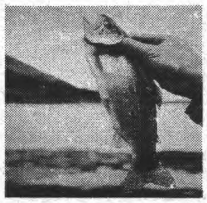
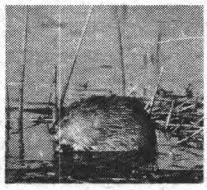
Canada Alberta Northwest Territories

Northern River Basins Study





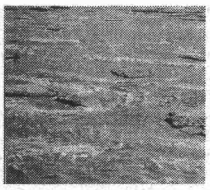



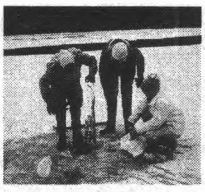


NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 90

ANALYSES OF DEHYDRORETINOL, RETINOL, RETINYL PALMITATE AND TOCOPHEROL IN FISH

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






Prepared for the
Northern River Basins Study
under Project 3144-D4

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 — 14 Feb 96
(Dr. Fred J. Wrona, Science Director) (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.
SUPPLEMENTAL COMMENTARY HAS BEEN ADDED TO THIS PUBLICATION: [ ] Yes [ ] No

P. A. Larkin — 14 Feb 1996
(Dr. P. A. Larkin, Ph.D., Chair) (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 — 14/02/96
(Lucille Partington, Co-chair) (Date)

Robert McLeod — 14/02/96
(Robert McLeod, Co-chair) (Date)

ANALYSES OF DEHYDRORETINOL, RETINOL, RETINYL PALMITATE AND TOCOPHEROL IN FISH PEACE, ATHABASCA AND SLAVE RIVERS, SEPTEMBER TO DECEMBER, 1994

STUDY PERSPECTIVE

For the majority of vertebrate species, a variety of important physiological processes depend upon adequate levels of vitamin A (retinoids) and vitamin E (tocopherol). Fish obtain much of their vitamin A and E directly from their diet, and under normal physiological conditions most vitamin A compounds are stored in the liver. These compounds have received increasing attention from scientists as possible indicators of exposure to a variety of environmental pollutants. Exposure to some environmental contaminants (e.g., PCBs, dioxins and furans) can alter vitamin A metabolism in both mammals and fish. The aquatic fauna of northern rivers in Alberta are exposed to pulp mill effluent, and other types of industrial and municipal effluents. A bench mark program was seen as the first step in assessing the utility of detecting possible pollutant effects by measuring vitamin stores in fish.

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?*

The objective of this project was to examine vitamin A and E indices in fish collected from the Peace, Athabasca and Slave rivers and their major tributaries in 1994, part of a multi-faceted study into altered fish physiological function as a result of environmental contaminants. The fish species targeted for collection and analyses were burbot (primary target species), northern pike and longnose sucker. Biochemical analyses were conducted in the laboratory to determine the levels of retinol, dehydroretinol, retinyl palmitate (three storage forms of vitamin A), β-carotene (precursor form of vitamin A), and tocopherol in the liver of each fish specimen. Additional retinyl palmitate analysis was performed on longnose suckers collected from the upper Athabasca River in the fall, 1992.

Fish were collected from sites located on the Peace, Smoky, Little Smoky, Wapiti, Wabasca, Athabasca, McLeod, Pembina, Lesser Slave, Clearwater and Slave Rivers. For comparison purposes, results from the collection sites were organized into reference (upstream locations and tributaries receiving no inputs from pulp mills), near-field (<100 km downstream of a pulp mill source) and far-field groups (>100 km downstream of a pulp mill source), where sample sizes were adequate. In mature burbot, retinoid levels were similar between reference, near-field and far-field locations, although there were some noticeable site specific differences. Tocopherol levels in male and immature burbot tended to be higher in near-field collection sites, possibly reflecting the enhanced nutrient loading in these areas. For both sexes of longnose sucker, retinoid and tocopherol levels were similar and showed no consistent pattern between field locations, but there were noticeable site specific differences. Liver retinyl palmitate stores in female longnose suckers collected from the Hinton area, in 1992, showed the levels decreasing downstream of the combined effluent, although the magnitude of the response was small. Too few northern pike were sampled to provide interpretation of field or site related differences.

The concentrations of retinoid stores in fish from this study were greater than the values found in fish exposed to environmental contaminants in other locations in Canada, indicative of possible reduced contaminant effects in these rivers. Data from these fish will 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 aquatic fauna in the Northern River Basins Study Area are subject to exposure to pulp mill, domestic and industrial effluents from a variety of sites located throughout the region. Contaminant studies (Pastershank and Muir 1995; Swanson et al. 1993) show that dioxins and furans are elevated in fish near some of the effluent sources. Associated studies (Lockhart et al. 1995; Swanson et al. 1993) indicate greater activity of hepatic biotransformation enzymes (MFO) in mountain whitefish and longnose sucker. Decreases in the levels of both circulating retinoids and tissue storage forms are also found in fish experimentally treated with MFO inducing contaminants (Delorme et al. 1994; Palace and Brown 1994; Ndayibagira et al. 1995). Lower retinoids have also been reported in white suckers and sturgeon inhabiting polluted areas in the St. Lawrence River (Spear et al. 1992; Branchaud et al. 1995; Ndayibagira et al. 1995) and in fish exposed to pulp mill effluent (Friesen et al. 1994; Brown and Munkittrick unpublished data). Therefore it seems possible that imbalances in hepatic vitamins may also occur in fishes near effluent sources in the Northern River Basins Study Area.

The objective of the reported analyses was to examine vitamin A indices and tocopherol in fish collected at sites located throughout the study area. In accordance with the terms of reference for Project 3144-D4, fish liver samples from the 1994 Fall Basin-Wide Fish Collection in the Peace, Athabasca and Slave Rivers (EnviResource Consulting 1995) were analyzed. The design for statistical analysis of the data was predetermined by the Northern River Basins Study Science Directors and the Contaminant 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. The premise behind the monitoring approach is for early detection of possible pollutant effects.

Generally, measures of vitamin A storage compounds in fish from the Peace and Athabasca drainages were greater than the low values reported in fish exposed to environmental contaminants (Spear et al. 1992; Friesen et al. 1994; Palace and Brown 1994; Branchaud et al. 1995). Fish deprived of dietary vitamin A typically display poor growth and condition, (Taveekijkarn et al. 1994). In support of the contention that vitamin A reserves are sufficient, growth and condition of fish were similar between reference and near- or far-site locations in the Peace and Athabasca drainages (Brown et al. 1996).

The lower levels of retinyl palmitate in longnose suckers collected from the region downstream of Hinton in 1992 is consistent with responses observed in other species exposed to pulp mill inputs. However, the overall magnitude of the response was much smaller than observed elsewhere (Friesen et al. 1994; Brown and Munkittrick unpublished data).

The low levels of tocopherol and high levels of free retinol and dehydroretinol in immature and male burbot at the PR3 site are atypical of findings at other sites. The cause of this response is unknown but it may be indicative of the presence of some form of oxidative stressor(s). Further investigation of fish from the PR3 location is advised.

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1.0 INTRODUCTION

A variety of important physiological processes depend upon adequate levels of retinoids (vitamin A compounds) and tocopherol (vitamin E). The best studied function of vitamin A is in the visual process. Although less well understood, vitamin A serves other physiological functions which are of considerable significance. These functions include roles in growth and differentiation of epithelial cells, general growth, reproduction, immunocompetence, hepatic pathology and bone metabolism (Halver 1982; Taveekijkarn et al. 1994). Many of the roles are supported by vitamin A metabolites (e.g. retinoic acid, dehydroretinoic acid). Normal vitamin A homeostasis depends on adequate stores of the vitamin, a tightly regulated supply of the vitamin to target tissues and the ability of cells to produce the functionally active forms (Zile 1992). Tocopherol is considered as an important cellular compound preventing oxidative damage (Serbinova et al. 1991; Roberfroid and Calderon 1995). It forms part of cellular defense mechanisms against damaging free radical generation and lipid peroxidation. Additionally, retinol and ß-carotene have been recognized as having antioxidant activity (Palozza and Krinsky 1991; Ribera et al. 1991; Roberfroid and Calderon 1995).

Fish obtain vitamins A and E directly from the diet or in the case of vitamin A by conversion of some dietary pro-vitamin carotenoid produced by plants (Halver 1982). Carotenoid bioconversion into vitamin A is complicated by the fact that there are two forms of vitamin A (A_1 - retinol and A_2 - dehydroretinol). Canthaxanthin is considered a provitamin A_1 compound because via echinenone, fish can convert canthaxanthin to ß-carotene (Guillou et al. 1989). ß-Carotene is a dimer of retinaldehyde which is readily converted to retinol. Dehydroretinol comes from the oxidative conversion of retinol (Schiedt et al. 1985; 1986) or by the direct conversion from the provitamin carotiniods, astaxanthin or zeaxanthin (Katsuyama and Matsuno 1988). Under normal physiological conditions, most body vitamin A compounds (90 %) are stored in the liver as esters of long-chain fatty acids (e.g. palmitate, stearate, oleate). Thus measurement of retinoid ester concentration in the liver of animals gives a good indication of total body reserves (Brewster 1984). Retinyl palmitate is a predominate storage form of vitamin A in many animals. Both dehydroretinol and retinol support the physiological roles of vitamin A (Bowmaker 1990; Giguere 1994). Thus, all forms require consideration when assessing the vitamin A status of animals (Stancher and Zonata 1984). To support its physiological roles concentrations of retinol and dehydroretinol are fairly tightly regulated by a series of intracellular and extracellular binding proteins. It is generally the concentrations of the storage forms that show the greatest sensitivity toward exposure to environmental pollutants and nutritional factors (Zile 1992).

Retinoids and tocopherol have received increasing attention as indicators of exposure to a variety of environmental contaminants (Peakall 1992). It is well established that dietary exposure to a variety of organic pollutants, particularly chemicals (coplanar-PCBs, dioxins and furans) that interact with the *Ah* receptor, produce severe disturbances in vitamin A metabolism. These disturbances can be characterized as an accelerated metabolism and breakdown of target tissue retinoids and metabolites that results in a greater demand for vitamin A in both mammals (Zile 1992) and fish (Gilbert et al. 1995). The higher demand likely increases mobilization of hepatic stores of vitamin A esters. Thus, a consequence of chronic exposure to planar halogenated aromatic hydrocarbons (PHAH) is severely depleted body stores of vitamin A (Zile 1992).

Feeding vitamin A deficient diets containing polybrominated biphenyl caused clinical signs of vitamin A deficiency sooner than in animals fed solely a vitamin A deficient diet (Darjono et al. 1983). In the Great Lakes basin, low levels of hepatic retinoids are found in fish-eating birds from contaminated sites indicating that the homeostasis of vitamin A is impaired (Fox 1993). In fish, dietary tocopherol supplementation lowers the mutagenicity resulting from exposure to the *Ah*-inducer, benzo(*a*)pyrene (BaP). Depletion of liver tocopherol has been associated with greater susceptibility of trout to BaP toxicity (Williams et al. 1992). Therefore assessment of hepatic vitamin A and tocopherol storage represent sensitive markers of exposure to certain organic pollutants as well as nutritional status.

Decreases in the levels of both circulating retinoids and tissue storage forms were found in trout experimentally treated with coplanar PCB (Palace and Brown 1994; Ndayibagira et al. 1995) or pentachlorodibenzofuran (Delorme et al. 1994). In Atlantic tomcod exposed to effluent from an industrialized area, retinoids were negatively correlated with the levels of organic contaminants (Fairchild et al. 1994). Lower retinoids have also been reported in white suckers and sturgeon inhabiting polluted areas in the St. Lawrence River (Spear et al. 1992; Branchaud et al. 1995; Ndayibagira et al. 1995). Recent investigations show that fish exposed to pulp mill effluent also have diminished stores of hepatic retinoids (Friesen et al. 1994; Brown and Munkittrick unpublished data). Hepatic tocopherol concentrations were depleted in lake trout experimentally exposed to coplanar PCB (Palace et al. 1996).

The aquatic fauna in the Northern River Basins Study Area are subject to exposure to pulp mill, domestic and industrial effluents from a variety of sites located throughout the region. Contaminant studies (Pastershank and Muir 1995; Swanson et al. 1993) show that dioxins and furans are elevated in fish near some of the effluent sources. Associated studies (Lockhart et al. 1995; Swanson et al. 1993) indicate greater activity of hepatic biotransformation enzymes (MFO) in mountain whitefish and longnose sucker. Therefore it seems possible that imbalances in hepatic vitamins may also occur in fishes near effluent sources in the Northern River Basins Study Area.

The objective of the reported analyses was to examine vitamin A indices and tocopherol in fish collected at sites located throughout the study area. In accordance with the terms of reference for Project 3144-D4, fish liver samples from the 1994 Fall Basin-Wide Burbot Collection in the Peace, Athabasca and Slave River drainages (EnviResource Consulting 1995) were analyzed. The design for statistical analysis of the data was predetermined by the Northern River Basins Study Science Directors and the Contaminant 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. The field locations and sites are summarized in Table 1. The premise behind the monitoring approach is for early detection of possible pollutant effects.

2.0 METHODS

2.1 Fish Samples and Collection Sites

Liver samples of Burbot (*Lota lota*), longnose sucker (*Catostomus catostomus*), northern pike (*Esox lucius*) and flathead chub (*Platygobio gracilis*) were obtained from various sites in the Peace, Athabasca and Slave River basins by EnviResource Consulting Ltd., Calgary, Alberta. Frozen tissue samples were subsequently sent to the Freshwater Institute for analysis of vitamin compounds (retinoids & tocopherol).

Collection sites were preselected by the Northern River Basins Study Science Directors and the Contaminant Component Leader and descriptions are detailed in the Fall and Winter Fish Collections From the Peace, Athabasca and Slave Rivers (EnviResource Consulting 1995). Designated sites are outlined in Figure 1. We analyzed liver samples from burbot, longnose sucker and northern pike collected during the fall 1994 Basin-Wide Fish Collection. Frozen liver samples from flathead chub (N = 24) were small (< 500 mg) and were not analyzed for vitamins because the entire tissue sample was required to perform MFO analyses (W.L. Lockhart pers. comm,). Northern River Basins study sample numbers have been used in listing data.

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

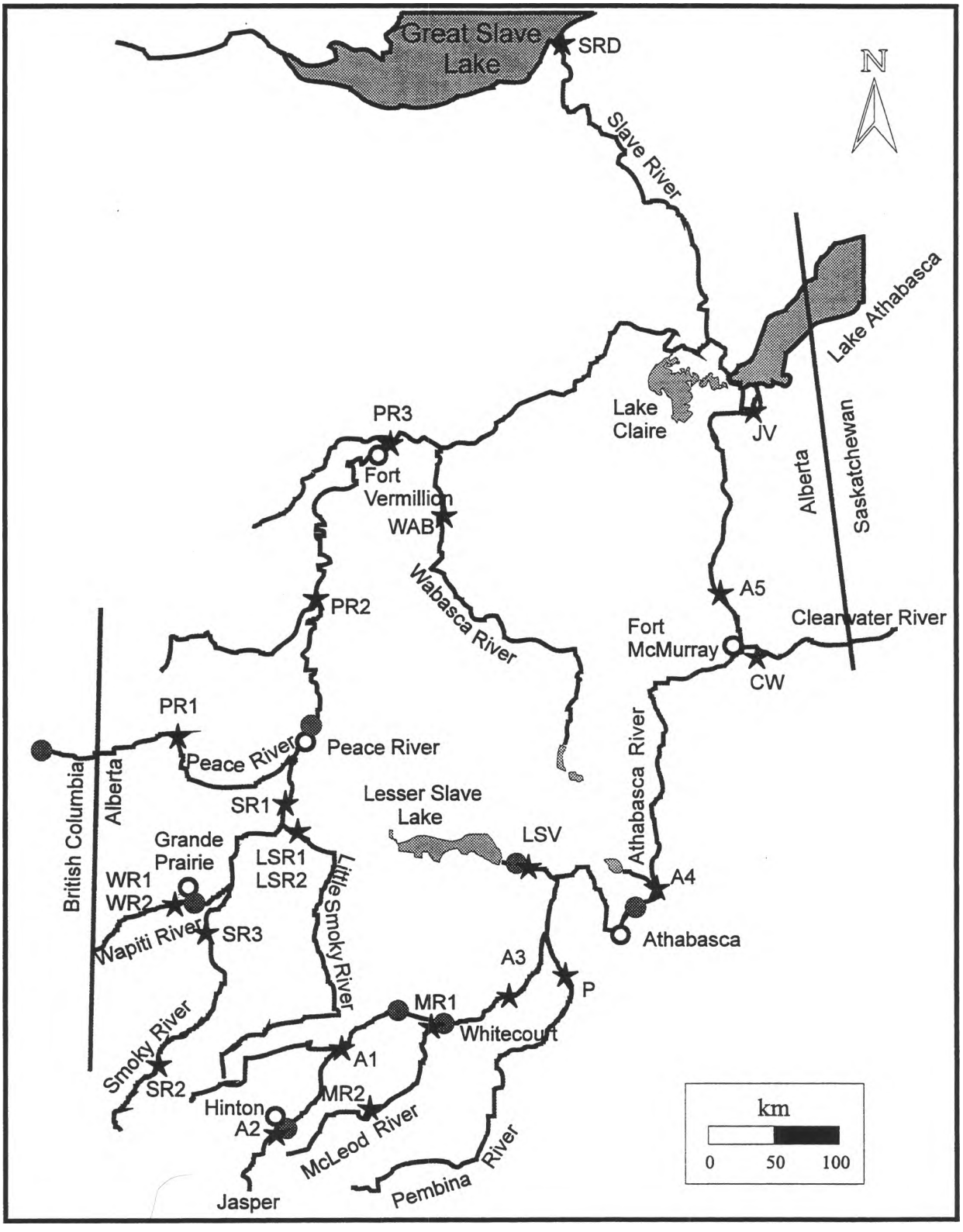

Figure 1. Sampling sites (stars) for the 1994 Basin-Wide Fish Collection. Towns (open circles) and pulp mill (filled circles) locations are also indicated

2.2. Vitamin Assay

Retinoids and tocopherol were measured in liver tissue by isocratic HPLC (Palace and Brown 1993). The samples were kept in subdued light and on ice throughout the homogenization and extraction procedure. Briefly, tissue (approx. 100 mg) was weighed and homogenized (Polytron) with 2.0 mL of distilled deionized (Milli Q, Millipore Inc.) water. To precipitate proteins after homogenization, 200 µL of HPLC grade ethanol was added to 200 µL of tissue homogenate. Liver samples were extracted with 500 µL of (3:2, v/v) ethyl acetate/hexane. Residues from the ethyl acetate/hexane extracts were redissolved in mobile phase and injected (20 µL) onto a 3 µm bead size Adsorbosphere HS C_{18} column, 4.6 mm i.d., 150 mm length, with attached 10 mm Adsorbosphere guard column (Alltech Associates Inc.). The HPLC system consisted of two model 302 solvent pumps, a model 231 automatic sample injector, a model 704 system controller, a four-channel model 620 data module (Gilson Medical Electronics). A Gilson model 116 dual channel UV absorbance detector was set at 325 nm for dehydroretinol and dehydroretinyl ester detection and at 292 nm, for tocopherol and tocopherol acetate detection. A Shimadzu model RF-535 fluorometric detector was set at 330 nm excitation wavelength and at 480 nm emission wavelength for retinol, retinyl acetate and retinyl palmitate. By monitoring UV absorbance at 450 nm (Waters Model 484), ß-carotene (retinol precursor) was detectable in longnose sucker and northern pike samples. The column was thermostated to 26 °C and samples and standards were eluted isocratically with acetonitrile/methanol/water (70:20:10, v/v/v) delivered at a flow rate of 1.0 ml/min. Standard retinol, retinyl acetate, retinyl palmitate, tocopherol, tocopherol acetate and ß-carotene were purchased from Sigma Chemical Co. (St. Louis, Mo.) and 3,4-dehyroretinol was a generous gift from Dr. H. Keller (F. Hoffmann-La Roche Ltd., Basle, Switzerland). As with previous work, there was no loss of these compounds after tissue storage at -110±1 °C for 180 days.

