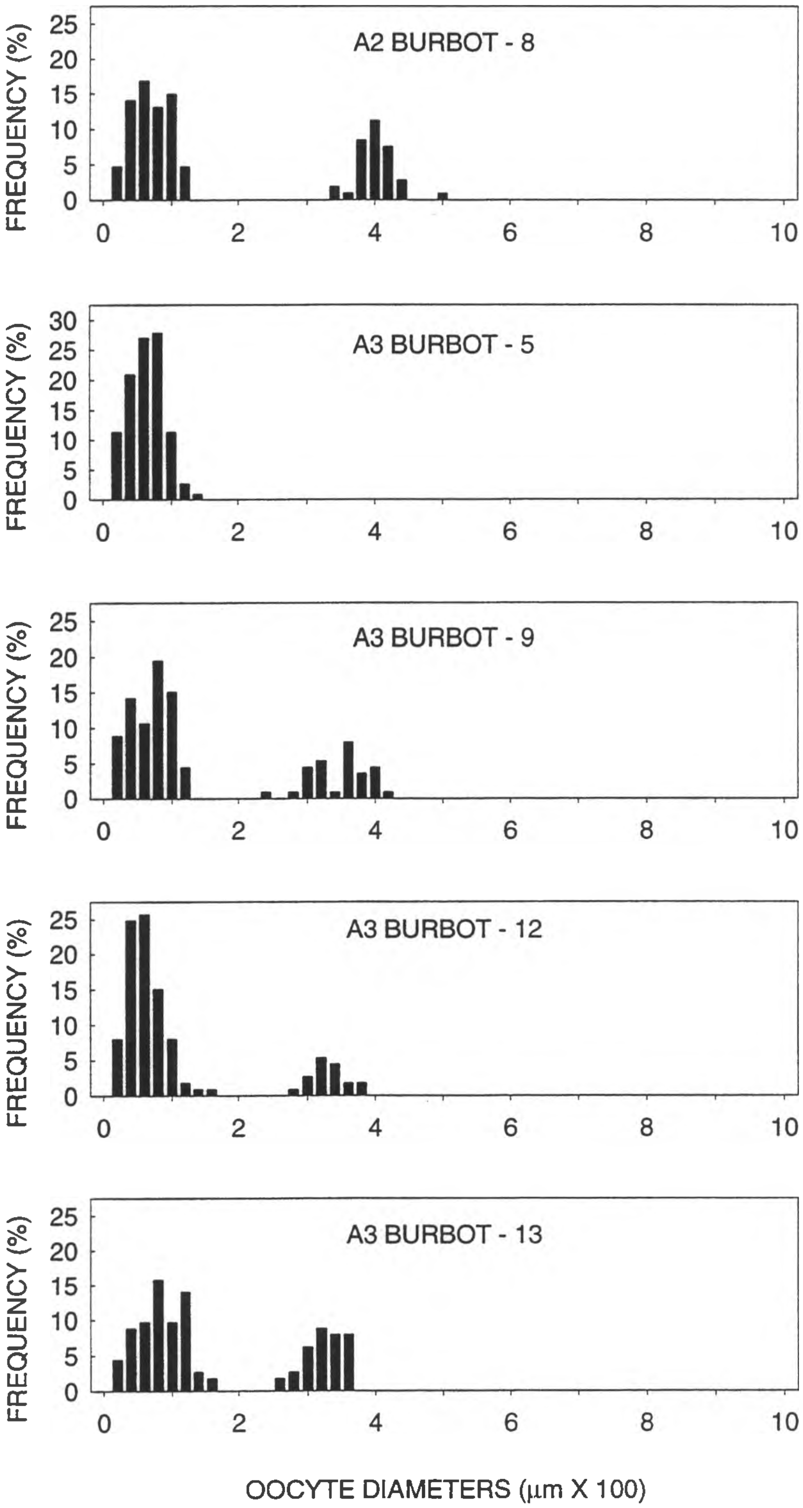
Unique ID	Day	NRBS#	Fish#	Site	Data Group	Length (mm)	Weight (g)	Gonad Weight (g)	Condition Factor	GSI (%)	Sex	Age (y)	Plasma Ktest (ng/mL)	Plasma Test (ng/mL)	Plasma E2 (ng/mL)	Clutch Oocyte Wt (mg)	Clutch Oocyte Diam (µm)	Amount Clutch (%)	Absolute Fecundity (# oocytes)	Relative Fecundity (oocytes/ g)	Maturity Index
90015	256	SR1-FLCH-1	1	SR1	SR1	287	230	8.0	0.939	3.60	F	8		0.204	0.689	0.441	887	15.3	18149	82	10
90022	257	SR1-FLCH-2	2	SR1	SR1	245	156	4.0	1.034	2.63	F	8				0.409	890	17.4	9775	64	10
90023	257	SR1-FLCH-3	3	SR1	SR1	300	274	5.7	0.994	2.12	F	8		0.733	1.319	0.332	792	15.4	17189	64	10
90028	259	SR1-FLCH-4	4	SR1	SR1	275	206	10.0	0.942	5.10	F	7		0.438	2.613	0.585	944	14.3	17088	87	10
90026	259	SR1-FLCH-5	5	SR1	SR1	264	191	5.0	1.011	2.69	F	8		0.261	0.158	0.454	909	11.9	11013	59	10
90030	259	SR1-FLCH-6	6	SR1	SR1	196	77	1.0	1.009	1.32	F	4		0.285	4.175	0.204	680	10.3	4902	64	10
90044	261	SR1-FLCH-12	12	SR1	SR1	200	79	1.5	0.969	1.94	F	5		0.195	0.111	0.308	803	14.0	4870	63	10
90040	261	SR1-FLCH-8	8	SR1	SR1	242	136	0.4	0.957	0.29	M	8	2.76	0.314							2
90038	261	SR1-FLCH-7	7	SR1	SR1	162	42				FI		0.50	1.463	0.455						7
90039	261	SR1-FLCH-9	9	SR1	SR1	153	31	1.0	0.838	3.33	FI	3		0.459	0.625						7
90045	261	SR1-FLCH-10	10	SR1	SR1	176	51	0.0	0.935		FI	4		0.101	0.435						7
90046	261	SR1-FLCH-11	11	SR1	SR1	200	75	0.3	0.934	0.40	MI	5	0.50	0.786							1
90073	263	LSR2-FLCH-1	1	LSR2	LSR	165	47	2.9	0.982	6.58	FI	3		0.306	0.205						7
90109	268	WR1-FLCH-2	2	WR1	WR	146	26	0.2	0.829	0.78	M	3	2.93	0.727							2
90108	268	WR1-FLCH-3	3	WR1	WR	158	36	0.2	0.908	0.56	M	4	2.49	0.445							2
90111	268	WR1-FLCH-4	4	WR1	WR	134	22	0.1	0.910	0.46	M	3	3.03	0.258							2
90242	284	WAB1-FLCH-1	1	WB1	WB	191	75	4.0	1.019	5.63	MI	4	0.50	0.699							1
90082	265	WR1-FLCH-1	1	WR1	WR	150	26	0.4	0.759	1.56	FI	3		1.024	1.022						7
90110	268	WR1-FLCH-5	5	WR1	WR	132	21	0.1	0.909	0.48	I	3	0.50	0.227	0.001						
90180	275	PR2-FLCH-1	1	PR2	PR	270	225	7.0	1.108	3.21	F	7				0.429	904	15.5	16309	75	10
90184	276	PR2-FLCH-2	2	PR2	PR	261	164	3.0	0.906	1.86	F	6		0.834	0.870	0.320	807	23.1	9363	58	10
90193	277	PR2-FLCH-3	3	PR2	PR	294	285	8.0	1.090	2.89	F	8		1.283	1.601	0.402	878	9.3	19881	72	10
90195	277	PR2-FLCH-4	4	PR2	PR	264	207	6.0	1.092	2.99	F	6				0.543	923	13.2	11050	55	10
90160	273	PR1-FLCH-1	1	PR1	PR	151	33.2				I	2	0.50	0.787	0.001						

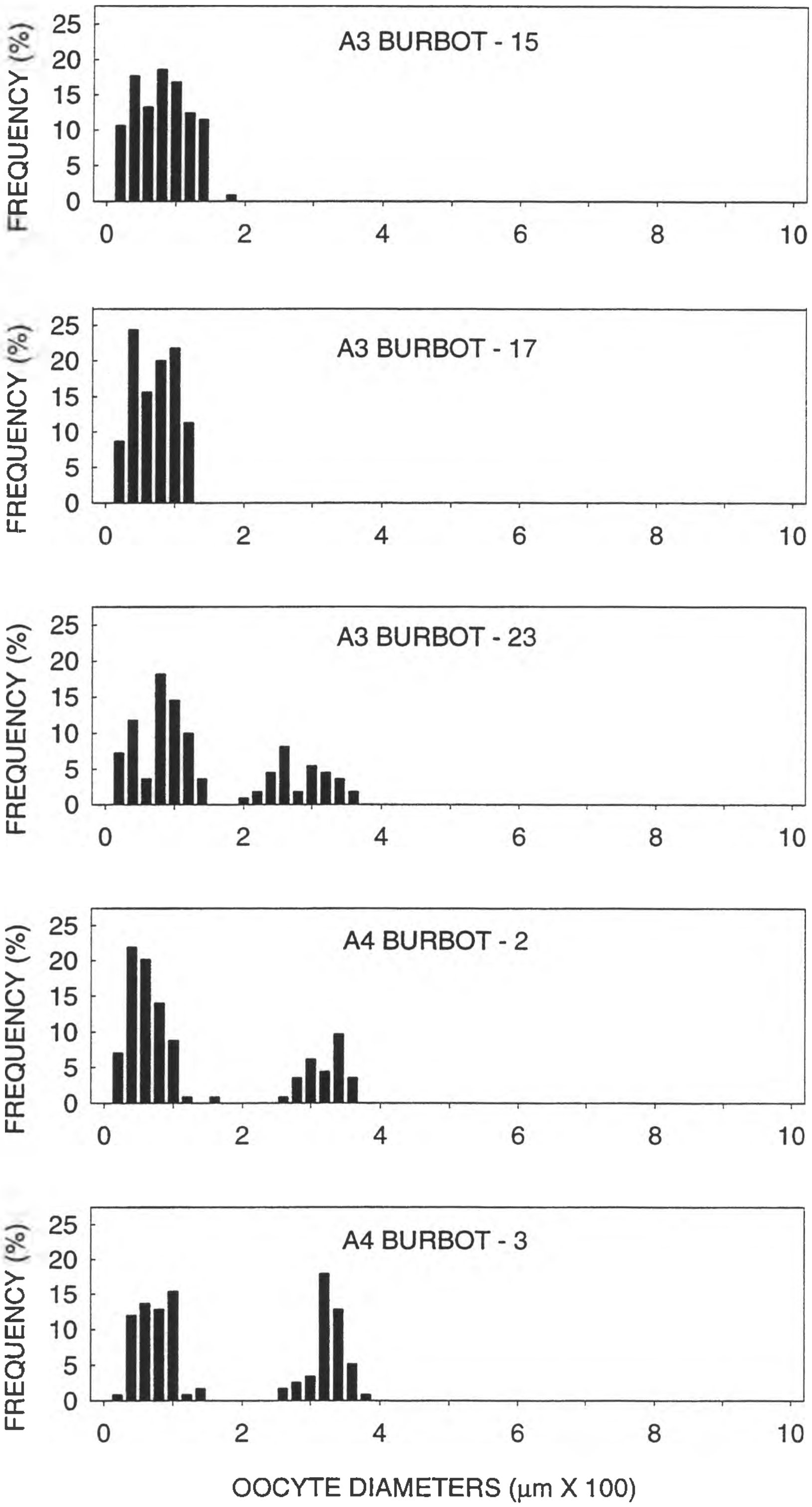
APPENDIX C

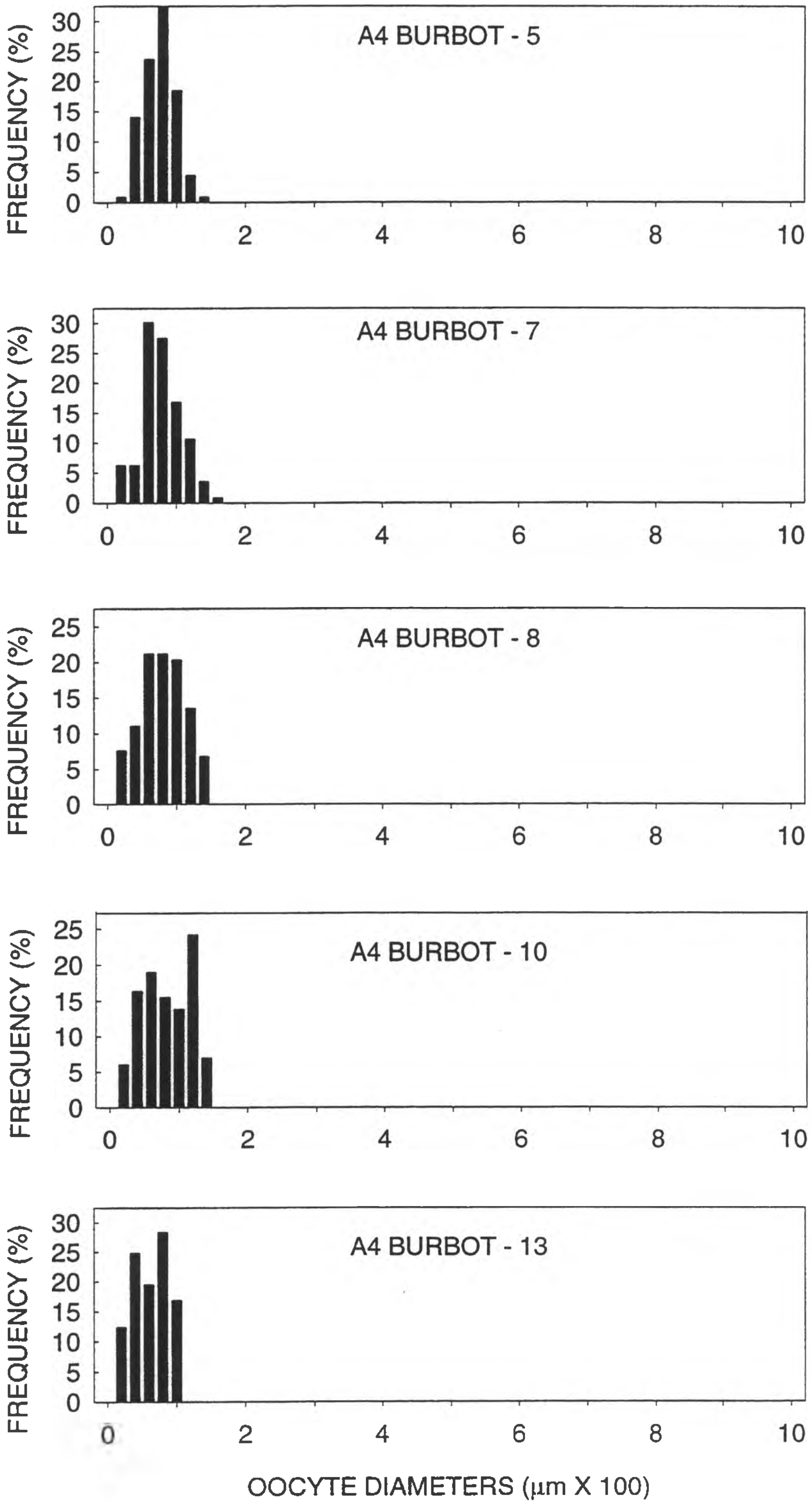
Burbot Oocyte Diameter Frequency Distributions

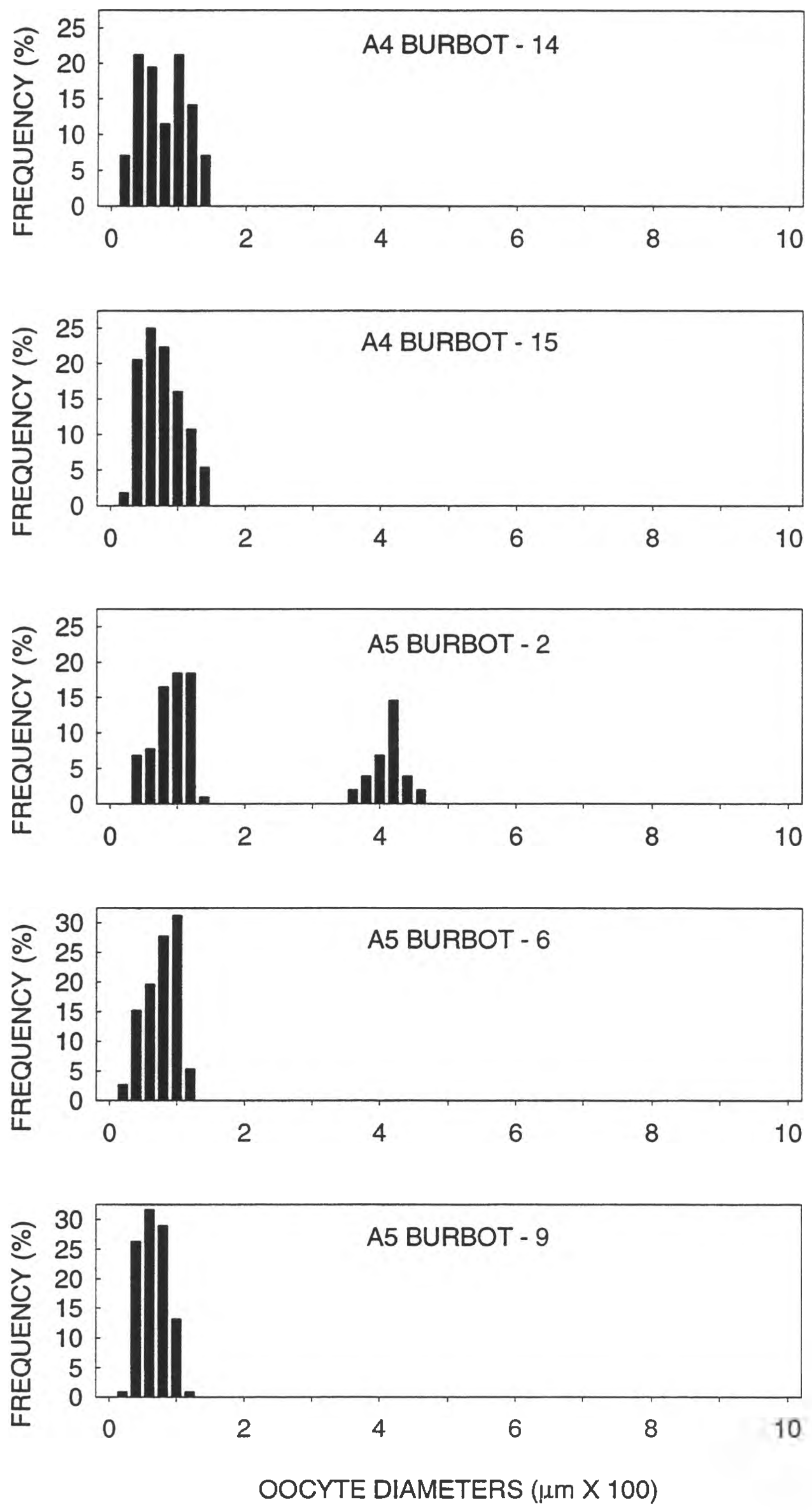

A1 BURBOT - 6
A1 BURBOT - 7
A1 BURBOT - 8
A1 BURBOT - 9
A1 BURBOT - 10
FREQUENCY (%)
0
5
10
15
20
25
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

