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Vitello-lipid and vitello-protein deposition in environmentally stressed and non-stressed populations of Morone saxatilis (striped bass)

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VITELLO-LIPID AND VITELLO-PROTEIN DEPOSITION IN

ENVIRONMENTALLY STRESSED AND NON-STRESSED POPULATIONS

OF <u>MORONE</u> <u>SAXATILIS</u> (STRIPED BASS)

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VITELLO – LIPID AND VITELLO – PROTEIN DEPOSITION IN ENVIRONMENTALLY STRESSED AND NON-STRESSED POPULATIONS OF <u>MORONE</u> <u>SAXATILIS</u> (STRIPED BASS)

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ABSTRACT

Summer somatic indices of three and four year old striped bass (Morone saxatilis) from Lake Anna (LA), Virginia, a cooling water reservoir, indicated prey ingestion was not sufficient to maintain growth during summer months when striped bass metabolism was elevated by thermal and hypoxic stress. Vitello-lipid and vitello-protein concentrations of stressed LA striped bass were compared to those of non-stressed striped bass from Smith Mountain Lake (SML), Virginia, a hydroelectric reservoir. Seasonal comparisons of LA and SML striped bass vitello-lipid concentrations and vitello-protein concentrations showed no significant differences between sites ($P \leq 0.05$). Vitello nutrients were not utilized as metabolic substrates by stressed LA striped bass, indicating striped bass vitello-nutrient deposition is not significantly affected by thermal, hypoxic, and starvation stresses of eight to ten weeks duration.

INTRODUCTION

The decline of striped bass (<u>Morone</u> <u>saxatilis</u>) fisheries along the Atlantic Coast since 1973 is well documented (Boreman and Austin 1985; Stevens et al. 1985). Among the factors associated with the decline are decreased egg production and larval survival rates (Chadwick 1979; Stevens et al. 1985). A correlation between reduced fecundity and chronic environmental stresses, e.g. thermally induced hypoxia and reduced prey availability, has been identified by several studies (Coutant 1985; Price et al. 1985; Stevens et al. 1985; Zale et al. 1990). Coutant (1987) hypothesized that thermal and hypoxic stress could be disruptive of oogenesis due to the increased energy requirements of adult fish. It is well known that stress-induced increases of corticosteroids and catacholamines can result in catabolism of fish tissues (Hoar and Randall 1969; Mazeaud et al. 1977; Mazeaud and Mazeaud 1981; Davis et al. 1982). Hence, the use of vitello-nutrients as an emergency source of metabolic substrates may cause ovarian stores of lipid and protein to be inadequate for normal egg and larval development.

The objective of this study was to test Coutant's hypothesis by determining if vitello-lipids and vitello-proteins of thermally and hypoxically stressed striped bass in Lake Anna (LA), a Virginia cooling water reservoir, differ from those of non-stressed striped bass in Smith Mountain Lake (SML), a Virginia hydroelectric reservoir.

SITES AND POPULATIONS

Both LA (York River Drainage) and SML (Roanoke River Drainage) striped bass are spawned from a common brood stock (C. Sledd, Virginia Commission of Game and Inland Fisheries, personal communication) reducing the influence of population genetics on the data. As clupeids have been shown to have similar nutritional qualities (Strange and Pelton 1987), the clupeid forage bases of the two lakes minimized dietary influences on the data. Successful spawning has not been documented for either striped bass population (C. Sledd, personal communication).

<u>Lake Anna, VA</u> (stressing summer habitat for striped bass)

The main reservoir of LA is 3,885 hectares. The reservoir adjoins a 1,376 hectare "waste heat treatment facility" at the lower end of the lake. The lake proper, is an elongated impoundment with a two-armed upper section formed by the North Anna River and Pamunkey Creek (Figure 1). It was created in 1972 to supply cooling water for Virginia Power's North Anna Nuclear Power Station (Virginia Power 1986).

The pelagic forage fish complex is dominated by gizzard shad (<u>Dorosoma</u> <u>cepedianum</u>) and threadfin shad (<u>Dorosoma</u> <u>petenese</u>). Blueback herring (<u>Alosa</u> <u>aestivalis</u>) also are present in low densities (Virginia Power 1986). Annual stockings of striped bass in LA since 1973 by the Virginia Commission of Game and Inland Fisheries (VCGIF) have

established a productive sport fishery.

Smith Mountain Lake, VA (control)

Smith Mt. Lake is a 8,337 hectare impoundment of the Roanoke River. It consists of two tributary arms formed by the Roanoke and Blackwater rivers and a deep, lower section above the dam (Figure 2). The lake, created in 1963, is operated by Appalachian Power Company as a run of the river and pump storage hydroelectric facility (Ney et al. 1988).

The pelagic forage fish complex is dominated by gizzard shad and alewife (Alosa pseudoharengus). Annual stockings of striped bass by the VCGIF since 1970 (Ney et al. 1988) has created a fishery that is the top producer of citation size striped bass in Virginia (C. Sledd, VCGIF, personal communication).

METHODS AND MATERIALS

Female striped bass were collected from LA and SML, in April and May (spring), and July and August (summer) in 1987 and 1988. Collections were made using electrofishing, gill netting, and cooperating anglers who were instructed in specimen handling. Total length and weight were recorded for each fish to determine fish condition, length at age, and weight at age. These somatic indices were used as stress indicators in conjunction with habitat data.

Fish condition (K) was determined using the formula developed by Carlander (1977):

$$K = (W \times 10^5) / (L \times 10^3)$$

where: W = weight in grams

               L = length in millimeters

               10^5 = unity factor for K.

Scales, used to determine the age of fish (Hile 1941), were collected from one scale row below the lateral line and anterior of the tip of the pectoral fin when it was laid flat along this scale row. Cellulose acetate impressions of collected scales were magnified 15X, and the number of annuli on each scale was recorded. Subsamples of scale mounts were read by a second reader for verification. Only striped bass three years old and older were used in the study because, for undetermined reasons, younger striped bass are tolerant of high ($\geq$ 27°C) temperatures (Schaich and Coutant 1978; Coutant and Caroll 1980; Coombs and Peltz 1982; Moss 1985). Sample pairing between reservoirs provided equal numbers of the same age fish for annual and seasonal comparisons but further limited the data set to 10 three year olds and 24 four year olds.

Summer striped bass habitat quality was determined by quantifying the percent of each reservoir's water column having temperatures $\leq$ 25°C and dissolved oxygen (D.O.) levels $\geq$ 2 ppm. These parameter values are non-stressing for age three and older striped bass (Schaich and Coutant 1978; Coombs and Peltz 1982; Lewis 1983; Moss 1985). Habitat data were collected from lower, middle, and upper lake stations to spatially identify available habitat

(Virginia Power 1986; Virginia Water Control Board 1988) (Figures 1-2).

Ovaries were collected, weighed, and stored at -20°C until assayed. Total egg lipid was determined with Packer's (1967) gravimetric procedure. Ovarian membrane was removed from ovarian stroma (Pudney 1987) while frozen. Ovarian stroma was sampled from the anterior, middle, and posterior sections of the ovary, homogenized in distilled water to a 20% homogenate, treated with chloroform - methanol, and heated at 60°C for 30 min. The solution was separated in a separatory funnel, the chloroform layer removed, washed with 1 M NaCl, and separated a second time. The washed chloroform layer was removed to a tared beaker, the solvent evaporated at 40°C under nitrogen, and the beaker reweighed for total lipid weight. The lipid residue was re-suspended in chloroform for thin layer chromatography analysis.

Lipid classes were identified with thin layer chromatography (Skipski and Barclay 1969). Total lipid extracts and lipid class standards were spotted on activated silica gel "G" plates and developed in a 1:1 hexane:diethylether solvent for 20 min at room temperature. Chromatograph lanes used in quantification work were covered with aluminum foil before oxidizing the plate in iodine vapor. Oxidized sample and standard lipid spots were marked for Rf calculation and to identify lipid containing areas in the non-oxidized lanes.

Lipid classes were quantified by dichromate reduction (Skipski and Barclay 1969). Non-oxidized chromatograph lipids were scraped into a 5% sodium dichromate - 95% sulfuric acid solution and heated at 100°C for 45 min. Mixtures were centrifuged to remove silica gel and 0.5 ml of supernatant was diluted in 5.0 ml distilled water. Absorbance at 350 nm was converted to ug lipid with a standard curve (Appendix I).

Phospholipid was quantified by perchloric acid digest (Dittmer and Wells 1969). Chloroform was evaporated from 10 ul of total lipid and 0.4 ml perchloric acid was added. The solution was refluxed at 180°C for one h, treated with ammonium molybdate, reduced with "Fiske - SubbaRow reducing agent" then heated at 100°C for 10 min. Absorbance at 830 nm was converted to ug phosphorus with a standard curve (Appendix I).

Total egg protein was determined with Bradford's (1976) colorimetric assay. The 20% homogenate of ovarian stroma was treated with 0.1% SDS, incubated at room temperature and diluted to 10%. A 250 ul subsample was mixed with 2.5 ml coomassie blue and incubated 20 min at room temperature. Absorbance at 595 nm was converted to ug protein with a standard curve (Appendix I). Additional 20% homogenate was prepared for electrophoretic studies by heating at 100°C for 3 min with SDS and DTT reagents (Appendix II), then mixed with a dilute sucrose solution. Final protein concentration

of the mixture was 4 ug/ul. Preparations were stored at
-20°C until assayed.