Figure 2 shows chromatographic profiles from a mix of authentic standards. Dehydroretinol and retinol compounds were detected and quantified by their UV absorbance at 325 nm (Fig. 2A) (Stancher and Zonta 1984). Dehydroretinoids and retinoids were also apparent at 292 nm (Fig. 2B) with dehydroretinol showing greater absorbance than retinol. The retinol, retinyl acetate and retinyl palmitate standards were more efficiently detected by fluorescence (Fig. 2C) (Rettemaier and Schuep 1992). In contrast, dehydroretinol did not show appreciable fluorescence. These differences in absorbance/fluorescence combined with their characteristic UV absorbance spectra were used to verify the presence of dehydroretinol, retinol and retinyl palmitate in the various liver extracts.

Tocopherol and tocopherol acetate were routinely quantified by their UV absorbance at 292 nm (Fig. 2B) (Nelis et al. 1983). Fluorescence at 295 nm excitation wavelength and at 330 nm emission was used to verify concentrations in samples with low tocopherol levels (Rettemaier and Schuep 1992). ß-Carotene was detected and quantified by its UV absorbance at 450 nm (Fig. 2D) (Guillou et al. 1993).

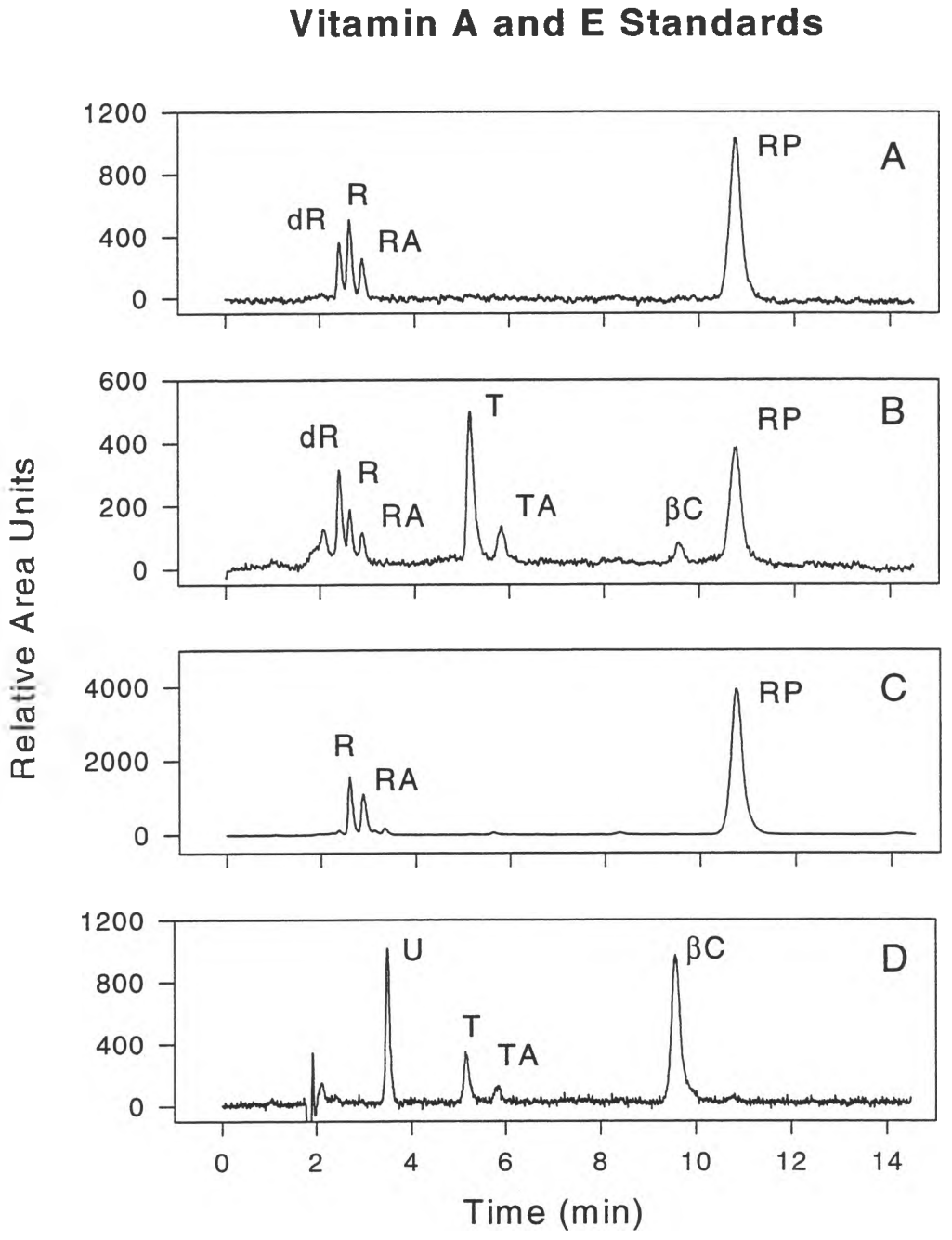

Figure 2. Separation of vitamin standard mix containing 4 ng dehydroretinol (dR), 2.5 ng retinol (R), 1.3 ng retinol acetate (RA), 90 ng tocopherol (T), 9 ng tocopherol acetate (TA), 3.3 ng ß-carotene and 28.8 ng retinyl palmitate (RP): (A) UV absorbance at 325 nm, (B) UV absorbance at 292 nm, (C) fluorescence at 330 nm excitation wavelength and at 480 nm emission and (D) UV absorbance at 450 nm.. An unknown substance (U) with absorbance at 450 nm was detectable in the standard mix.

The different absorbance/fluorescence properties can be exploited to infer that some unidentified chromatographic peaks were also vitamin A compounds (Spear et al. 1992; Ndayibagira et al. 1995). Based on their absorbance and fluorescence characteristics at least three dehydroretinyl esters and a retinyl ester were also present in many chromatographic profiles. Chromatographic peaks eluting at 8.3, 8.7 and 12.2 minutes likely represent dehydroretinyl esters while a peak eluting at 10.2 min likely represents a retinyl ester (see Fig. 2). Due to the lack of exact standards, precise quantification of these esters was not possible. For comparative purposes the major dehydroretinyl ester (dRE2) level was approximated. The estimates were based on the retinyl palmitate standards and a correction factor for the different UV absorbance of dehydroretinol and retinol at 325 nm (Stancher and Zonta 1984).

Reproducibility of the method was evaluated by 10 measurements of retinoids, tocopherol and ß-carotene in the same tissue. Both retinyl acetate and tocopherol acetate were used as an internal standard, with the recovery of known spikes used to correct for the efficiency of each extraction. Recovery efficiencies were determined by spiking known amounts of retinoids (8-400 ng), tocopherol (875-4375 ng) and ß-carotene (5-200 ng). Varying amounts of tissue (2.5-50 mg) were analyzed to examine the correlation between the amount of tissue and the concentration of retinoids, tocopherol and ß-carotene.

2.3. 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 Contaminant 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. Due to differences in some of the measured variables between sexes and state of maturity at some sites, data were analyzed as mature females, mature males and immature fish for each location. Differences between groups of fish collected at each site for any given parameter were tested by analysis of variance (MGLH) computed using the Systat statistical package (Wilkinson et al. 1992). The Bartlett or the Hartley tests were used to test for homogeneity of variance and, where necessary, data were log transformed to obtain more uniform variances. Pairwise comparisons were conducted by applying the LSD or Dunnett's test to the least squared means produced by the ANOVAs. A probability level of <0.05 was considered significant. For clarity of presentation arithmetic means with standard errors have been used in the figures.

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 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 River, 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 sites one (PR1), two (PR2) and three (PR3). Athabasca River fish are few and indicated for comparative purposes. To provide greater information for comparative purposes, we also analyzed hepatic retinyl palmitate levels in female longnose suckers collected by the Northern River Basins Study on the upper Athabasca River in the fall of 1992 (Barton et al. 1992). The sites sampled were site G (Near Entrance - upstream of Hinton), site H (Weldwood Haul Bridge at Hinton), site J (Obed Mountain Coal Bridge), site L (below Berland River confluence) and site M (Windfall Bridge).

Northern Pike. To simplify presentation, data for northern pike collected on the Peace drainage were grouped according to capture site: Wapiti River (WR1 & WR2), Smokey 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 1 with the data for each fish. Due to the small numbers no statistical analysis was undertaken.

3.0 RESULTS AND DISCUSSION

3.1. Vitamin Analyses

When known amounts of retinoids, tocopherol or ß-carotene were added to liver samples recovery of the spikes was 93.0 to 94.8%, 87.7 to 98.5% and 90.1 to 95.6 %, respectively. Retinyl acetate and tocopherol acetate, used as an internal standard, corrected for differences in sample extraction efficiencies (79.3±3.9%) in liver tissue. The precision of the method was evaluated by repeated measures of the same tissue sample. Ten separate extractions of 10 mg of tissue led to percent standard error measures of 1.14% for tocopherol, 2.18-4.21% for the retinoids and 3.21% for ß-carotene, respectively.

3.2 Burbot.

Extracts of burbot liver contained quantifiable levels of dehydroretinol, retinol, retinyl palmitate and tocopherol (Fig. 3). Based on absorbance and fluorescence characteristics there were also significant quantities of three dehydroretinyl esters (dRE1, dRE2, dRE3) and a retinyl ester (RE). Both dehydroretinyl esters and retinyl esters have also been identified in other feral fish species (Spear et al. 1992; Ndayibagira et al. 1995). ß-Carotene was generally near or below detection in all burbot samples.

Females (Figs. 4 & 5). In maturing female burbot, liver dehydroretinol, retinol, dRE2 and retinyl palmitate concentrations were similar between the reference, near-field and far-field locations (Fig. 4). Tocopherol tended to be higher at near-field sites relative to reference sites. On an individual

site basis, mean values ranged from, 0.36-2.65, 0.65-4.51, 61-208 and 43-435 ug/g liver for dehydroretinol, retinol, dRE2 and tocopherol, respectively. Distinct site specific differences were evident in the hepatic concentration of retinol and retinyl palmitate (Fig. 5). Levels of retinol were highest at reference sites A2 and P while retinyl palmitate concentrations were highest at sites P and SD. Relative to males and immature fish, female burbot tended to have lower levels of dehydroretinol. There were no other sex related differences in hepatic retinoid and tocopherol levels

Males (Figs. 6 & 7). While there were no field related differences in concentrations of hepatic retinoids and tocopherol (Fig. 6), there was considerable variability for dehydroretinol and retinol within the far-field locations. When examined from a site specific basis, with the exception of the single specimen collected in the PR3 group, hepatic retinol and dehydroretinol levels did not differ between collection sites (Fig. 7). The retinol and dehydroretinol levels in the single specimen collected at the PR3 site were remarkable and represent the highest recorded level. However firm conclusions regarding this observation cannot be made without a greater sample size. There were site specific differences in the retinoid esters. In the Peace River system, the highest levels of dRE2 and retinyl palmitate were found in the mainstream sites (PR1, PR2 & PR3) and the WB site. In the Athabasca River system, burbot from site A5 had the lowest levels of retinyl palmitate. Liver retinyl palmitate was highest in the fish collected at the SD location. Hepatic tocopherol was similar between all sites except for the very low value in the single fish collected at PR3.

Immature fish (Fig. 8 & 9). Immature fish showed no field related differences in the concentrations of retinol, dRE2 or retinyl palmitate (Fig. 8). Dehydroretinol was highest in the far-field locations but this was not different from the reference locations. Similar to female fish the levels of tocopherol were elevated in the near-field category. On a site-specific basis dehydroretinol was elevated in burbot collected at the PR3 site. Levels of retinol did not differ between sites. Location related differences were evident in the hepatic concentration of dehydroretinyl ester and retinyl palmitate. Lowest ester levels were found in immature burbot from the SR1 sites in the Peace river system and from the A5 site in the Athabasca system (Fig. 9). Low levels of tocopherol were observed in fish from the PR3 and A5 site. There were too few immature burbot collected from reference areas to facilitate comparisons.

While there are no comparative data available for burbot, hepatic concentrations of retinoids and tocopherol in burbot from the Peace and Athabasca drainages were similar to measurements in other feral freshwater species from reference areas (Spear et al. 1992; Friesen et al. 1994; Delorme et al. 1994). The very low to non-detectable levels of ß-carotene could indicate that burbot, like salmonids (Schiedt et al. 1985), do not readily absorb this compound from dietary sources. Also, it is worth noting that retinyl palmitate concentrations tended to be higher in burbot collected from the Slave Delta (SD). These fish were also in significantly better condition than the fish captured from the Peace and Athabasca locations (Brown et al. 1996), thereby emphasizing the importance of nutritional factors. The reason for the elevated tocopherol in burbot from near-field locations may reflect enhanced nutrient loadings in these areas. The very low levels of tocopherol in immature and the male fish at the PR3 site could be indicative of the presence of some type of oxidative stress. The higher levels of dehydroretinol and retinol in these same fish is possibly the result of impaired liver storage found during tocopherol deficiency (Wolf, 1984).

3.3 Longnose Sucker

Extracts of longnose sucker liver contained quantifiable levels of dehydroretinol, retinol, retinyl palmitate, tocopherol and ß-carotene (Fig. 10). Based on absorbance and fluorescence characteristics there were also significant quantities of one dehydroretinyl ester (dRE2) and a retinyl ester (RE). However, the major vitamin A storage form was retinyl palmitate. Few longnose sucker were captured on the Athabasca drainage.

Female Fish (Fig. 11). Dehydroretinol and retinol differed between collection sites (Fig. 11). However, there was no pattern with respect to reference, near-field and far-field locations. The retinoid esters (dRE2 and retinyl palmitate) were higher at the SR1 site than the others. Hepatic tocopherol levels tended to be the highest at the PR3 and SR1 sites. The retinol precursor ß-carotene was somewhat lower in fish collected from the near- and far-field locations but did not differ from fish at the WR reference site.

Male Fish (Figs.12). Few differences were apparent in male fish. Dehydroretinol showed some site related differences but these were unrelated to field location. There were no site specific differences in other retinoids, ß-carotene or tocopherol (Fig. 12).

Immature Fish (Fig. 13). Dehydroretinol and retinol levels tended to be elevated at the WR and PR3 sites (reference and far, respectively). However levels in fish from the LSR and PR3 sites were similar to the near-field site (Fig. 13). Concentrations of dRE2 and retinyl palmitate were elevated in liver samples from longnose sucker collected at the SR1 site (Fig. 13). Hepatic concentrations of ß-carotene were variable and did not differ between sites.

Fish Collected in Fall 1992 Upper Athabasca River (Fig. 14). Hepatic retinyl palmitate stores in female longnose suckers collected in 1992 (Fig. 14) were similar to those measured in female longnose suckers collected from the Peace River sites (Figs. 11 & 12). Highest retinoid levels occurred in reference fish collected upstream of the town of Hinton. Levels of retinyl palmitate were lower at the sites downstream of the combined input from the town of Hinton and the adjacent pulp mill.

There are few direct comparative data for concentrations of hepatic retinoids and tocopherol in longnose suckers. The liver levels of retinoids and tocopherol in longnose sucker from the Peace and Athabasca drainages were roughly similar to measurements in white suckers captured from reference areas (Spear et al. 1992; Friesen et al. 1994; Delorme et al. 1994). While some differences in retinoid stores were apparent, overall, the concentrations in longnose suckers were greater than the values reported for white suckers from polluted areas near Montreal (Spear et al. 1992; Branchaud et al. 1995). Lower levels of retinyl palmitate in the region downstream of Hinton is consistent with responses observed near a pulp mill in Manitoba (Friesen et al. 1994). The tocopherol levels found in the livers of longnose suckers were lower than those observed in burbot or trout (Palace and Brown 1994) . Despite lower levels of the pro-vitamin ß-carotene in females and immature fish at the PR and SR1 locations there seemed to be no repercussions on the retinoids.