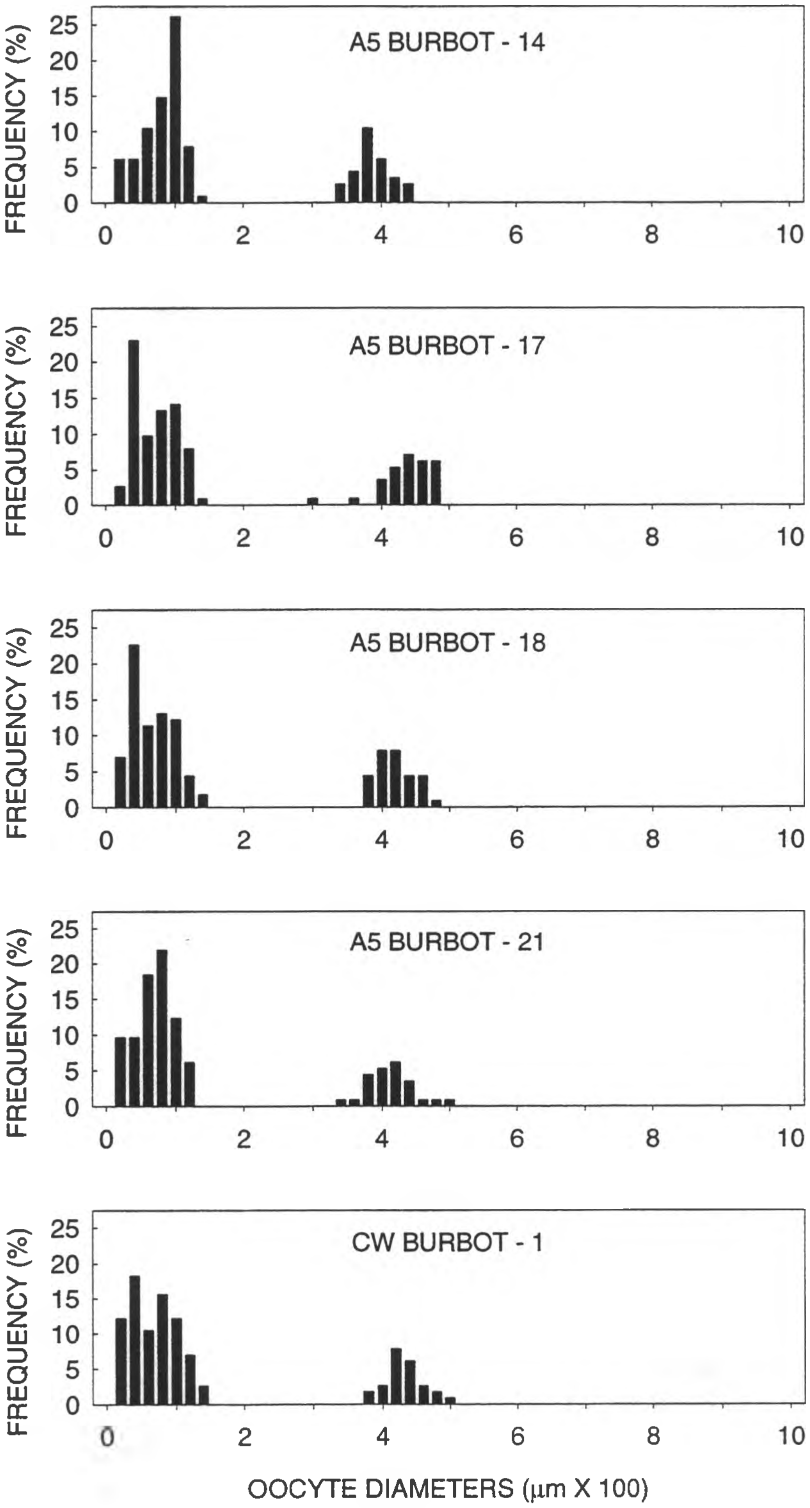

A2 BURBOT - 1
A2 BURBOT - 2
A2 BURBOT - 3
A2 BURBOT - 4
A2 BURBOT - 7
FREQUENCY (%)
0
5
10
15
20
25
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

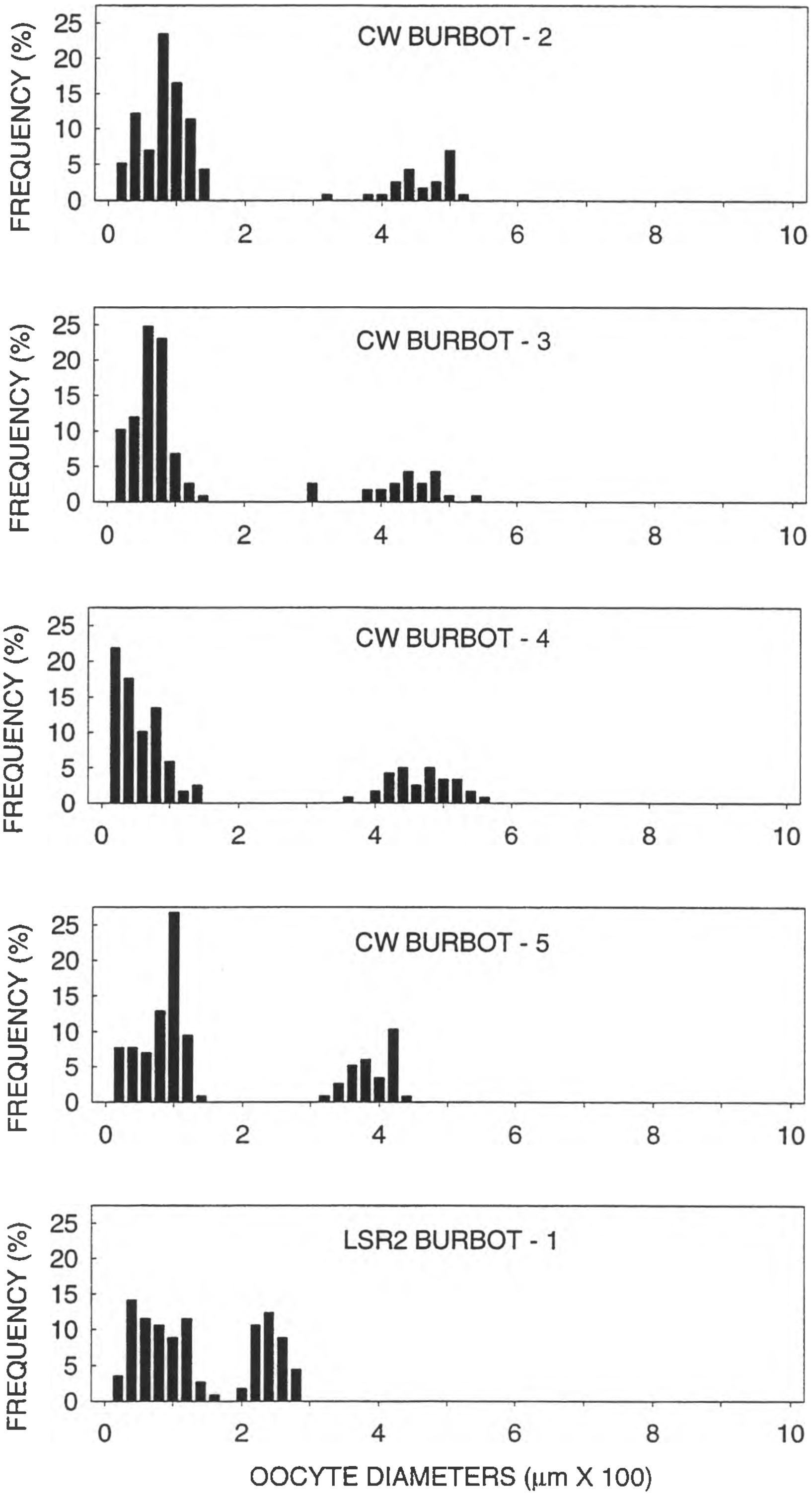

A2 BURBOT - 8
A3 BURBOT - 5
A3 BURBOT - 9
A3 BURBOT - 12
A3 BURBOT - 13
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

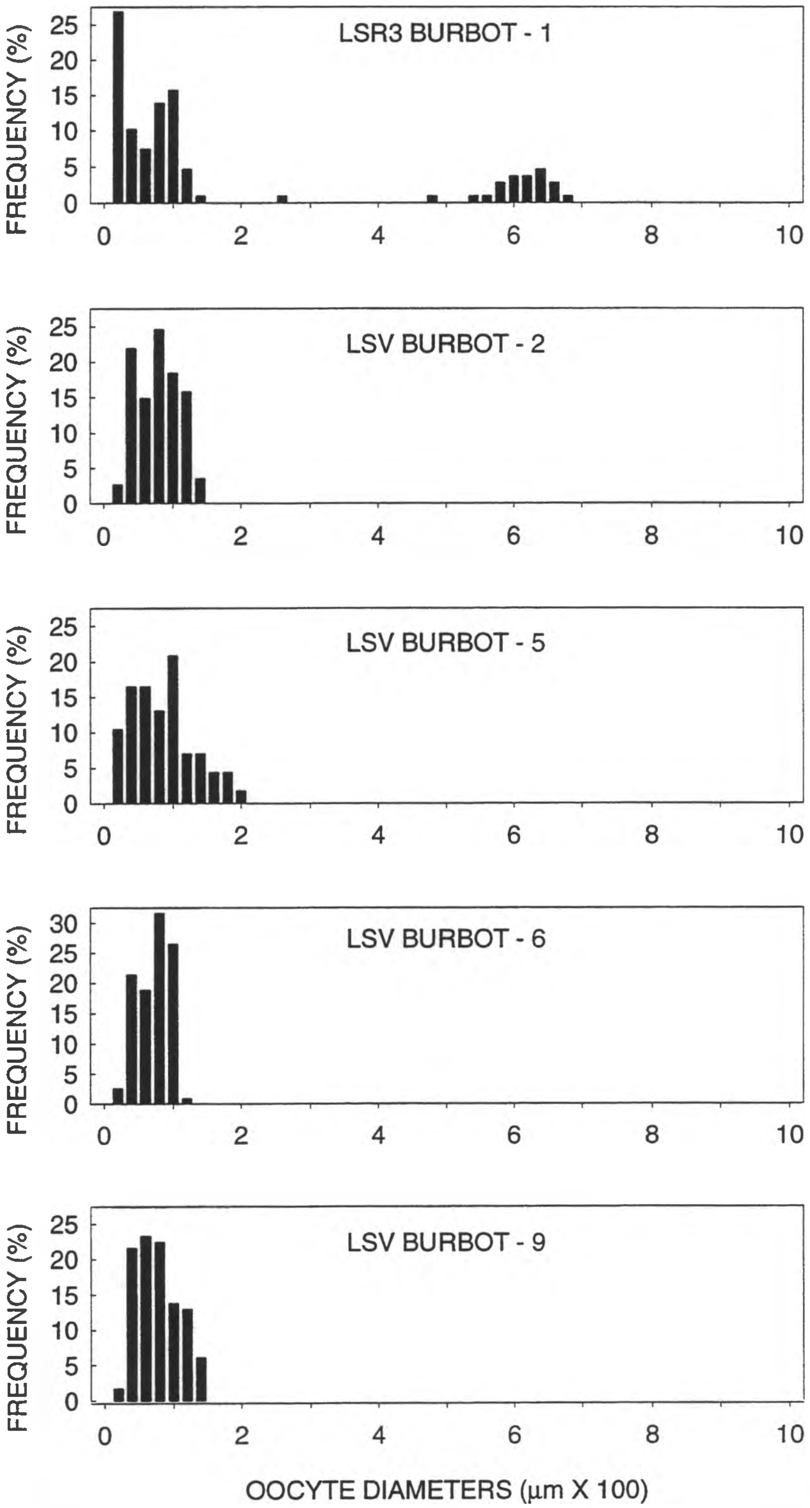

A3 BURBOT - 15
A3 BURBOT - 17
A3 BURBOT - 23
A4 BURBOT - 2
A4 BURBOT - 3
FREQUENCY (%)
0
5
10
15
20
25
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

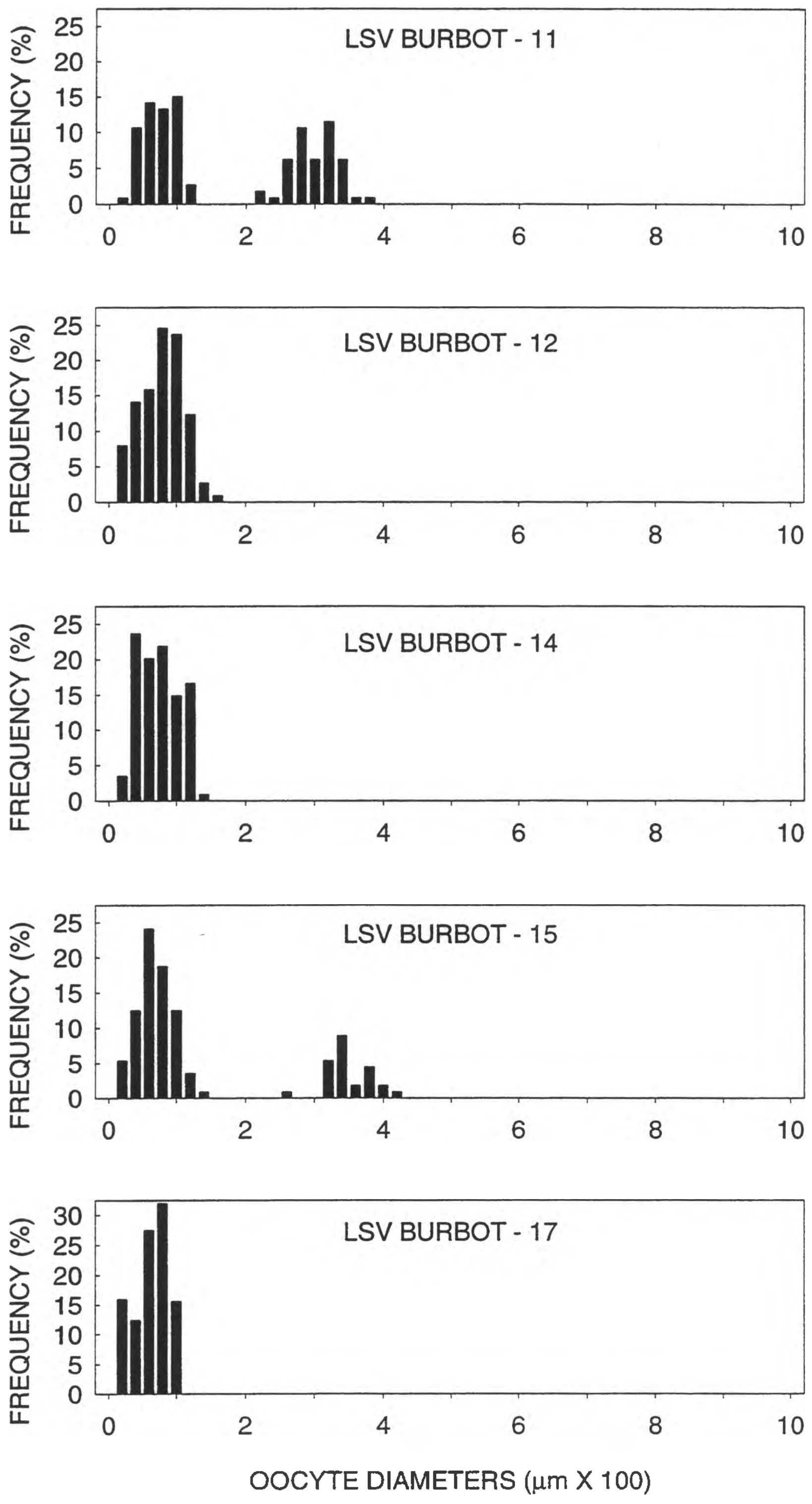

A4 BURBOT - 5
A4 BURBOT - 7
A4 BURBOT - 8
A4 BURBOT - 10
A4 BURBOT - 13
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

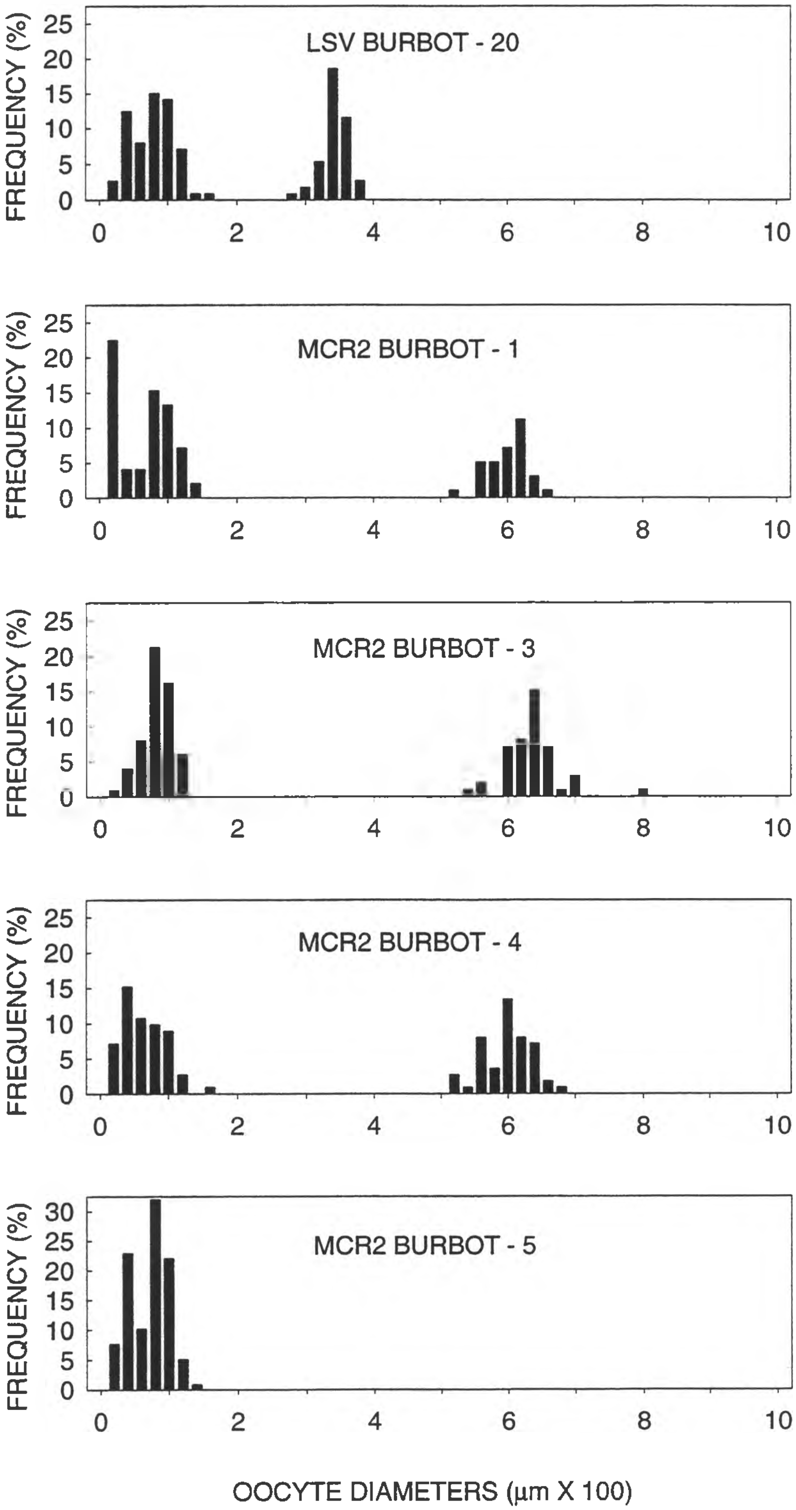

A4 BURBOT - 14
A4 BURBOT - 15
A5 BURBOT - 2
A5 BURBOT - 6
A5 BURBOT - 9
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