Polyacrylamide gel electrophoresis (Laemmli 1970) was
used to profile component proteins. Proteins were run on
15% and 11% gels to increase profile detail of small and
large component proteins. The 17 cm gels were run for 12 hs
at 90 volts in a glycine - tris buffer (pH = 8.6). Gels
were fixed in 12% TCA, stained in 0.25% coomassie blue and
cleared with 10% acetic acid. Sample and molecular weight
standard proteins were quantified with a scanning
densitometer set for absorbance at 620 nm and their Rf
values determined. Mean Rf values of the molecular weight
standards were used to group sample protein bands for
comparative analyses as standard Rf values were in good
agreement with their theoretical Rf values (Appendix I).

Stepwise multivariate regression analysis was used to
identify variables contributing to the variation in total
lipid and protein. Means of variables identified as
significant ($P \leq 0.05$) were compared with the Wilcoxin
t-test.

RESULTS AND DISCUSSION

Somatic indices and habitat quality

Four year old female striped bass from SML were
significantly longer and heavier than four year old female
striped bass from LA (Table 1). Lengths and weights of
three year old female striped bass were not significantly

different between sites (Table 1).

Summer K values from SML striped bass were significantly higher than summer K values from LA striped bass (Table 1). The K values were not significantly different between sites for fish collected during the spring (Table 1).

Seasonal ovary weights were not significantly different between sites (Table 1).

Striped bass habitat quality in SML during August 1988 was similar to that of previous years (Ney et al. 1988). Water temperatures were $\leq 25^\circ C$ and D.O. was ≥ 2.0 ppm in the lower and middle lake areas (Figure 3).

Striped bass habitat quality in LA during summer months has been documented as poor (Grimes 1986). Habitat temperatures are $\geq 27^\circ C$ at the upper margin of an anoxic hypolimnion and $\geq 28^\circ C$ 1 m above this margin. This stress-inducing habitat for age three and older striped bass persists for eight to ten weeks during summer months.

Several authors (Dorfman and Westman 1970; Bryce and Shelton 1982; Coutant 1985; Zale et al. 1990) have reported that water temperatures in excess of $25^\circ C$ slow striped bass growth by decreasing conversion efficiencies while increasing standard metabolic rate. Somatic indices of SML striped bass show the summer temperature - D.O. regimen of SML keeps striped bass metabolism at a level maintainable by available forage (Ney et. al 1988) and contributes to

exceptionally high SML striped bass growth (Smith and Kaufman 1982). Conversely, somatic indices of LA striped bass show the summer temperature - D.O. regimen of LA elevates striped bass metabolism to a level where a significant amount of body protein and lipid is used for metabolic fuel rather than growth. Lake Anna striped bass growth is below the Virginia mean (Smith and Kaufman 1982).

Catabolism of body tissues is common in carnivorous fish during starvation (Mazeaud et al. 1977). Proteins and lipids from muscle and other tissues are used as substrates for aerobic metabolism as carbohydrate is poorly utilized (Walton and Cowey 1982; Waarde 1982; Millikin 1982; Walton 1985; Gapp 1987). As striped bass metabolism increases exponentially with temperature, starvation catabolism is more severe in summer months (Kruger and Brocksen 1978; Love 1980; Waarde 1982; Weatherley and Gill 1987). High summer rates of catabolism may reduce the amounts of lipids and proteins available for oocyte deposition. Temperature also may directly effect oogenesis by interfering with the activity and timing of stenothermal enzyme systems (Miller 1979; Elliott 1981).

Lipids

Collection season was significantly correlated with total lipid weight (Table 2), as lipid weight was significantly higher in spring samples from both sites (Table 3). Therefore, site comparisons of significant lipid

data were made on a seasonal basis (spring/summer).
Triglyceride-ester concentration, phosphorus concentration,
and ovary storage time also were significantly correlated
with total lipid weight (Table 2). However, no significant
differences were found for phosphorus or triglyceride-ester
concentrations (Tables 3-4). Both classes of lipids were
present in similar relative amounts in spring and summer
samples from each site (Table 5). Ovary storage time was
correlated with lipid weight through a sampling pattern
relationship. Early in the study collections were summer
samples with predictably low total lipid weight, later
collections were spring samples with predictably high total
lipid weight.

Unidentified lipids between the chromatogram origin and
the cholesterol standard (unknown-1) were not significantly
correlated with total lipid weight (Table 2), and showed no
significant differences between sites (Table 4). However,
summer unknown-1 concentrations were significantly higher
than spring unknown-1 concentrations at both sites
(Table 3). High summer concentrations of unknown-1 lipids
suggests these polar lipids are important in early
oogenesis. Phospholipids, which remain near chromatogram
origins (Skipski and Barclay 1969) as do the unknown-1
lipids, are particularly important in cellular activity as
they regulate major metabolic processes, biomembrane
function, and Balbiani body activity (Love 1968; Wallace and

Selman 1981; Leger et al. 1981; Watanabe 1981; Kanazawa
1981; Tocher and Sargent 1984; Fremont et al. 1984;
Weatherley and Gill 1987; Scott 1987; Moodie et al. 1989).

Absences of significant site differences in seasonal
total lipid and lipid class concentrations do not support a
vitello-lipid depletion mechanism for Coutant's hypothesis
(1987).

Proteins

Season was significantly correlated with total protein
weight (Table 6) as total protein weight was significantly
higher in spring samples from both sites (Table 7).
Therefore, site comparisons of significant protein data were
made on a seasonal basis (spring/summer).

Proteins between 15,000-100,000 daltons (15-100 Kd)
were found in nearly all samples and proteins between 100-
206 Kd were found in 55% of the samples (Table 8). Proteins
above 206 Kd or below 15 Kd, which were present in less than
half the samples, contributed little to total protein weight
(Table 8) and were not used in analyses.

Concentrations of 100-206 Kd proteins were
significantly higher in spring samples from LA (Table 7) but
were not significantly different between sites (Table 10).
Concentrations of 68-100 Kd proteins were higher in spring
samples (Tables 7, 9); however, the difference was
significant only in samples from SML (Table 7) and site
differences were not significant (Table 10).

Concentrations of 42-68 Kd proteins were significantly higher in summer at both sites (Table 7) and significantly higher in spring SML samples than in spring LA samples (Table 10). Concentrations of 25-42 Kd proteins were higher in summer samples (Table 7); however, the difference was significant only in samples from LA and site differences were not significant (Table 10). Concentrations of 18-25 Kd proteins, while higher in the summer, were not significantly different between seasons or sites (Tables 7, 10). Concentrations of 15-18 Kd proteins were higher in spring samples (Tables 7, 9); however, the difference was significant only in samples from LA (Table 7) and site differences were not significant (Table 10).

The significant site differences in the spring concentrations of the 42-68 Kd proteins show this class of proteins was not as prevalent in LA striped bass eggs during the spring. This study did not determine the functions of these proteins and could not assess the importance of the between site differences. This should be of concern in future studies.

Absences of significant site differences in seasonal total protein concentration do not support a vitello-protein depletion mechanism for Coutant's hypothesis (1987). However, significant differences in some spring component protein concentrations do suggest a vitello-protein alteration mechanism.

CONCLUSIONS

Coastal populations of striped bass have shown considerable fluctuations in abundance since the middle 1800's (Raney 1952). Such fluctuations are characteristic of species subject to year class dominance, the production of such large numbers of young in a particular year that individuals from the year class dominate catches for several years (Merriman 1941). Dominant year classes appear at lengthy intervals as environmental factors must be perfectly coordinated to promote exceptional levels of oogenesis, spawning and larval survival. Environmental perturbations along the Atlantic Coast, ranging from eel grass blight in the 1930's to damming of rivers, have been both shown and speculated to be responsible for periods of low striped bass reproduction and recruitment (Merriman 1941; Raney 1952; Mansueti 1962; Coutant 1985). Summer stress conditions described in this study are similar to summer conditions that have developed and are currently present in the Chesapeake Bay (Coutant 1985; Price et al. 1985). Because the Chesapeake Bay is undoubtedly a more complex system than a freshwater impoundment, extrapolations of results from this study must be made with prudence. Results of this study do indicate, however, that summer thermal, hypoxic, and starvation stresses are not sufficient to stimulate significant catabolism of vitello-lipids and vitello-proteins in striped bass.

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Table 1.   Wilcoxin t-test comparisons of female

             striped bass somatic data from Lake Anna

             (LA) and Smith Mountain Lake (SML), Virginia.

Parameter	Z	P	df	Larger mean
Age 4 lengths	-2.34	0.02	23	SML
Age 3 lengths	-0.63	0.53	9	SML
Age 4 weights	-2.40	0.02	23	SML
Age 3 weights	1.35	0.18	8	SML
Spring K factors	-0.52	0.60	14	LA
Summer K factors	2.36	0.02	16	SML
Summer ovary weight	-1.68	0.09	17	SML
Spring ovary weight	0.97	0.33	13	SML

Table 2.　　　Backward, stepwise multiple regression of
Lake Anna and Smith Mt. Lake, Virginia total
striped bass egg lipid versus collection
season, egg [phosphorus], egg [triglyceride-
esters], egg unknown-1 [lipid], collection
site, and ovary storage time.

Step	Variable	Model R^2	F	Correlation
1	Collection site	0.74	0.91	
2	Egg unknown-1 [lipid]	0.70	2.68	
3	Egg [phosphorus]	0.70	15.09 **	−
4	Egg [triglyceride-ester]	0.70	8.76 *	+
5	Collection season	0.70	31.80 ***	
6	Ovary storage time	0.70	4.28 x	−

x $P \leq 0.05$,　* $P \leq 0.01$,　** $P \leq 0.001$,　*** $P \leq 0.0001$

Table 3.   Within site Wilcoxin t-test comparisons of
                total lipid weight and lipid class
                concentration of striped bass eggs from
                spring and summer collections in Smith
                Mountain Lake (SML) and Lake Anna (LA),
                Virginia.