3.4 Northern Pike

Extracts of northern pike liver contained quantifiable levels of dehydroretinol, retinyl palmitate, tocopherol and ß-carotene (Fig. 15). Based on absorbance and fluorescence characteristics there were also significant quantities of dehydroretinyl esters (dRE1, dRE2, dRE3) and lesser amounts of a retinyl ester (RE). Because retinol and retinyl palmitate were near detection limits by UV absorbance, both were routinely quantified by the more sensitive fluorescence detection. Observed means for female, male and immature fish are given in Figures 16, 17 and 18, respectively. Too few pike were sampled to provide interpretation of field or site related differences. In contrast to the burbot and longnose suckers, the dehydroretinoids formed the bulk of hepatic vitamin A compounds. Additionally, hepatic tocopherol levels tended to be lower than observed in the other species. These findings are similar to observations in brown bullheads collected in the Great Lakes Basin (L. Arcand and S.Brown unpublished data). At present, the reason for the species difference in type of vitamin A compounds is unclear. The ultimate cause may relate to different dietary/trophic factors as well as to differences in the amount of oxidative stress experienced by certain species.

4.0 CONCLUSIONS

There were species differences in the storage forms of hepatic vitamin A from burbot, longnose sucker and northern pike. Burbot livers contained approximately equivalent amounts of retinyl and dehydroretinyl esters. Livers from longnose suckers contained predominantly retinyl esters while northern pike contained mostly dehydroretinyl esters.

With few exceptions the levels of dehydroretinols and retinol were relatively high and fell within ranges observed in reference fish from other studies (Friesen et al. 1994; Gilbert et al. 1995; Palace and Brown 1994; Palace et al. 1995). While some differences I retinoid stores were apparent between field locations and site, the concentrations in fish from this study were greater than the very low values found in fish exposed to environmental contaminants in other locations (Spear et al. 1992; Friesen et al. 1994; Palace and Brown 1994; Branchaud et al. 1995). White sucker collected from polluted areas (Spear et al. 1992; Friesen et al. 1994) or lake trout treated with MFO inducing PCBs in the laboratory (Palace and Brown 1994) have minimal levels of retinyl palmitate (<20 ug/g). Burbot collected from the A5 site, near the tar sands development (SUNCOR) displayed mildly elevated hepatic MFO concentrations in male and immature fish (W. L. Lockhart pers. Com.). Hepatic levels of retinyl palmitate were marginal and ear 20 ug/g in fish at the A5 location, but they did not differ significantly from those found in burbot collected from some reference locations. Thus, the mild MFO induction observed in burbot from both the A5 and WB sites was probably insufficient to drastically lower hepatic vitamin stores. The lower levels of retinyl palmitate in longnose suckers collected from the region downstream of Hinton in 1992 is consistent with responses observed in white suckers near a pulp mill in Manitoba (Friesen et al. 1994). However, the overall magnitude of the response is much smaller than observed in other species collected near pulp mill effluent (Friesen et al. 1994; Brown and Munkittrick unpublished data). The very low levels of tocopherol in immature and male burbot at the PR3 site may be indicative of the presence of some form of oxidative stressor(s). The higher levels of dehydroretinol and retinol in these fish is possibly the result of impaired liver storage found during

tocopherol deficiency (Wolf, 1984). Although, the effect was not readily apparent in longnose sucker from the PR3 site, fish collected from this site warrant further scrutiny. The elevated tocopherol levels found in burbot collected from the near-field locations seems likely to be related to nutritional factors in the near-field region.

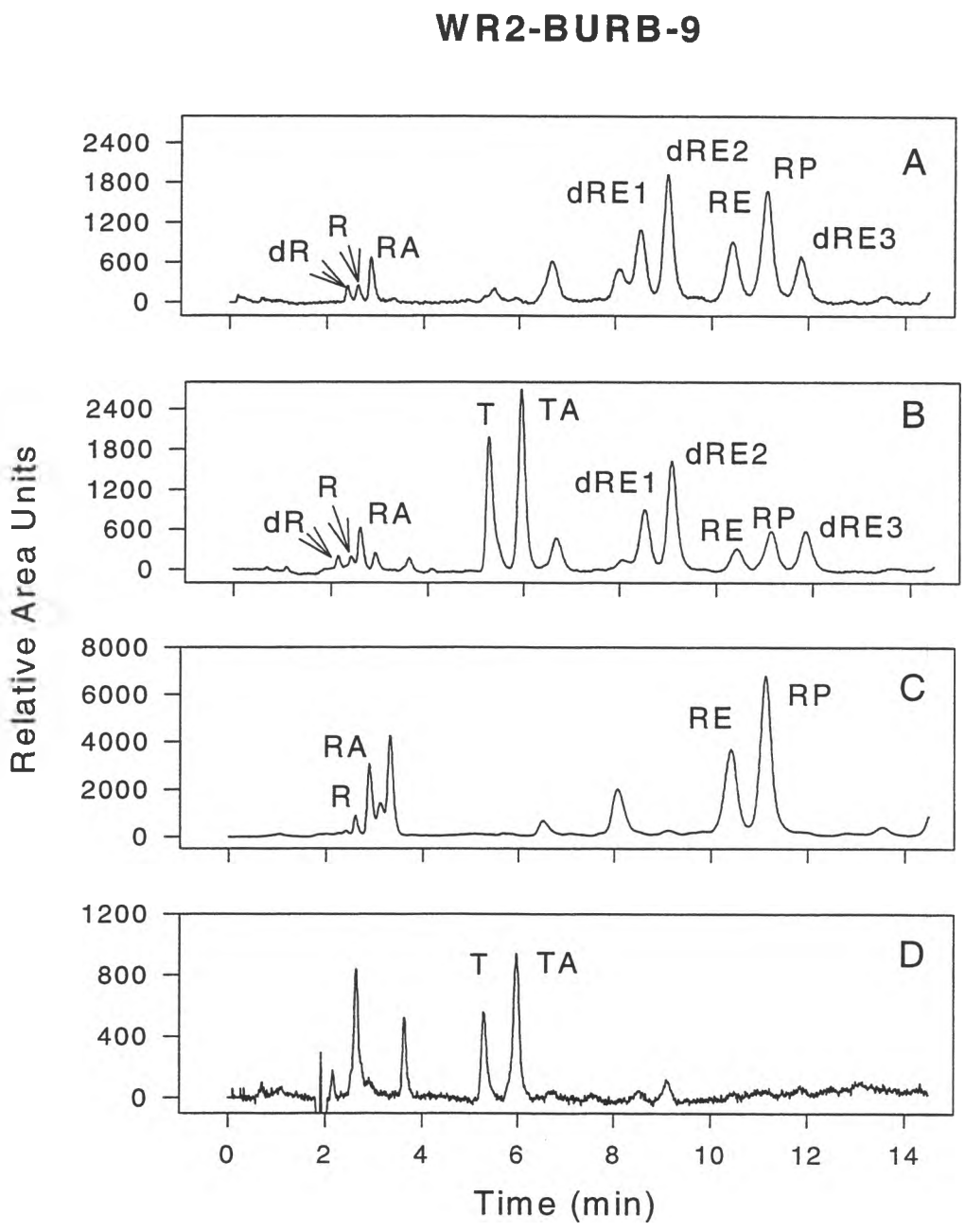

Figure 3. Elution profile of liver extract from a burbot (WR2-BURB-9): (A) UV absorbance at 325 nm, (B) UV absorbance at 292 nm, (C) fluorescence at 330 nm excitation wavelength and at 480 nm emission and (D) UV absorbance at 450 nm. Peaks routinely quantifiable in burbot include dehydroretinol (dR), retinol (R), tocopherol (T), and retinyl palmitate (RP). Internal standards retinyl acetate (RA) and tocopherol acetate (TA) were used to correct for extraction efficiency. Three dehydroretinyl esters (dRE1. dRE2, dRE3) and a retinyl ester (RE) are also present.

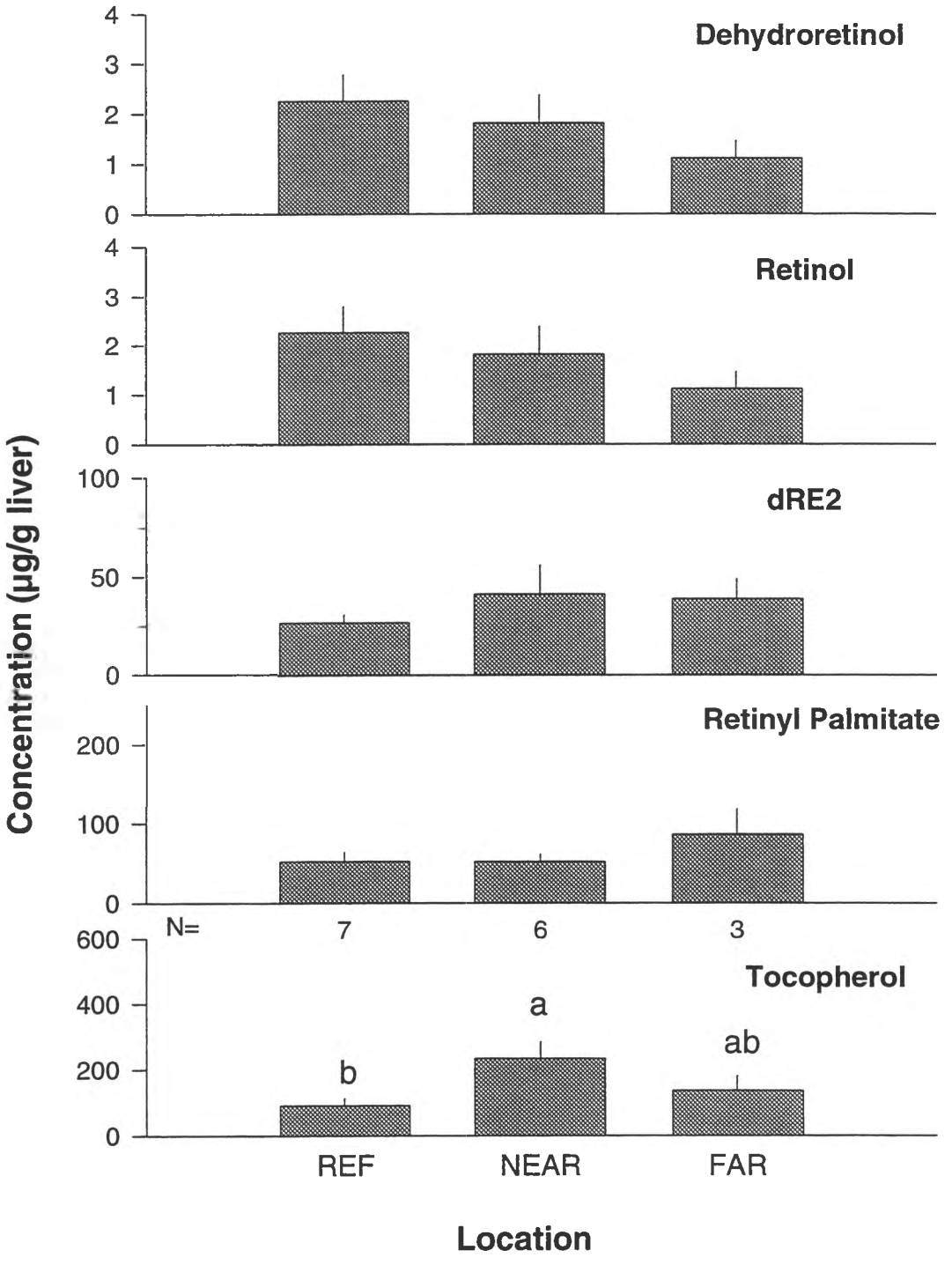

Figure 4. Mean values for retinoids and tocopherol in livers of female burbot from reference, near-field and far-field locations. Histogram bars represent mean and SEM. The same letters above the bars indicate similar means ($P<0.05$). Number of sites used in the analysis are indicated after N=.

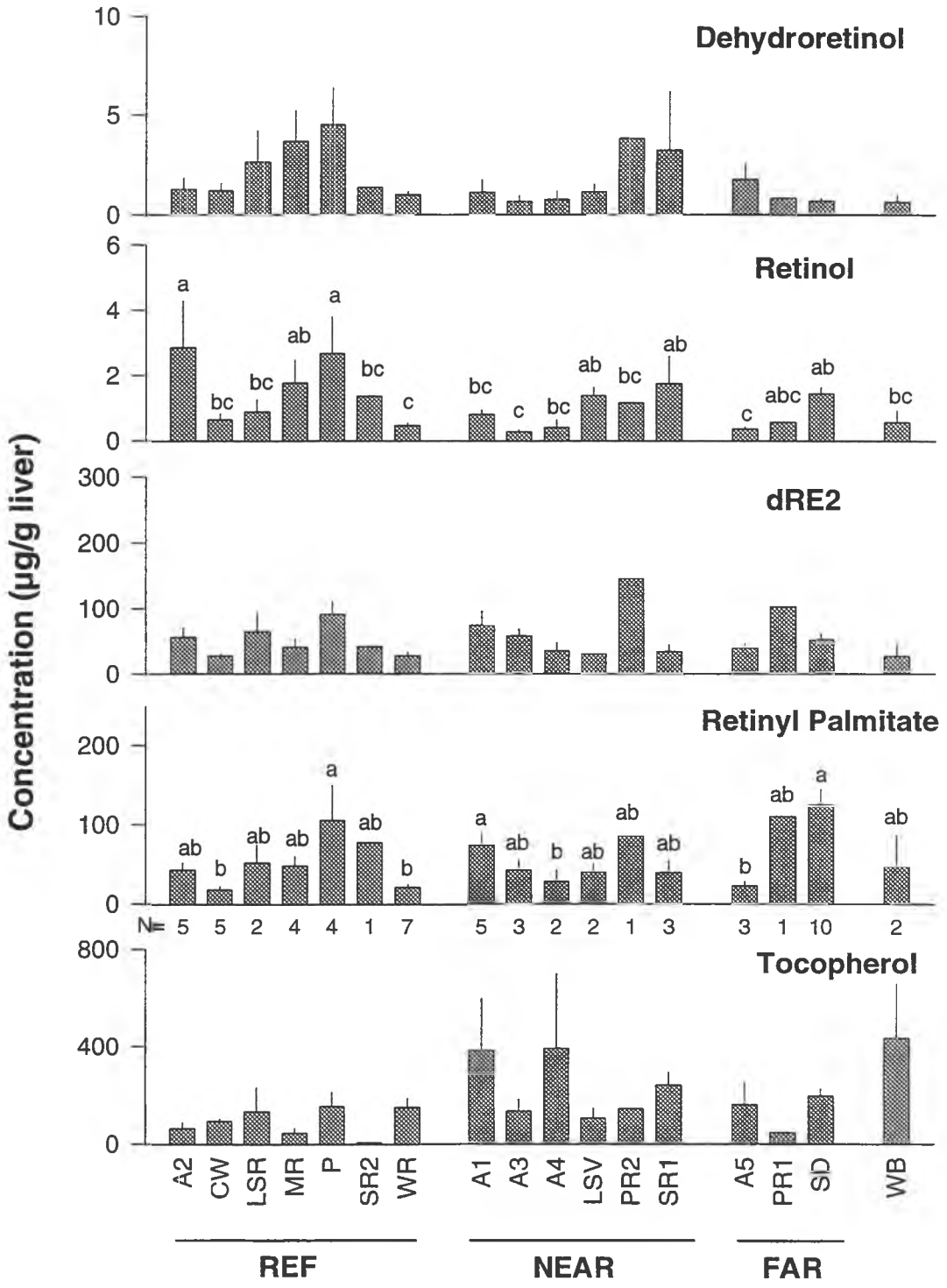

Figure 5. Retinoids and tocopherol in livers of female burbot from the various sample sites. Histogram bars represent mean and SEM. The same letters above the bars indicate similar means ($P<0.05$). Sample sizes are indicated after N=.

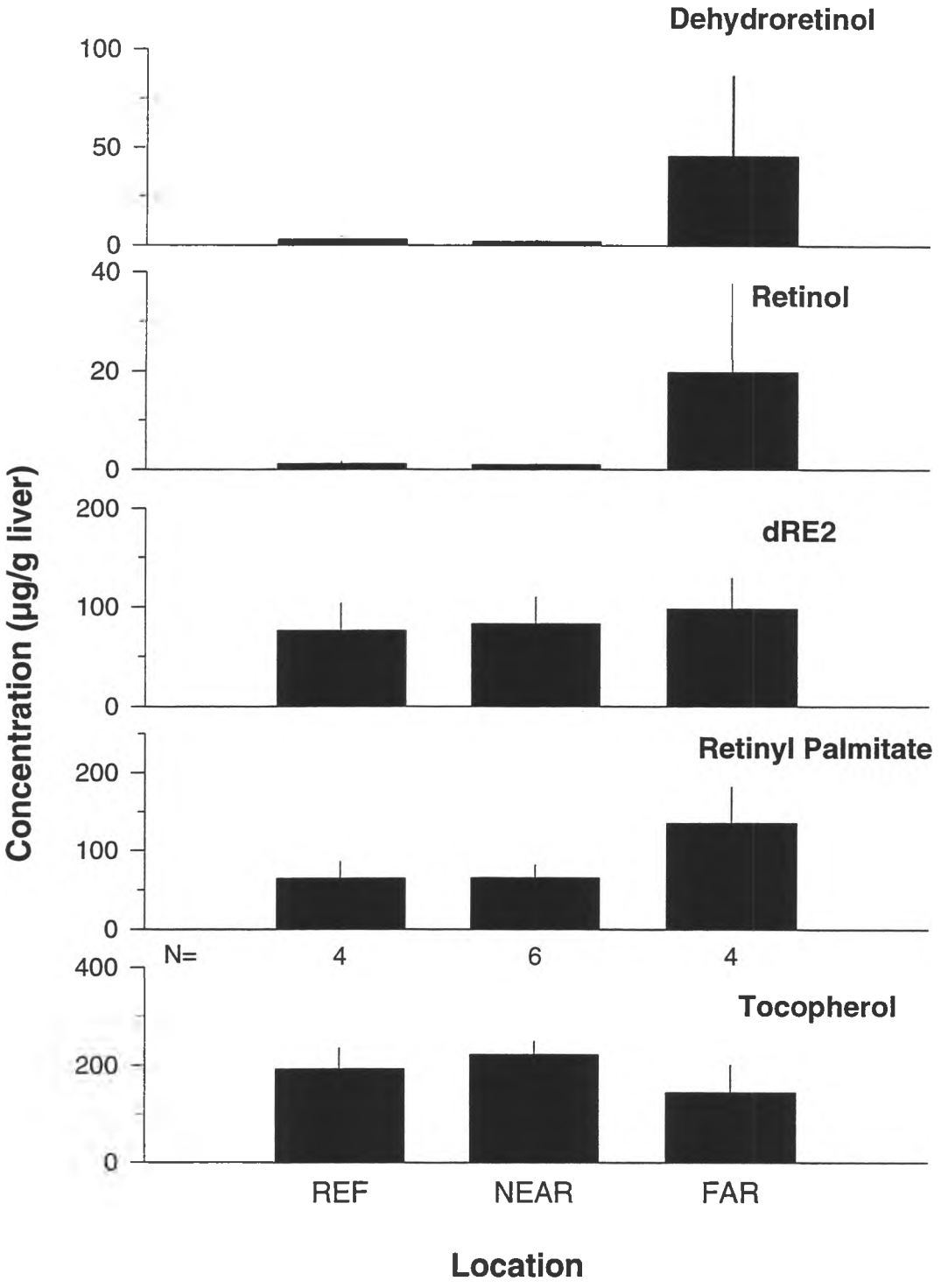

Figure 6. Mean values for retinoids and tocopherol in livers of male burbot from reference, near-field and far-field locations. Histogram bars represent mean and SEM. The same letters above the bars indicate similar means ($P<0.05$). Number of sites used in the analysis are indicated after N=.