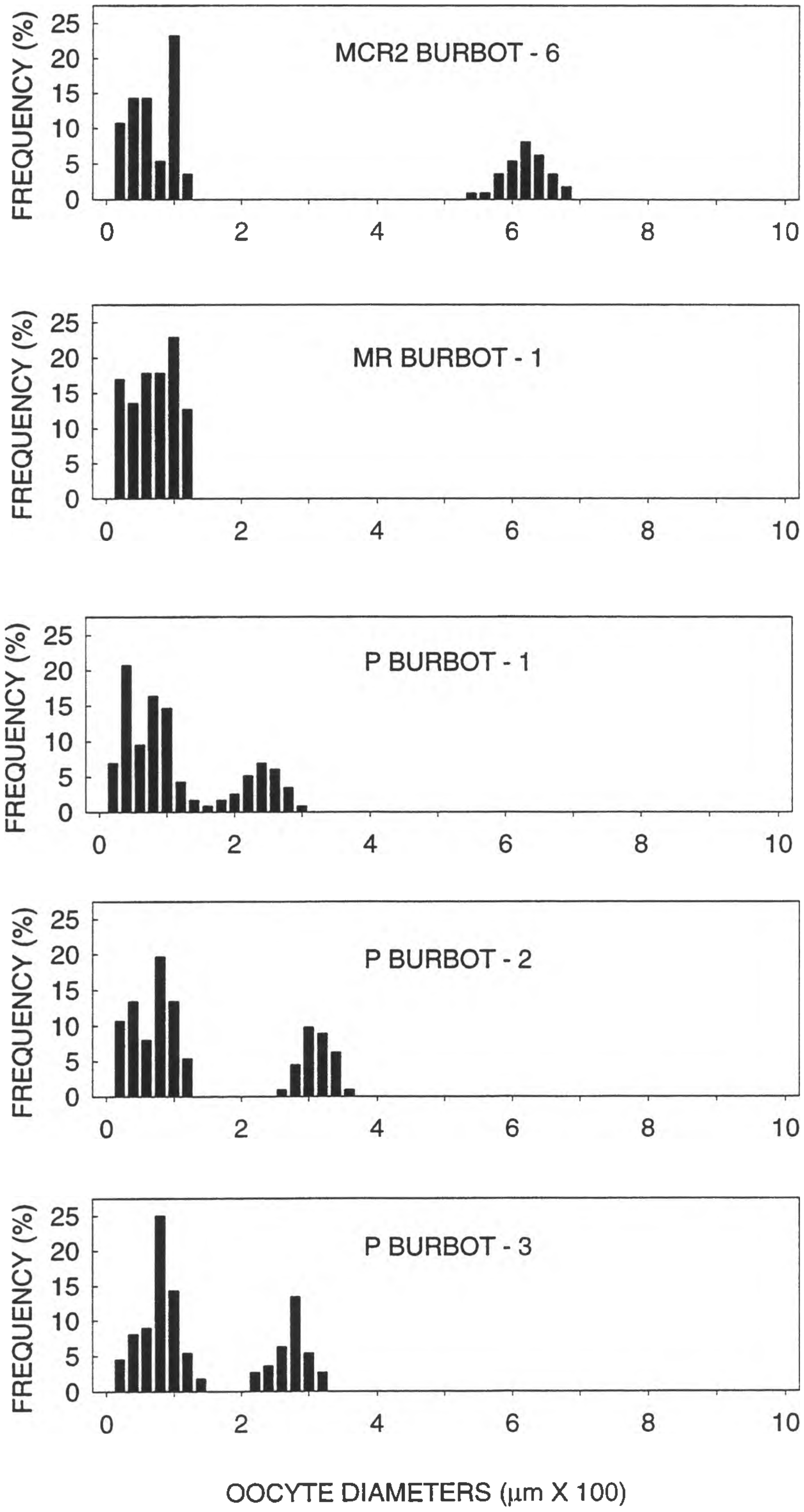

A5 BURBOT - 14
A5 BURBOT - 17
A5 BURBOT - 18
A5 BURBOT - 21
CW BURBOT - 1
FREQUENCY (%)
0
5
10
15
20
25
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

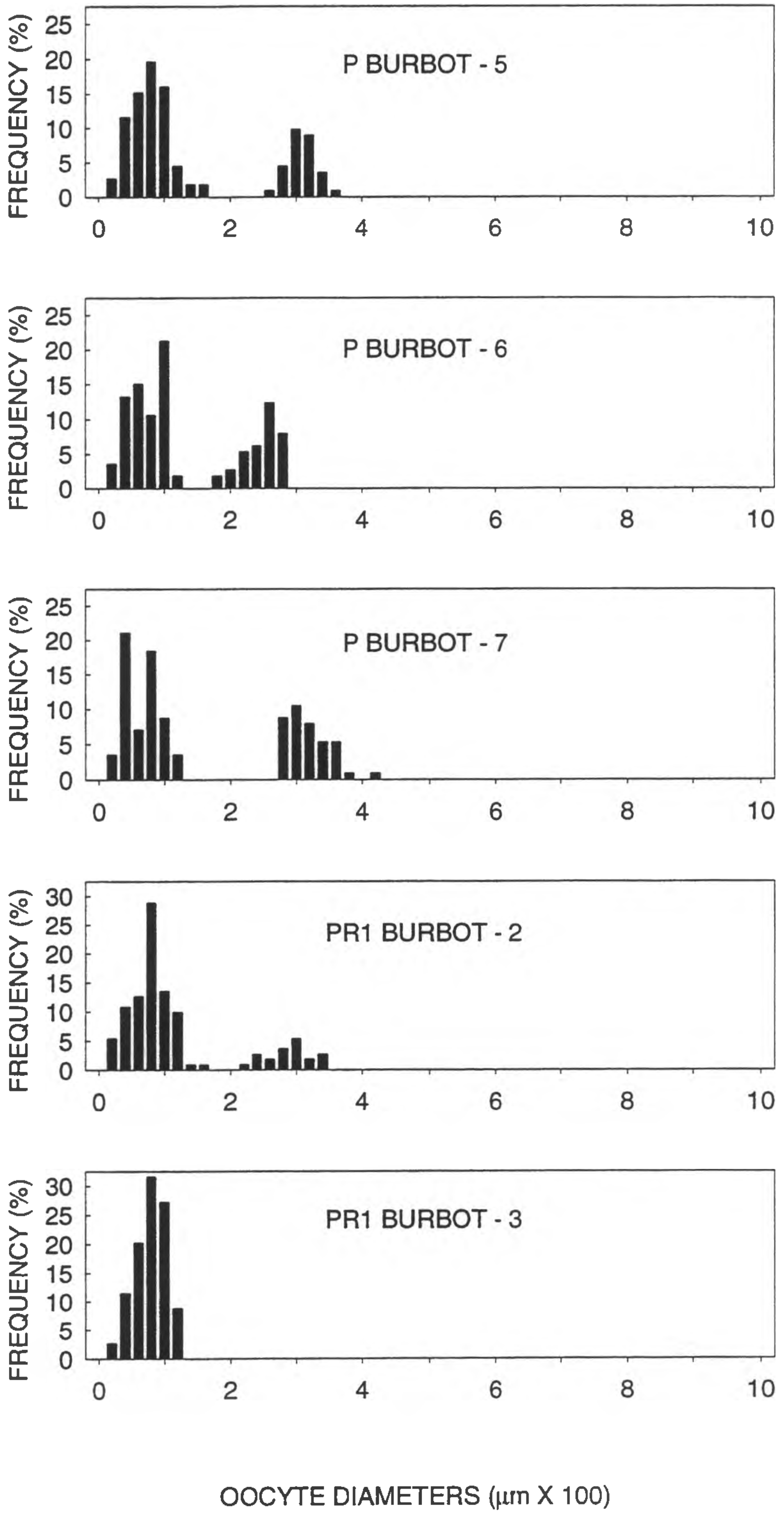

CW BURBOT - 2
CW BURBOT - 3
CW BURBOT - 4
CW BURBOT - 5
LSR2 BURBOT - 1
FREQUENCY (%)
0
5
10
15
20
25
0
2
4
6
8
10
OOCYTE DIAMETERS (μm X 100)

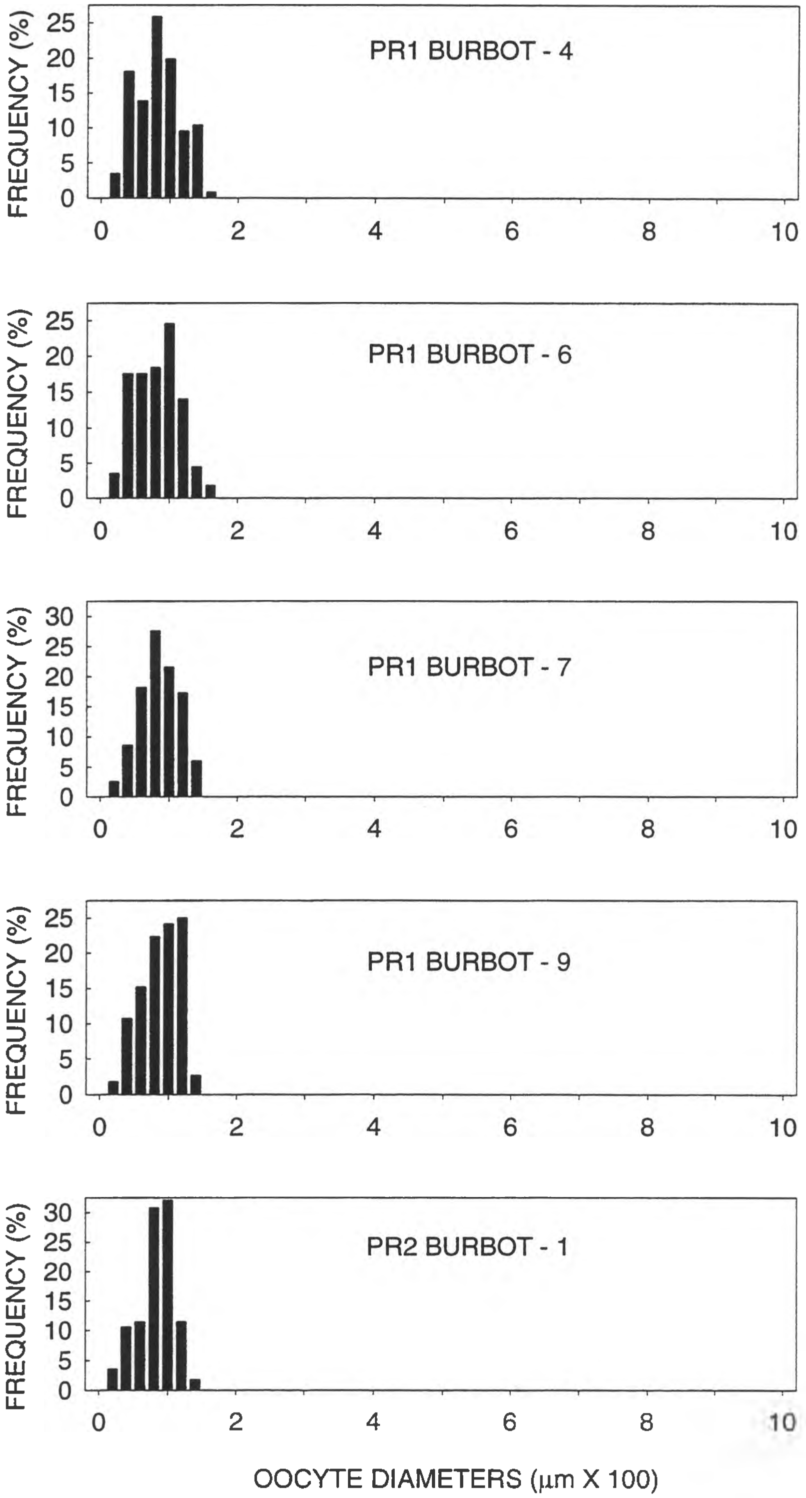

LSR3 BURBOT - 1
LSV BURBOT - 2
LSV BURBOT - 5
LSV BURBOT - 6
LSV BURBOT - 9
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

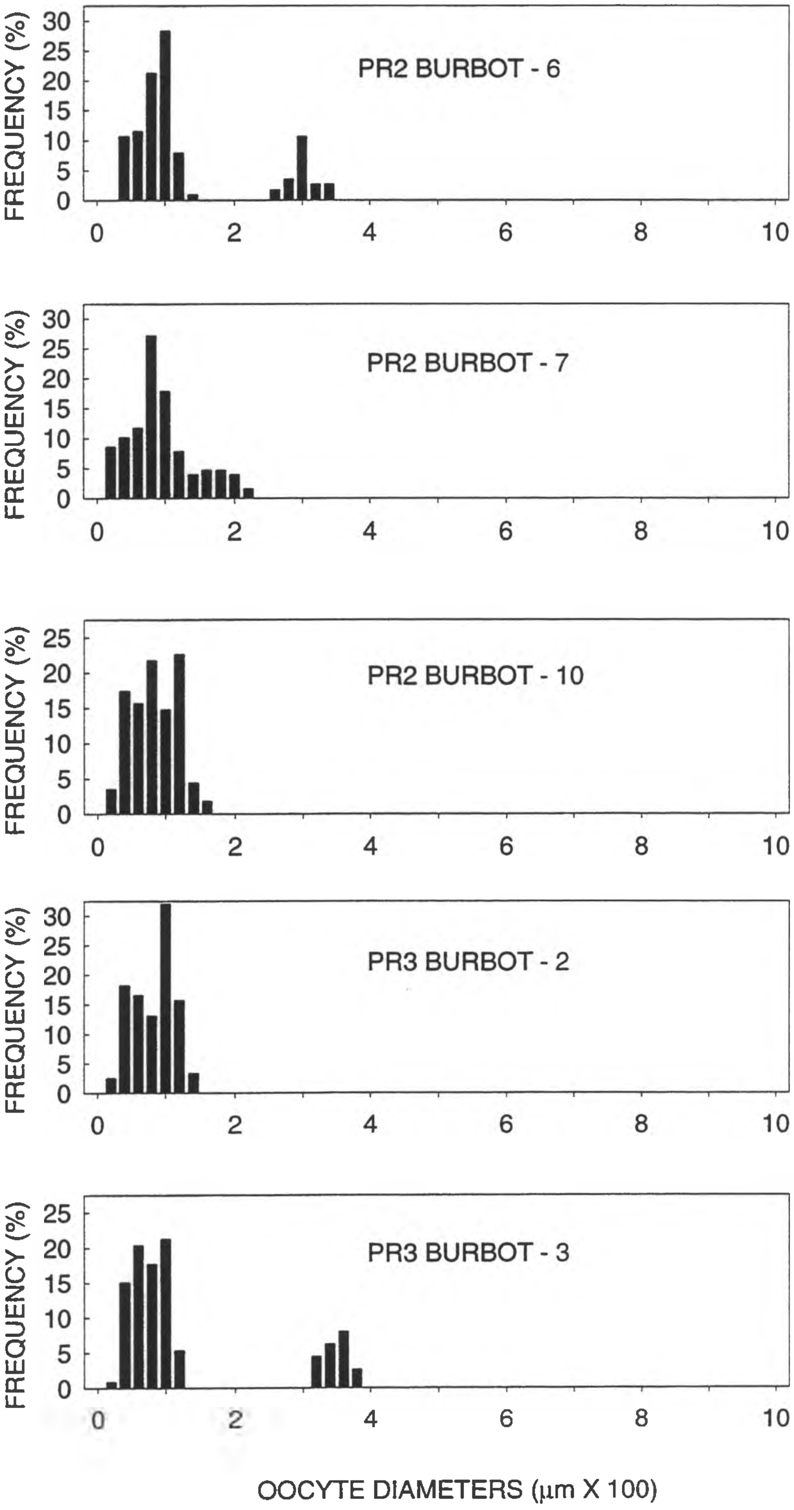

LSV BURBOT - 11
LSV BURBOT - 12
LSV BURBOT - 14
LSV BURBOT - 15
LSV BURBOT - 17
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

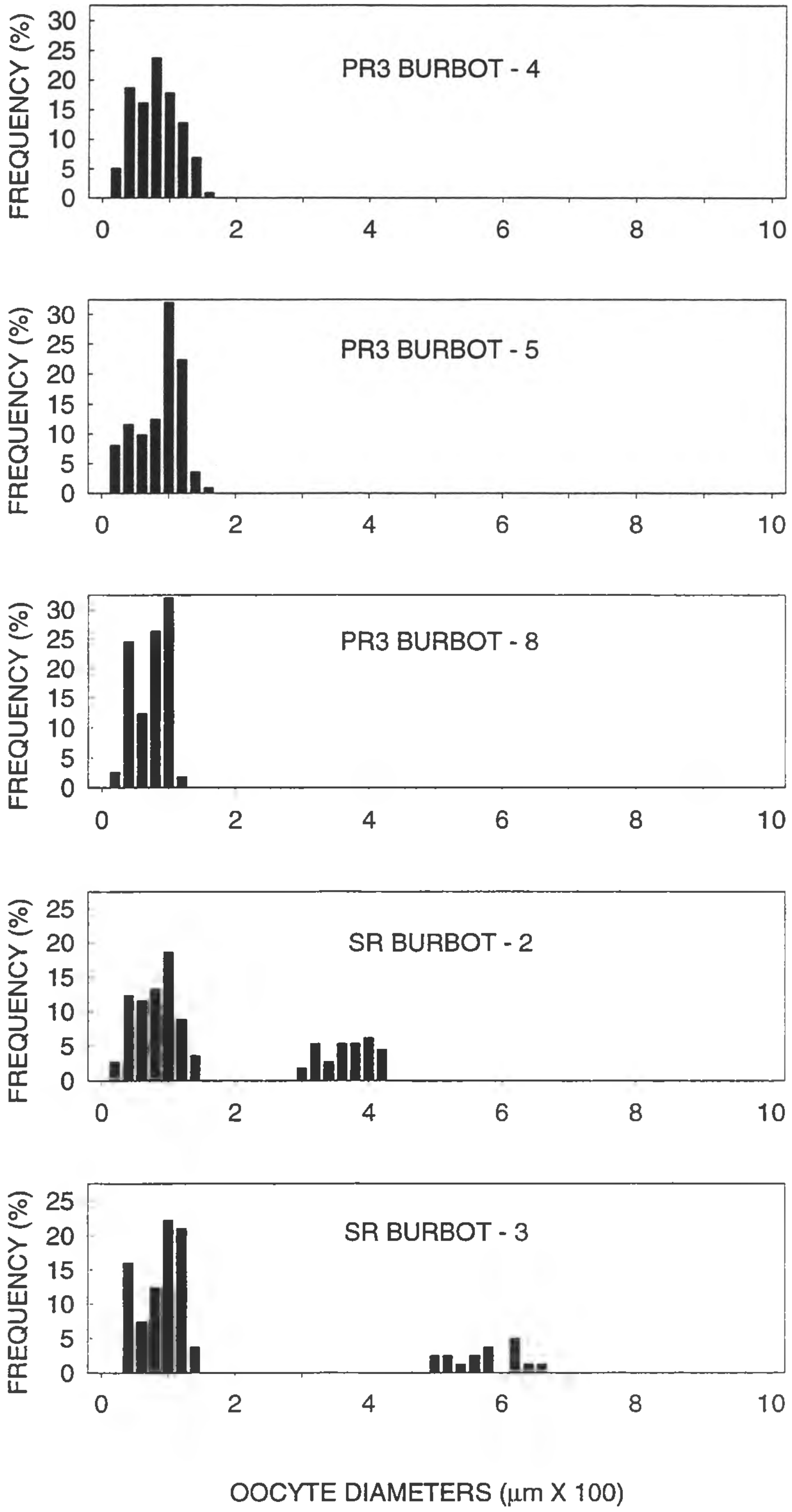

LSV BURBOT - 20
MCR2 BURBOT - 1
MCR2 BURBOT - 3
MCR2 BURBOT - 4
MCR2 BURBOT - 5
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

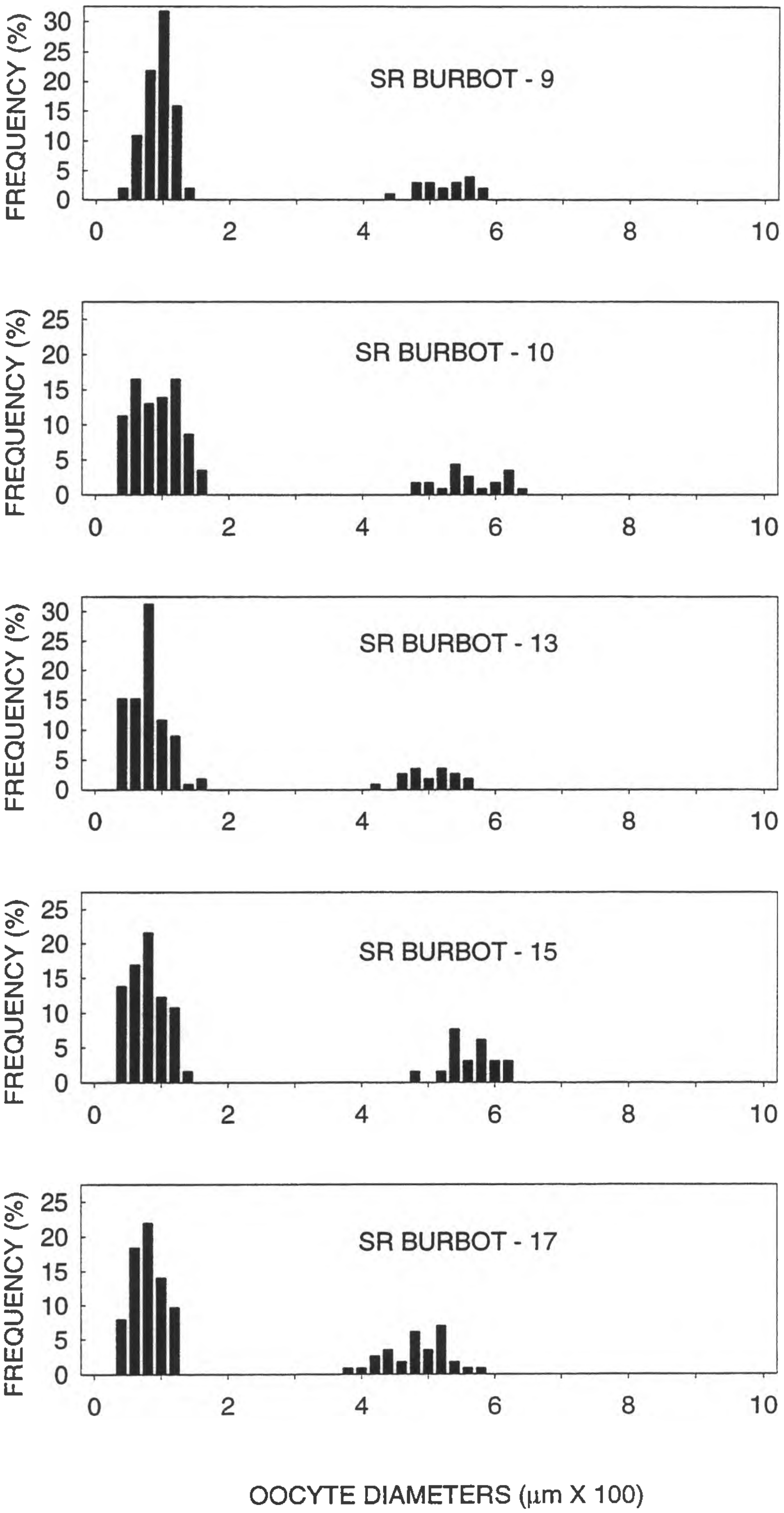

MCR2 BURBOT - 6
MR BURBOT - 1
P BURBOT - 1
P BURBOT - 2
P BURBOT - 3
FREQUENCY (%)
0
5
10
15
20
25
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