Parameter	Z	P	df	Larger mean
LA total lipid weight	2.65	0.008	16	Spring
SML total lipid weight	3.42	0.001	16	Spring
LA [phosphorus]	0.67	0.500	16	Spring
SML [phosphorus]	0.05	0.960	16	Spring
LA unknown-1 [lipid]	2.21	0.030	16	Summer
SML unknown-1 [lipid]	3.32	0.001	16	Summer
LA [trig.-ester]	0.43	0.670	16	Summer
SML [trig.-ester]	-0.24	0.810	16	Spring

Table 4.　　　Seasonal Wilcoxin t-test comparisons of total lipid weight and lipid class concentraion of striped bass eggs from Lake Anna (LA) and Smith Mountain Lake (SML), Virginia.

Parameter	Z	P	df	Larger mean
Summer				
Total lipid weight	0.71	0.480	17	LA
[Phosphorus]	-0.27	0.790	17	SML
Unknown-1 [lipid]	0.71	0.480	17	LA
[Trig.-ester]	-0.62	0.540	17	LA
Fish length	-2.21	0.030	17	SML
Fish weight	-2.96	0.003	17	SML
Spring				
Total lipid weight	-0.16	0.870	15	SML
[Phosphorus]	0.47	0.640	15	LA
Unknown-1 [lipid]	-0.68	0.490	15	SML
[Trig.-ester]	0.68	0.490	15	SML
Fish length	-1.00	0.320	15	SML
Fish weight	0.99	0.320	14	SML

Table 5.   Seasonal lipid profiles for striped bass
             ovaries collected from Lake Anna and Smith
             Mountain Lake, Virginia.   Mean concentrations
             are reported with +/- one standard deviation
             and N.

Lipid component	Spring	Summer
Lake Anna		
Total lipid:	129.0 +/- 31.8 (8) *	41.2 +/- 20.6 (8)
Phosphorus:	0.7 +/- 0.4 (8) **	0.6 +/- 0.4 (9)
Triglycerides-esters	187.0 +/- 49.4 (7) ***	190.7 +/- 109.1 (8)
Unknown-1 lipid:	13.3 +/- 1.3 (7) ***	69.0 +/- 17.9 (8)
Smith Mountain Lake		
Total lipid:	133.5 +/- 48.4 (8) *	41.0 +/- 21.5 (9)
Phosphorus:	0.5 +/- 0.2 (8) **	0.5 +/- 0.2 (9)
Triglycerides-esters	219.7 +/- 94.5 (7) ***	192.3 +/- 69.2 (8)
Unknown-1 lipid:	4.0 +/- 0.2 (8) ***	139.0 +/- 63.9 (8)

* mg / gram wet tissue
** ug / 80 ug wet tissue
*** ug / 320 ug wet tissue

Table 6.   Backward, stepwise multiple regression of
            Lake Anna and Smith Mt. Lake, Virginia total
            egg protein versus collection season,
            collection site, ovary storage time and
            concentration of molecular weight
            (expressed as Kilo daltons) groups: 15-18 Kd,
            18-25 Kd, 25-42 Kd, 42-68 Kd, 68-100 Kd,
            100-206 Kd.

Step	Variable	Model R^2	F
1	15-18 Kd	0.67	0.00
2	42-68 Kd	0.67	0.00
3	Collection site	0.67	0.06
4	100-206 Kd	0.66	0.39
5	Ovary storage time	0.64	1.03
6	68-100 Kd	0.59	2.67
7	25-42 Kd	0.51	1.53
8	18-25 Kd	0.48	1.15
9	Collection season	0.48	21.38 *

* $P \leq 0.0001$

Table 7.   Within site Wilcoxin t-test comparisons of
Lake Anna (LA) and Smith Mt. Lake (SML),
Virginia total protein weight and component
protein percentage of striped bass eggs from
spring and summer collections.

Parameter	Z	P	df	Larger mean
LA total protein weight	2.74	0.01	16	Spring
SML total protein weight	3.08	0.00	16	Spring
LA 100-206 Kd [protein]	2.24	0.03	13	Spring
SML 100-206 Kd [protein]	-1.84	0.07	11	Spring
LA 68-100 Kd [protein]	1.16	0.24	15	Spring
SML 68-100 Kd [protein]	2.36	0.02	16	Spring
LA 42-68 Kd [protein]	-3.03	0.00	16	Summer
SML 42-68 Kd [protein]	-3.42	0.00	16	Summer
LA 25-42 Kd [protein]	-1.97	0.05	16	Summer
SML 25-42 Kd [protein]	-1.49	0.14	16	Summer
LA 18-25 Kd [protein]	-0.24	0.81	16	Summer
SML 18-25 Kd [protein]	-0.19	0.85	16	Summer
LA 15-18 Kd [protein]	2.46	0.01	16	Spring
SML 15-18 Kd [protein]	1.20	0.23	16	Spring

Table 8.   Percent of striped bass egg samples from Lake
            Anna and Smith Mt. Lake, Virginia containing
            protein bands in indicated molecular weight
            groups (molecular weights are expressed as
            Kilo daltons) and molecular weight group
            contribution to total protein weight.

| Molecular weight	Sample percent	Weight percent
> 206	41	7
100-206	55	18
68-100	93	14
42-68	100	18
25-42	100	14
18-25	100	8
15-18	100	15
< 15	41	6

Table 9.   Seasonal molecular weight group contribution to total protein weight of striped bass eggs from Lake Anna and Smith Mt. Lake, Virginia. Mean percentages are reported with +/- one standard deviation and N. Total protein is reported as percent wet tissue weight.

Molecular Weight	Spring %	+/-	(N)	Summer %	+/-	(N)
100-206	36.4	23.7	13	6.0	5.2	13
68-100	21.6	20.3	15	5.6	3.5	18
42-68	3.8	1.9	16	27.8	12.5	18
25-42	8.0	5.0	16	16.6	11.8	18
18-25	6.9	4.4	16	6.7	2.5	18
15-18	18.0	9.2	16	10.1	9.6	18
Total protein	4.7	1.4	16	2.6	0.8	18

Table 10.   Seasonal Wilcoxin t-test comparisons of
             total protein weight and component protein
             percentage of striped bass eggs from Lake
             Anna (LA) and Smith Mountain Lake (SML),
             Virgina.

Parameter	Z	P	df	Larger mean
Summer				
Total protein	0.00	1.00	17	LA
100-206 Kd	0.64	0.52	12	SML
68-100 Kd	-0.48	0.63	17	SML
42-68 Kd	-0.35	0.72	17	SML
25-42 Kd	1.77	0.08	17	LA
18-25 Kd	-0.97	0.33	17	SML
15-18 Kd	-1.02	0.31	17	SML
Spring				
Total protein	-0.68	0.50	15	SML
100-206 Kd	-0.50	0.62	12	LA
68-100 Kd	-1.10	0.27	14	SML
42-68 Kd	-2.05	0.04	15	SML
25-42 Kd	0.37	0.71	15	LA
18-25 Kd	-0.05	0.96	15	SML
15-18 Kd	-1.42	0.16	15	SML

Figure 1. Lake Anna, Virginia showing locations of
           striped bass habitat monitoring stations.
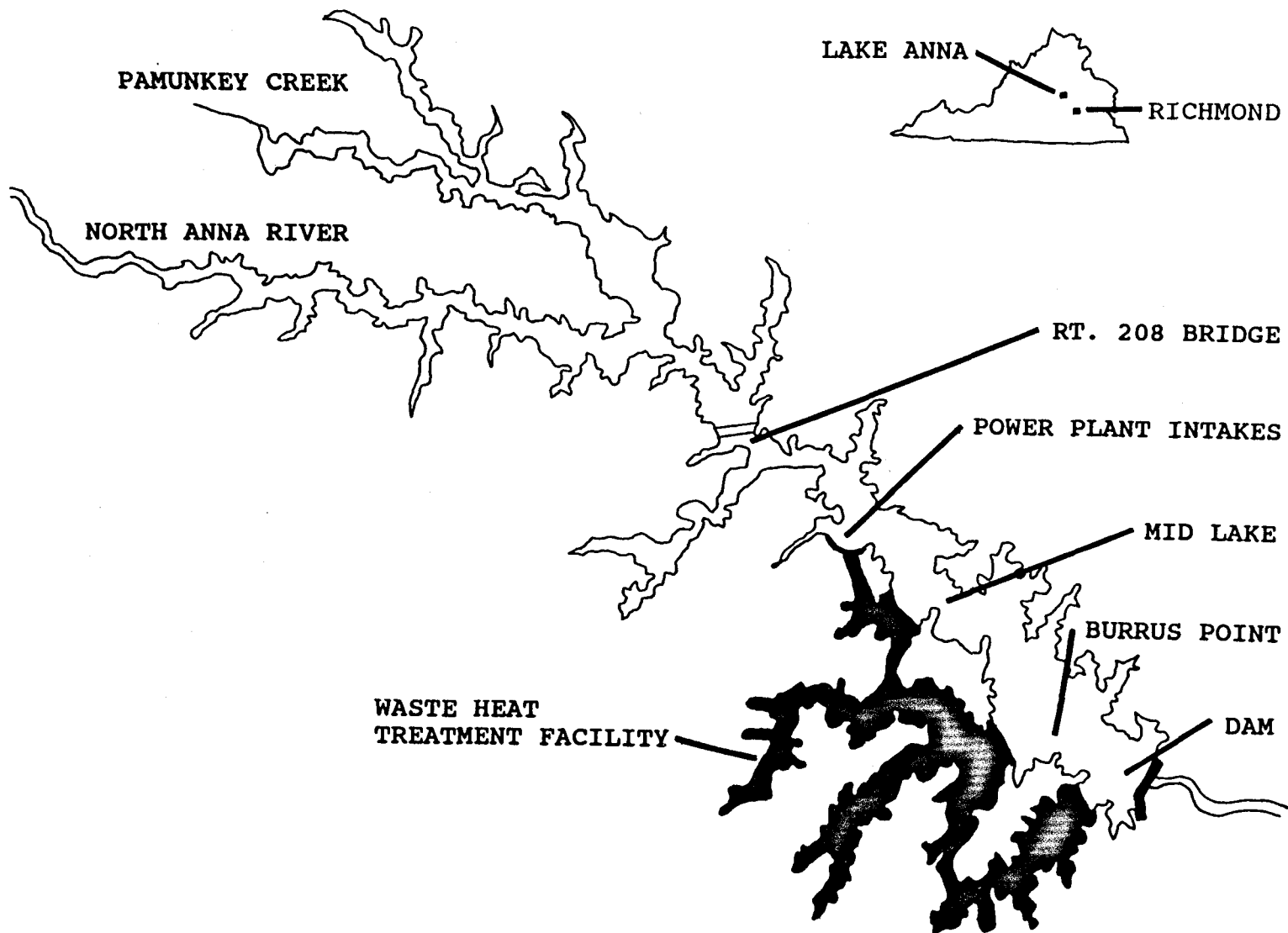
PAMUNKEY CREEK