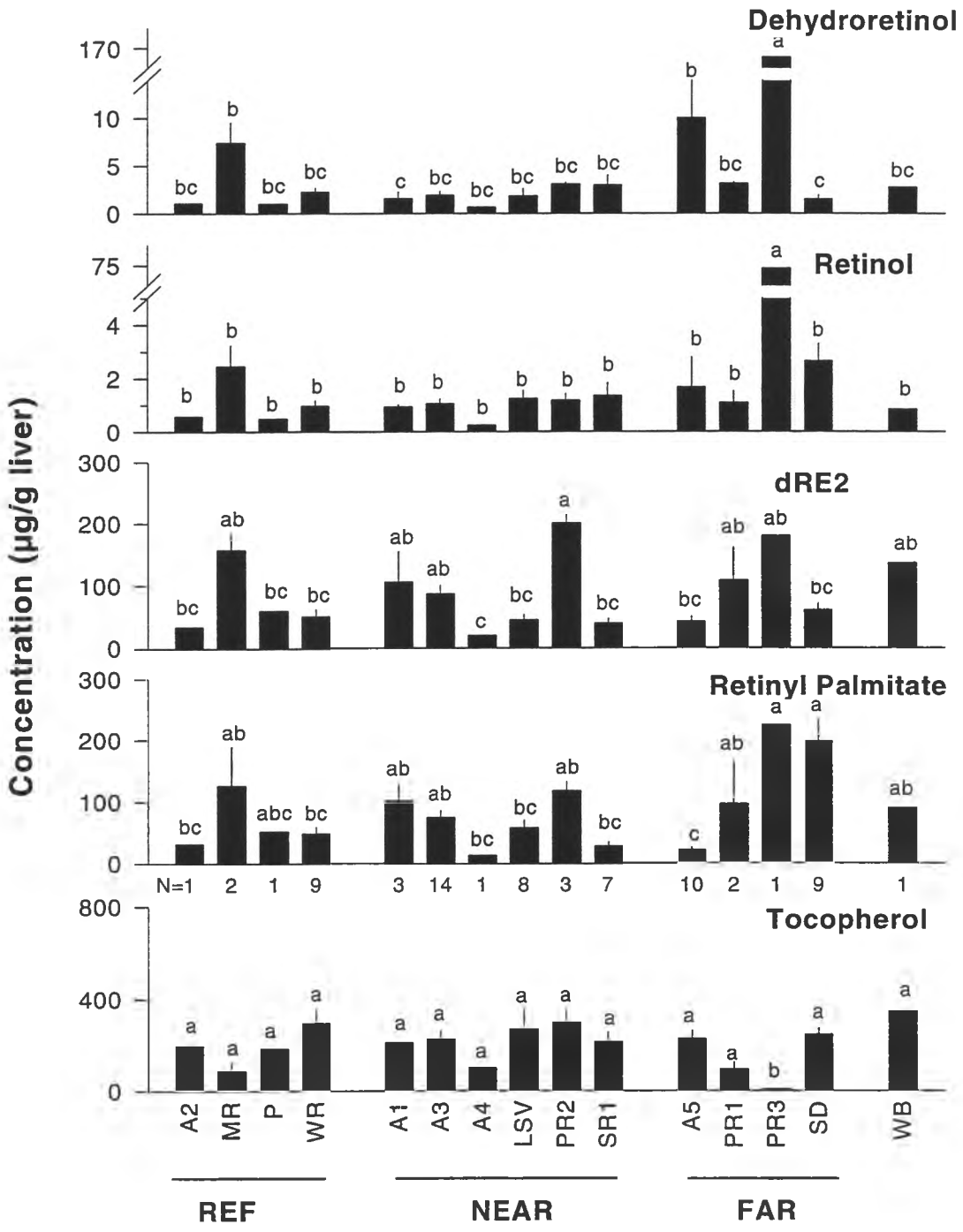

Figure 7. Retinoids and tocopherol in livers of male burbot from the various sample sites. Histogram bars represent mean and SEM. The same letters above the bars indicate similar means ($P<0.05$). Sample sizes are indicated after N=.

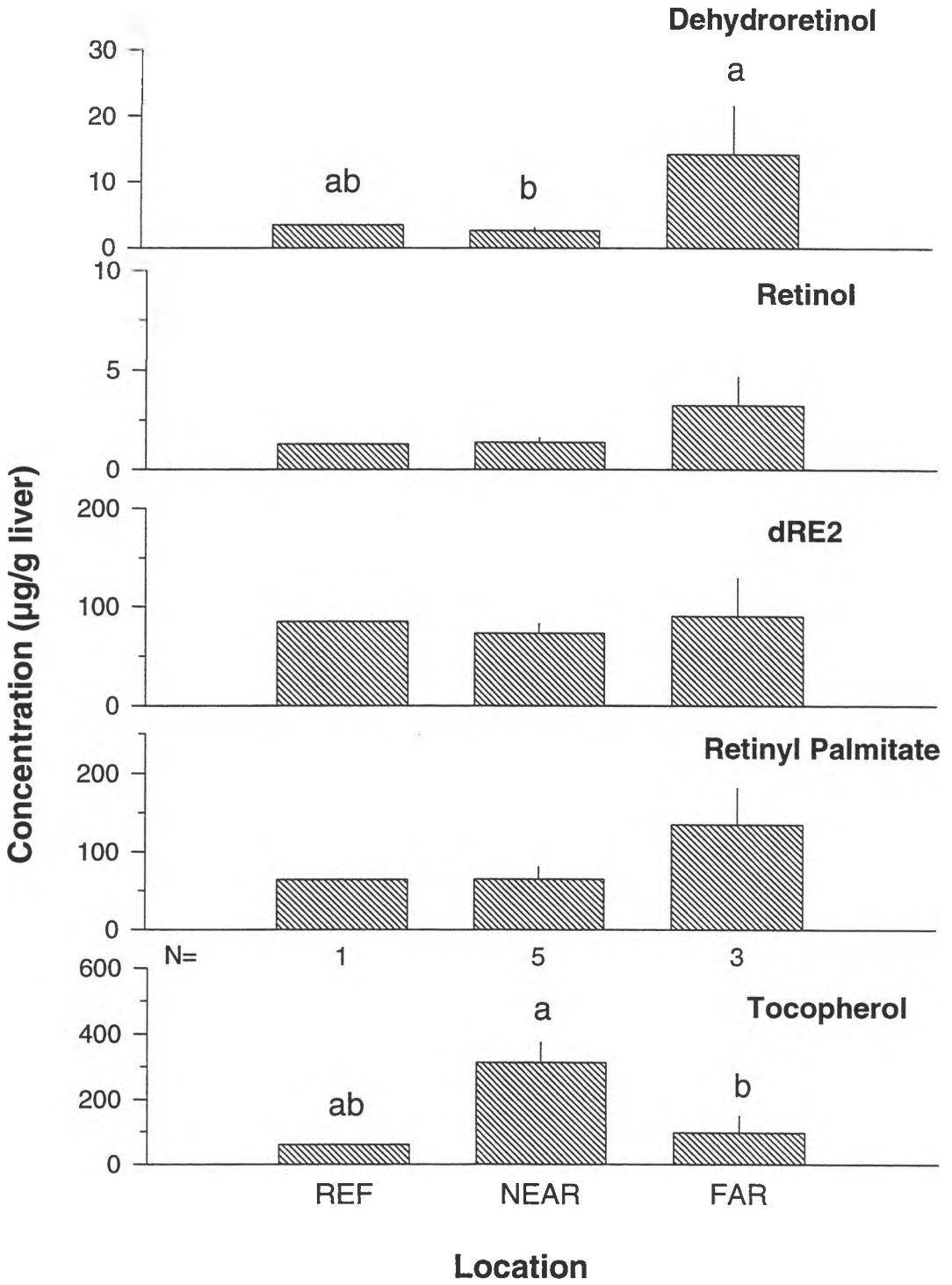

Figure 8. Means values retinoids and tocopherol in livers of immature burbot from reference, near-field and far-field locations. Histogram bars represent mean and SEM. The same letters above the bars indicate similar means ($P<0.05$). Number of sites used in the analysis are indicated after N=.

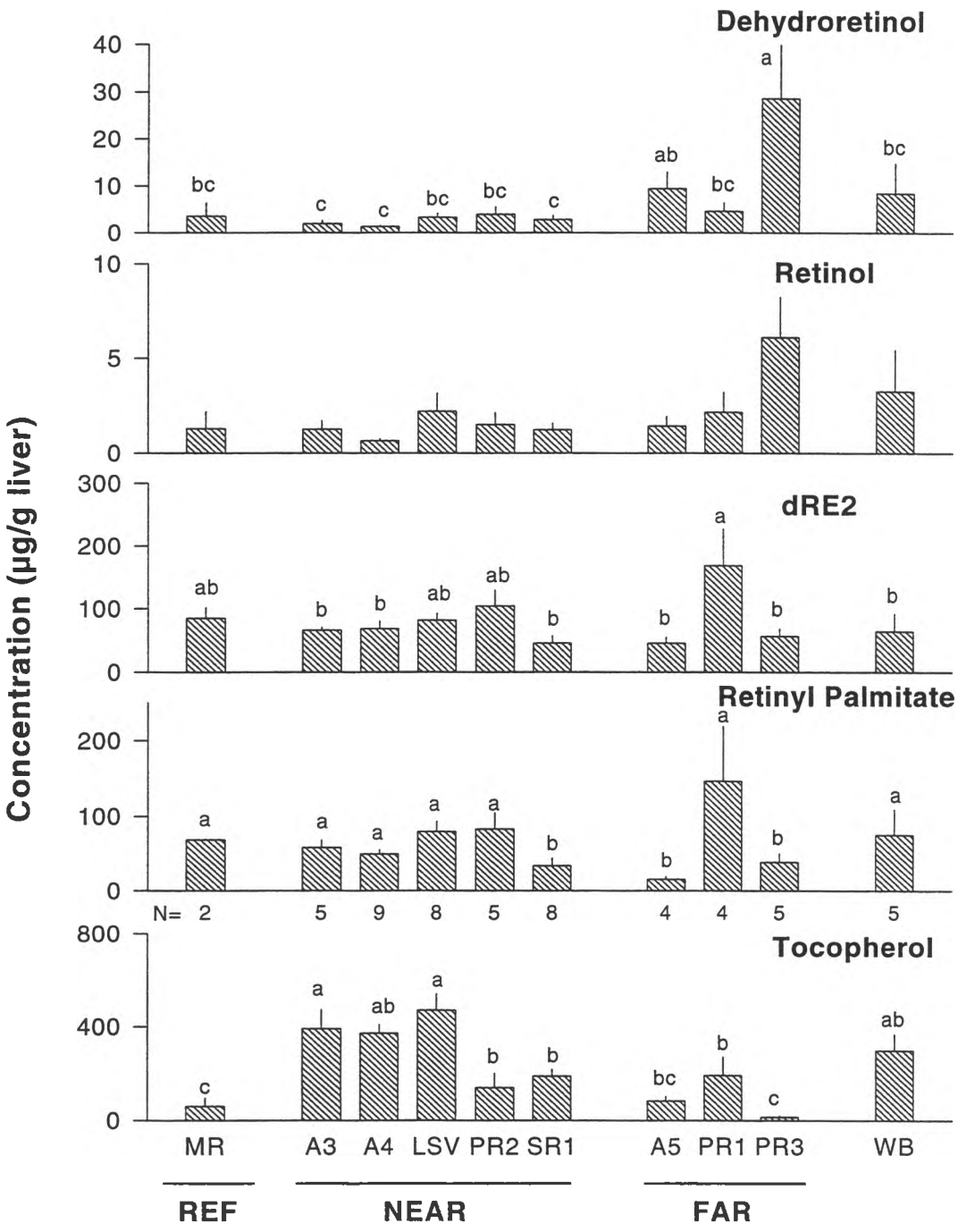

Figure 9. Retinoids and tocopherol in livers of immature burbot from the various sample sites. Histogram bars represent mean and SEM. The same letters above the bars indicate similar means ($P<0.05$). Sample sizes are indicated after N=.

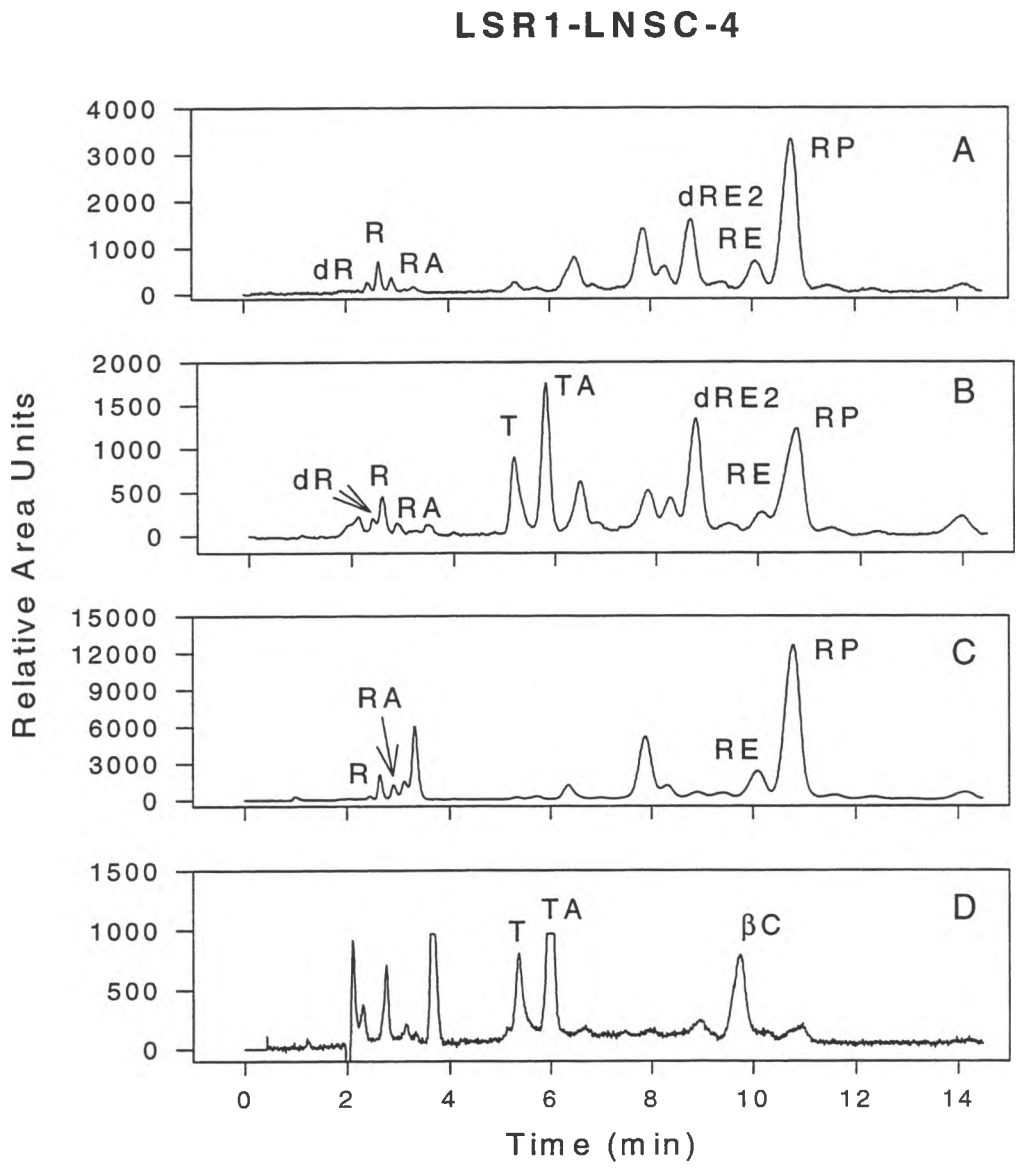

Figure 10. Elution profile of liver extract from a longnose sucker (LSR1-LNSC-4): (A) UV absorbance at 325 nm, (B) UV absorbance at 292 nm, (C) fluorescence at 330 nm excitation wavelength and at 480 nm emission and (D) UV absorbance at 450 nm. Peaks routinely quantifiable in longnose sucker include dehydroretinol (dR), retinol (R), tocopherol (T), retinyl palmitate (RP) and ß-carotene (ßC). Internal standards retinyl acetate (RA) and tocopherol acetate (TA) were used to correct for extraction efficiency. Based on absorbance and fluorescence characteristics a dehydroretinyl ester (dRE2) and a retinyl ester (RE) are also present.