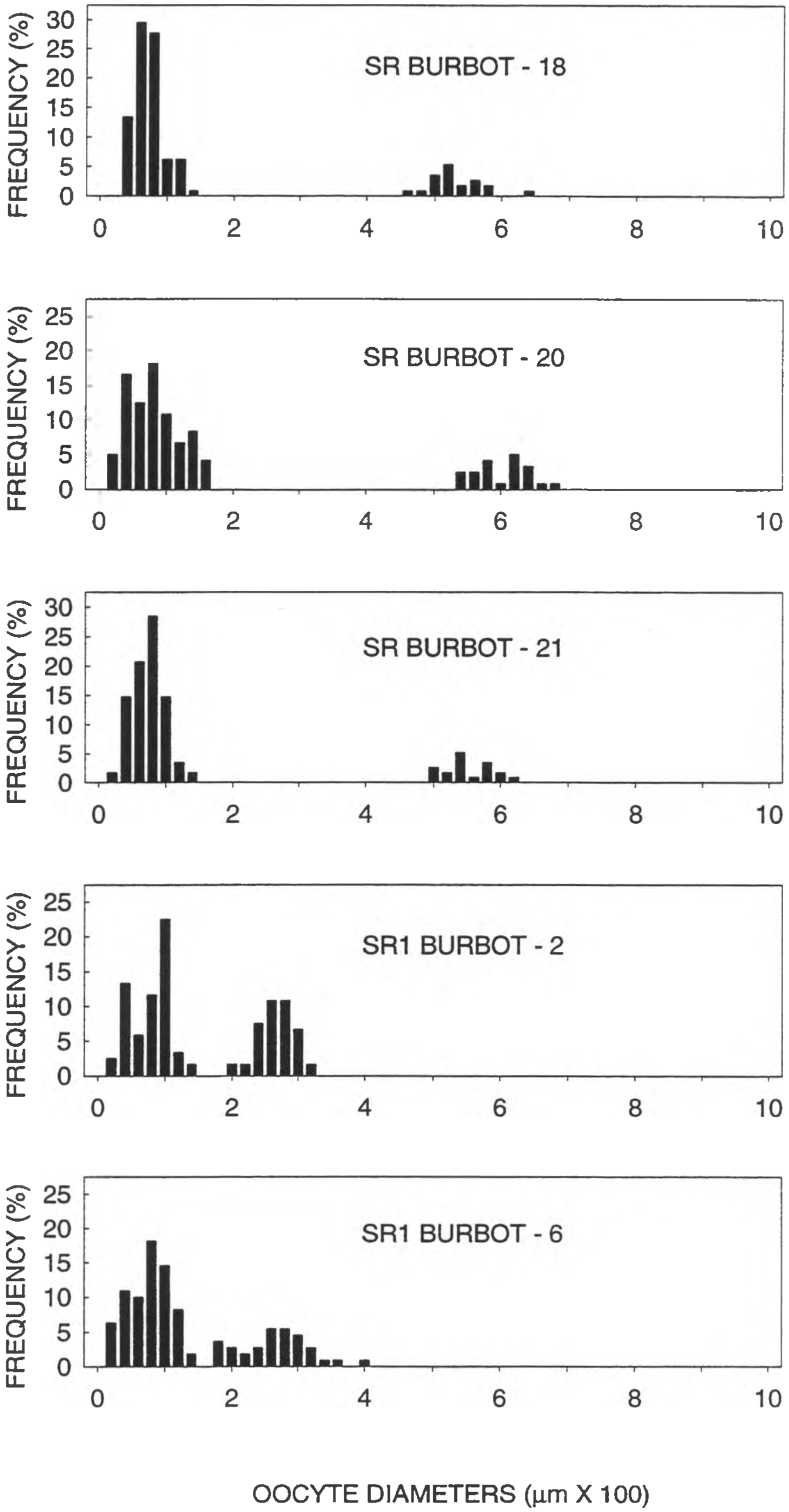

P BURBOT - 5
P BURBOT - 6
P BURBOT - 7
PR1 BURBOT - 2
PR1 BURBOT - 3
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

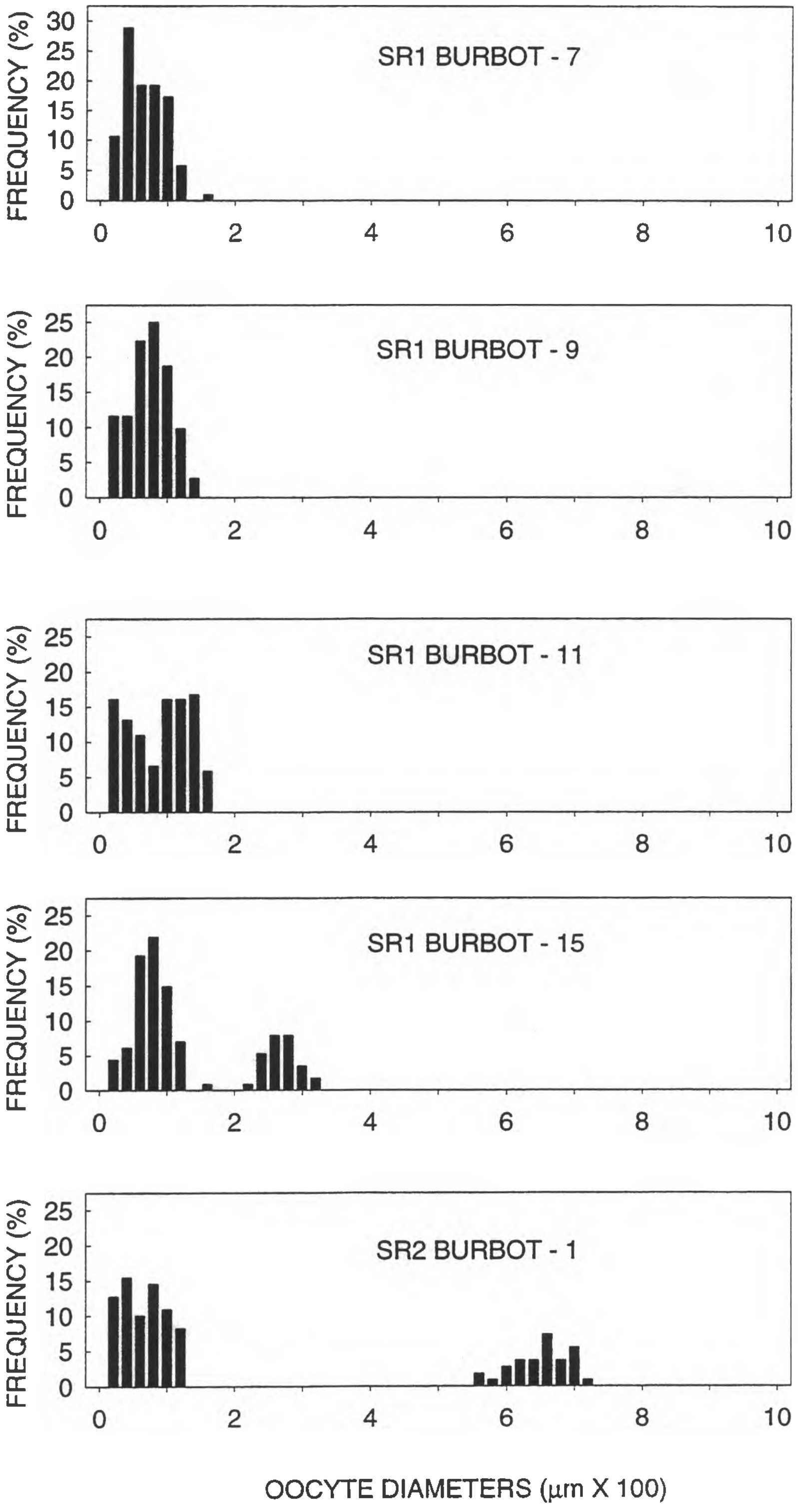

FREQUENCY (%)
PR1 BURBOT - 4
PR1 BURBOT - 6
PR1 BURBOT - 7
PR1 BURBOT - 9
PR2 BURBOT - 1
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

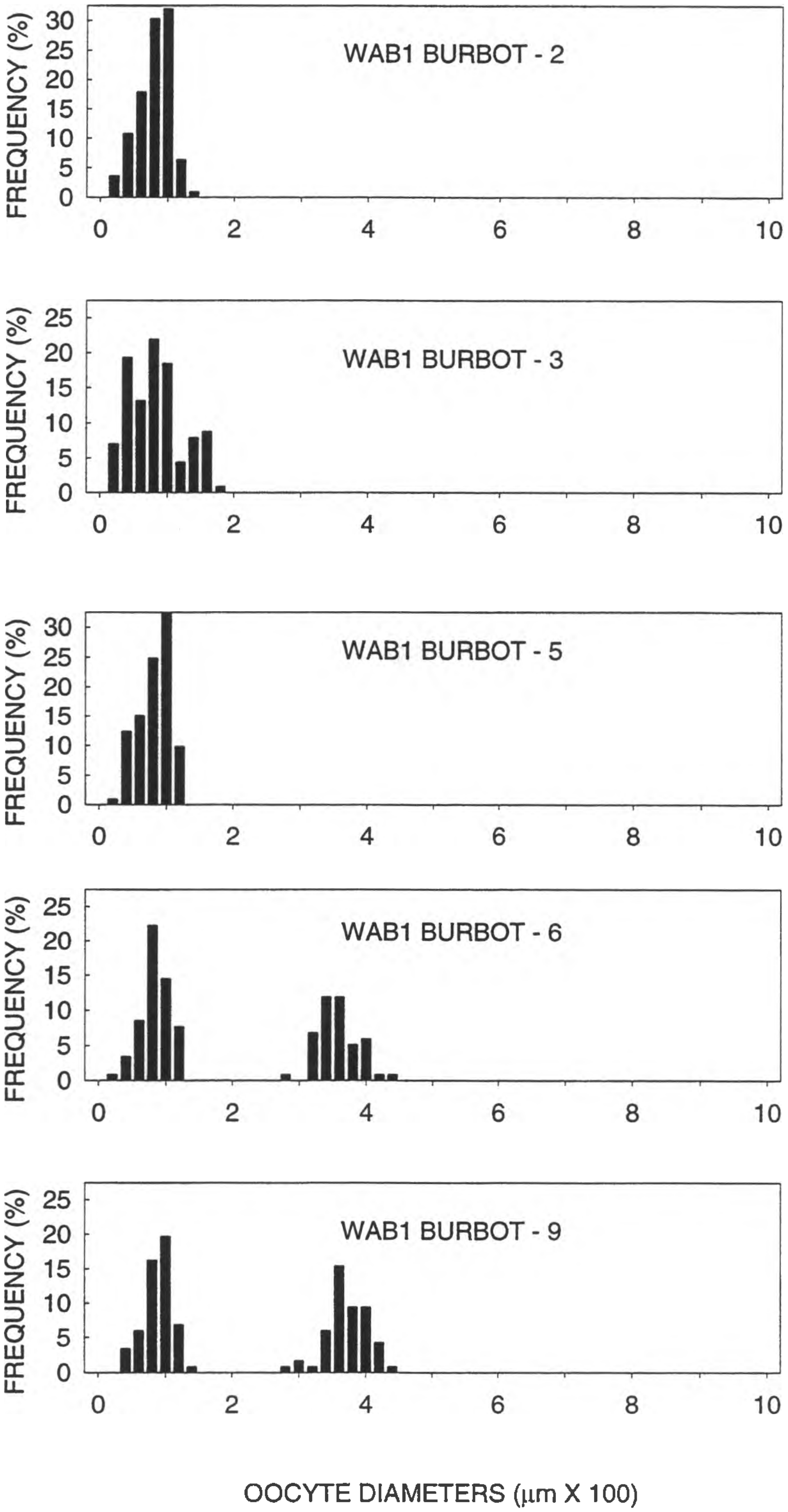

PR2 BURBOT - 6
PR2 BURBOT - 7
PR2 BURBOT - 10
PR3 BURBOT - 2
PR3 BURBOT - 3
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

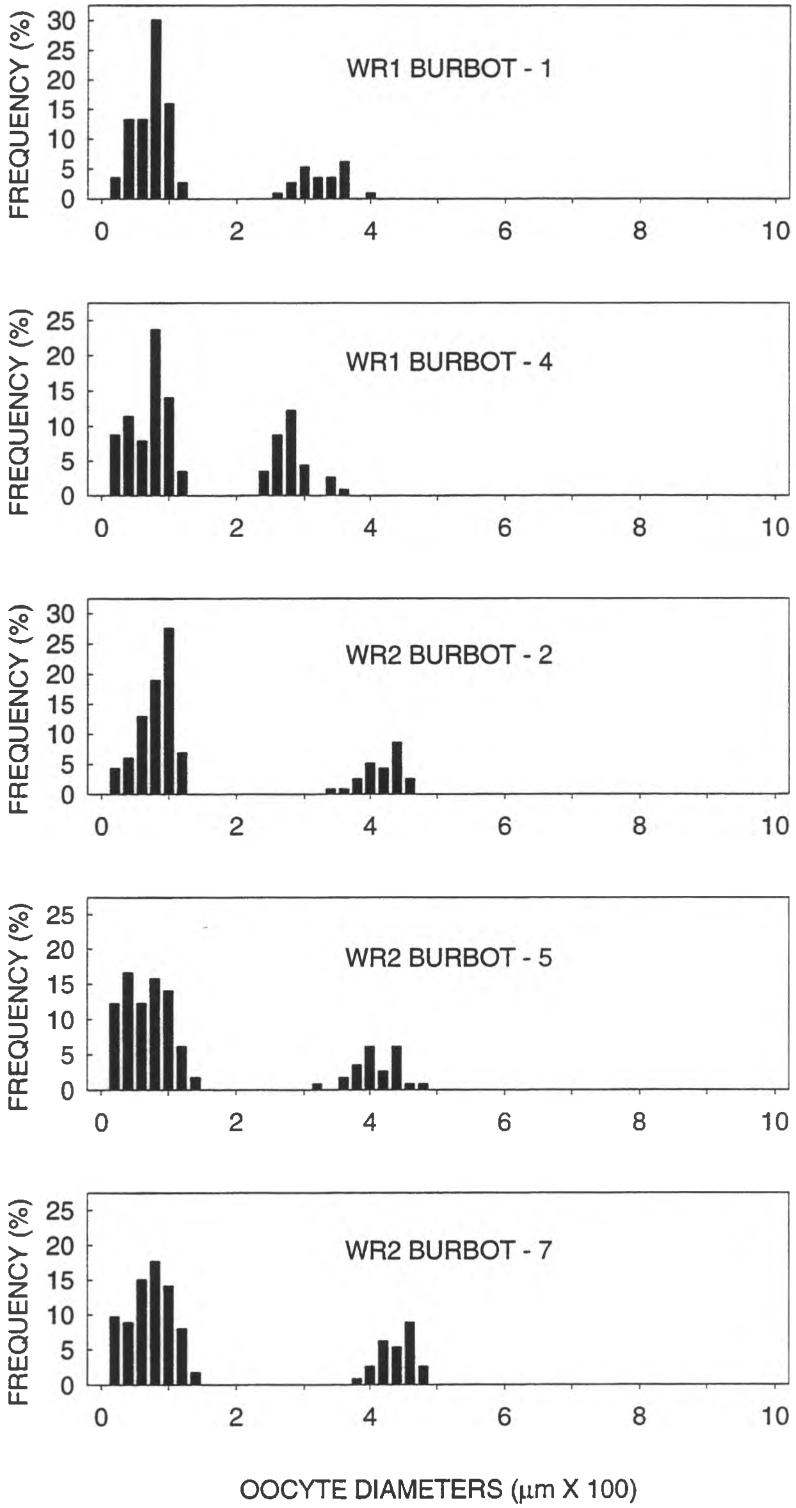

PR3 BURBOT - 4
PR3 BURBOT - 5
PR3 BURBOT - 8
SR BURBOT - 2
SR BURBOT - 3
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

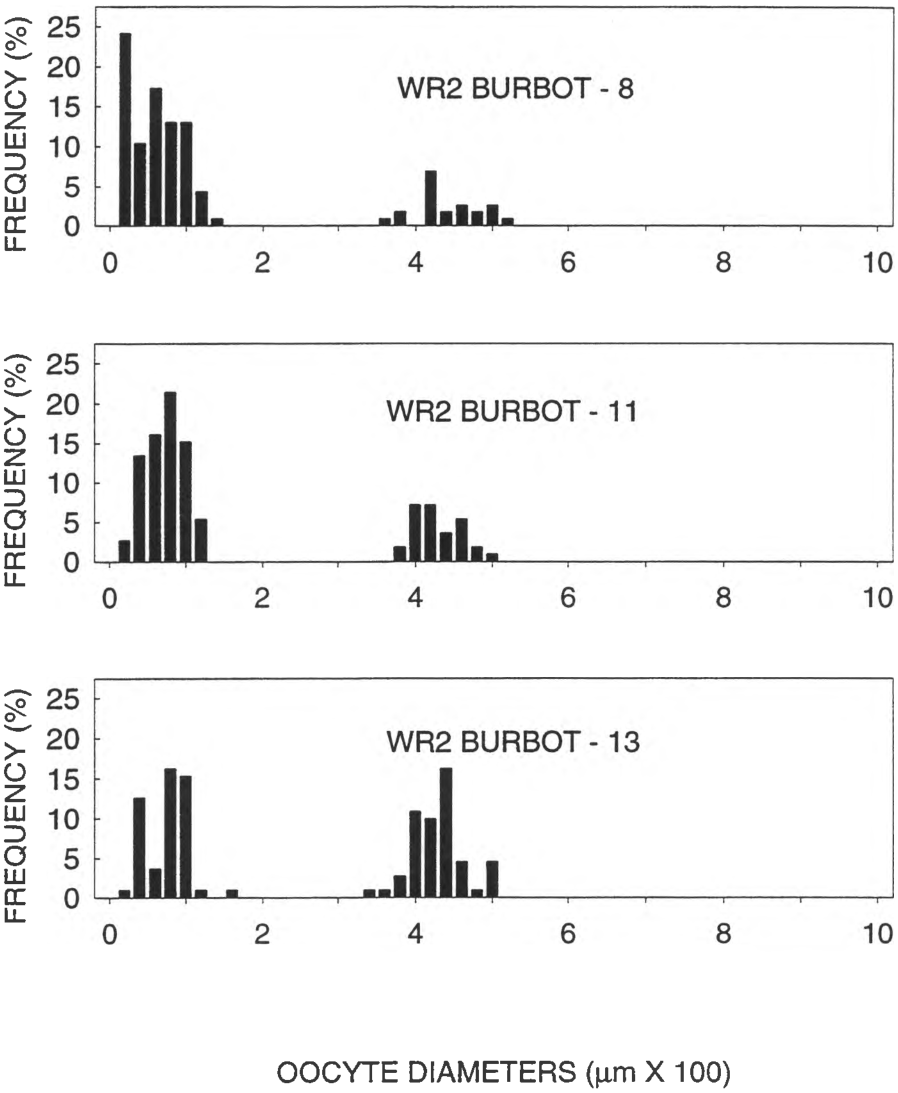

SR BURBOT - 9
SR BURBOT - 10
SR BURBOT - 13
SR BURBOT - 15
SR BURBOT - 17
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)