NORTH ANNA RIVER

LAKE ANNA

RICHMOND

RT. 208 BRIDGE

POWER PLANT INTAKES

MID LAKE

BURRUS POINT

WASTE HEAT
TREATMENT FACILITY

DAM

Figure 2. Smith Mt. Lake, Virginia showing locations of striped bass habitat monitoring stations.
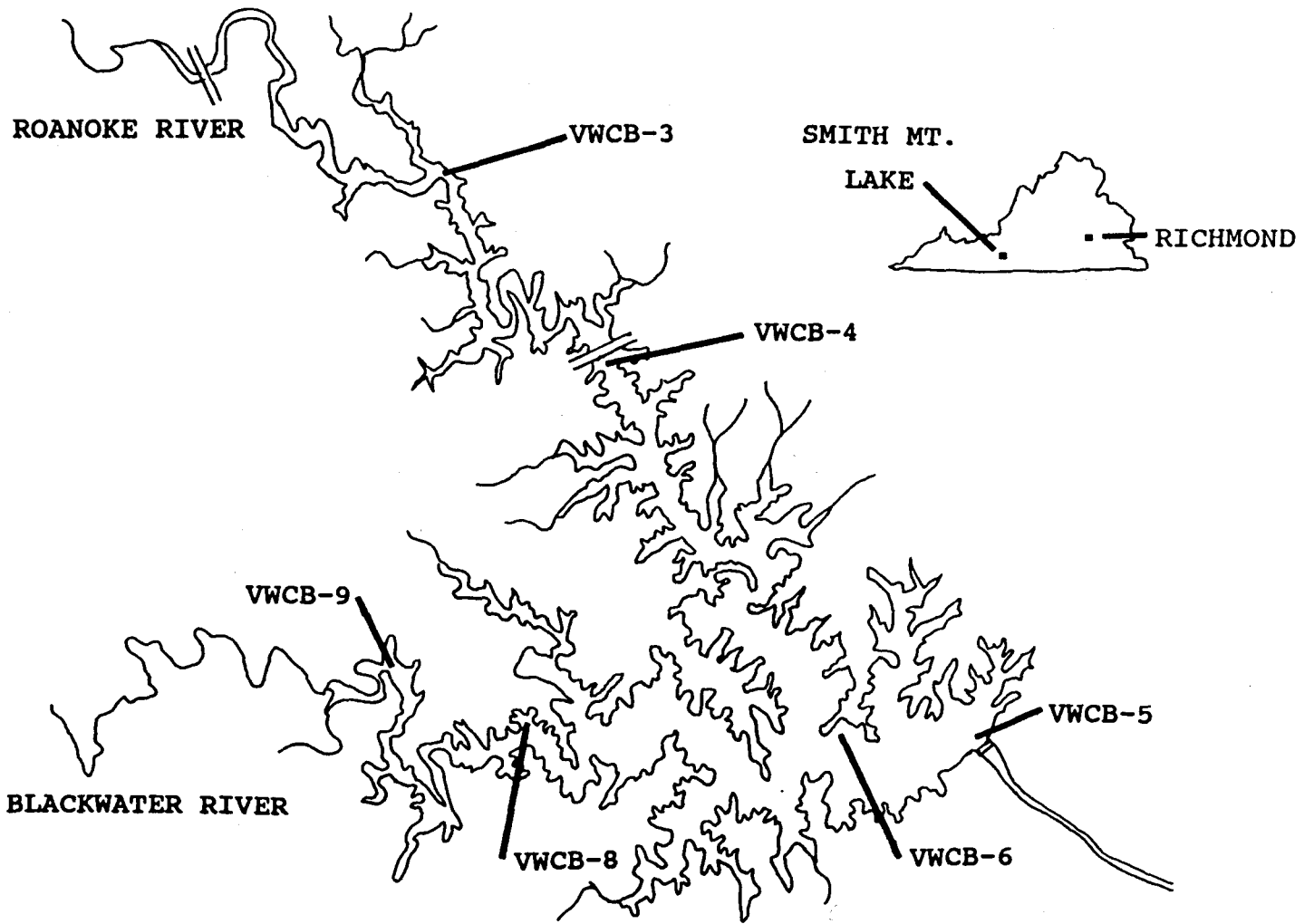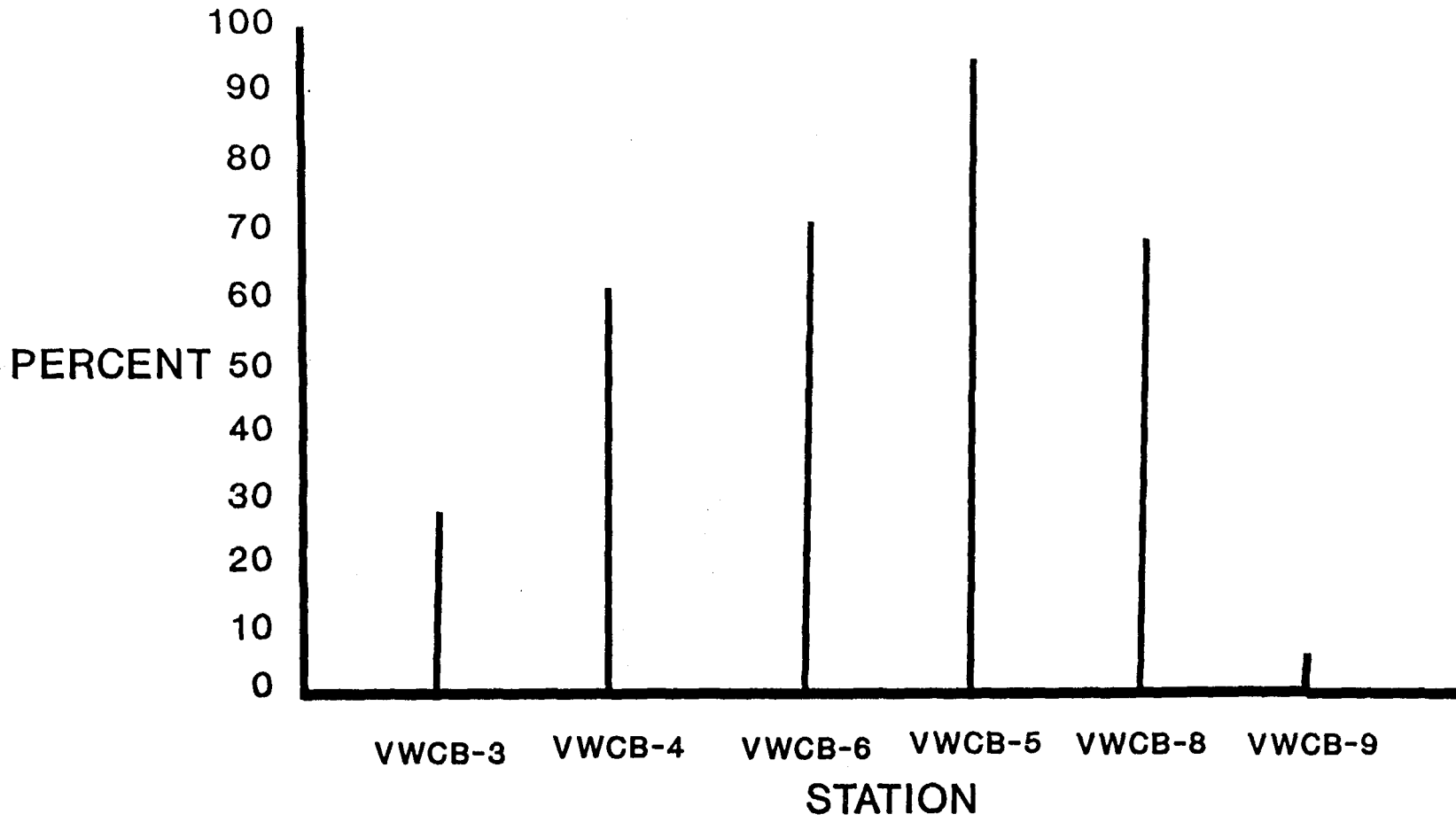
ROANOKE RIVER — VWCB-3