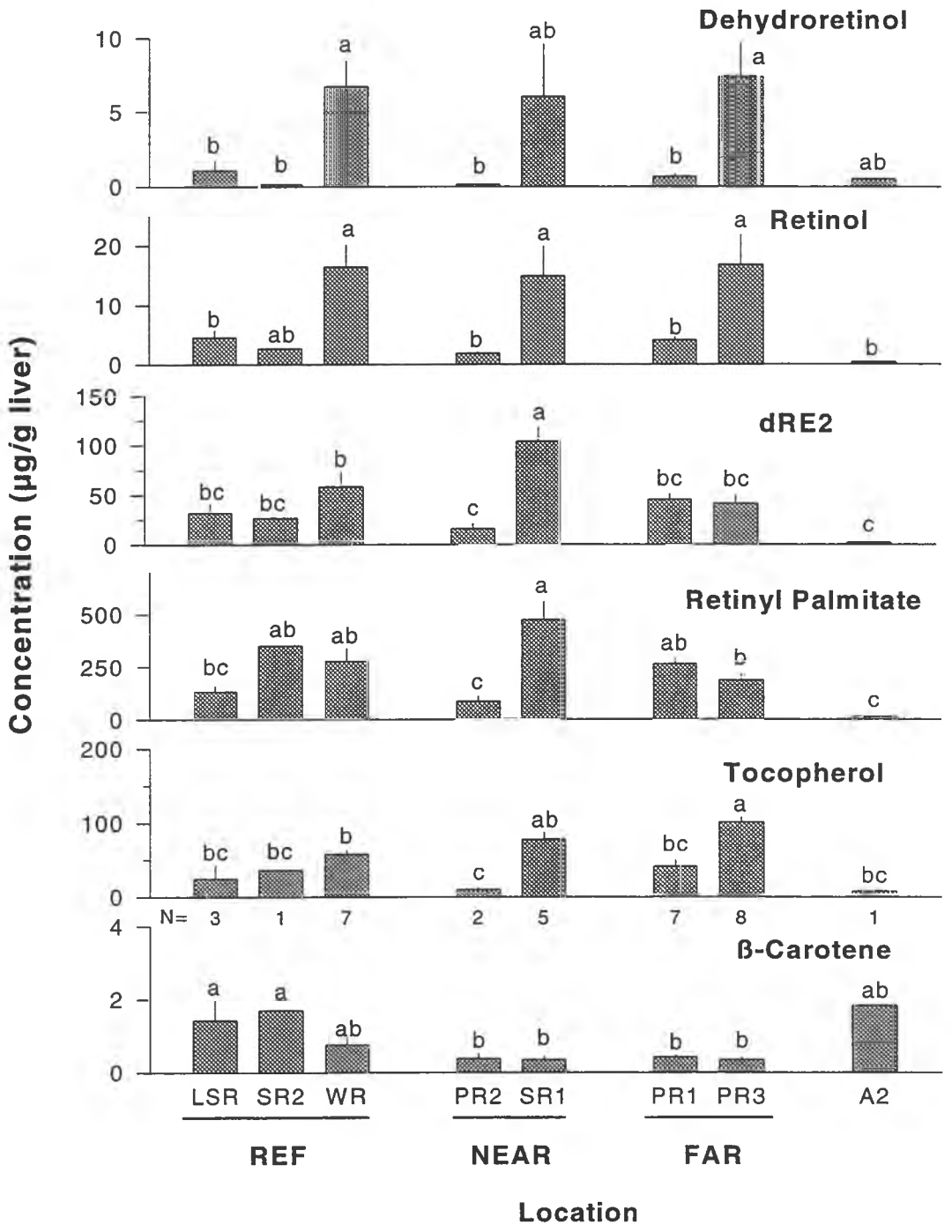

Figure 11. Retinoids, tocopherol and ß-carotene in livers of female longnose sucker from the various collection sites. Histogram bars represent mean and SEM. The same letters above the bars indicate similar means ($P<0.05$). Sample sizes are indicated after N=.

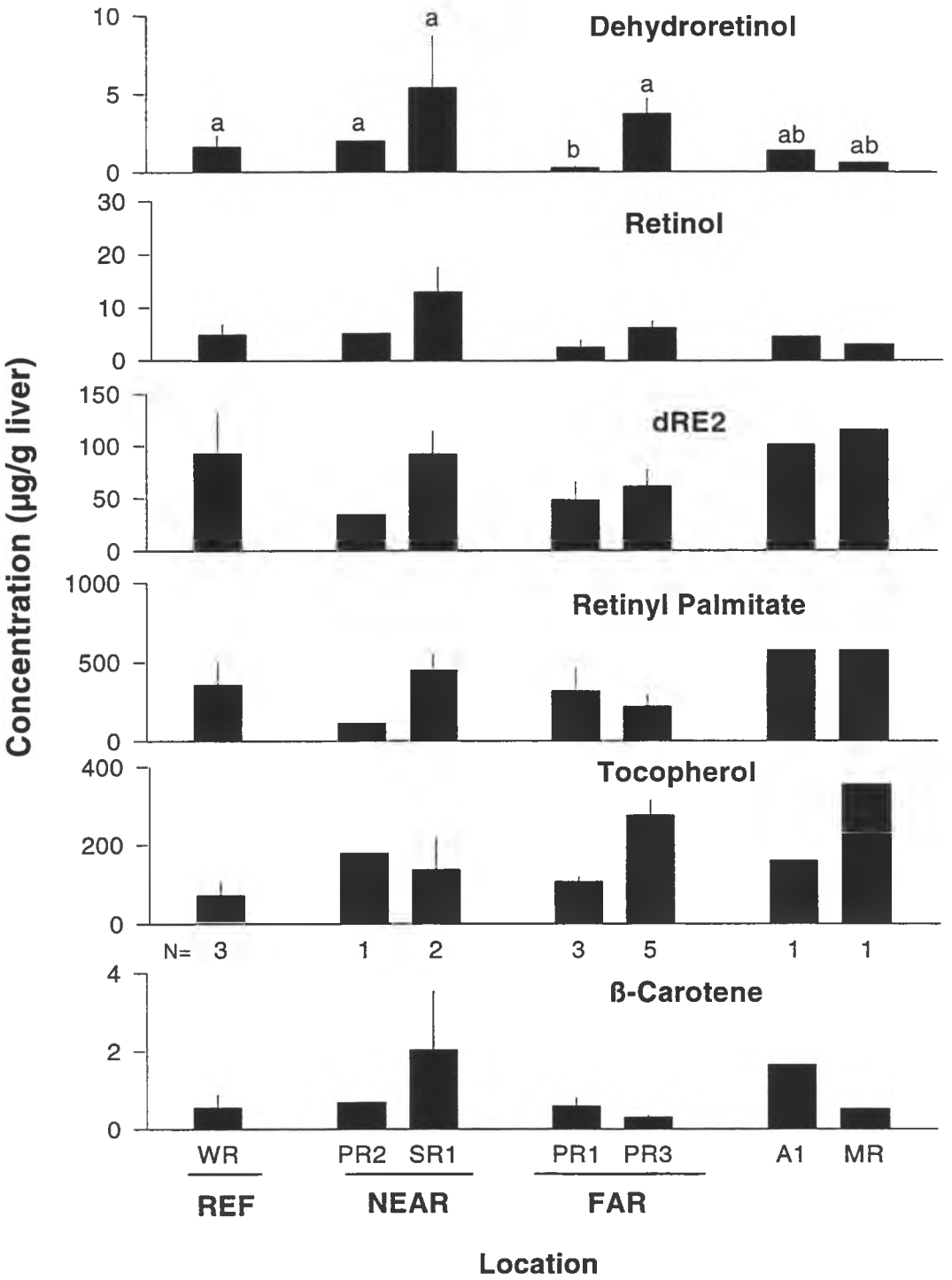

Figure 12. Retinoids, tocopherol and ß-carotene in livers of male longnose sucker from the various collection sites. Histogram bars represent mean and SEM. The same letters above the bars indicate similar means ($P<0.05$). Sample sizes are indicated after N=.

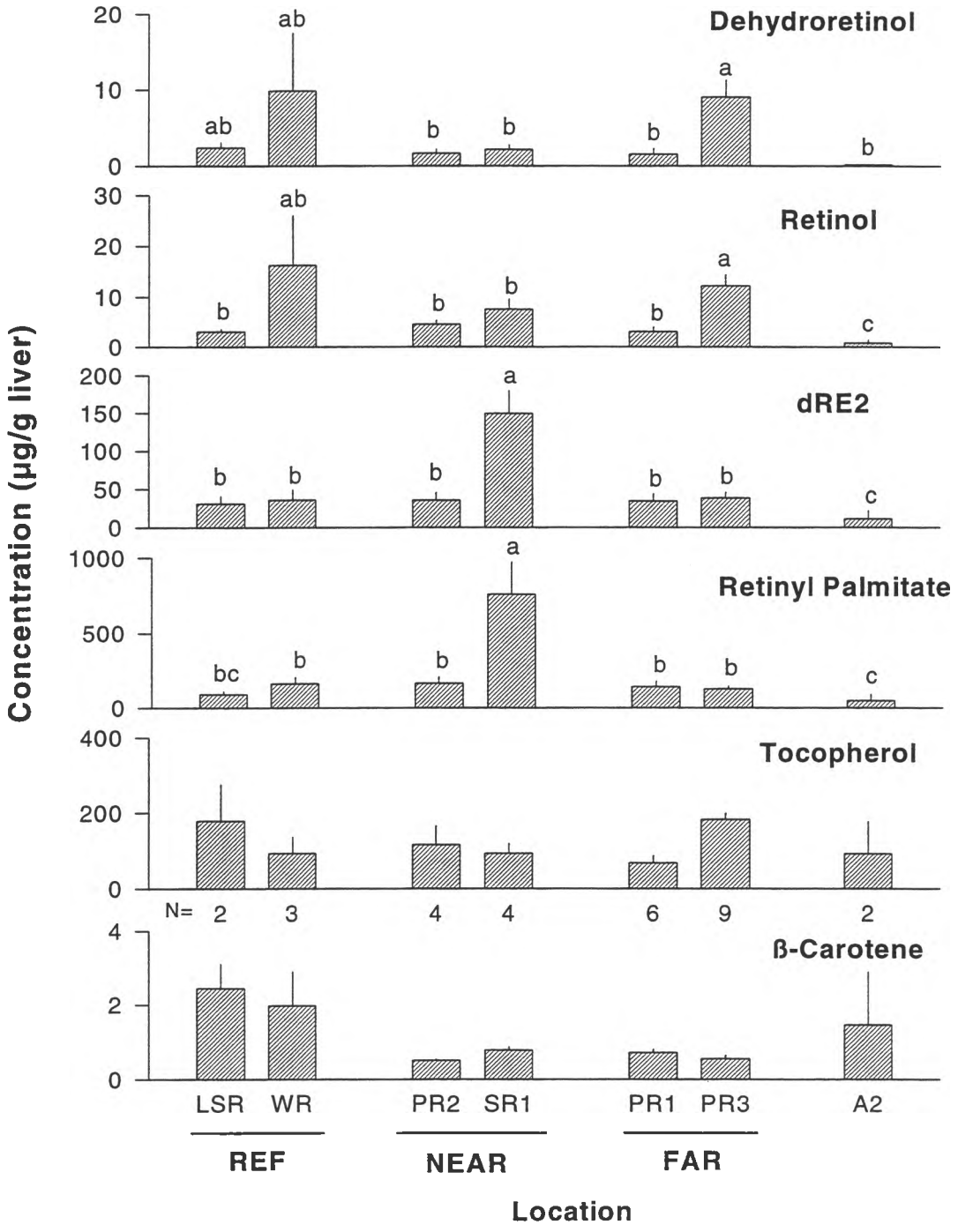

Figure 13. Retinoids, tocopherol and ß-carotene in livers of immature longnose sucker from the various sites. Histogram bars represent mean and SEM. The same letters above the bars indicate similar means ($P<0.05$). Sample sizes are indicated after N=.

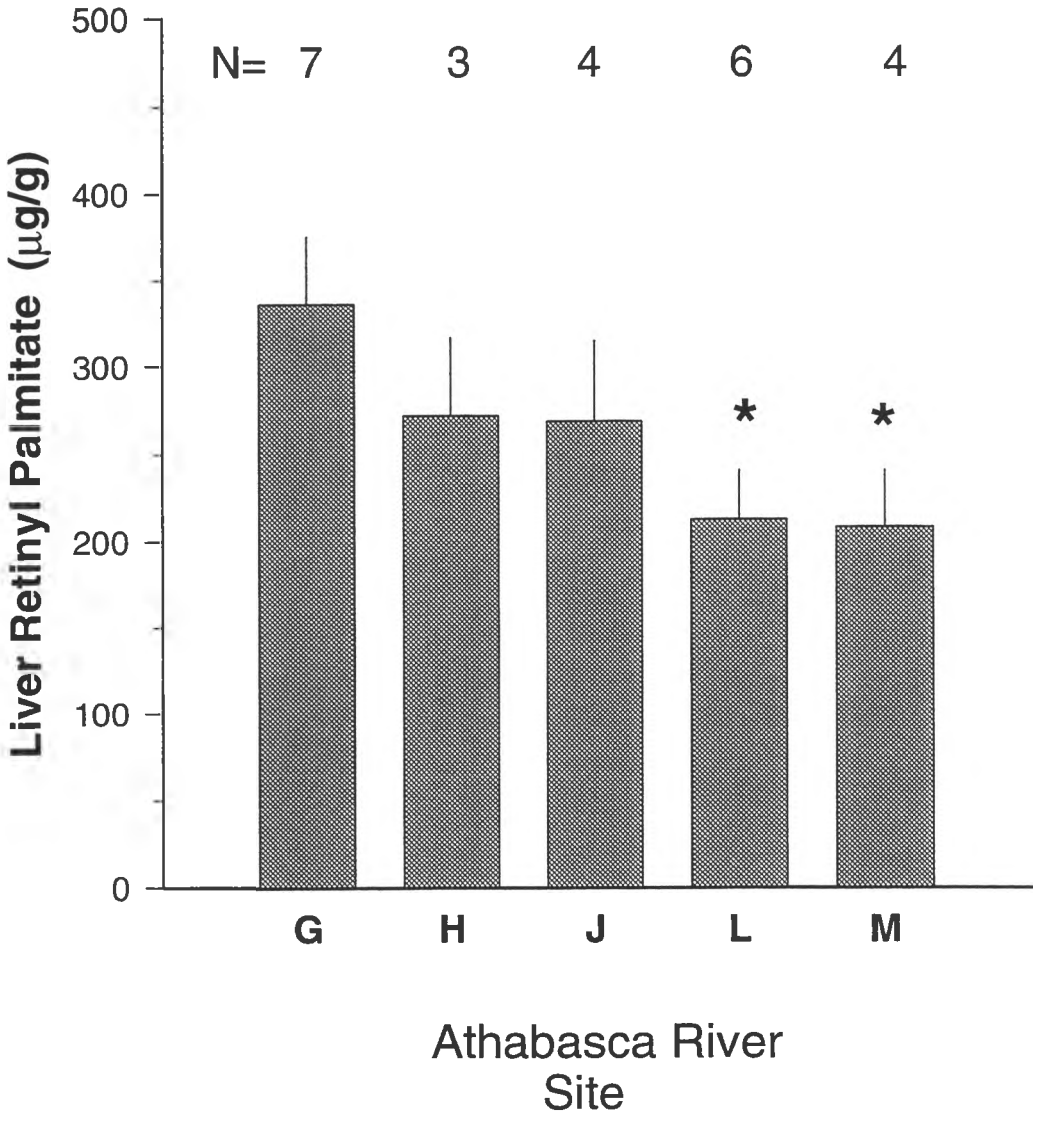

Figure 14. Retinyl palmitate in female longnose sucker collected in the fall 1992 at various sites on the Upper Athabasca River. Site G was located upstream of effluent outfall at Hinton while sites H, J, L and M are located downstream of inputs from Hinton (see Barton et al. 1992). Asterisk indicate groups significantly different from G group by Dunnett's test ($P<0.05$). Sample sizes are indicated after N=.

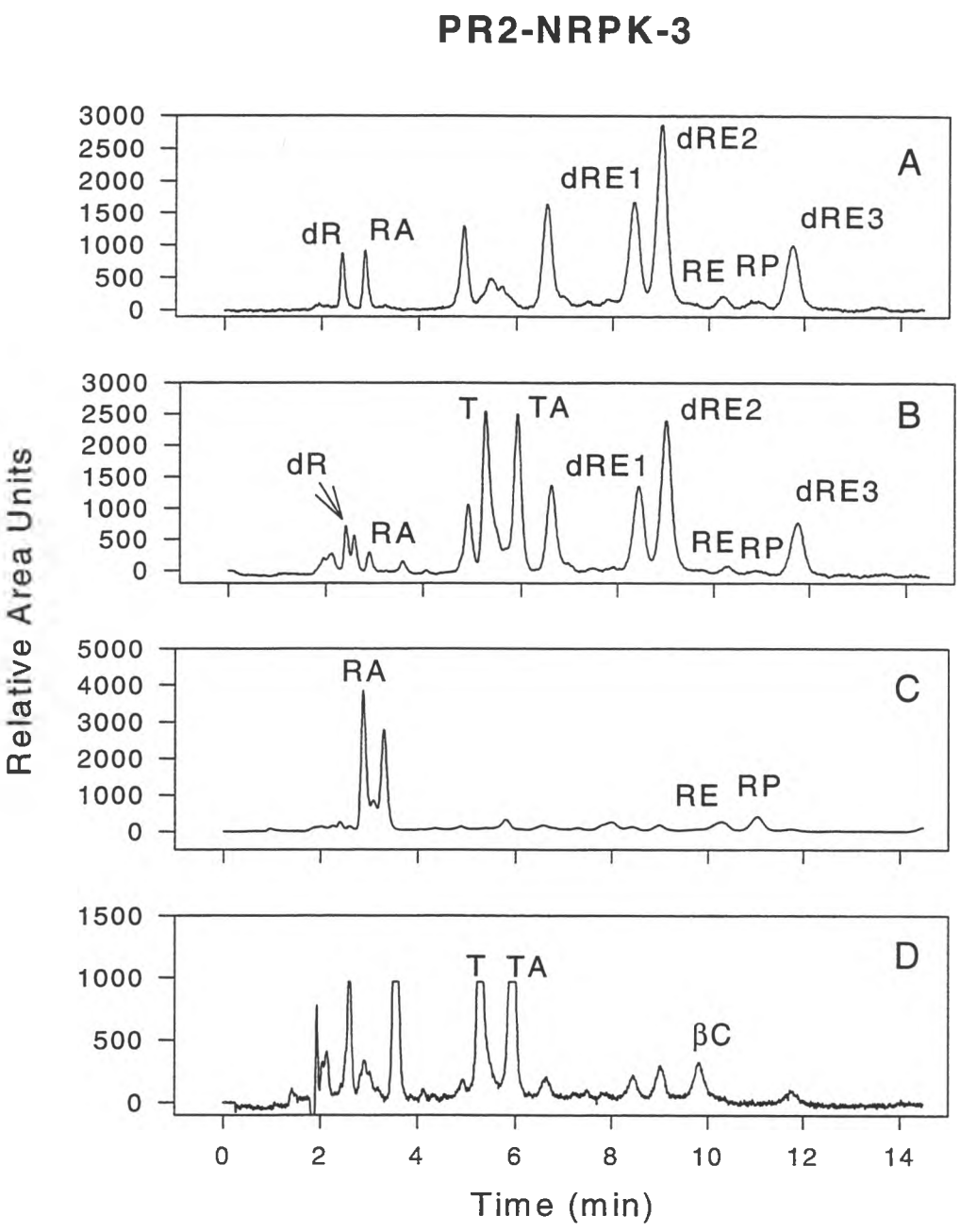

Figure 15. Elution profile of liver extract from a northern pike (PR2-NRPK-3): (A) UV absorbance at 325 nm, (B) UV absorbance at 292 nm, (C) fluorescence at 330 nm excitation wavelength and at 480 nm emission and (D) UV absorbance at 450 nm. Peaks routinely quantified in northern pike include dehydroretinol (dR), tocopherol (T), retinyl palmitate (RP) and ß-carotene (ßC). Internal standards retinyl acetate (RA) and tocopherol acetate (TA) were used to correct for extraction efficiency. Based on absorbance and fluorescence characteristics at least three dehydroretinyl esters (dRE1. dRE2, dRE3) and a retinyl ester (RE) are also present.