SR BURBOT - 18
SR BURBOT - 20
SR BURBOT - 21
SR1 BURBOT - 2
SR1 BURBOT - 6
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)


SR1 BURBOT - 7
SR1 BURBOT - 9
SR1 BURBOT - 11
SR1 BURBOT - 15
SR2 BURBOT - 1
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

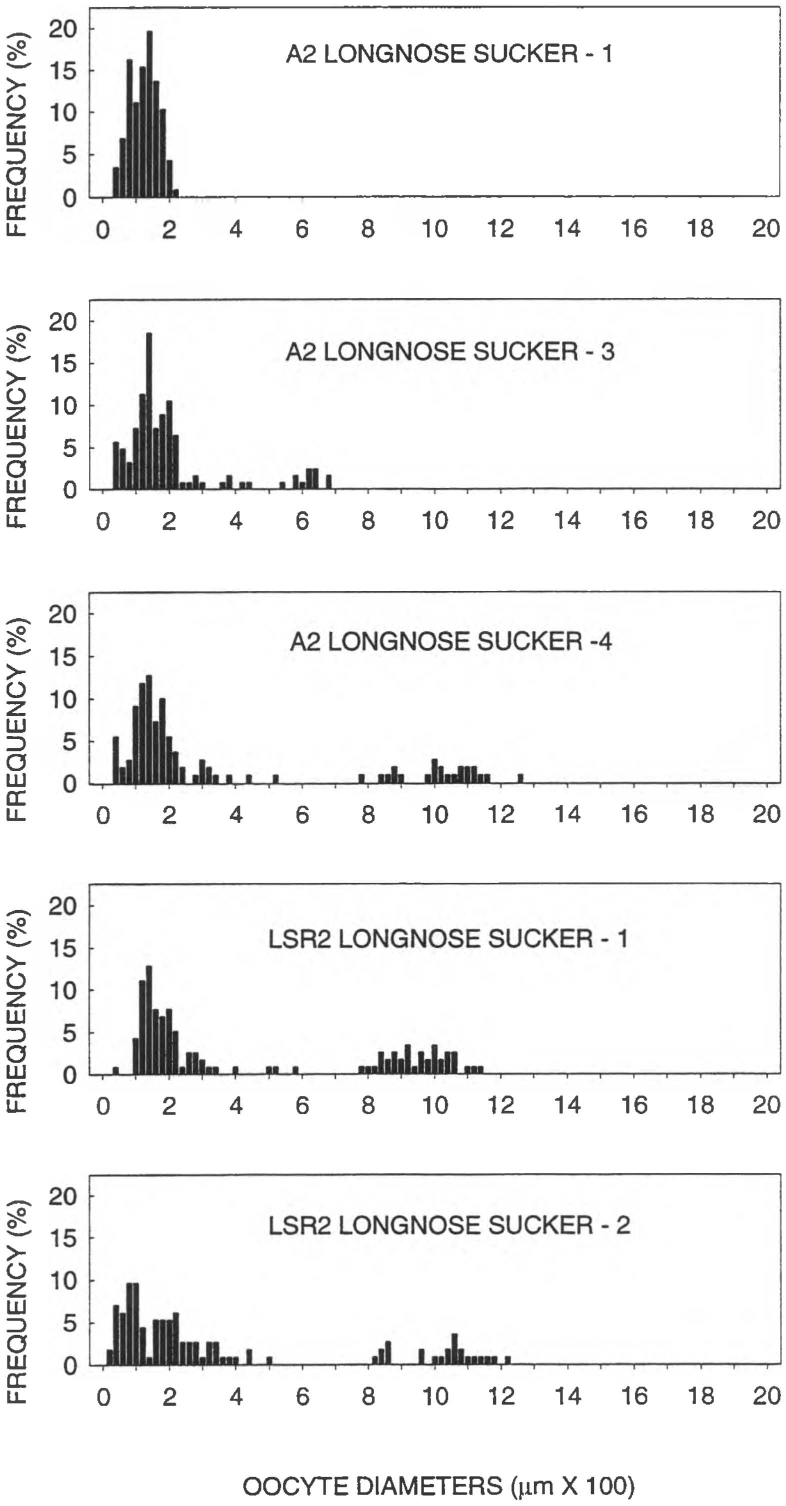

WAB1 BURBOT - 2
WAB1 BURBOT - 3
WAB1 BURBOT - 5
WAB1 BURBOT - 6
WAB1 BURBOT - 9
FREQUENCY (%)
0
5
10
15
20
25
30
0
2
4
6
8
10
OOCYTE DIAMETERS (μm X 100)

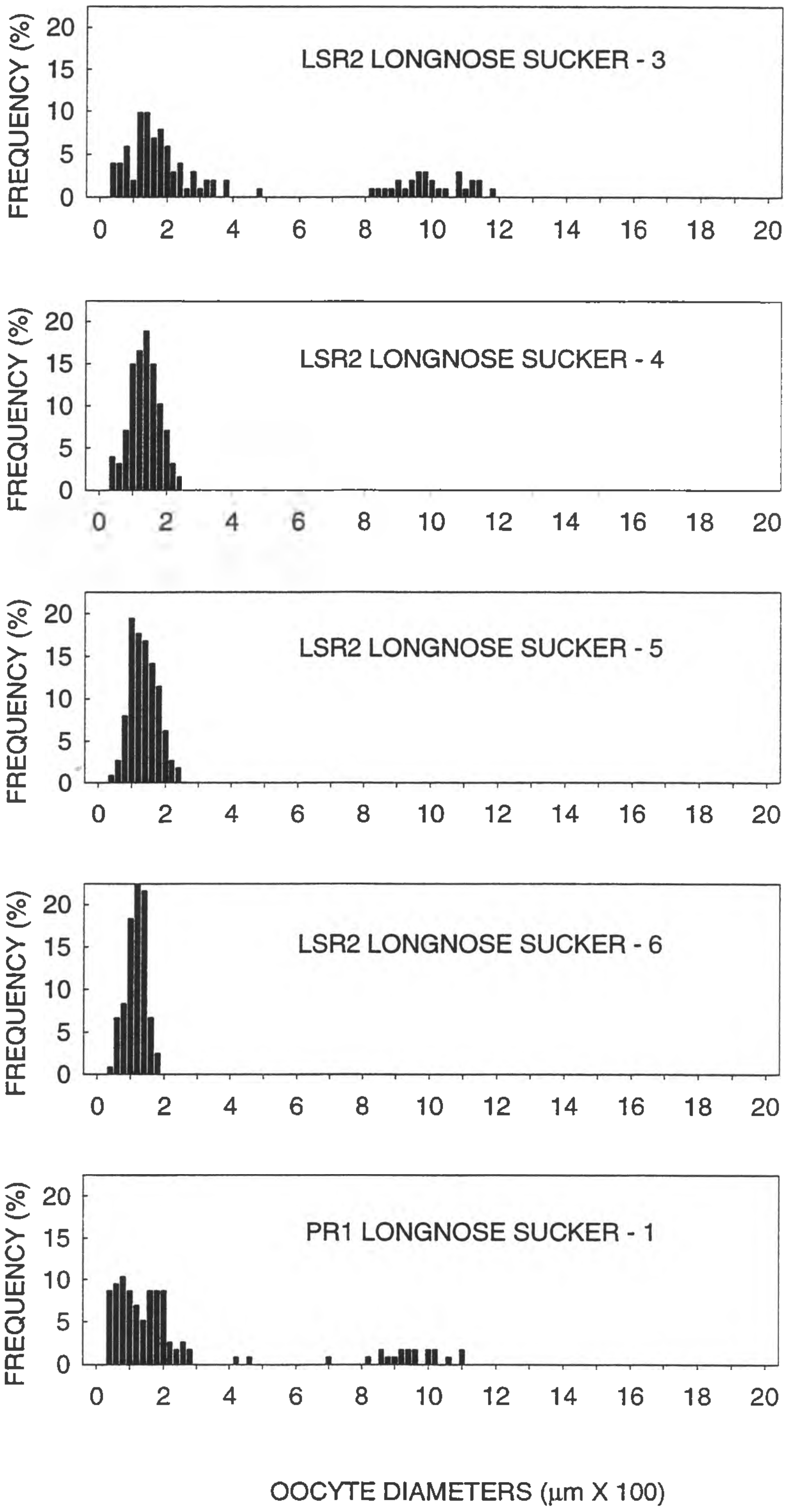

FREQUENCY (%)
30
25
20
15
10
5
0
WR1 BURBOT - 1
0
2
4
6
8
10
FREQUENCY (%)
25
20
15
10
5
0
WR1 BURBOT - 4
0
2
4
6
8
10
FREQUENCY (%)
30
25
20
15
10
5
0
WR2 BURBOT - 2
0
2
4
6
8
10
FREQUENCY (%)
25
20
15
10
5
0
WR2 BURBOT - 5
0
2
4
6
8
10
FREQUENCY (%)
25
20
15
10
5
0
WR2 BURBOT - 7
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)

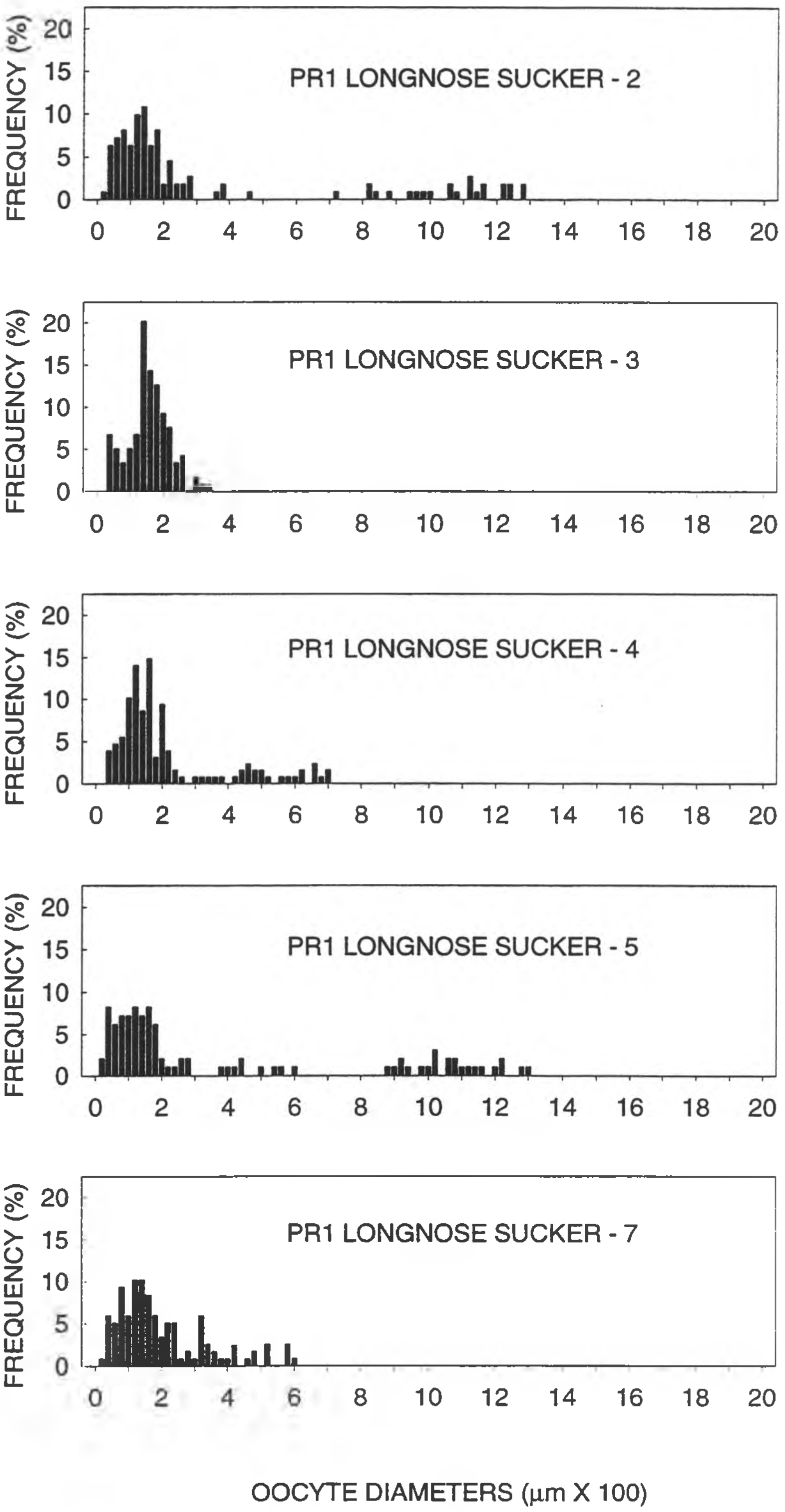

FREQUENCY (%)
25
20
15
10
5
0
WR2 BURBOT - 8
0
2
4
6
8
10
FREQUENCY (%)
25
20
15
10
5
0
WR2 BURBOT - 11
0
2
4
6
8
10
FREQUENCY (%)
25
20
15
10
5
0
WR2 BURBOT - 13
0
2
4
6
8
10
OOCYTE DIAMETERS (μm X 100)

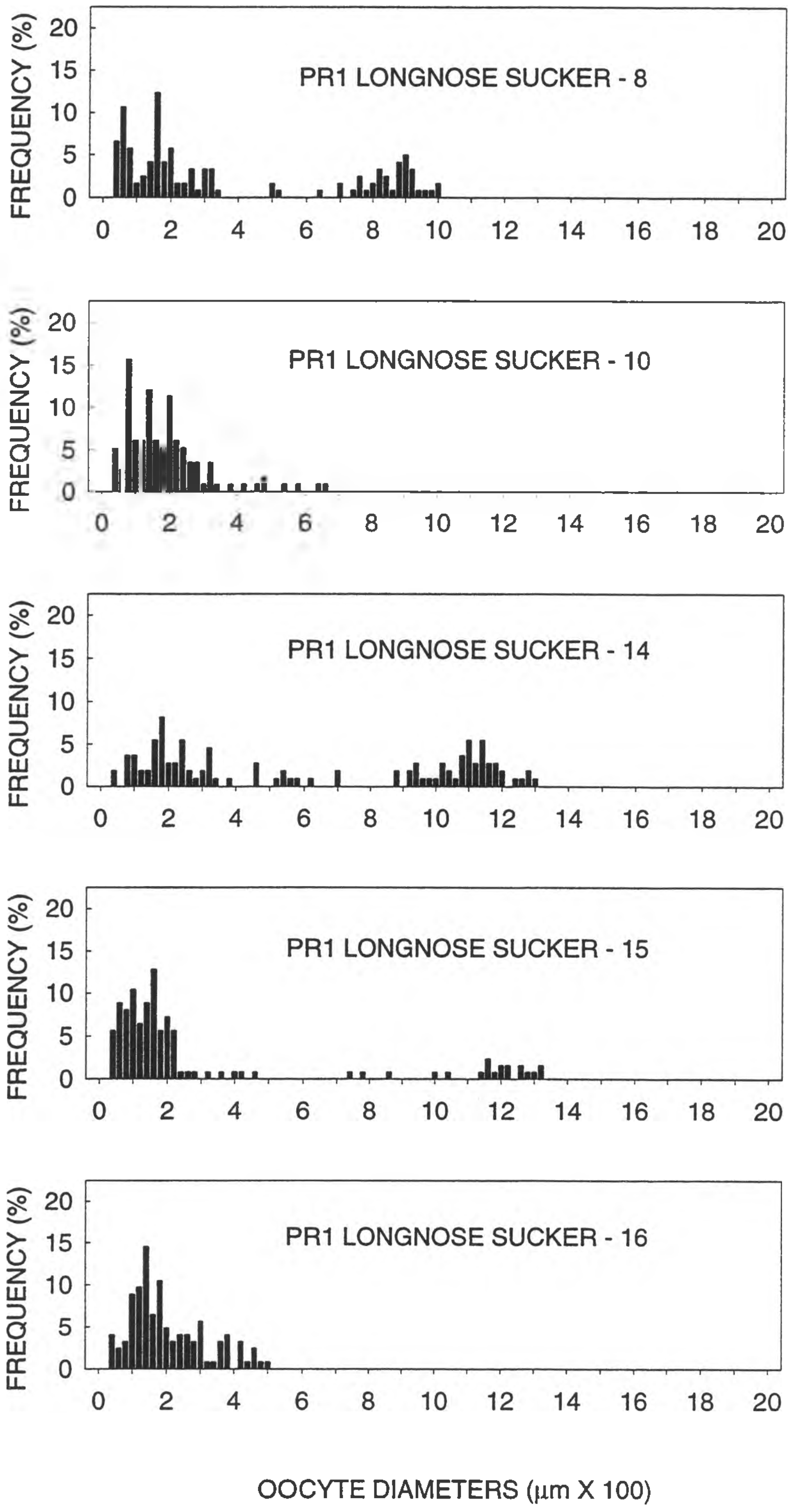
APPENDIX D

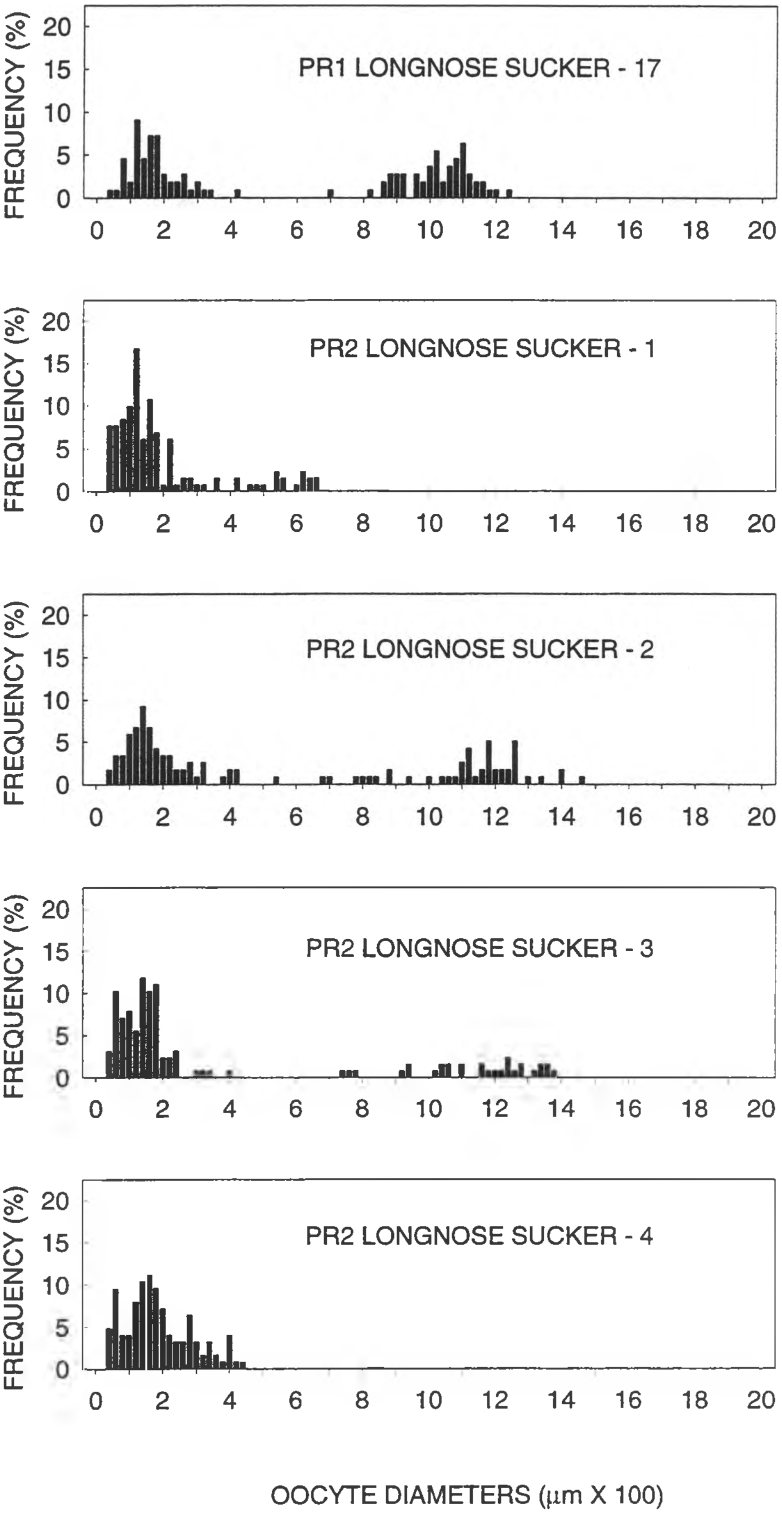
Longnose Sucker Oocyte Diameter Frequency Distributions