SMITH MT.
LAKE — RICHMOND

VWCB-4

VWCB-9

VWCB-5

BLACKWATER RIVER

VWCB-8

VWCB-6

Figure 3. Percent of water column at striped bass
          habitat monitoring stations in Smith Mt.
          Lake, Virginia with temperatures $\leq$ 25°C and
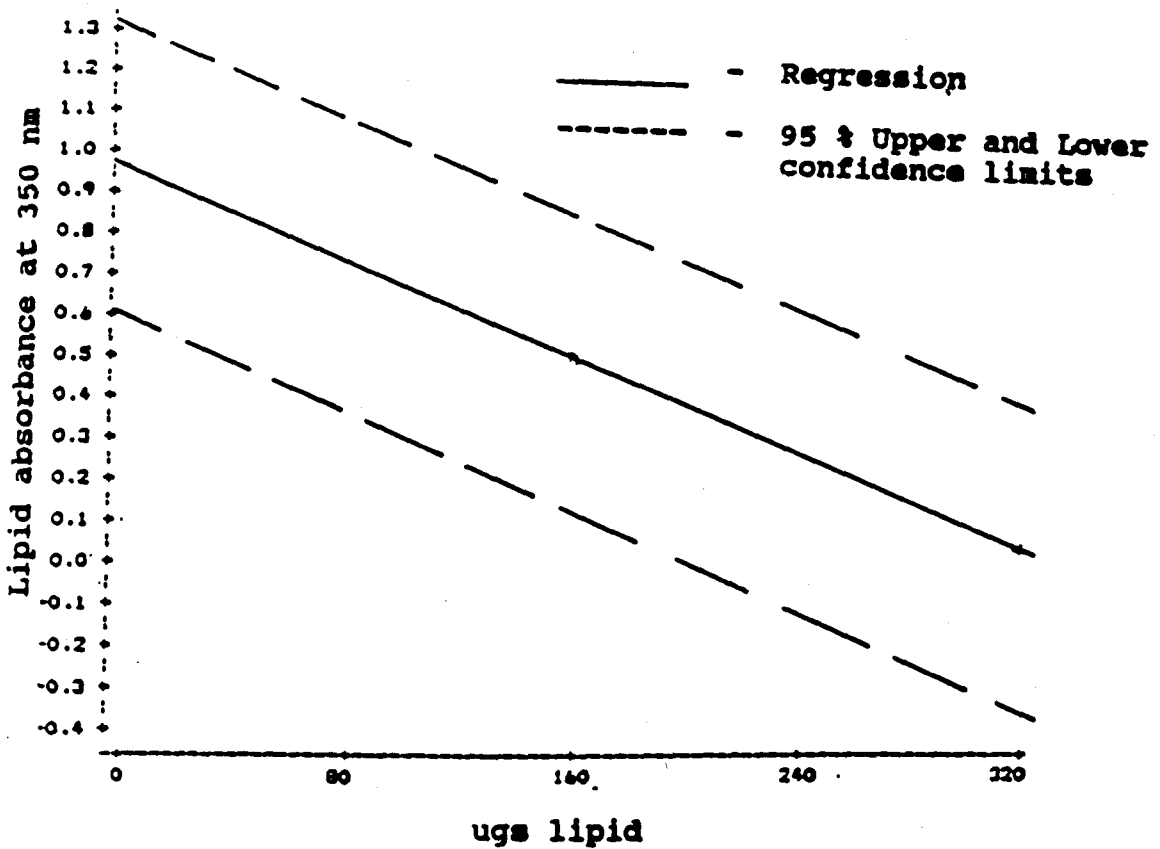   D.O. $\geq$ 2ppm.

APPENDIX I

Data Pertinent to Statistical Analyses

Figure 1. Regression data for lipid class standards.

ug Lipid	N	Mean Absorbance (350 nm)	Standard Deviation	Range
0	34	0.96	0.15	0.64-1.50
80	28	0.71	0.22	0.21-1.10
160	35	0.43	0.21	0.01-1.00
320	15	0.06	0.03	0.03-0.12

Regression
equation: Lipid absorbance = -0.003(ugs lipid) + 0.939

Figure 2.   Regression data for phosphorus standards.

ug Phosphorus	N	Mean Absorbance (830 nm)	Standard Deviation	Range
0.31	12	0.07	0.01	0.05-0.08
0.62	17	0.15	0.01	0.11-0.17
1.24	17	0.30	0.02	0.27-0.33
2.48	17	0.61	0.03	0.57-0.65
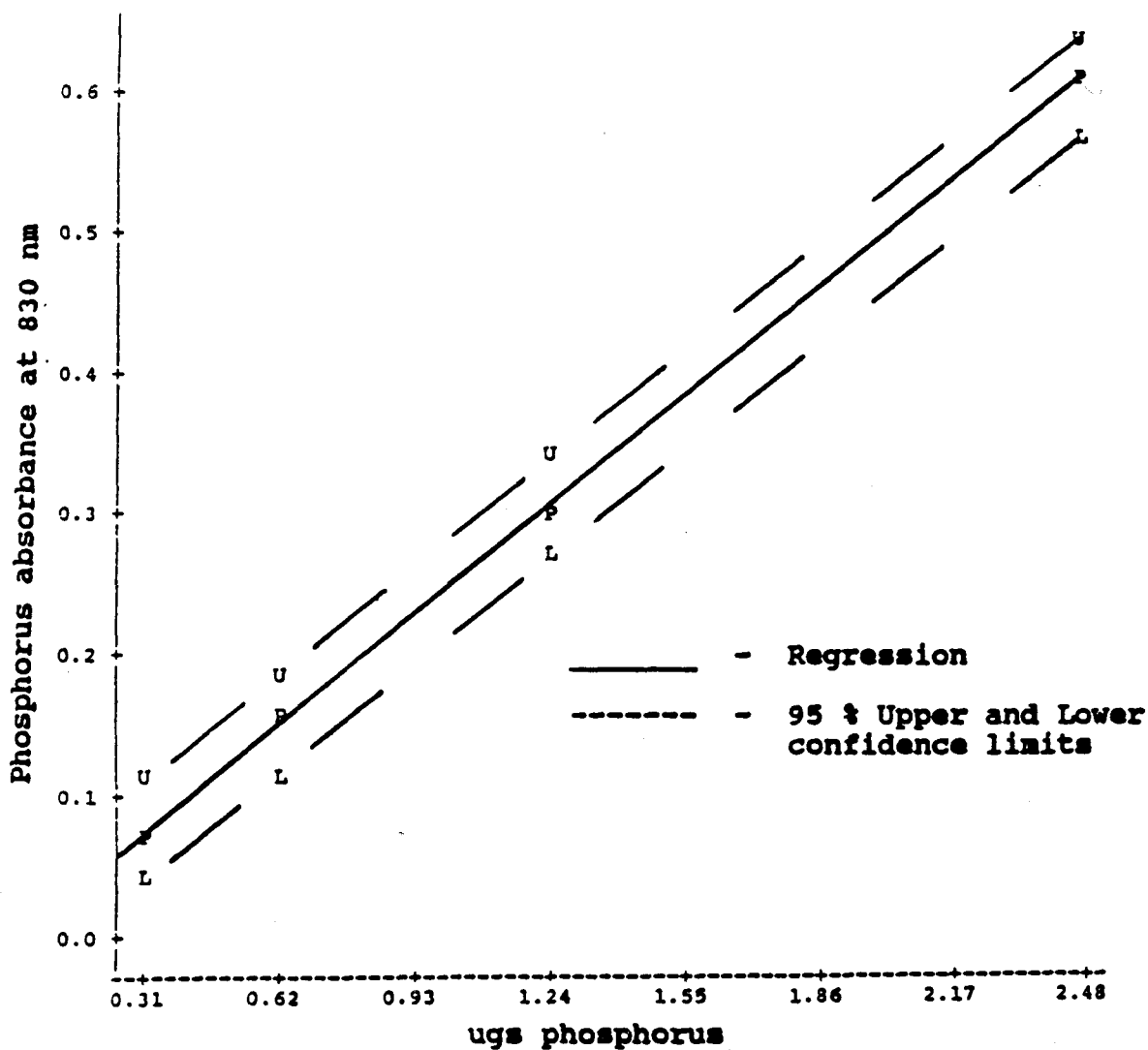
Regression
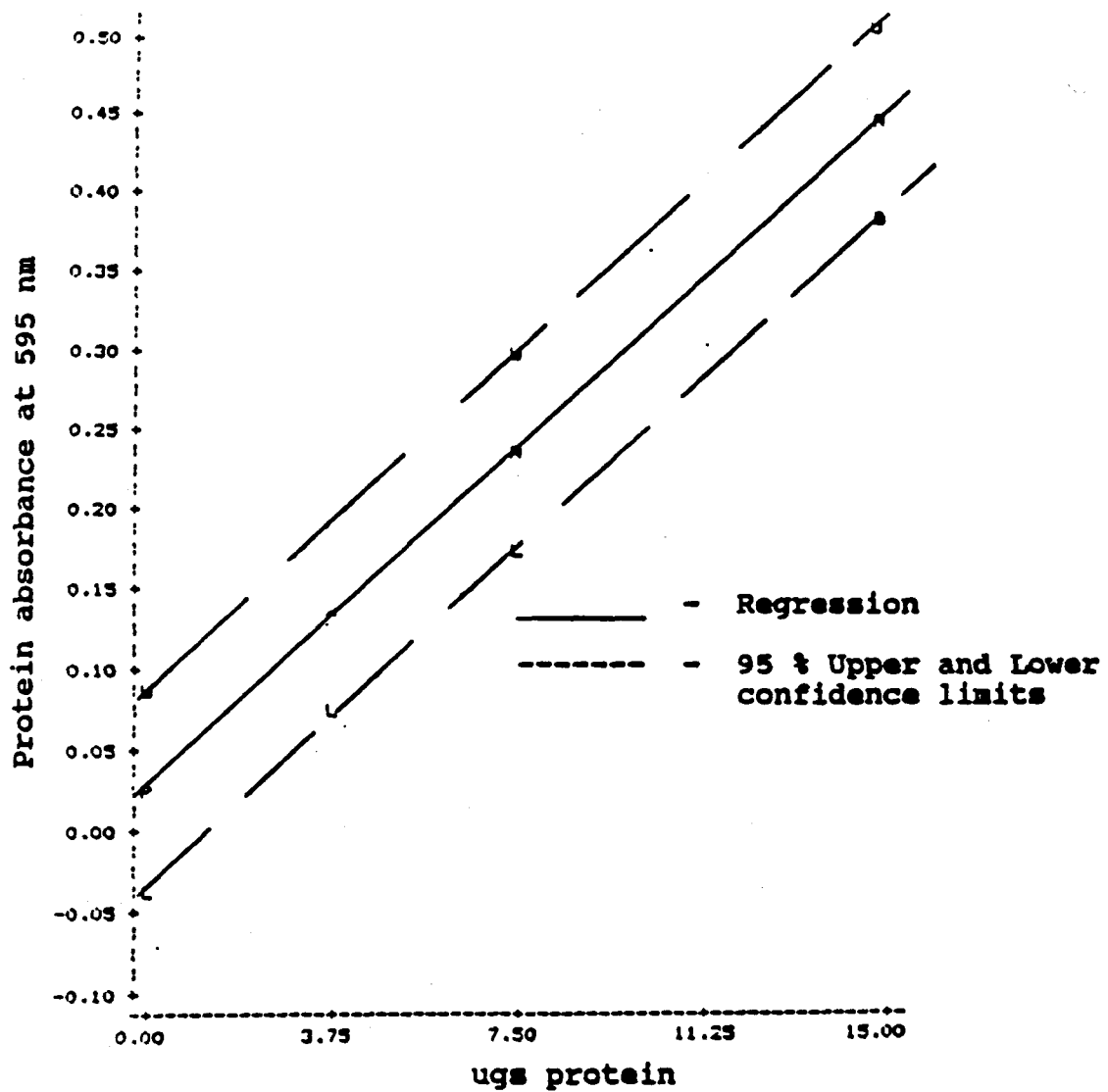equation: Phosphorus absorbance = 0.25(ugs phosphorus) + 0.0