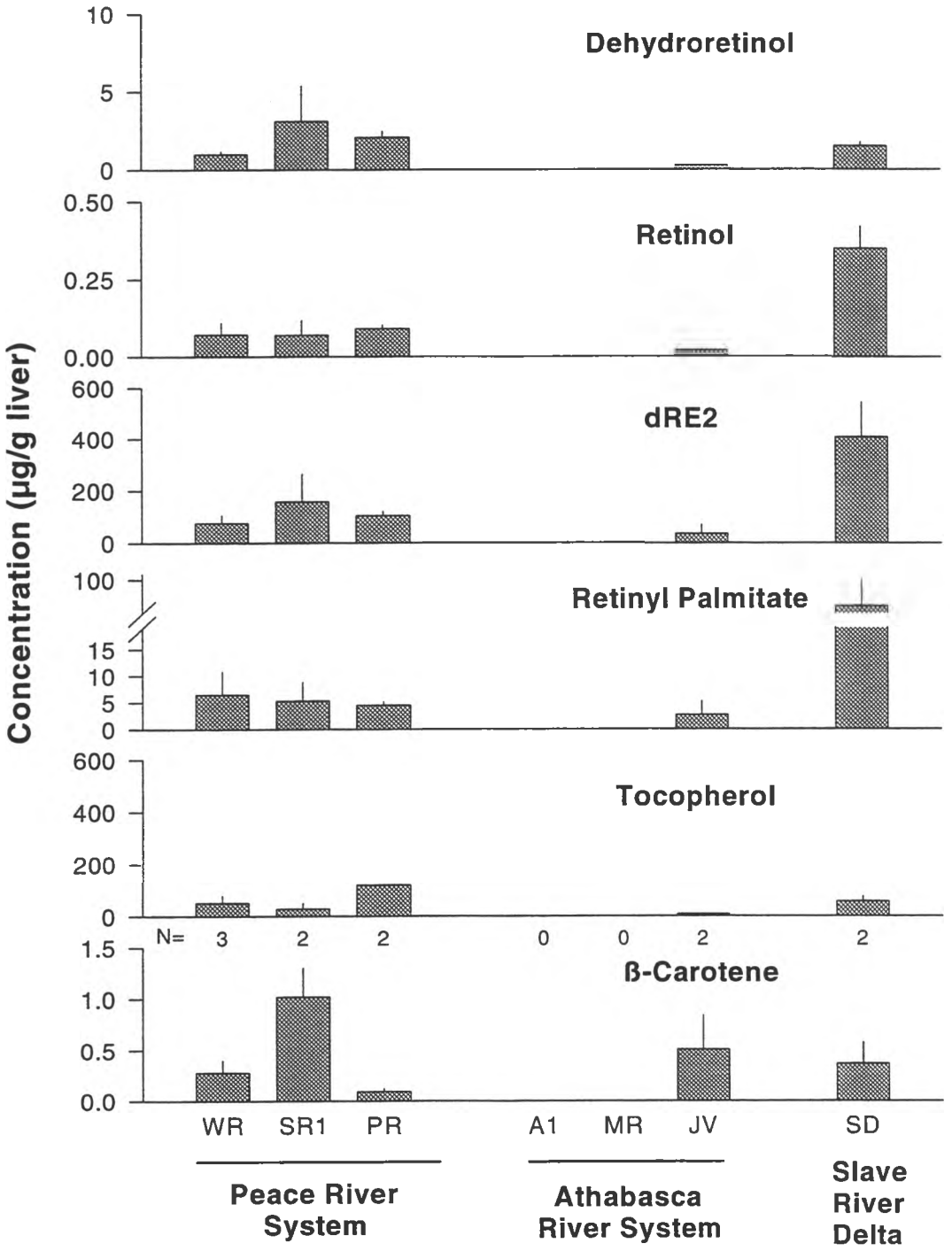

Figure 16. Retinoids, tocopherol and ß-carotene in livers of female northern pike collected at the various sites in the Peace/Athabasca/Slave river systems. Histogram bars represent mean and SEM. Sample sizes are indicated after N=.

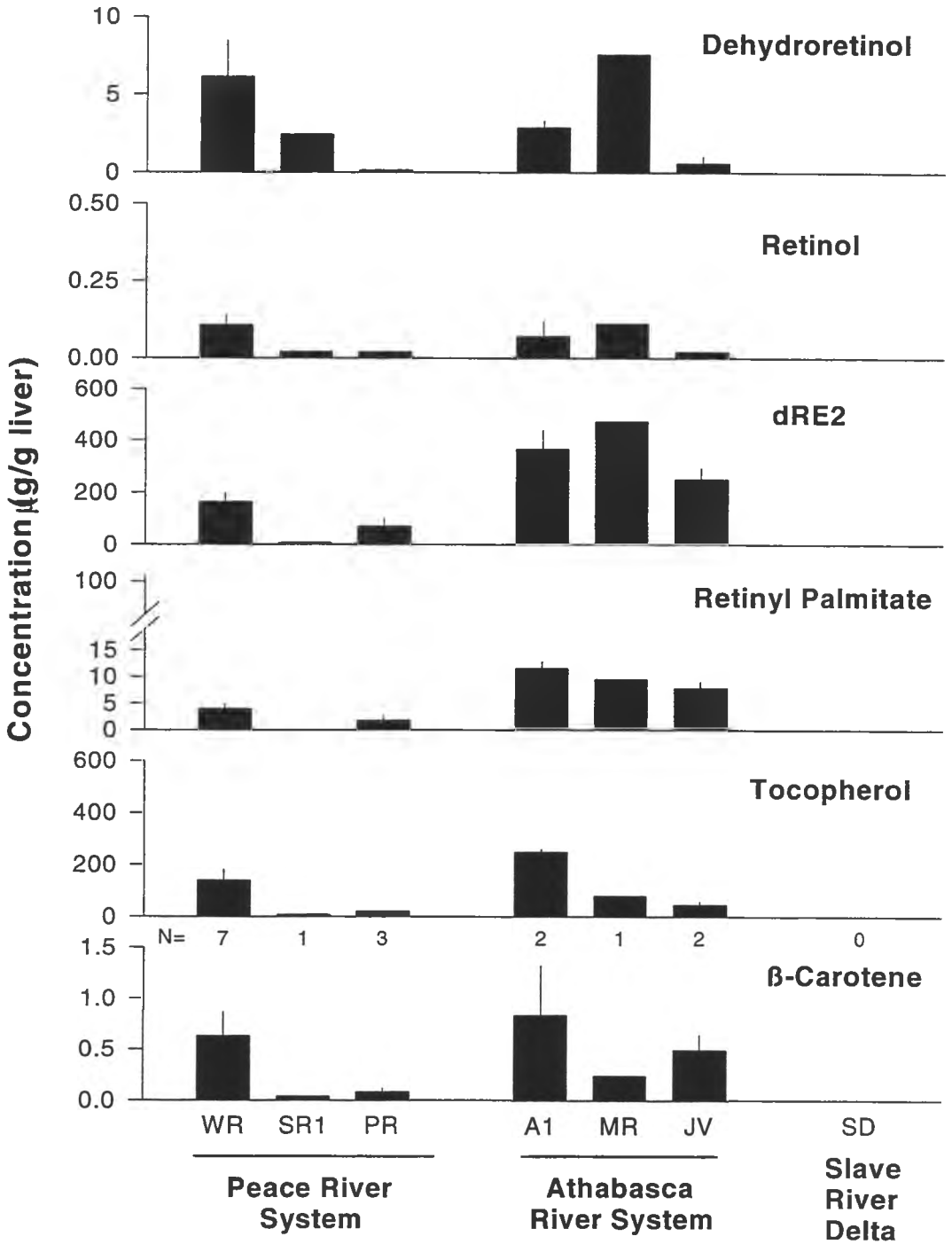

Figure 17. Retinoids, tocopherol and ß-carotene in livers of male northern pike collected at the various sites in the Peace/Athabasca/Slave river systems. Histogram bars represent mean and SEM. Sample sizes are indicated after N=.

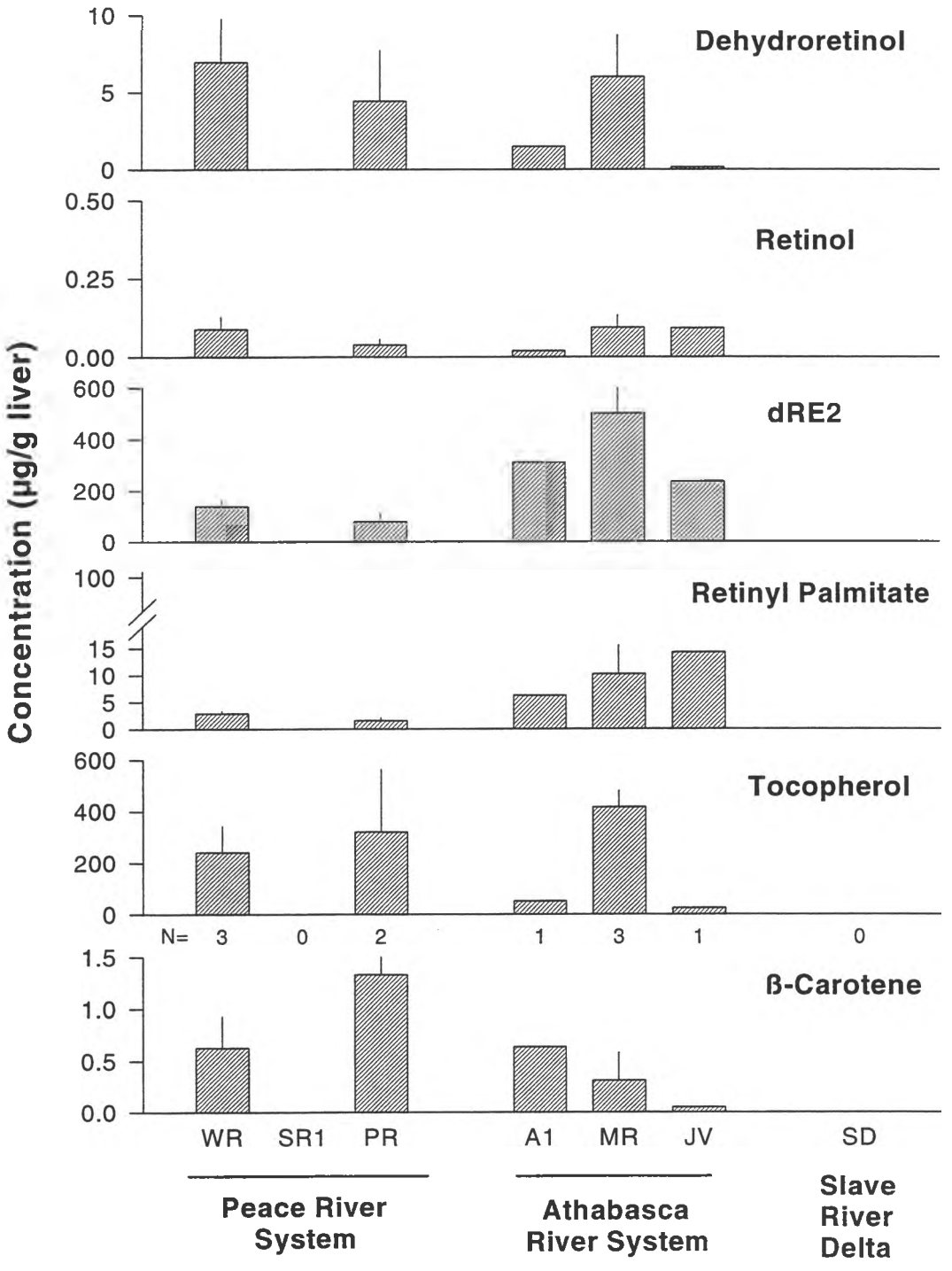

Figure 18. Retinoids, tocopherol and ß-carotene in livers of immature pike collected at the various sites in the Peace/Athabasca/Slave river systems. Histogram bars represent mean±SEM. Sample sizes are indicated after N=.

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APPENDIX A

NORTHERN RIVER BASINS STUDY

SCHEDULE A - TERMS OF REFERENCE

Project 3144-D4: 1994 Fall Basin-Wide Burbot Collection - Retinol, Dehydroretinol and Retinyl Palmitate Analyses

I. BACKGROUND & OBJECTIVES

A variety of important physiological processes related to development, regeneration and reproduction depend on adequate levels of retinol (vitamin A). Imbalances of vitamin A has been associated with a variety of anomalies including reproductive impairment, embryonic mortality, growth retardation and bone deformities. In fish, retinol deficiency has been associated with loss of vision, edema, depigmentation and impaired growth.

Because vitamin A is an essential dietary element it has recently received increasing attention as a possible indicator of exposure to a variety of environmental contaminants. In birds and mammals, alterations of vitamin A are known to be caused by a variety of pollutants, including both polynuclear aromatics and PHAHs that bind to the Ah receptor. Alterations of vitamin A has also frequently, but not always, been correlated to mixed function oxygenase (MFO) induction. Information about retinoids stores in fish exposed to organic contaminants is, however, limited. In studies of Lake Char, Palace and Brown (1994) showed that liver retinol, dehydroretinol and retinyl palmitate concentrations declined and kidney palmitate levels increased in PCB-exposed fish. They concluded that tissue retinoid concentrations provide sensitive indicators of coplanar PCB exposure in lake char. Recent studies by Brown (unpublished data) also show that retinoid levels are altered in fish exposed to pulp mill effluents.

The aquatic fauna of the northern river basins are exposed to pulp mill and other types of municipal and industrial effluents. Contaminant studies have shown elevated concentrations of dioxins and furans in tissues of fish collected near these effluent sources. In the spring and fall of 1992 the Northern River Basins Study (NRBS) also 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.* 1992a&b). These fish were analyzed for hepatic MFO induction. The results of these analyses were somewhat inconclusive, however, mountain whitefish from sites downstream of Hinton showed small increases in liver microsomal enzyme activity relative to fish collected from upstream sites (Lockhart *et al.* 1993). Swanson (1993) also reported elevated EROD activity in mountain whitefish and longnose suckers exposed to pulp mill effluents in the Wapiti/Smoky river system.

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 behaviour, 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 liver concentrations of retinol, dehydroretinol and retinyl palmitate in burbot, northern pike, longnose sucker and flathead chub collected in the fall of 1994.

II. GENERAL REQUIREMENTS

1. Various sample sizes of fish species were collected at different sites in the fall of 1994. The contractor is to conduct retinoid analyses on livers from 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 liver samples for each of the four fish species.

2. Liver samples, stored and transported at -60°C have been supplied to Don Metner by EnviResource Consulting Limited, Calgary. The contractor is expected to maintain these tissue samples in a suitable condition to allow for retinoid analyses. Analyses of retinol, dehydroretinol and retinyl palmitate will be conducted in accordance with the methods outlined in Palace and Brown (1994).

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

4. 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 retinol, dehydroretinol and retinyl palmitate levels in the livers of burbot, longnose sucker, northern pike and flathead chub are to conform to the HPLC methodology outlined in Palace and Brown (1994). Specifically, the methodology is as follows:

Liver tissues are to be homogenized using distilled deionized water as a homogenizing medium. After homogenization, proteins are to be precipitated using 200 L HPLC-grade ethanol added to

the liver sample. Liver samples are then to be extracted using 500 μL (3:2, v,v) ethyl acetate/hexane. Residues from the ethyl/acetate hexane extracts are then to be redissolved in mobile phase and injected (20 μL) onto a 3-μm-bead-size Adsorbosphere HS C_{18} HPLC column (4.6 mm i.d., 150 mm length) with attached 10 mm Adsorbosphere guard column. The column is to be thermostated to 26°C, and samples and standards of retinol, retinyl palmitate and 3,4-dehydroretinol are to be eluted isocratically with aceronitrile:methanol:water (70:20:10, v/v/v) delivered at a flow rate of 1.0 mL/min.

A complete standard curve, using at least five concentrations, is to be run with each batch of samples. Retinyl acetate and tocopherol acetate are to be used as internal standards, with the recovery of known spikes used to correct for the recovery efficiency of each extraction. All samples, blanks and standards are to be run in duplicate. Final concentration levels are to be based on the average of the two tests on each sample.

IV. REPORTING REQUIREMENTS

1. Prepare a comprehensive report outlining the results of the retinoid 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 detailed description of the analytical methods employed and a summary of assay performance characteristics are to be included for retinoid investigated.
    b) an appendix or tables indicating the mean retinoid concentrations from replicate analyses of each 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 are to be submitted to the Component Coordinator by **March 31st, 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 Eøø, 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 is to comprise of one original and four duplicates of each slide.