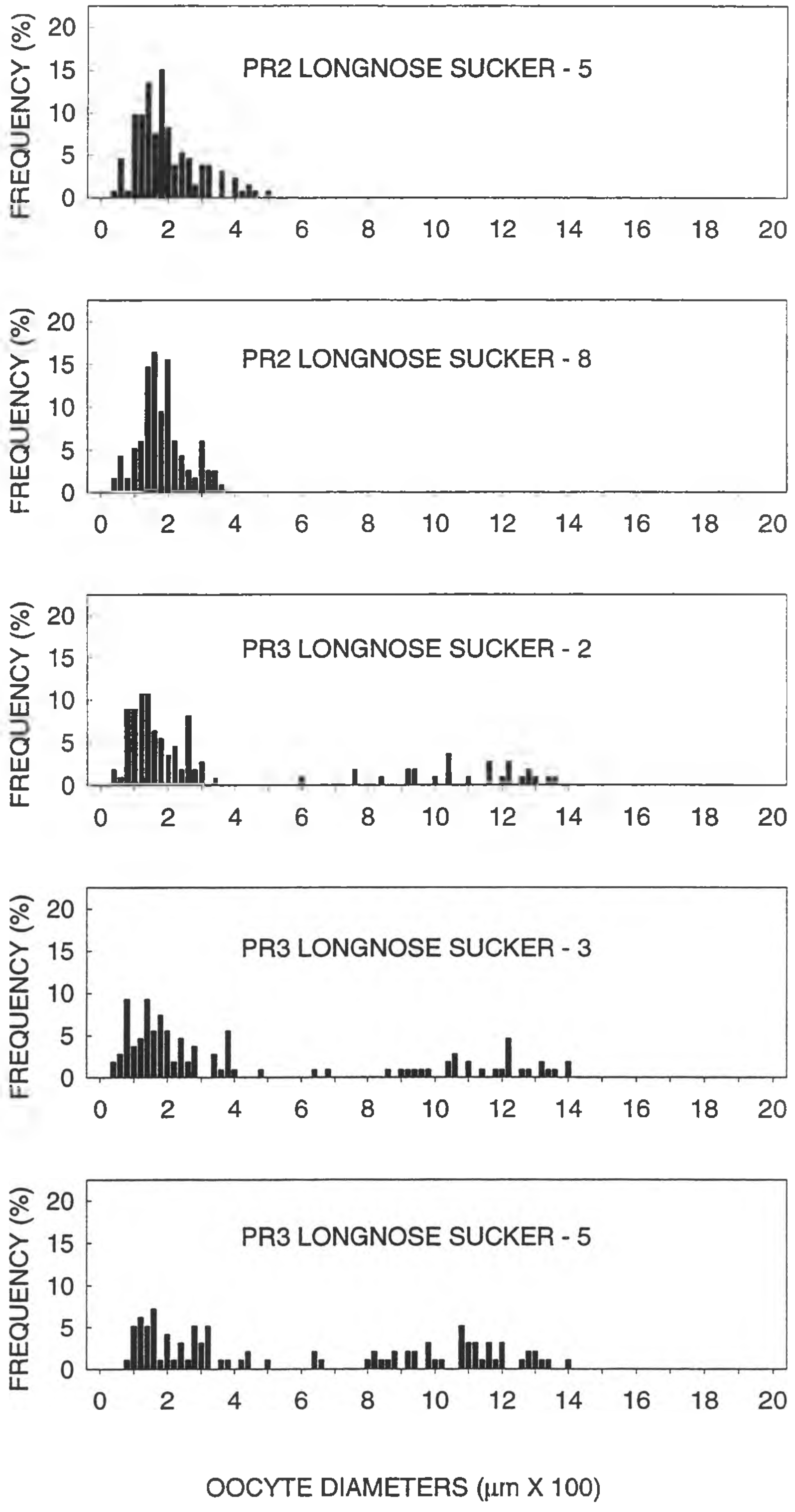

A2 LONGNOSE SUCKER - 1
A2 LONGNOSE SUCKER - 3
A2 LONGNOSE SUCKER -4
LSR2 LONGNOSE SUCKER - 1
LSR2 LONGNOSE SUCKER - 2
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
20
OOCYTE DIAMETERS (µm X 100)

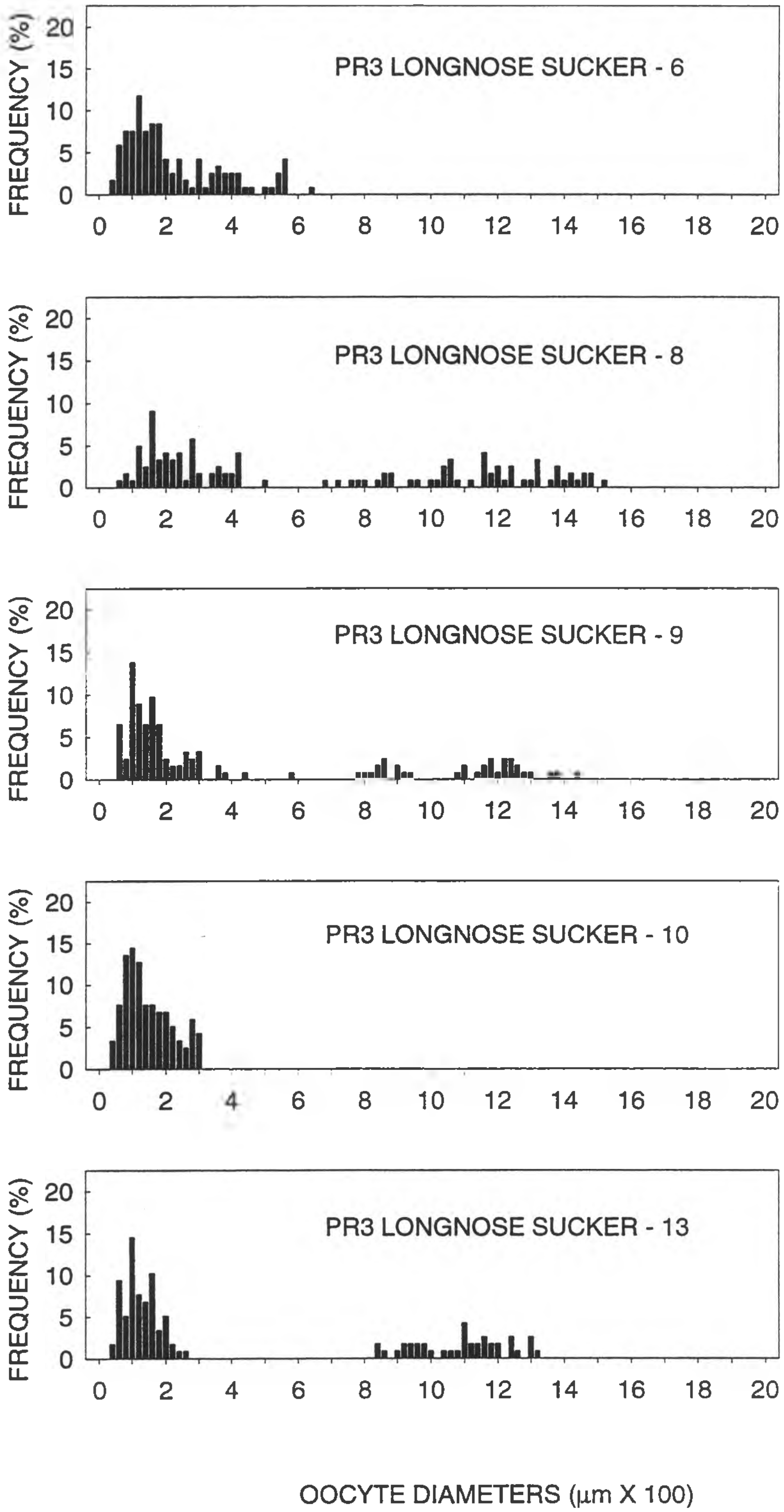

LSR2 LONGNOSE SUCKER - 3
LSR2 LONGNOSE SUCKER - 4
LSR2 LONGNOSE SUCKER - 5
LSR2 LONGNOSE SUCKER - 6
PR1 LONGNOSE SUCKER - 1
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
20
OOCYTE DIAMETERS (µm X 100)

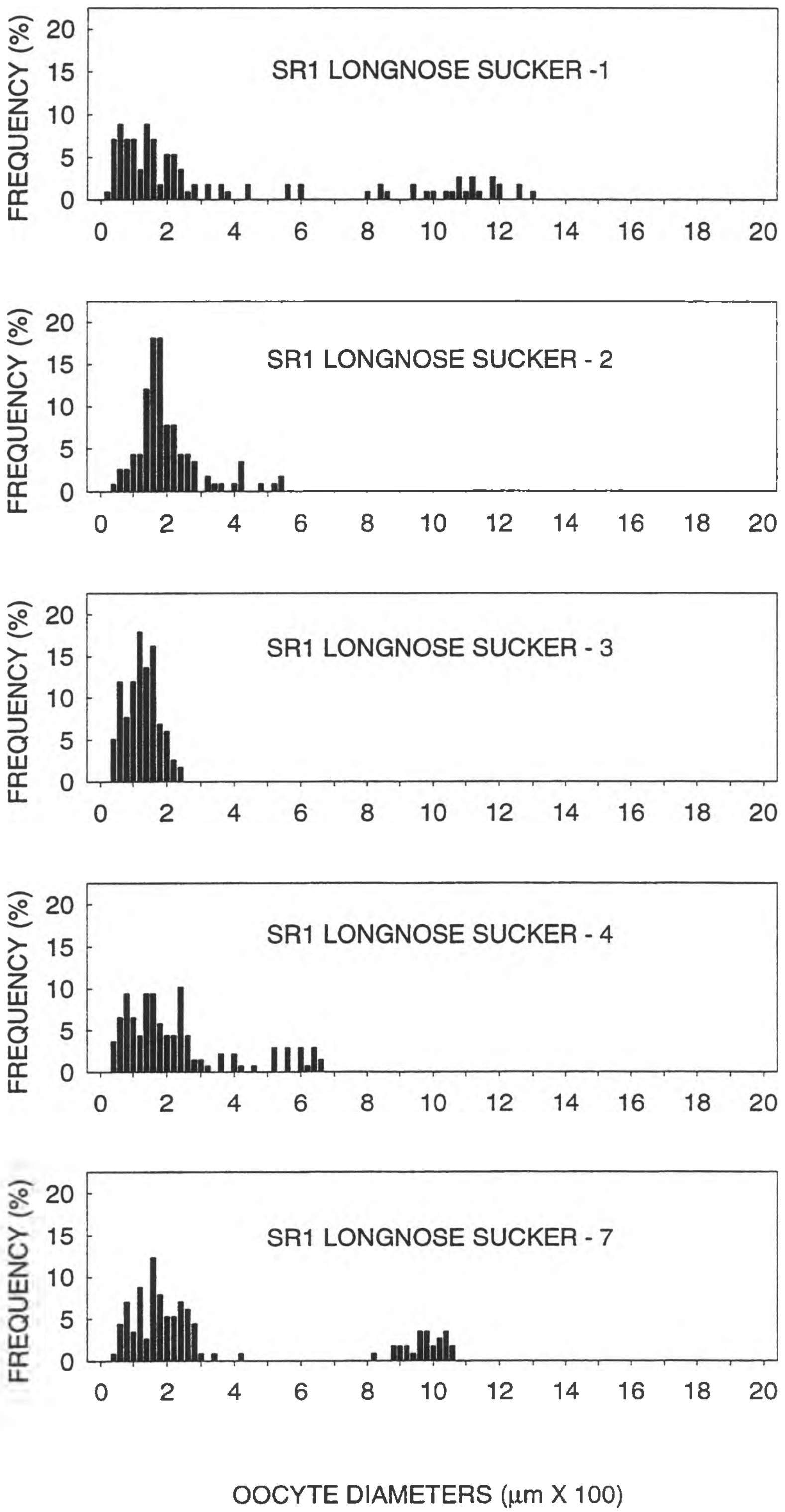

PR1 LONGNOSE SUCKER - 2
PR1 LONGNOSE SUCKER - 3
PR1 LONGNOSE SUCKER - 4
PR1 LONGNOSE SUCKER - 5
PR1 LONGNOSE SUCKER - 7
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
20
OOCYTE DIAMETERS (µm X 100)

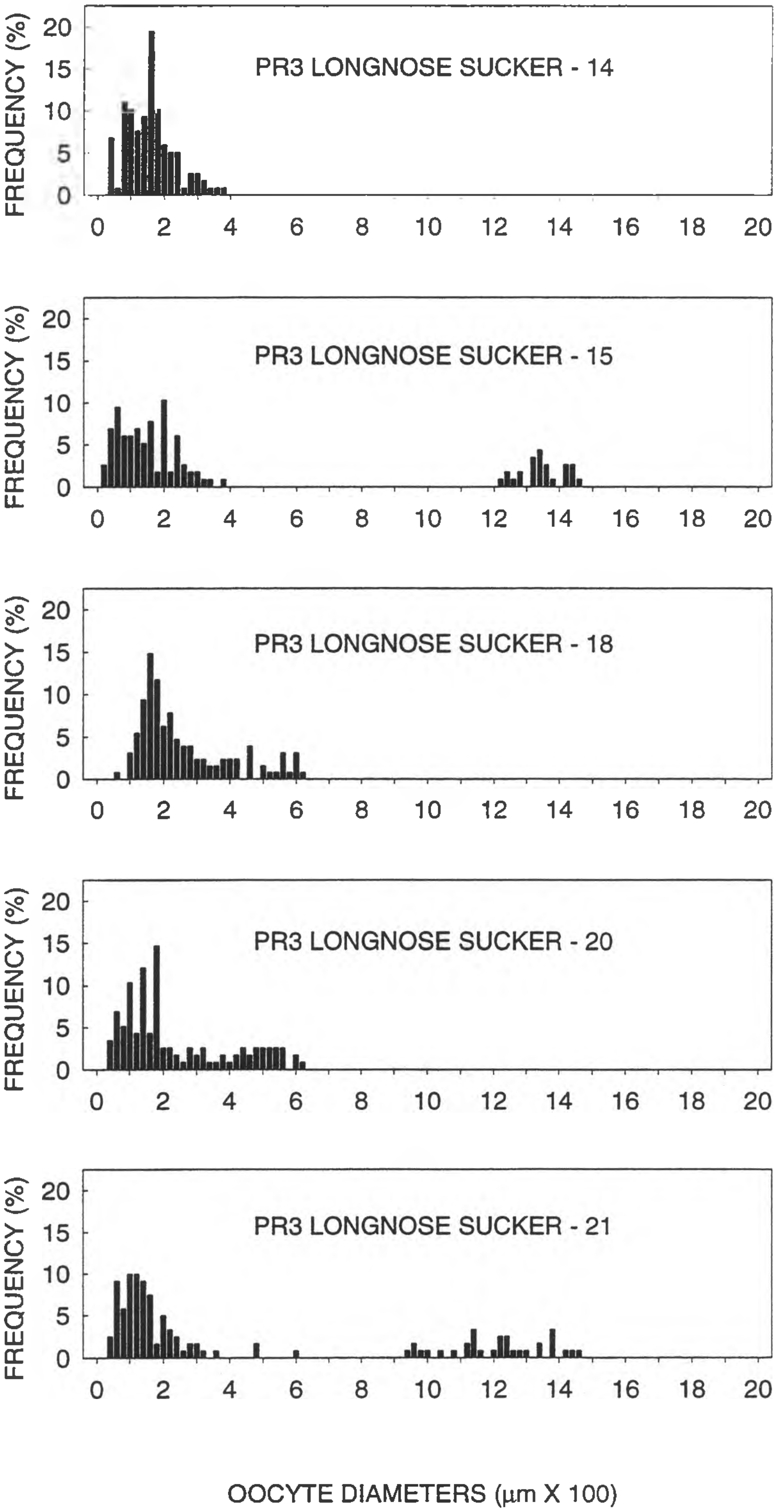

PR1 LONGNOSE SUCKER - 8
PR1 LONGNOSE SUCKER - 10
PR1 LONGNOSE SUCKER - 14
PR1 LONGNOSE SUCKER - 15
PR1 LONGNOSE SUCKER - 16
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
20
OOCYTE DIAMETERS (µm X 100)

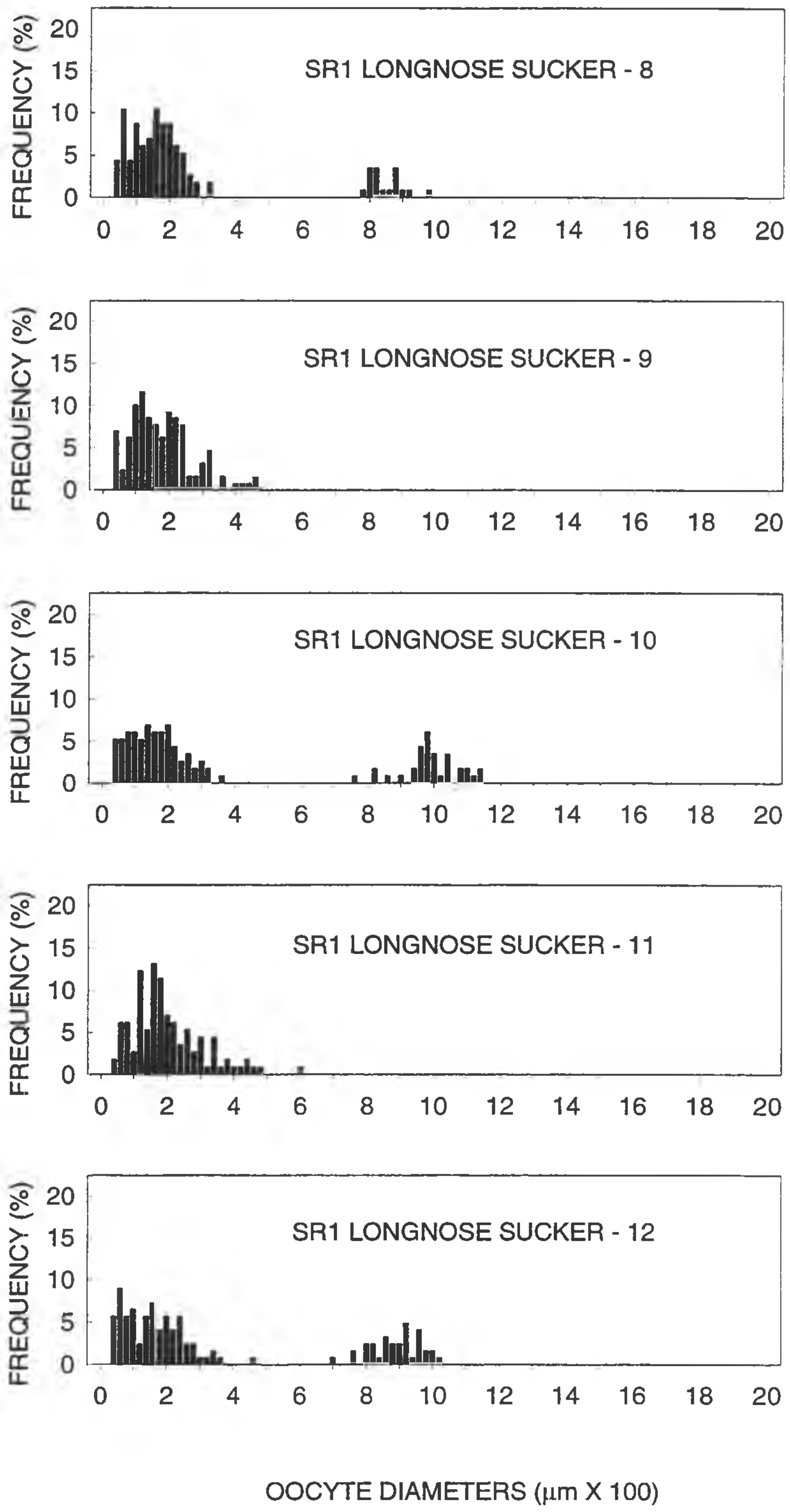

PR1 LONGNOSE SUCKER - 17
PR2 LONGNOSE SUCKER - 1
PR2 LONGNOSE SUCKER - 2
PR2 LONGNOSE SUCKER - 3
PR2 LONGNOSE SUCKER - 4
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
20
OOCYTE DIAMETERS (μm X 100)