Figure 3. Regression data for total protein standards.

ug Protein	N	Mean Absorbance (595 nm)	Standard Deviation	Range
3.75	19	0.13	0.02	0.10-0.17
7.50	19	0.26	0.02	0.22-0.32
15.0	19	0.44	0.04	0.35-0.50

Regression equation: Protein absorbance = 0.028(ugs protein) + 0.03

Figure 4. Goodness of fit data for protein

molecular weight standards.

standard's molecular weight (Kilo daltons)	N	Mean Rf	Standard Deviation	Range
15	15	0.04	0.01	0.02-0.06
18	15	0.16	0.01	0.13-0.17
25	15	0.26	0.02	0.24-0.30
42	15	0.41	0.02	0.38-0.45
68	15	0.66	0.04	0.61-0.72
100	15	0.89	0.02	0.85-0.94
206	15	1.00	0.00	0.99-1.00

P — Regression
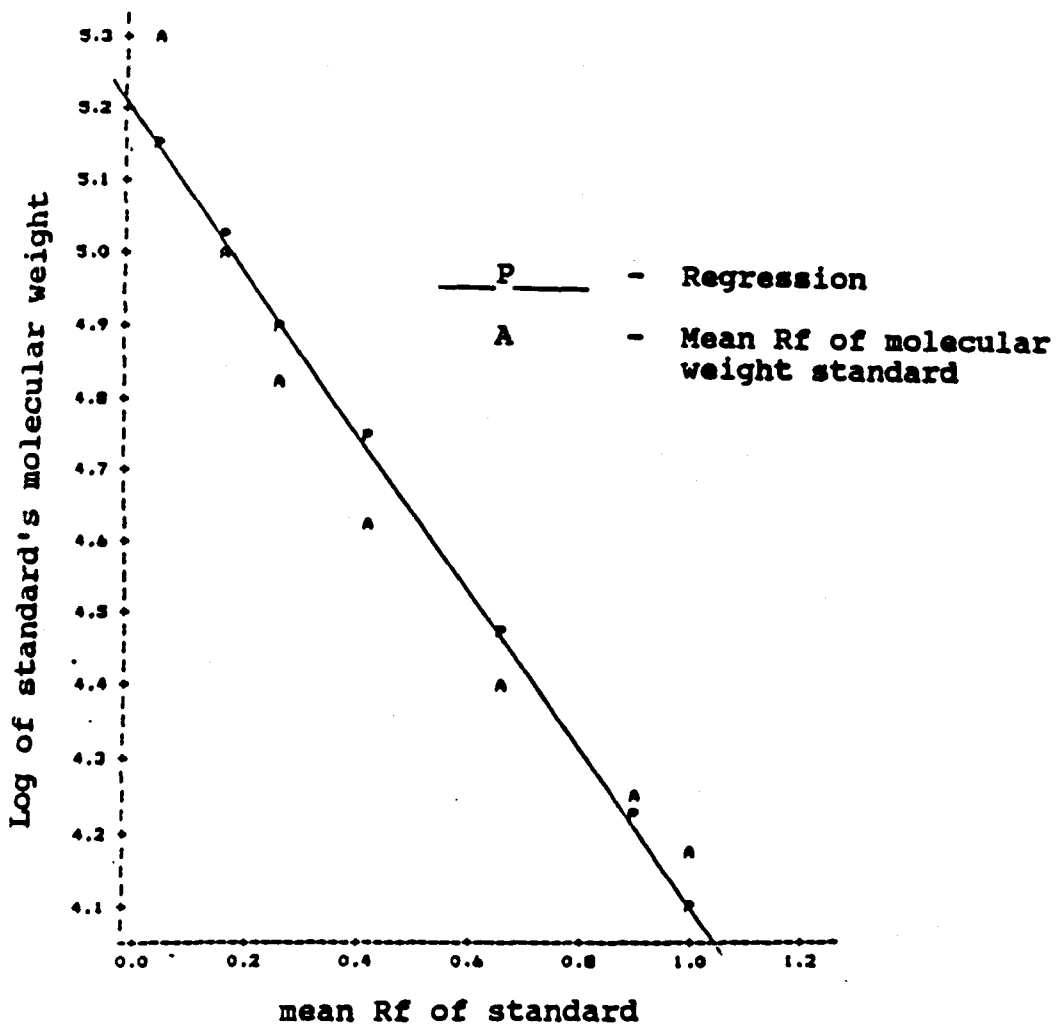
A — Mean Rf of molecular weight standard

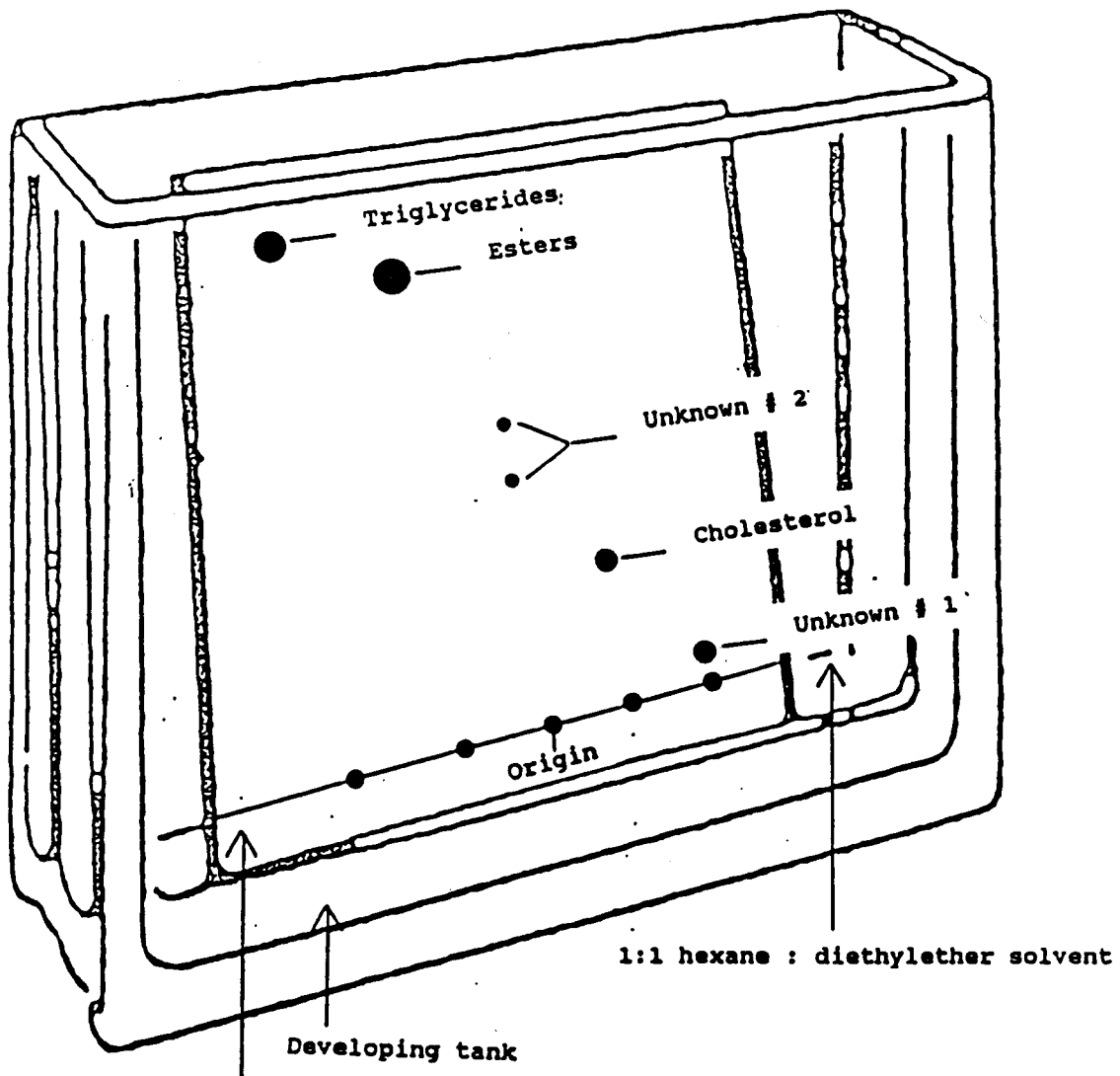Figure 5. Generalized chromatogram of striped bass
          ovary lipids. Spot size indicates the
          relative amount of each lipid class present.