V. DELIVERABLES

1. A data interpretation report that includes the methods and results of retinol, dehydroretinol and retinyl palmitate analyses for NRBS fish samples collected in the fall 1994.

2. 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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T5J 3N4
phone: (403) 427-1742
fax: (403) 422-3055

VII. LITURATURE CITED

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APPENDIX B

Table 2. Liver concentrations of dehydroretinol (dR), retinol (R), dehydroretinyl ester (dRE2), retinyl palmitate, (RP), tocopherol (T) and ß-carotene (ßC) in individual burbot (BURB), longnose sucker (LNSC) and northern pike (NRPK) collected in the NRBS study area.

UniqID	Year Day Sampled	NRBS Code	Site	Species	Fish No.	dR (µg/g)	R (µg/g)	dRE2 (µg/g)	RP (µg/g)	T (µg/g)	ßC (µg/g)
90287	255	A1-BURB-1	A1a	BURB	1	0.62	1.05	65.0	57.0	194.0	
90288	255	A1-BURB-2	A1b	BURB	2	2.86	0.97	450.1	151.8	210.5	
90289	255	A1-BURB-6	A1b	BURB	6	0.93	0.63	242.2	111.2	163.6	
90290	255	A1-BURB-7	A1b	BURB	7	0.14	0.53	107.2	75.7	156.6	
90291	256	A1-BURB-8	A1b	BURB	8	0.14	0.63	115.0	69.3	162.7	
90292	256	A1-BURB-9	A1b	BURB	9	3.59	1.16	342.1	89.8	1252.9	
90293	256	A1-BURB-10	A1b	BURB	10	0.90	1.06	42.8	25.8	175.0	
90294	257	A1-BURB-11	A1b	BURB	11	1.14	0.78	215.4	95.5	228.4	
90315	263	A2-BURB-1	A2	BURB	1	1.08	0.23	67.9	19.0	63.5	
90317	263	A2-BURB-3	A2	BURB	3	1.30	7.50	73.7	32.8	90.1	
90318	264	A2-BURB-4	A2	BURB	4	0.14	4.78	145.5	41.5	7.4	
90319	265	A2-BURB-5	A2	BURB	5	1.06	0.58	79.7	31.7	199.3	
90321	265	A2-BURB-7	A2	BURB	7	0.44	0.56	104.1	71.4	15.3	
90322	266	A2-BURB-8	A2	BURB	8	3.42	1.13	246.8	51.4	136.1	
90350	269	A3-BURB-1	A3	BURB	1	1.22	0.70	62.2	26.0	179.4	
90351	269	A3-BURB-2	A3	BURB	2	3.69	1.14	322.4	96.3	192.2	
90352	269	A3-BURB-3	A3	BURB	3	1.12	0.55	109.8	50.9	322.4	
90353	269	A3-BURB-4	A3	BURB	4	2.70	1.13	170.4	44.4	94.8	
90354	269	A3-BURB-5	A3	BURB	5	1.09	0.45	192.4	49.1	619.9	
90355	269	A3-BURB-6	A3	BURB	6	0.47	0.52	44.3	14.0	160.1	
90356	269	A3-BURB-7	A3	BURB	7	2.45	1.35	286.4	112.9	105.3	
90357	269	A3-BURB-8	A3	BURB	8	0.95	1.09	83.9	71.2	265.0	
90358	269	A3-BURB-9	A3	BURB	9	1.23	0.36	83.6	17.7	45.9	
90359	269	A3-BURB-10	A3	BURB	10	2.78	0.98	362.4	119.9	217.2	
90360	269	A3-BURB-11	A3	BURB	11	0.56	0.51	133.3	49.0	162.8	
90361	269	A3-BURB-12	A3	BURB	12	0.62	0.30	132.8	50.9	221.2	
90362	269	A3-BURB-13	A3	BURB	13	0.14	0.15	178.8	59.1	137.1	
90363	269	A3-BURB-14	A3	BURB	14	0.47	0.29	162.2	44.5	157.6	
90364	269	A3-BURB-15	A3	BURB	15	1.58	1.89	158.6	98.4	412.7	
90365	269	A3-BURB-16	A3	BURB	16	4.21	2.74	127.4	57.6	499.0	
90366	269	A3-BURB-17	A3	BURB	17	2.28	0.74	145.0	38.8	261.1	
90367	269	A3-BURB-18	A3	BURB	18	3.90	2.78	225.5	137.9	497.1	
90368	269	A3-BURB-19	A3	BURB	19	1.03	1.27	233.8	101.2	260.1	
90369	269	A3-BURB-20	A3	BURB	20	1.10	0.49	100.1	20.2	274.4	
90370	269	A3-BURB-21	A3	BURB	21	0.14	0.86	231.9	78.5	7.4	
90371	269	A3-BURB-22	A3	BURB	22	4.73	1.70	406.7	118.6	445.6	
90406	280	A4-BURB-1	A4	BURB	1	1.93	0.44	274.9	44.1	425.1	
90405	280	A4-BURB-2	A4	BURB	2	0.33	0.18	45.7	12.9	82.2	
90407	280	A4-BURB-3	A4	BURB	3	1.19	0.62	113.5	43.5	700.6	
90408	280	A4-BURB-4	A4	BURB	4	1.56	0.39	107.0	30.7	395.1	
90409	280	A4-BURB-5	A4	BURB	5	0.25	0.39	105.9	33.2	293.3	
90410	280	A4-BURB-6	A4	BURB	6	0.66	0.25	45.2	13.1	100.6	
90411	280	A4-BURB-7	A4	BURB	7	1.64	0.61	174.3	58.6	168.2	
90412	280	A4-BURB-8	A4	BURB	8	0.77	0.67	91.5	41.7	275.1	
90413	280	A4-BURB-9	A4	BURB	9	0.58	0.53	99.3	45.0	526.2	

UniqID	Year Day Sampled	NRBS Code	Site	Species	Fish No.	dR (µg/g)	R (µg/g)	dRE2 (µg/g)	RP (µg/g)	T (µg/g)	ßC (µg/g)
90414	280	A4-BURB-10	A4	BURB	10	2.42	0.86	139.8	40.0	445.3	
90418	281	A4-BURB-14	A4	BURB	14	1.32	1.39	114.4	63.5	387.9	
90419	281	A4-BURB-15	A4	BURB	15	1.28	0.65	311.8	90.7	436.2	
90442	286	A5-BURB-1	A5	BURB	1	1.41	0.68	44.7	12.0	47.5	
90443	286	A5-BURB-2	A5	BURB	2	0.27	0.24	63.5	21.5	39.8	
90444	286	A5-BURB-3	A5	BURB	3	12.65	1.58	98.4	11.4	45.7	
90445	286	A5-BURB-4	A5	BURB	4	2.48	1.07	191.0	55.6	345.3	
90446	287	A5-BURB-5	A5	BURB	5	2.46	0.62	100.7	16.4	302.5	
90447	287	A5-BURB-6	A5	BURB	6	4.24	0.52	71.9	6.7	52.5	
90448	287	A5-BURB-7	A5	BURB	7	86.17	11.66	208.0	26.7	213.4	
90449	287	A5-BURB-8	A5	BURB	8	0.66	0.17	44.6	14.3	135.3	
90450	287	A5-BURB-9	A5	BURB	9	17.79	2.79	163.3	24.1	131.0	
90451	287	A5-BURB-10	A5	BURB	10	3.13	0.87	87.3	19.9	100.3	
90452	287	A5-BURB-11	A5	BURB	11	1.38	0.54	113.0	24.4	375.6	
90453	287	A5-BURB-12	A5	BURB	12	2.19	0.74	78.4	21.0	271.6	
90454	287	A5-BURB-13	A5	BURB	13	0.80	0.21	93.1	14.7	141.3	
90455	287	A5-BURB-14	A5	BURB	14	2.73	0.41	114.7	34.3	341.0	
90456	287	A5-BURB-15	A5	BURB	15	0.92	0.41	37.1	7.8	348.3	
90457	287	A5-BURB-16	A5	BURB	16	1.44	0.65	65.3	18.5	94.7	
90458	287	A5-BURB-17	A5	BURB	17	2.38	0.42	91.3	12.5	100.1	
90429	283	CW-BURB-1	CW	BURB	1	0.79	0.50	79.5	27.3	109.3	
90430	283	CW-BURB-2	CW	BURB	2	0.85	0.26	70.2	16.0	93.8	
90431	283	CW-BURB-3	CW	BURB	3	1.47	0.83	44.1	13.9	64.1	
90432	283	CW-BURB-4	CW	BURB	4	0.39	0.37	68.5	7.9	113.0	
90433	285	CW-BURB-5	CW	BURB	5	2.54	1.23	50.1	28.0	83.3	
90072	262	LSR2-BURB-1	LSR2	BURB	1	4.23	1.26	213.6	75.1	35.0	
90511	351	LSR3-BURB-1	LSR2	BURB	1	1.06	0.49	82.6	29.0	227.3	
90383	275	LSV-BURB-1	LSV	BURB	1	0.14	1.70	101.6	93.1	184.1	
90384	275	LSV-BURB-2	LSV	BURB	2	2.94	1.79	134.3	79.4	366.6	
90385	275	LSV-BURB-3	LSV	BURB	3	0.57	0.89	68.4	41.0	214.7	
90386	275	LSV-BURB-4	LSV	BURB	4	6.28	2.40	172.8	66.7	788.3	
90387	275	LSV-BURB-5	LSV	BURB	5	3.42	3.36	59.1	105.1	282.8	
90389	276	LSV-BURB-7	LSV	BURB	7	4.06	1.45	116.9	42.8	129.6	
90390	276	LSV-BURB-8	LSV	BURB	8	0.42	0.42	48.1	28.0	293.7	
90391	276	LSV-BURB-9	LSV	BURB	9	2.85	0.86	215.1	42.9	551.8	
90392	276	LSV-BURB-10	LSV	BURB	10	1.04	0.68	248.5	61.9	694.5	
90393	276	LSV-BURB-11	LSV	BURB	11	1.53	1.62	72.7	49.9	150.6	
90394	276	LSV-BURB-12	LSV	BURB	12	2.26	0.71	218.6	69.0	640.7	
90395	276	LSV-BURB-13	LSV	BURB	13	1.80	2.36	188.4	123.3	73.9	
90396	276	LSV-BURB-14	LSV	BURB	14	2.31	0.84	286.7	78.3	609.8	
90397	276	LSV-BURB-15	LSV	BURB	15	0.77	1.13	68.0	32.0	60.6	
90398	276	LSV-BURB-16	LSV	BURB	16	0.99	0.60	106.3	35.6	415.9	
90399	276	LSV-BURB-17	LSV	BURB	17	9.01	8.44	169.0	157.1	135.0	
90400	276	LSV-BURB-18	LSV	BURB	18	2.04	0.99	167.5	44.0	479.3	
90401	276	LSV-BURB-19	LSV	BURB	19	0.14	0.17	27.1	27.1	67.4	
90308	259	MR-BURB-1	MR1	BURB	1	6.32	2.19	234.5	69.6	24.9	
90504	348	MCR2-BURB-1	MR2	BURB	1	0.59	0.44	61.5	48.3	35.3	

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90505	348	MCR2-BURB-2	MR2	BURB	2	5.29	3.23	301.8	188.6	51.6	
90506	348	MCR2-BURB-3	MR2	BURB	3	3.96	2.90	123.9	82.4	102.8	
90507	348	MCR2-BURB-4	MR2	BURB	4	7.80	3.06	29.2	25.6	11.1	
90508	348	MCR2-BURB-5	MR2	BURB	5	0.72	0.39	155.7	68.5	95.2	
90509	348	MCR2-BURB-6	MR2	BURB	6	2.40	0.63	157.3	36.4	26.1	
90510	349	MCR2-BURB-7	MR2	BURB	7	9.54	1.69	421.7	63.3	121.7	
90373	271	P-BURB-1	P	BURB	1	9.92	5.83	205.2	83.0	7.4	
90374	271	P-BURB-2	P	BURB	2	1.66	0.57	191.1	54.8	277.7	
90375	272	P-BURB-3	P	BURB	3	2.51	2.39	337.2	237.1	185.9	
90376	272	P-BURB-4	P	BURB	4	1.01	0.49	138.5	51.4	184.2	
90377	273	P-BURB-5	P	BURB	5	3.97	1.84	100.9	46.4	135.7	
90145	271	PR1-BURB-2	PR1	BURB	2	0.84	0.56	236.2	110.3	47.3	
90148	272	PR1-BURB-3	PR1	BURB	3	2.93	0.91	230.1	54.7	211.6	
90158	272	PR1-BURB-4	PR1	BURB	4	2.19	1.44	255.5	109.7	388.3	
90157	272	PR1-BURB-5	PR1	BURB	5	2.98	0.65	126.5	27.6	125.1	
90161	273	PR1-BURB-6	PR1	BURB	6	9.97	5.28	791.2	361.9	7.4	
90162	273	PR1-BURB-7	PR1	BURB	7	3.46	1.04	271.8	57.2	165.9	
90163	273	PR1-BURB-8	PR1	BURB	8	3.28	1.53	372.3	164.9	61.6	
90171	275	PR2-BURB-1	PR2	BURB	1	2.74	0.87	258.1	67.9	99.8	
90176	275	PR2-BURB-2	PR2	BURB	2	8.51	3.15	428.9	143.8	17.8	
90173	275	PR2-BURB-4	PR2	BURB	4	0.14	0.37	143.8	82.3	7.4	
90175	275	PR2-BURB-5	PR2	BURB	5	2.83	1.10	445.1	139.8	308.9	
90172	275	PR2-BURB-6	PR2	BURB	6	3.85	1.16	336.3	86.2	145.2	
90183	276	PR2-BURB-7	PR2	BURB	7	1.03	0.22	100.6	13.6	290.1	
90181	276	PR2-BURB-8	PR2	BURB	8	2.97	0.76	414.3	89.7	201.7	
90182	276	PR2-BURB-9	PR2	BURB	9	3.40	1.65	522.6	120.0	385.9	
90194	277	PR2-BURB-10	PR2	BURB	10	7.22	2.95	267.5	108.6	282.1	
90208	279	PR3-BURB-2	PR3	BURB	2	79.68	13.36	131.5	27.8	7.4	
90214	279	PR3-BURB-4	PR3	BURB	4	18.51	3.79	87.7	22.4	29.8	
90213	279	PR3-BURB-5	PR3	BURB	5	4.12	1.43	184.7	62.6	21.1	
90209	279	PR3-BURB-6	PR3	BURB	6	168.17	73.56	414.4	224.2	7.4	
90231	281	PR3-BURB-8	PR3	BURB	8	18.86	3.38	50.7	10.4	7.4	
90230	281	PR3-BURB-9	PR3	BURB	9	22.13	8.39	193.6	70.5	7.4	
90479	287	SR-BURB-1	SRD1	BURB	1	1.47	2.45	165.2	298.0	246.4	
90478	287	SR-BURB-2	SRD1	BURB	2	1.00	0.90	171.0	136.7	349.0	
90477	287	SR-BURB-3	SRD1	BURB	3	1.38	2.16	294.5	258.9	50.4	
90476	287	SR-BURB-4	SRD1	BURB	4	1.47	2.49	77.7	79.1	158.7	
90483	288	SR-BURB-6	SRD2	BURB	6	0.87	2.50	180.9	262.9	292.5	
90481	288	SR-BURB-7	SRD2	BURB	7	0.71	1.48	94.9	79.4	181.2	
90493	288	SR-BURB-8	SRD2	BURB	8	4.04	6.67	314.0	358.7	178.5	
90486	288	SR-BURB-9	SRD2	BURB	9	0.61	0.72	78.0	113.9	7.4	
90484	288	SR-BURB-10	SRD2	BURB	10	0.58	1.22	117.5	187.5	174.7	
90485	288	SR-BURB-11	SRD2	BURB	11	0.14	0.02	85.4	193.0	176.6	
90494	288	SR-BURB-12	SRD2	BURB	12	0.46	1.08	88.3	86.2	435.4	
90482	288	SR-BURB-13	SRD2	BURB	13	0.44	1.53	31.5	55.0	256.1	
90487	288	SR-BURB-14	SRD2	BURB	14	1.34	2.98	105.9	275.8	276.0	
90497	288	SR-BURB-15	SRD2	BURB	15	0.14	0.93	80.3	84.5	238.5	