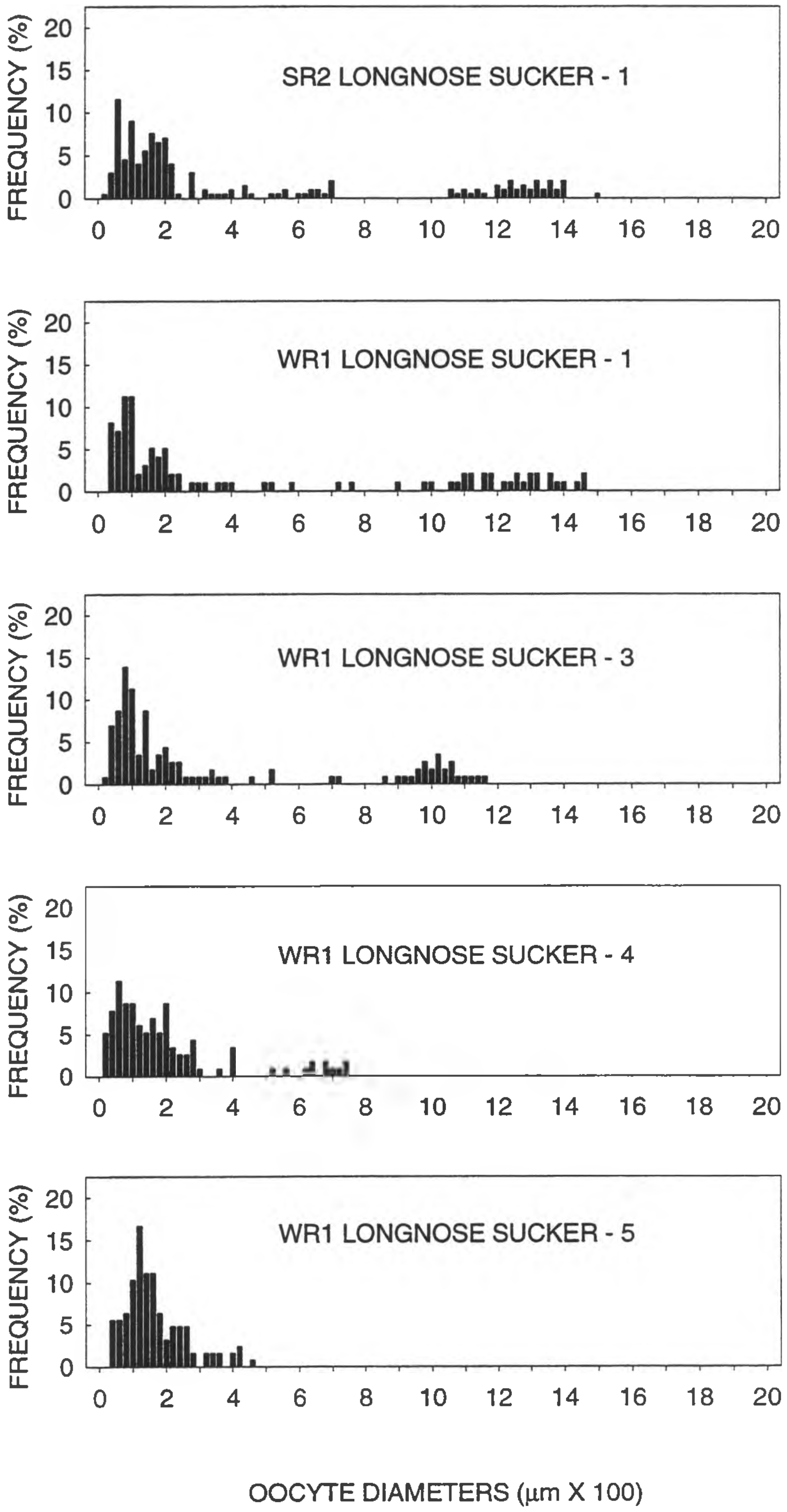

PR2 LONGNOSE SUCKER - 5
PR2 LONGNOSE SUCKER - 8
PR3 LONGNOSE SUCKER - 2
PR3 LONGNOSE SUCKER - 3
PR3 LONGNOSE SUCKER - 5
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
20
OOCYTE DIAMETERS (µm X 100)

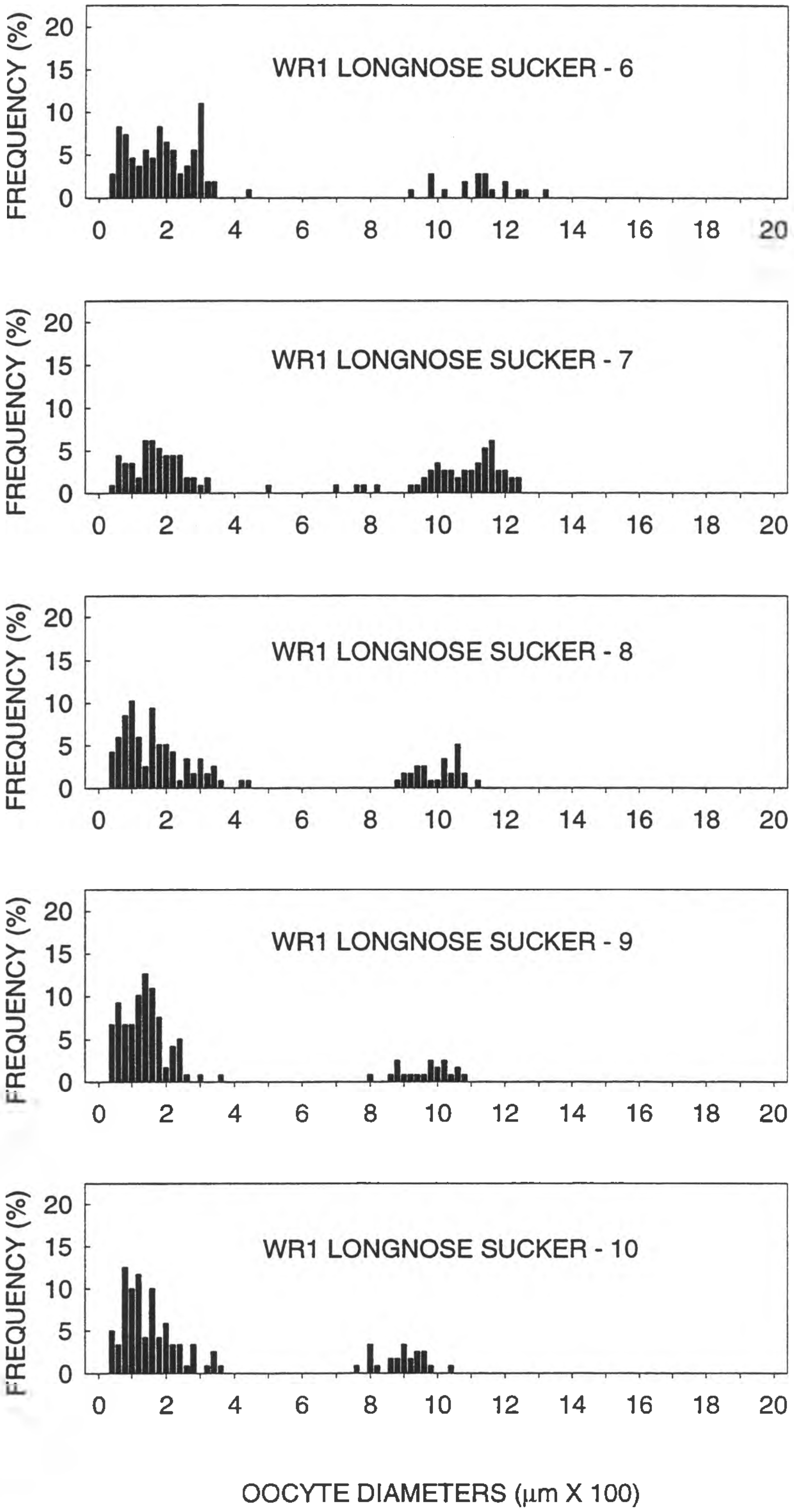

PR3 LONGNOSE SUCKER - 6
PR3 LONGNOSE SUCKER - 8
PR3 LONGNOSE SUCKER - 9
PR3 LONGNOSE SUCKER - 10
PR3 LONGNOSE SUCKER - 13
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
20
OOCYTE DIAMETERS (µm X 100)


SR1 LONGNOSE SUCKER -1
SR1 LONGNOSE SUCKER - 2
SR1 LONGNOSE SUCKER - 3
SR1 LONGNOSE SUCKER - 4
SR1 LONGNOSE SUCKER - 7
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
20
OOCYTE DIAMETERS (µm X 100)


FREQUENCY (%)
0
5
10
15
20
PR3 LONGNOSE SUCKER - 14
PR3 LONGNOSE SUCKER - 15
PR3 LONGNOSE SUCKER - 18
PR3 LONGNOSE SUCKER - 20
PR3 LONGNOSE SUCKER - 21
0
2
4
6
8
10
12
14
16
18
20
OOCYTE DIAMETERS (µm X 100)

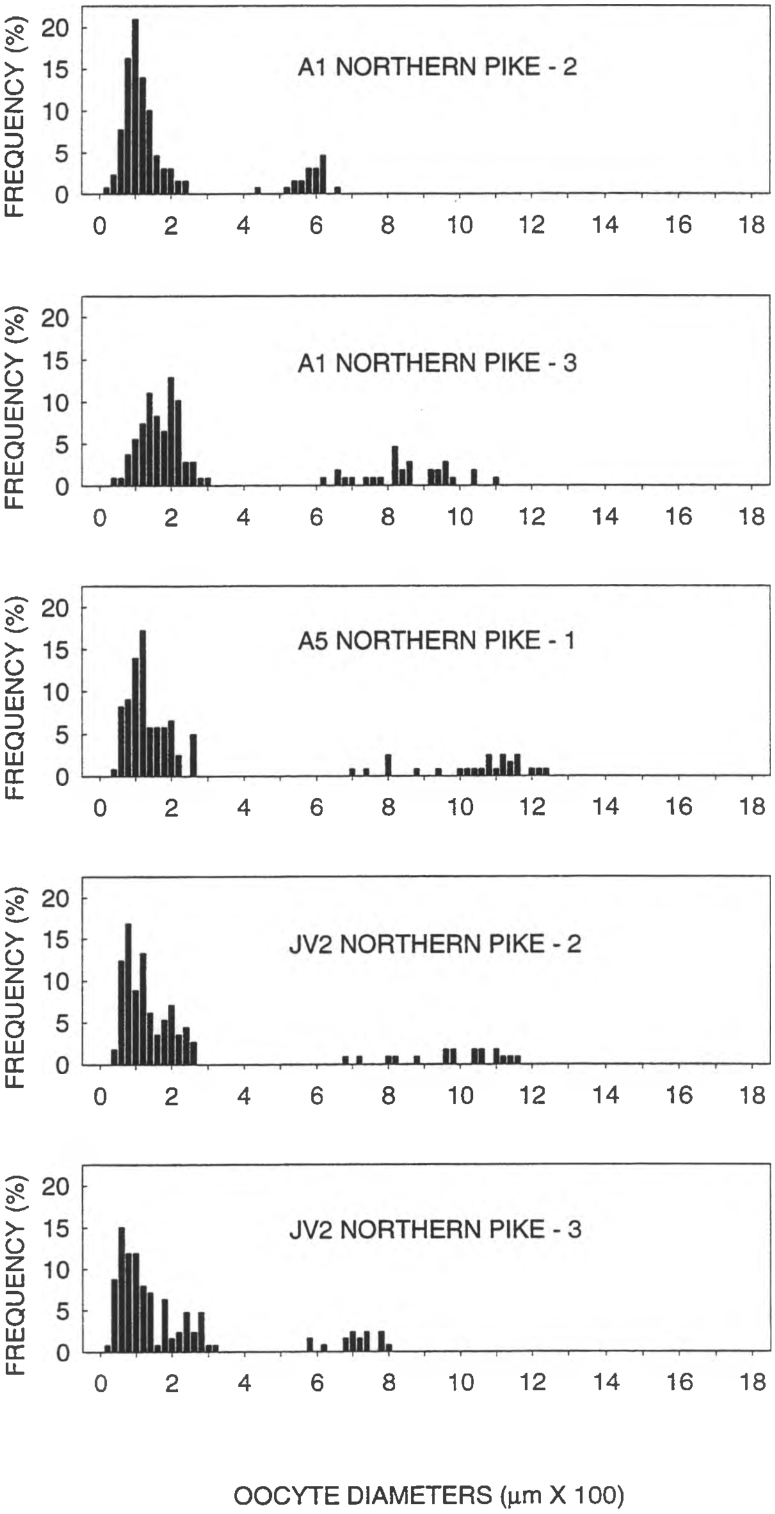

SR1 LONGNOSE SUCKER - 8
SR1 LONGNOSE SUCKER - 9
SR1 LONGNOSE SUCKER - 10
SR1 LONGNOSE SUCKER - 11
SR1 LONGNOSE SUCKER - 12
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
20
OOCYTE DIAMETERS (µm X 100)

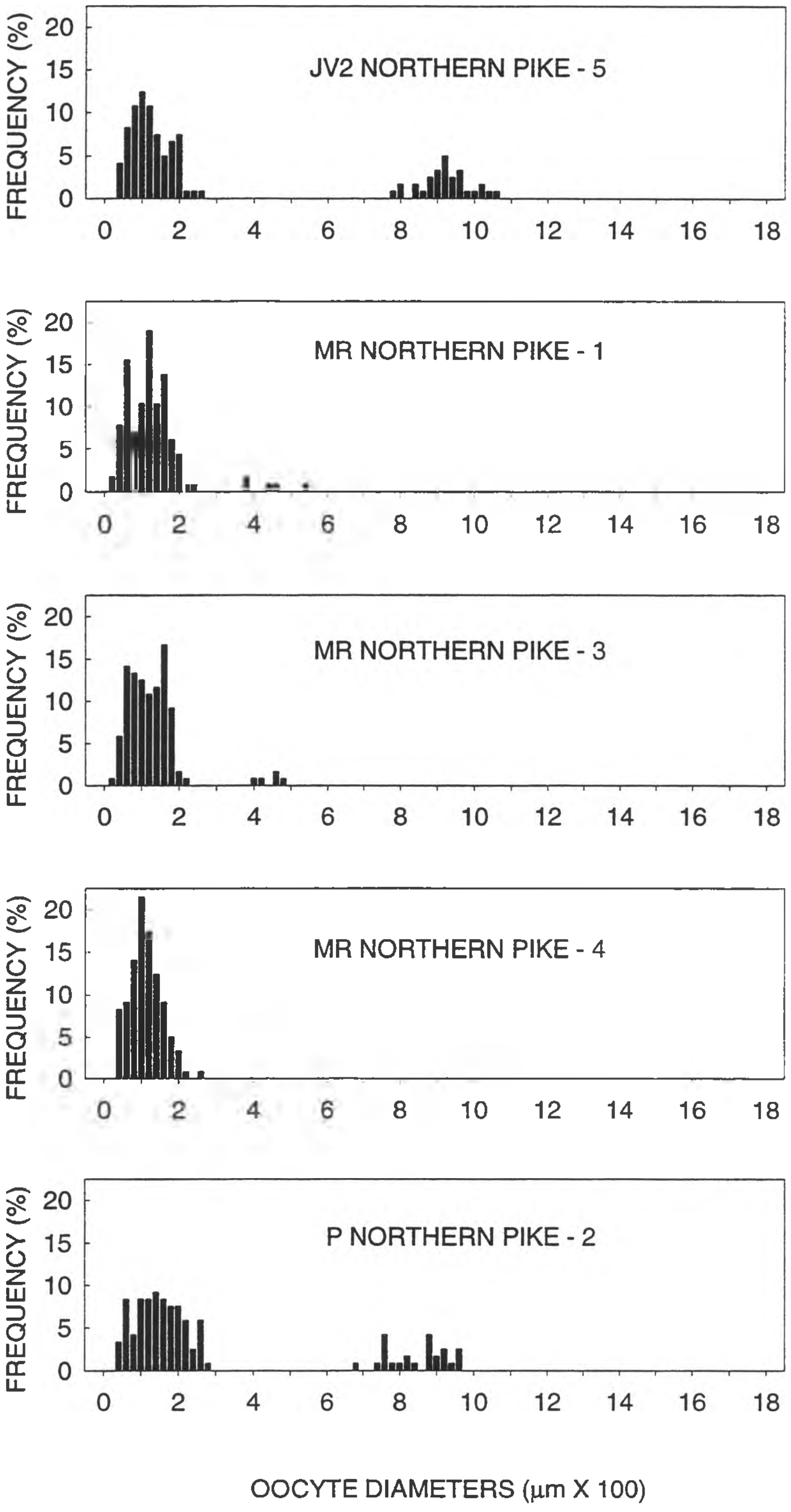

SR2 LONGNOSE SUCKER - 1
WR1 LONGNOSE SUCKER - 1
WR1 LONGNOSE SUCKER - 3
WR1 LONGNOSE SUCKER - 4
WR1 LONGNOSE SUCKER - 5
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
20
OOCYTE DIAMETERS (µm X 100)

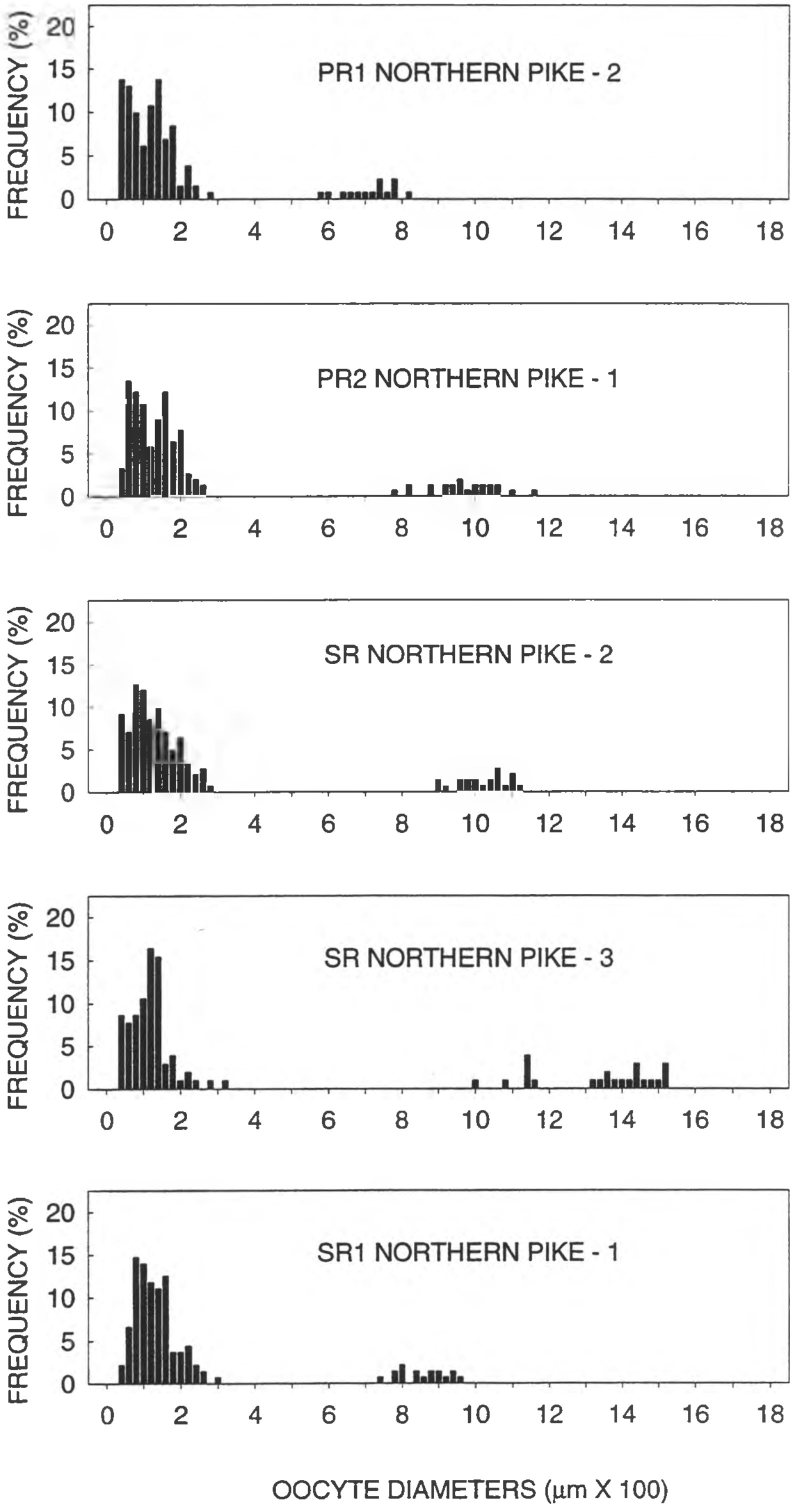

WR1 LONGNOSE SUCKER - 6
WR1 LONGNOSE SUCKER - 7
WR1 LONGNOSE SUCKER - 8
WR1 LONGNOSE SUCKER - 9
WR1 LONGNOSE SUCKER - 10
FREQUENCY (%)
0
5
10
15
20
0 2 4 6 8 10 12 14 16 18 20
OOCYTE DIAMETERS (µm X 100)