Triglycerides·

Esters

Unknown # 2

Cholesterol

Unknown # 1

Origin

1:1 hexane : diethylether solvent

Developing tank

Silica gel ·'G' chromatography plate

APPENDIX II

Methodologies for Homogenate Preparation and Lipid

and Protein Analyses

The following methodologies were used to prepare homogenates and extracts, and analyze lipids and proteins. Equipment and reagent guidelines are available in Packer (1967). All assays were performed on a minimum of three replicates.

Preparing Homogenates

Store whole ovaries at -20°C or colder until ready to homogenize. To prepare a 20% homogenate, slice the ovary into sections and remove the ovarian membrane from the ovarian stroma while the ovary is still frozen. Weigh out 6 g of ovarian stroma, taking tissue from the anterior, middle and posterior sections of the ovary, and rinse into a pre-iced Rotler-Elvejhem homogenizer with 10 ml distilled water. Continue grinding on ice until the tissue is completely homogenized, rinse the homogenate into a pre-iced graduated cylinder and bring the total volume to 30 ml with distilled water. Keep the homogenate on a ice bath while assaying for lipids and proteins.

Total Lipid Determination

Add 44 ml of 2:1 chloroform - methanol reagent to 5 ml of homogenate, heat in a 60°C water bath for 30 min stirring occasionally. Filter the mixture through a sintered glass funnel into a clean beaker and add 15 ml of 1 M NaCl solution to the filtrate (note: clean sintered glass funnels with chloroform - methanol only as other reagents will solubilize the residue and clog the filter). Separate the

mixture in a separatory funnel and remove the bottom chloroform layer containing the lipids, into the filtrate - NaCl beaker. Discard the remaining layer in the separatory funnel and rinse the separatory funnel with chloroform - methanol. Add 5 ml of 1 M Nacl solution to the removed chloroform layer, separate the mixture in a separatory funnel and remove the bottom chloroform layer into a tared beaker. Evaporate the chloroform solvent under nitrogen using a $40^{\circ}C$ hot plate, cool the beaker under N_2 and reweigh the dried beaker. The weight difference represents the weight of the lipid in 1 g of tissue.

To prepare the lipid extract for thin layer chromatography and phospholipid assay work add the total weight of the lipid in the replicate beakers and resuspend the residue in a glass stoppered flask with a volume of chloroform that will yield a [lipid] of 8 ug lipid/ul. Label the flask with the specimen identification number, contents and date of extraction, store the stoppered flask at $-20^{\circ}C$.

Thin Layer Chromatography and Dichromate Quantification

of Component Lipids

Silica gel "G" plates are marked to delineate six 2 cm wide lanes: three for running sample replicates for lipid class quantification, one for running lipid standards for standards quantification, one for running sample lipids for iodine oxidation and one for running standard lipids for

iodine oxidation. A 0.5 cm lane scraped clean between the lanes to be oxidized and the lanes to be protected from iodine oxidation will facilitate taping down the aluminum foil used to protect lanes used for lipid quantification work. A mark at the edge of each lane, 2 cm from the bottom of the plate, is used to establish the origin of each lane (Appendix II, Figure 1).

The silica gel "G" plates are activated at 110°C for 45 min and cooled in a desiccator. Spot the lipid extract in each of the four sample lanes and spot lipid class standards in each of the two standard lanes (change standard amounts spotted with successive runs to generate standard curve data). Let the chloroform solvent evaporate from the chromatogram, develop the plates at room temperature for 20 min in a tank lined with filter paper and wetted with the developing solvent (1:1 hexane - diethylether). Remove the plate and cover the sample and standard lanes to be quantified with aluminum foil, tape down loose edges with masking tape. Place the plate in a tank of iodine vapor until the lipid spots appear, then remove the plate. Remove the aluminum foil from the plate carefully so as not to scrape off silica gel. Using the oxidized spots as a guide mark the upper and lower boundaries of the lipid spots in the sample and standards quantification lanes (Appendix II, Figure 2). Heat the plate at 40°C under nitrogen for 15 min to drive off the solvent.

Label a centrifuge tube for each sample and standard to be quantified plus one additional tube for the control. Pipette 2 ml of the dichromate reagent (1.25 g $NaCr_2$ in 500 ml of 36 N H_2SO_4) into each centrifuge tube.

Remove the chromatogram from the heat, keep it under a nitrogen hood. Quickly take the plate out of the nitrogen hood and scrape a sample spot or standard spot from the non-oxidized lanes onto cellophane paper and place the plate back in the nitrogen hood. Transfer the scrapings to the appropriate centrifuge tube and stir briefly with a glass stirring rod (the stirring rod is now dedicated to that tube). Repeat the scraping procedure for all non-oxidized sample and standard spots and a blank control spot of approximately the same size as the standard spots (use the oxidized sample spots to determine where to get a blank scraping). Place the centrifuge tubes in a 100°C water bath for 45 min and stir the tubes two or three times during the incubation to keep the silica gel in suspension. Remove the tubes and centrifuge them for 15 min. Pipette 0.5 ml of the supernatant to a 13 x 100 mm culture tube containing 5 ml distilled water. Let the culture tubes cool to room temperature and clean the outside of all tubes. Calibrate the colorimeter with distilled water (= 100 transmittance). Cover the culture tube with a parafilm square, invert briefly and place in the colorimeter. Read sample and standard absorbances at 350 nm. Absorbance is converted to

ug lipid using a standard curve (Appendix I).

Phospholipid Quantification

Evaporate the chloroform from 10 ul of the sample lipid extract by streaming nitrogen into a 13 x 100 mm culture tube. Evaporate the water from 20, 40, 80 and 160 ul volumes of the phosphorus standard (KH_2PO_4). Add 0.4 ml perchloric acid to samples and standards and prepare a control blank of 0.4 ml perchloric acid. Reflux mixtures for 1 h at 180°C, cool the test tubes, add 2.4 ml ammonium molybdate and 1.0 ml Fisk - Subarrow reducing agent. Vortex the mixture, heat in a boiling water bath for 10 min, then cool. Calibrate the colorimeter (control blank = 100% transmittance). Read sample and standard absorbance at 830 nm. Absorbance is converted to ug phosphorus using a standard curve (Appendix I).

Total Protein Determination

Place 0.1 ml of homogenate in a 13 x 100 mm culture tube, add 2.5 ml of 0.1% SDS and incubate the mixture for 10 min at room temperature. Dilute the homogenate - SDS mixture 10:1 with distilled water and vortex to mix. Place 250 ul of the diluted homogenate - SDS mixture into a 13 x 100 mm culture tube and add 250 ul distilled water.

Prepare a series of human albumin protein standards, bring the volume of the standards to 0.5 ml with distilled water. Use 0.5 ml of distilled water as a blank control.

Add 2.5 ml of the coomassie blue dye reagent (20% Sigma

reagent in distilled water) to samples, standards and control, vortex to mix and incubate the mixture 15 - 30 min at room temperature. Clean off the outside of the tubes, vortex to mix and read absorbances at 595 nm after calibrating the colorimeter (blank control = 100% transmittance). Absorbances are converted to ug protein with a standard curve (Appendix I).

Prepare homogenate for polyacrylamide gel electrophoresis by mixing the homogenate with 50% sucrose, 5% SDS and 0.4% DTT to obtain a protein concentration of 4 mg/ml, a sucrose concentration of 30%, a SDS concentration of 1% and a DTT concentration of 0.1%. Heat mixture 2 - 5 min at 100°C, vortex, cool and label with sample identification and date of extraction. Store at -20°C or colder.

Polyacrylamide Gel Electrophoresis of Component Proteins

Mix lower gel reagents as follows:

Mixing Order:	For one 15% gel	For one 11% gel
1 Lower Tris (pH=8.8)	11.9 ml	11.9 ml
2 Acrylamide	17.7 ml	23.0 ml
3 Distilled water	25.4 ml	20.2 ml
4 EDTA	0.63 ml	0.6 ml
5 SDS	0.63 ml	0.6 ml
6 Temed	63.0 ul	63.0 ul
7 APS (just prior to pouring)	6.3 ml	6.3 ml

Pour the lower gel into the mold; check that top margin is

level. Add a small amount of isobutyl alcohol to the top margin to eliminate air bubbles. Let lower gel polymerize, decant isobutyl alcohol and wash gel with distilled water. Mix upper gel as follows:

Mixing Order:	For one 5% gel
1 Upper Tris (pH=6.8)	0.72 ml
2 Acrylamide	4.00 ml
3 Distilled water	15.1 ml
4 EDTA	0.2 ml
5 SDS	0.2 ml
6 Temed	22.0 ul
7 APS -(just prior to pouring)	2.2 ml

Pour the upper gel into the mold until it begins to overflow; insert the well comb and let the upper gel polymerize. Do not use gels for 30 min or longer after polymerization.

Dilute 559 ml of stock running buffer (1350g glycine, 300ml 10% SDS, 300 ml 200 mM EDTA and 900 ml unadjusted Tris qs to 9 l) to 2 l with distilled water and adjust the pH to 8.6 with concentrated NaOH.