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90498	288	SR-BURB-16	SRD2	BURB	16	2.89	4.19	153.9	148.0	267.1	
90495	288	SR-BURB-17	SRD2	BURB	17	1.24	1.72	68.2	59.2	215.2	
90496	288	SR-BURB-18	SRD2	BURB	18	0.53	1.27	57.0	86.3	296.9	
90490	289	SR-BURB-20	SRD2	BURB	20	0.86	1.41	151.7	136.9	269.1	
90489	289	SR-BURB-21	SRD2	BURB	21	0.14	2.54	139.8	128.8	114.2	
90007	255	SR1-BURB-1	SR1	BURB	1	6.91	2.86	119.5	46.9	118.9	
90008	255	SR1-BURB-2	SR1	BURB	2	0.28	1.25	31.8	14.3	340.6	
90001	256	SR1-BURB-4	SR1	BURB	4	0.73	0.30	55.4	13.0	195.7	
90012	256	SR1-BURB-5	SR1	BURB	5	5.14	2.15	140.6	58.7	333.3	
90011	256	SR1-BURB-6	SR1	BURB	6	0.35	0.63	79.3	66.4	153.2	
90002	256	SR1-BURB-7	SR1	BURB	7	1.10	0.42	132.9	16.3	67.9	
90003	256	SR1-BURB-8	SR1	BURB	8	3.77	0.75	79.6	9.9	189.3	
90005	256	SR1-BURB-9	SR1	BURB	9	1.21	0.37	38.4	7.4	269.9	
90013	256	SR1-BURB-10	SR1	BURB	10	5.48	2.18	257.5	93.9	164.8	
90014	256	SR1-BURB-11	SR1	BURB	11	0.76	0.71	31.1	14.1	174.9	
90006	256	SR1-BURB-12	SR1	BURB	12	1.38	1.03	88.7	37.7	240.7	
90017	256	SR1-BURB-13	SR1	BURB	13	5.13	3.14	178.0	55.6	100.3	
90016	256	SR1-BURB-14	SR1	BURB	14	7.79	3.22	112.9	39.7	418.5	
90018	256	SR1-BURB-15	SR1	BURB	15	9.16	3.36	119.7	37.8	231.4	
90020	257	SR1-BURB-16	SR1	BURB	16	0.82	0.38	43.8	12.4	154.4	
90021	257	SR-BURB-17	SR1	BURB	17	1.26	0.72	55.9	18.3	205.6	
90019	257	SR1-BURB-18	SR1	BURB	18	0.51	0.43	63.9	14.5	174.4	
90031	259	SR1-BURB-19	SR1	BURB	19	1.01	0.67	79.3	28.0	218.1	
90512	354	SR3-BURB-1	SR3	BURB	1	1.37	1.36	94.8	77.5	7.4	
90235	282	WAB1-BURB-1	WB	BURB	1	33.51	11.60	398.4	200.1	170.8	
90236	283	WAB1-BURB-2	WB	BURB	2	6.10	3.32	150.6	90.2	403.7	
90239	283	WAB1-BURB-3	WB	BURB	3	0.51	0.33	45.8	15.8	168.7	
90237	283	WAB1-BURB-4	WB	BURB	4	0.78	0.50	68.0	24.3	516.5	
90241	283	WAB1-BURB-5	WB	BURB	5	1.28	0.59	73.6	46.4	236.1	
90238	283	WAB1-BURB-6	WB	BURB	6	0.98	0.91	102.9	87.2	209.0	
90243	284	WAB1-BURB-8	WB	BURB	8	2.72	0.84	314.5	90.5	348.0	
90244	284	WAB1-BURB-9	WB	BURB	9	0.32	0.20	20.3	8.3	661.1	
90093	266	WR1-BURB-1	WR1	BURB	1	0.61	0.37	32.6	21.4	177.0	
90092	266	WR1-BURB-2	WR1	BURB	2	2.01	0.79	197.7	115.9	52.6	
90106	268	WR1-BURB-3	WR1	BURB	3	3.64	0.86	235.5	48.5	456.4	
90107	268	WR1-BURB-4	WR1	BURB	4	1.55	0.51	106.9	24.4	79.9	
90278	291	WR2-BURB-1	WR2	BURB	1	3.24	1.33	227.0	77.5	242.2	
90280	291	WR2-BURB-2	WR2	BURB	2	1.12	0.51	109.6	34.9	318.3	
90279	291	WR2-BURB-3	WR2	BURB	3	1.95	1.14	82.2	37.0	308.2	
90275	291	WR2-BURB-4	WR2	BURB	4	0.51	0.29	23.2	5.2	247.0	
90281	291	WR2-BURB-5	WR2	BURB	5	1.30	0.59	51.7	15.2	189.5	
90284	291	WR2-BURB-6	WR2	BURB	6	1.40	0.47	72.6	19.1	166.2	
90274	291	WR2-BURB-7	WR2	BURB	7	0.53	0.35	23.3	9.3	40.1	
90276	291	WR2-BURB-8	WR2	BURB	8	1.28	0.74	68.1	32.3	91.1	
90277	291	WR2-BURB-9	WR2	BURB	9	2.08	1.03	80.7	32.5	557.6	
90282	291	WR2-BURB-10	WR2	BURB	10	4.32	2.38	84.8	65.5	114.5	
90273	291	WR2-BURB-12	WR2	BURB	12	0.83	0.38	68.0	30.3	517.6	

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90283	291	WR2-BURB-13	WR2	BURB	13	0.67	0.13	47.6	12.1	142.8	
90299	254	A1-LNSC-1	A1a	LNSC	1	1.35	4.49	232.5	576.6	159.1	1.63
90324	264	A2-LNSC-2	A2	LNSC	2	0.14	0.12	0.6	7.9	7.4	2.89
90325	266	A2-LNSC-3	A2	LNSC	3	0.14	1.41	50.3	88.6	176.7	0.04
90326	266	A2-LNSC-4	A2	LNSC	4	0.51	0.37	4.4	14.1	7.4	1.84
90071	262	LSR2-LNSC-1	LSR2	LNSC	1	0.86	5.90	100.3	166.3	7.4	2.39
90074	263	LSR2-LNSC-2	LSR2	LNSC	2	0.14	2.31	29.8	81.0	7.4	1.36
90075	263	LSR2-LNSC-3	LSR2	LNSC	3	2.25	5.54	90.0	149.6	58.7	0.52
90078	263	LSR2-LNSC-4	LSR2	LNSC	4	3.04	3.55	93.2	110.4	274.8	3.11
90077	263	LSR2-LNSC-5	LSR2	LNSC	5	1.66	2.49	49.1	66.6	81.4	1.77
90314	258	MR-LNSC-1	MR1	LNSC	1	0.57	3.05	265.0	572.6	355.8	0.51
90139	270	PR1-LNSC-1	PR1	LNSC	1	0.41	3.01	89.8	247.4	48.5	0.49
90135	270	PR1-LNSC-2	PR1	LNSC	2	0.58	3.10	88.0	207.8	76.7	0.74
90130	270	PR1-LNSC-3	PR1	LNSC	3	1.66	4.37	103.5	182.3	78.0	0.77
90133	270	PR1-LNSC-4	PR1	LNSC	4	0.92	2.51	56.3	136.3	60.4	0.89
90138	270	PR1-LNSC-5	PR1	LNSC	5	0.14	3.09	92.8	245.1	11.4	0.38
90142	270	PR1-LNSC-6	PR1	LNSC	6	0.68	0.95	51.8	94.5	12.2	1.00
90136	270	PR1-LNSC-7	PR1	LNSC	7	4.95	6.16	28.8	36.3	153.3	0.53
90131	270	PR1-LNSC-8	PR1	LNSC	8	0.14	4.06	98.2	290.3	29.2	0.39
90132	270	PR1-LNSC-9	PR1	LNSC	9	0.20	0.72	39.8	72.3	135.3	0.49
90141	270	PR1-LNSC-10	PR1	LNSC	10	0.14	0.55	56.2	106.2	33.0	0.42
90137	270	PR1-LNSC-12	PR1	LNSC	12	0.41	1.86	111.8	305.2	101.1	0.29
90143	270	PR1-LNSC-13	PR1	LNSC	13	0.14	5.07	180.0	573.5	82.5	0.98
90129	270	PR1-LNSC-14	PR1	LNSC	14	1.18	3.16	190.8	401.3	21.2	0.30
90147	272	PR1-LNSC-15	PR1	LNSC	15	1.00	6.73	63.5	201.0	47.2	0.27
90156	272	PR1-LNSC-16	PR1	LNSC	16	0.77	3.61	176.9	287.4	64.9	0.75
90146	272	PR1-LNSC-17	PR1	LNSC	17	1.26	5.60	106.4	256.3	52.2	0.39
90174	275	PR2-LNSC-1	PR2	LNSC	1	2.06	5.03	125.0	186.1	68.1	0.55
90167	275	PR2-LNSC-2	PR2	LNSC	2	0.14	1.68	26.1	62.7	12.3	0.23
90168	275	PR2-LNSC-3	PR2	LNSC	3	0.14	2.05	47.5	109.3	7.4	0.52
90166	275	PR2-LNSC-4	PR2	LNSC	4	0.14	2.15	34.6	100.2	16.9	0.46
90177	275	PR2-LNSC-5	PR2	LNSC	5	1.92	5.80	115.3	268.4	128.3	0.57
90191	277	PR2-LNSC-7	PR2	LNSC	7	1.96	5.15	79.2	122.3	178.2	0.68
90192	277	PR2-LNSC-8	PR2	LNSC	8	2.40	4.91	53.2	97.4	248.0	0.47
90200	279	PR3-LNSC-1	PR3	LNSC	1	2.94	8.98	208.4	456.4	272.4	0.15
90205	279	PR3-LNSC-2	PR3	LNSC	2	8.92	19.15	42.5	83.6	122.6	0.28
90201	279	PR3-LNSC-3	PR3	LNSC	3	11.62	24.44	194.8	333.6	112.4	0.24
90202	279	PR3-LNSC-4	PR3	LNSC	4	4.30	4.52	87.0	86.5	187.1	0.24
90198	279	PR3-LNSC-5	PR3	LNSC	5	20.19	47.27	91.9	205.3	105.0	0.37
90206	279	PR3-LNSC-6	PR3	LNSC	6	5.29	4.73	95.3	88.4	212.6	0.19
90210	279	PR3-LNSC-7	PR3	LNSC	7	0.81	1.67	34.3	60.8	88.9	0.41
90199	279	PR3-LNSC-8	PR3	LNSC	8	2.31	7.74	96.2	221.0	100.3	0.32
90207	279	PR3-LNSC-9	PR3	LNSC	9	4.54	8.05	87.2	124.7	79.9	0.67
90203	279	PR3-LNSC-10	PR3	LNSC	10	7.93	10.97	133.9	158.9	235.5	1.11
90204	279	PR3-LNSC-11	PR3	LNSC	11	0.75	2.55	54.9	88.4	288.8	0.39
90218	280	PR3-LNSC-12	PR3	LNSC	12	6.68	8.83	121.2	148.4	217.5	0.33
90217	280	PR3-LNSC-13	PR3	LNSC	13	0.52	3.98	128.0	277.9	66.2	0.23

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90224	280	PR3-LNSC-14	PR3	LNSC	14	11.23	14.87	56.7	88.8	133.1	0.56
90225	280	PR3-LNSC-15	PR3	LNSC	15	1.99	7.67	38.4	94.9	119.6	0.56
90219	280	PR3-LNSC-16	PR3	LNSC	16	4.84	11.68	68.7	157.3	155.3	0.41
90223	280	PR3-LNSC-17	PR3	LNSC	17	24.57	21.46	199.4	219.0	192.7	0.73
90221	280	PR3-LNSC-18	PR3	LNSC	18	11.57	21.42	104.9	191.6	221.8	0.34
90220	280	PR3-LNSC-19	PR3	LNSC	19	3.69	5.93	233.6	317.1	412.5	0.40
90222	280	PR3-LNSC-20	PR3	LNSC	20	6.78	12.09	55.5	103.2	228.2	0.57
90227	280	PR3-LNSC-21	PR3	LNSC	21	8.97	16.35	82.1	147.5	100.7	0.14
90226	280	PR3-LNSC-23	PR3	LNSC	23	8.35	10.30	39.4	51.2	170.9	0.71
90027	259	SR1-LNSC-1	SR1	LNSC	1	0.79	3.46	211.6	283.5	69.6	0.69
90025	259	SR1-LNSC-2	SR1	LNSC	2	3.15	11.08	455.5	1034.1	97.7	0.92
90036	261	SR1-LNSC-4	SR1	LNSC	4	1.37	7.02	345.7	1023.2	155.3	0.63
90032	260	SR1-LNSC-5	SR1	LNSC	5	8.68	17.48	263.3	541.1	217.2	3.54
90035	261	SR1-LNSC-6	SR1	LNSC	6	2.00	8.38	160.4	361.6	53.8	0.48
90033	260	SR1-LNSC-7	SR1	LNSC	7	3.88	16.80	297.3	776.8	89.5	0.26
90037	261	SR1-LNSC-8	SR1	LNSC	8	1.45	7.33	136.8	353.0	62.9	0.30
90043	261	SR1-LNSC-9	SR1	LNSC	9	3.16	10.03	424.6	840.4	89.5	0.62
90041	261	SR1-LNSC-10	SR1	LNSC	10	4.10	14.76	230.2	504.8	108.8	0.34
90042	261	SR1-LNSC-11	SR1	LNSC	11	0.83	1.94	149.0	130.9	28.6	0.98
90048	261	SR1-LNSC-12	SR1	LNSC	12	19.97	32.22	317.5	451.2	56.9	0.16
90245	288	SR2-LNSC-1	SR2	LNSC	1	0.14	2.66	61.4	351.0	36.0	1.69
90089	265	WR1-LNSC-1	WR1	LNSC	1	1.32	6.62	35.1	86.0	62.6	0.26
90088	265	WR1-LNSC-2	WR1	LNSC	2	0.66	3.89	85.1	202.7	16.1	0.21
90085	265	WR1-LNSC-3	WR1	LNSC	3	10.38	22.59	164.5	274.7	70.0	0.68
90086	265	WR1-LNSC-4	WR1	LNSC	4	1.63	6.21	30.9	99.3	7.4	0.34
90083	265	WR1-LNSC-5	WR1	LNSC	5	2.79	6.58	135.0	237.6	124.1	3.52
90087	265	WR1-LNSC-6	WR1	LNSC	6	6.36	21.37	160.2	436.8	62.6	1.79
90084	265	WR1-LNSC-7	WR1	LNSC	7	5.35	10.64	307.1	521.6	56.8	0.24
90091	265	WR1-LNSC-8	WR1	LNSC	8	13.23	24.02	79.7	181.2	35.6	0.86
90095	266	WR1-LNSC-9	WR1	LNSC	9	9.43	27.43	133.7	355.7	69.8	0.50
90094	266	WR1-LNSC-10	WR1	LNSC	10	0.99	2.34	60.8	84.9	45.0	0.98
90096	266	WR1-LNSC-11	WR1	LNSC	11	2.95	8.69	384.1	648.1	71.1	1.21
90097	266	WR1-LNSC-12	WR1	LNSC	12	25.07	35.77	81.5	147.2	146.9	2.06
90103	267	WR1-LNSC-13	WR1	LNSC	13	1.17	2.23	171.1	218.3	129.3	0.21
90295	255	A1-NRPK-1	A1b	NRPK	1	2.42	0.02	293.0	10.3	257.9	1.32
90296	255	A1-NRPK-2	A1b	NRPK	2	1.47	0.02	310.4	6.2	51.0	0.64
90298	257	A1-NRPK-4	A1b	NRPK	4	3.27	0.12	438.8	12.9	235.6	0.36
90499	291	JV1-NRPK-1	JV1	NRPK	1	1.02	0.02	291.4	9.1	57.1	0.35
90500	291	JV1-NRPK-2	JV1	NRPK	2	0.14	0.02	0.6	0.1	7.4	0.84
90501	291	JV1-NRPK-3	JV1	NRPK	3	0.14	0.09	234.9	14.2	23.8	0.05
90502	291	JV1-NRPK-4	JV1	NRPK	4	0.14	0.02	208.9	6.6	32.9	0.65
90503	291	JV1-NRPK-5	JV1	NRPK	5	0.42	0.02	70.8	5.2	7.4	0.19
90309	260	MR-NRPK-1	MR1	NRPK	1	4.44	0.15	593.6	20.8	369.0	0.04
90310	260	MR-NRPK-2	MR1	NRPK	2	7.56	0.11	472.5	9.5	78.8	0.24
90311	261	MR-NRPK-3	MR1	NRPK	3	11.14	0.12	303.0	4.5	541.8	0.84
90312	261	MR-NRPK-4	MR1	NRPK	4	2.38	0.02	607.5	5.2	337.3	0.04
90127	270	PR1-NRPK-1	PR1	NRPK	1	0.14	0.02	126.4	1.8	9.5	0.15

UniqID	Year Day Sampled	NRBS Code	Site	Species	Fish No.	dR (µg/g)	R (µg/g)	dRE2 (µg/g)	RP (µg/g)	T (µg/g)	ßC (µg/g)
90128	270	PR1-NRPK-2	PR1	NRPK	2	1.62	0.10	120.9	5.1	119.6	0.06
90140	270	PR1-NRPK-3	PR1	NRPK	3	0.14	0.02	64.5	3.5	39.2	0.04
90159	272	PR1-NRPK-4	PR1	NRPK	4	1.19	0.02	45.2	1.0	79.2	1.15
90169	275	PR2-NRPK-1	PR2	NRPK	1	2.43	0.08	88.8	3.9	118.0	0.12
90170	275	PR2-NRPK-2	PR2	NRPK	2	0.14	0.02	19.7	0.1	14.5	0.04
90178	275	PR2-NRPK-3	PR2	NRPK	3	7.68	0.06	111.3	2.1	562.0	1.50
90491	289	SR-NRPK-2	SRD2	NRPK	2	1.73	0.27	270.2	53.2	74.4	0.16
90492	289	SR-NRPK-3	SRD2	NRPK	3	1.20	0.42	539.5	99.6	37.9	0.57
90010	255	SR1-NRPK-1	SDR1	NRPK	1	5.37	0.12	263.3	8.8	7.4	1.31
90004	256	SR1-NRPK-2	SDR1	NRPK	2	2.39	0.02	6.2	0.1	7.4	0.04
90034	261	SR1-NRPK-3	SDR1	NRPK	3	0.76	0.02	52.0	1.8	49.2	0.74
90080	264	WR1-NRPK-1	WR1	NRPK	1	4.87	0.02	147.0	2.4	215.7	1.70
90081	264	WR1-NRPK-2	WR1	NRPK	2	0.14	0.02	214.9	5.2	7.4	0.78
90090	265	WR1-NRPK-3	WR1	NRPK	3	6.24	0.12	306.7	8.9	47.8	0.04
90098	267	WR1-NRPK-4	WR1	NRPK	4	0.60	0.15	135.5	15.1	12.7	0.04
90100	267	WR1-NRPK-5	WR1	NRPK	5	17.16	0.27	97.3	2.2	291.8	1.01
90099	267	WR1-NRPK-6	WR1	NRPK	6	1.86	0.10	83.5	3.1	138.9	0.04
90102	267	WR1-NRPK-7	WR1	NRPK	7	2.06	0.02	117.9	2.1	66.4	0.04
90101	267	WR1-NRPK-8	WR1	NRPK	8	11.90	0.09	182.2	3.8	420.7	0.88
90105	268	WR1-NRPK-9	WR1	NRPK	9	1.13	0.02	51.6	1.6	37.8	0.44
90104	268	WR1-NRPK-10	WR1	NRPK	10	1.30	0.08	62.9	1.5	41.5	0.27
90112	268	WR1-NRPK-11	WR1	NRPK	11	6.82	0.16	118.6	2.7	236.0	0.97
90113	268	WR1-NRPK-12	WR1	NRPK	12	11.33	0.13	236.5	4.3	229.5	0.57
90286	292	WR2-NRPK-1	WR2	NRPK	1	1.14	0.05	40.5	2.6	101.9	0.35