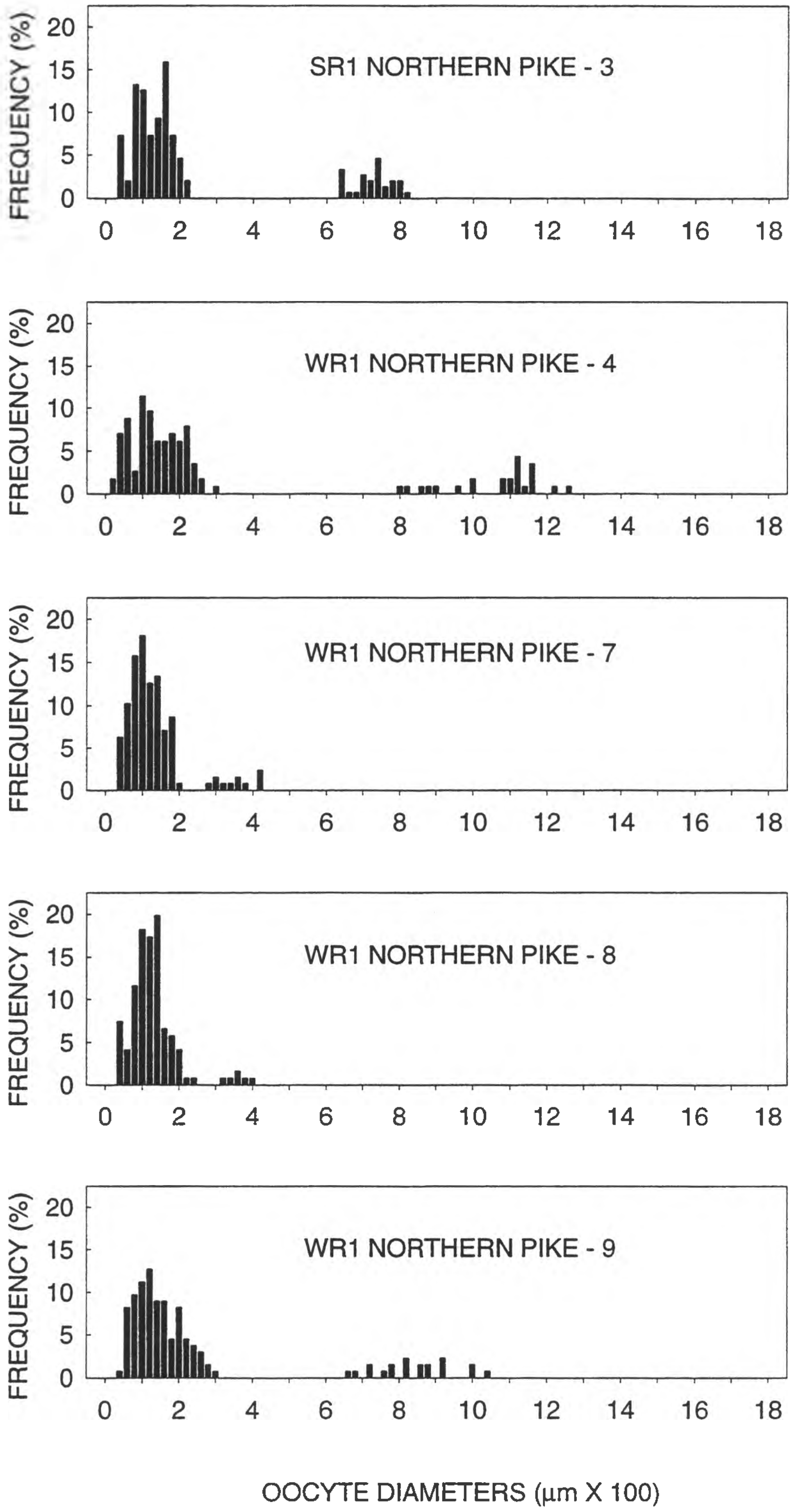
APPENDIX E

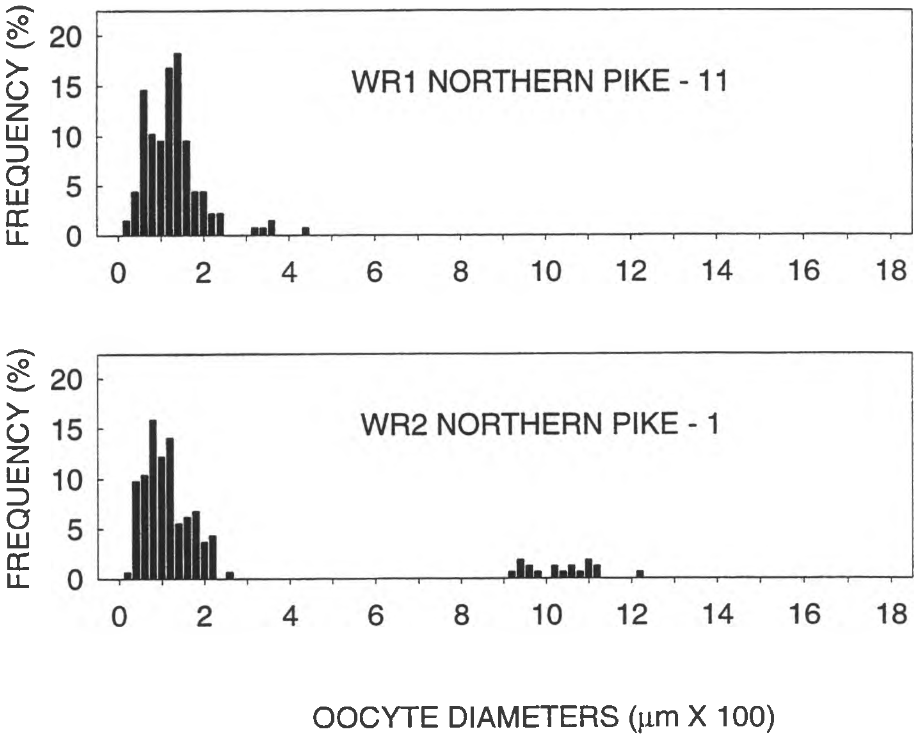
Northern Pike
Oocyte Diameter
Frequency Distributions


A1 NORTHERN PIKE - 2
A1 NORTHERN PIKE - 3
A5 NORTHERN PIKE - 1
JV2 NORTHERN PIKE - 2
JV2 NORTHERN PIKE - 3
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
OOCYTE DIAMETERS (µm X 100)


JV2 NORTHERN PIKE - 5
MR NORTHERN PIKE - 1
MR NORTHERN PIKE - 3
MR NORTHERN PIKE - 4
P NORTHERN PIKE - 2
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
OOCYTE DIAMETERS (µm X 100)

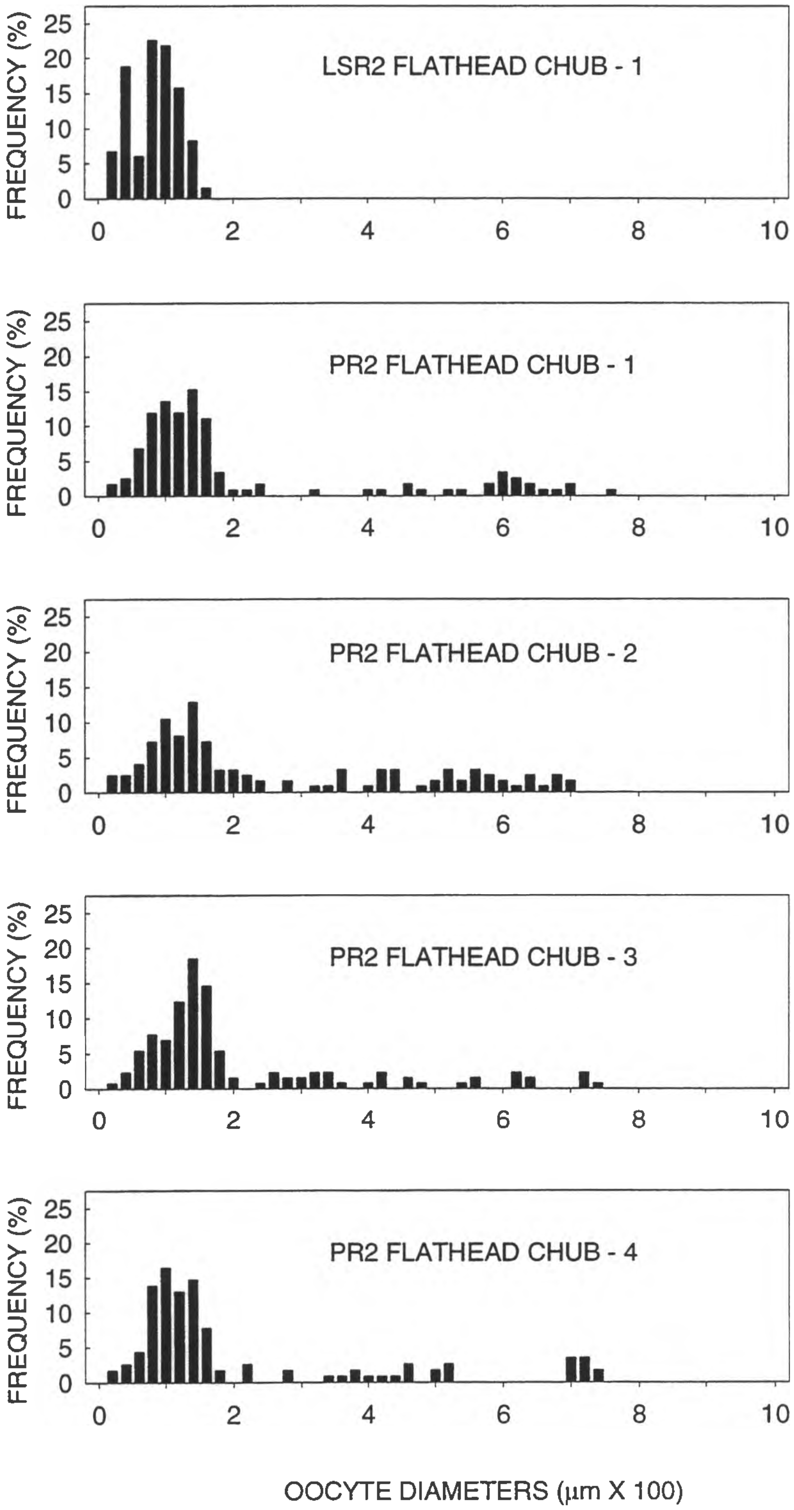

PR1 NORTHERN PIKE - 2
PR2 NORTHERN PIKE - 1
SR NORTHERN PIKE - 2
SR NORTHERN PIKE - 3
SR1 NORTHERN PIKE - 1
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
OOCYTE DIAMETERS (µm X 100)

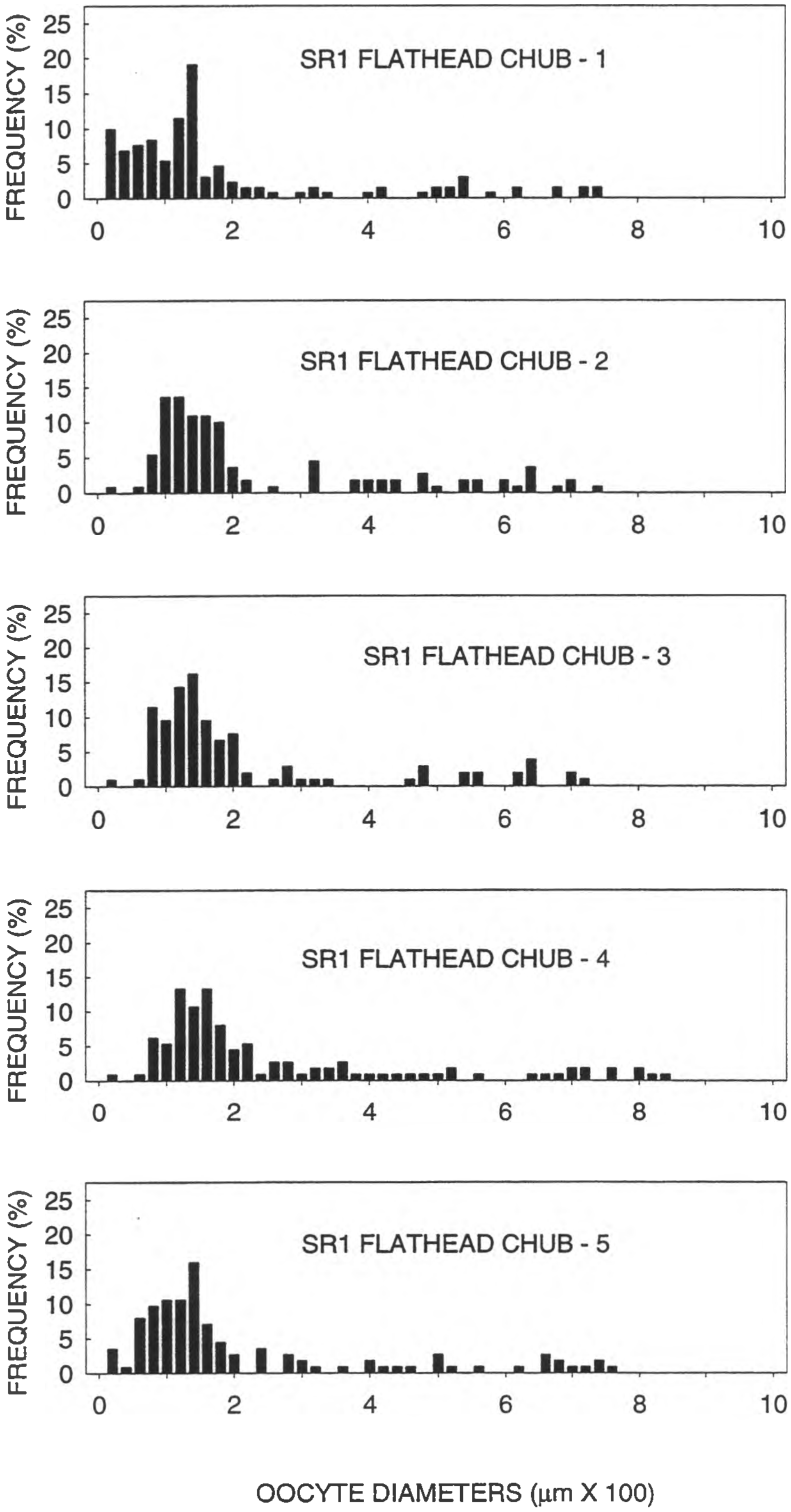

SR1 NORTHERN PIKE - 3
WR1 NORTHERN PIKE - 4
WR1 NORTHERN PIKE - 7
WR1 NORTHERN PIKE - 8
WR1 NORTHERN PIKE - 9
FREQUENCY (%)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
OOCYTE DIAMETERS (µm X 100)

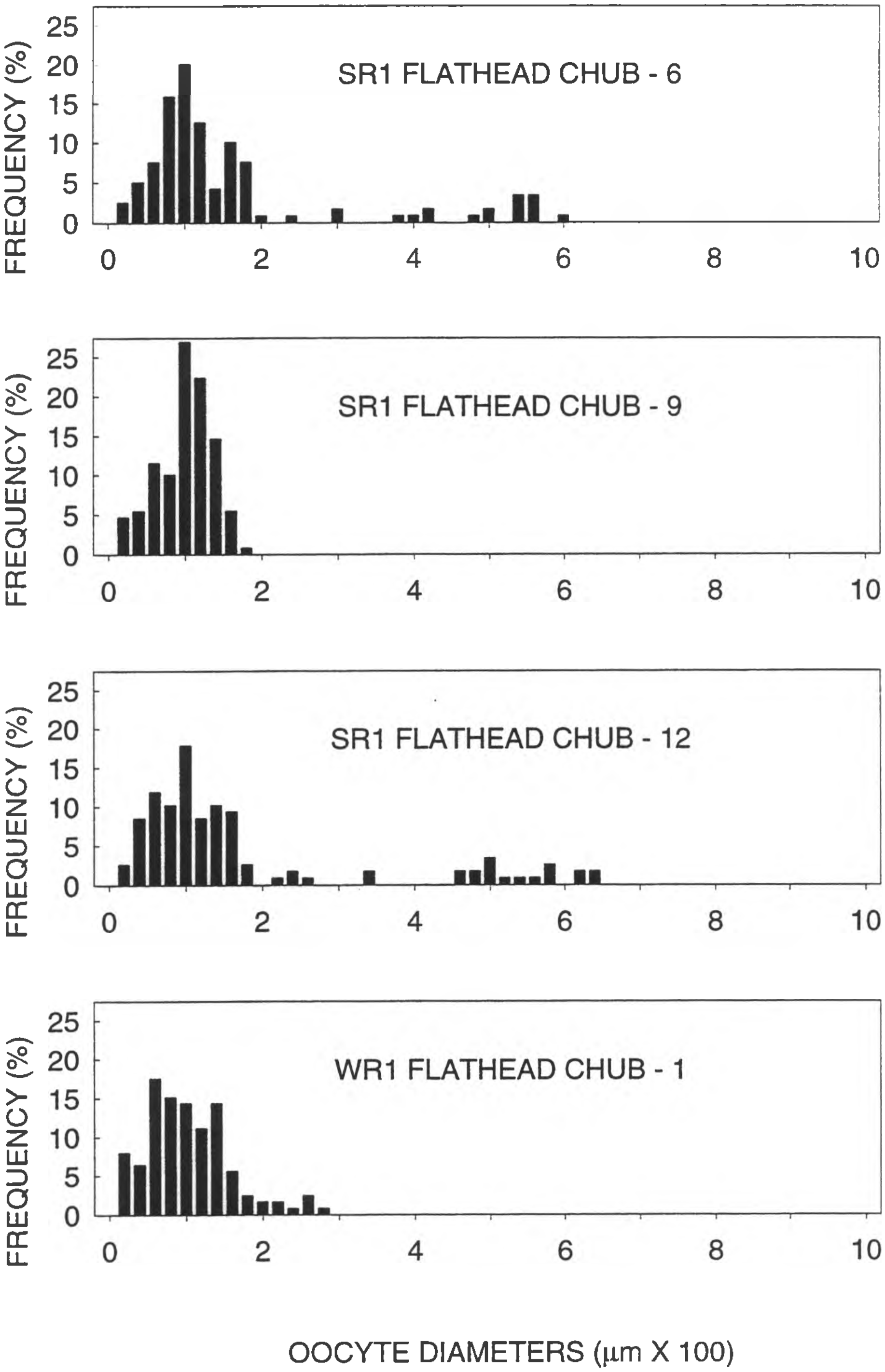

FREQUENCY (%)
20
15
10
5
0
WR1 NORTHERN PIKE - 11
0
2
4
6
8
10
12
14
16
18
FREQUENCY (%)
20
15
10
5
0
WR2 NORTHERN PIKE - 1
0
2
4
6
8
10
12
14
16
18
OOCYTE DIAMETERS (µm X 100)

APPENDIX F

Flathead Chub Oocyte Diameter Frequency Distributions


LSR2 FLATHEAD CHUB - 1
PR2 FLATHEAD CHUB - 1
PR2 FLATHEAD CHUB - 2
PR2 FLATHEAD CHUB - 3
PR2 FLATHEAD CHUB - 4
FREQUENCY (%)
0
5
10
15
20
25
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)


SR1 FLATHEAD CHUB - 1
SR1 FLATHEAD CHUB - 2
SR1 FLATHEAD CHUB - 3
SR1 FLATHEAD CHUB - 4
SR1 FLATHEAD CHUB - 5
FREQUENCY (%)
0
5
10
15
20
25
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)


SR1 FLATHEAD CHUB - 6
SR1 FLATHEAD CHUB - 9
SR1 FLATHEAD CHUB - 12
WR1 FLATHEAD CHUB - 1
FREQUENCY (%)
0
5
10
15
20
25
0
2
4
6
8
10
OOCYTE DIAMETERS (µm X 100)