Outline the wells in the upper gel with a permanent marker. Remove the well comb slowly while running distilled water over the gel and wash the wells with distilled water. Remove the bottom seal strip from the mold. Mount the mold assembly to the running box and load the running buffer. Load molecular weight standards and sample protein extract.

Connect the transformer (negative lead to top of running box, positive lead to the bottom of the running box). Remove air bubbles from the bottom of the plate. Run samples through the upper gel at 200 volts for 15 min. Turn down the voltage to 90 volts and run samples for 12 hs. Remove the gel from plate assembly by gently prying off one plate and immersing the remaining assembly in 12% TCA to float off the gel. Fix the gel in 12% TCA for 8 hs. Stain with 0.25% coomassie blue (in acetic acid) overnight and clear in 10% acetic acid. Read and quantify protein concentration from a scanning densitometer set for 620 nm.

Figure 1. Running lane configuration for
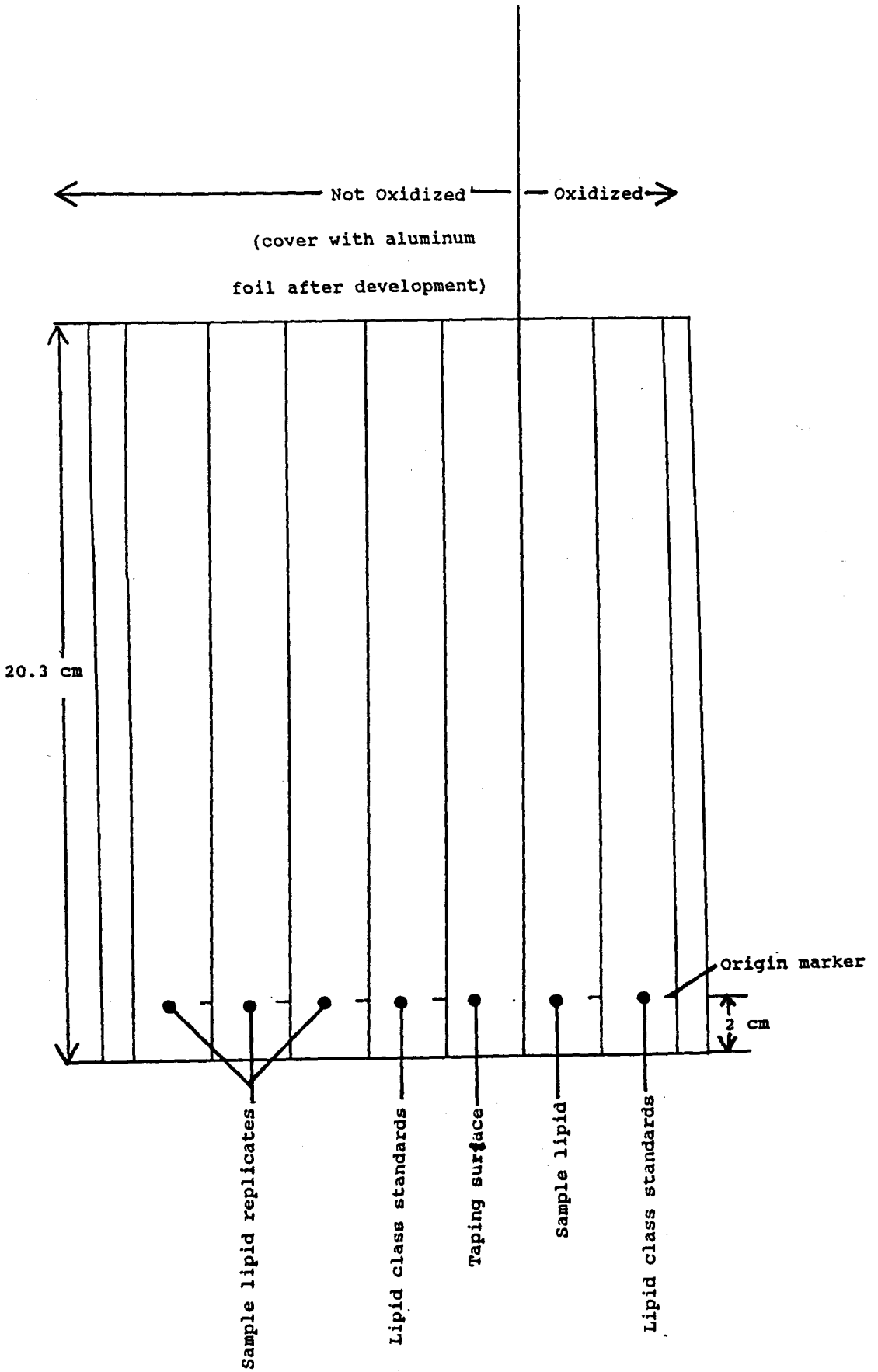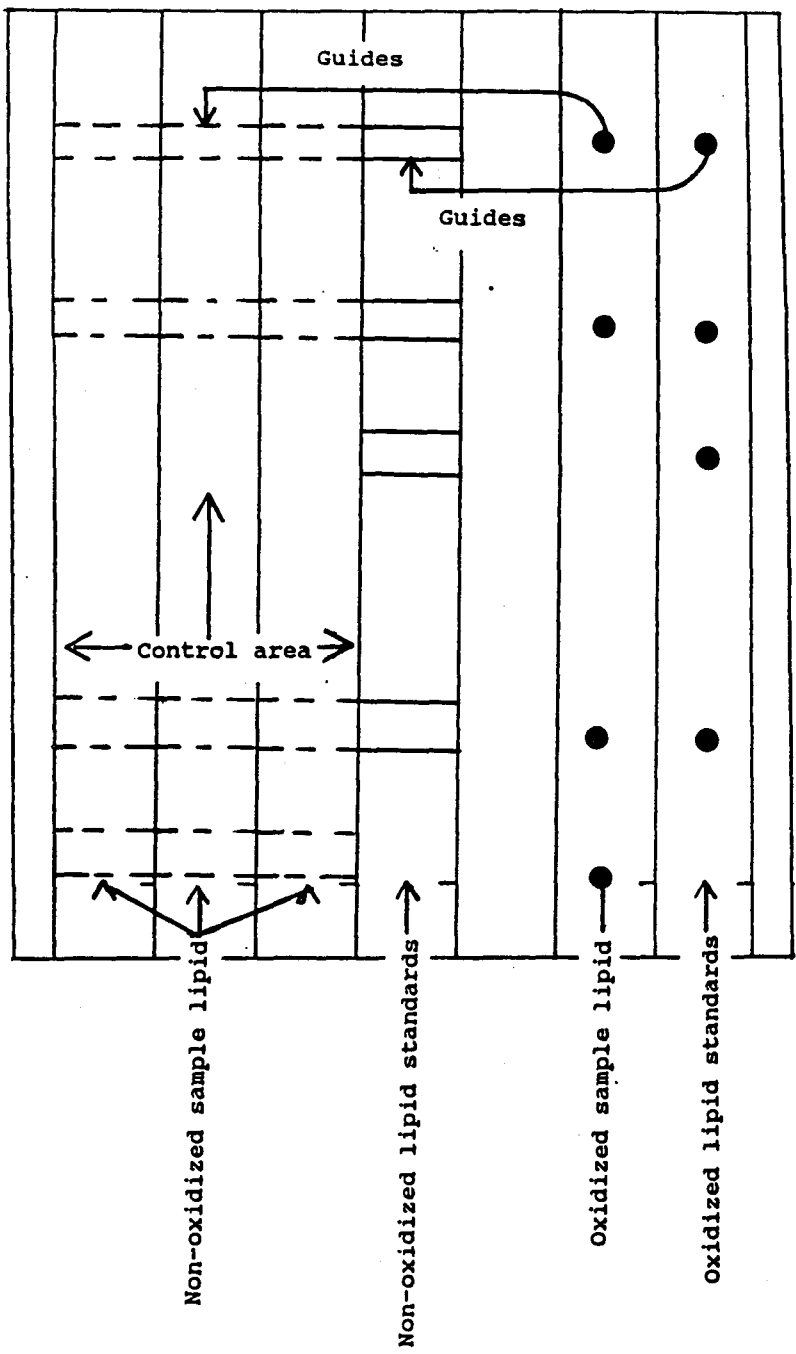chromatographic and dichromate analysis
of lipids.

Not Oxidized ◄──────────────────────┤├──► Oxidized

(cover with aluminum

foil after development)

20.3 cm

Origin marker

2 cm

Sample lipid replicates

Lipid class standards

Taping surface

Sample lipid

Lipid class standards

Figure 2.   Identification of chromatogram regions

containing lipid for dichromate

quantification.

Guides

Guides

Control area

Non-oxidized sample lipid

Non-oxidized lipid standards→

Oxidized sample lipid→
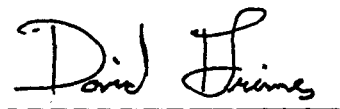
oxidized lipid standards→

VITA

David Van Grimes was born in Oak Park, Illinois on November 4, 1956. He completed elementary and high school in Spring Valley, New York in 1974. He graduated from the College of William and Mary with a Bachelor of Science degree in Biology in August, 1978. He was married in March 1985 to Laura E. Lyon and has a son, Brian V. Grimes, born in June 1988.

From 1979 through 1981 Mr. Grimes worked with James Reed and Associates collecting baseline data on macroinvertebrate and fish populations in stream and reservoir systems used for power production. Since 1981 Mr. Grimes has been employed by Virginia Power and has continued to work on monitoring the effects of power production on aquatic systems.

In 1986, he enrolled at the University of Richmond where he is currently a candidate for the Master of Science degree in Biology.

David V. Grimes