A Test of Hypotheses to Explain the Sigmoidal Relationship between Total Phosphorus and Chlorophyll *a* Concentrations in Canadian Lakes

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We tested five hypotheses to explain the sigmoidal relationship between total phosphorus (TP) and chlorophyll *a* (Chl *a*), two assuming that the nonlinearity is an artifact of various measurement biases and three assuming that it is based on underlying ecological interactions. Our first hypothesis was rejected; accounting for differences in extraction protocol of Chl *a* among published studies did not affect the sigmoidality. Our second hypothesis could not be rejected; there was an uncoupling between Chl *a* and PHYTO in oligotrophic lakes which may explain the initial nonlinearity. Our third hypothesis was upheld; the initial nonlinearity may be attributed to the presence of a disproportionately large fraction of unavailable phosphorus, since Chl *a* varied linearly with the proportion of total biologically active phosphorus in the TP fraction. The proportion of filamentous cyanophytes varied significantly with TP concentrations, and this was consistent with our fourth hypothesis that the higher Chl *a*:TP ratio at intermediate TP concentrations is attributable to reduced grazing impact of zooplankton in more productive lakes. Finally, Chl *a* varied linearly with total nitrogen, and this was consistent with our fifth hypothesis that the departure from linearity at extremely high phosphorus concentrations is indicative of nitrogen limitation.

Nous avons testé cinq hypothèses pour expliquer la relation sigmoïdale entre le phosphore total (PT) et la chlorophylle *a* (Chl *a*), dont deux supposant que la non-linérarité était un artefact de divers biais des mesures, et trois supposant qu'elle était basée sur des interactions écologiques sous-jacentes. La première hypothèse a été rejetée; le fait de tenir compte des différences des protocoles d'extraction de la Chl *a* entre les diverses études publiées ne modifiait pas la sigmoïdalité. La deuxième hypothèse n'a pu être rejetée; il existe un découplage entre la Chl *a* et PHYTO dans les lacs oligotrophes, qui pourrait expliquer la non-linéarité initiale. Notre troisième hypothèse a été maintenue; la non-linéarité initiale peut être attribuée à la présence d'une fraction proportionnellement importante de phosphore non disponible, étant donné que la teneur en Chl *a* variait de façon linéaire avec la proportion de phosphore total biologiquement actif de la fraction PT. La proportion de cyanophytes filamenteux variait de façon significative avec les concentrations de PT, et ceci était conforme à notre quatrième hypothèse, selon laquelle le rapport plus élevé Chl *a* : PT aux concentrations intermédiaires de PT peut être attribué à un impact réduit du broutage du zooplancton dans les lacs plus productifs. Enfin, la Chl *a* variait de façon linéaire avec l'azote total, et ceci correspondait à notre cinquième hypothèse, selon laquelle l'écart par rapport à la linéarité aux concentrations extrêmement élevées de phosphore peut indiquer une limite pour l'azote.

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In recent years, several groups of investigators have demonstrated that the relationship between algal chlorophyll *a* (Chl *a*) and the primary limiting nutrient in lakes, total phosphorus (TP), is best described by a sigmoidal rather than a linear function (McCauley et al. 1989; Prairie et al. 1989; Chow-Fraser 1991). This nonlinearity suggests that Chl *a* increases more rapidly with nutrients at intermediate concentrations compared with either high or low concentrations. Watson et al. (1992) have also shown that the TP – phytoplankton biomass (PHYTO) relationship is likewise sigmoidal by demonstrating that there is consistency in the Chl:PHYTO ratios across the trophic gradient.

To test the robustness and generality of their models, limnologists in previous studies deliberately used data from published sources that included a wide range of environmental conditions, a large geographical region, and a variety of sampling protocols. Logistical difficulties prevented concurrent measurements of all their variables in all cases and this may have led to invalid comparisons among different statistical models of disparate data subsets. A greater objection

is that analytical procedures for Chl *a* measurements varied from study to study and these procedures may have resulted in differential extraction efficiencies of chlorophyll, and thus contributed to some unknown bias towards evaluation of the nutrient–Chl curve. A brief review of the literature shows that the chemicals used to extract Chl *a* (see Table 1), the extraction duration, as well as the use of either a spectrophotometer or a fluorometer (e.g., SCOR-UNESCO 1966; Holm-Hansen and Riemann 1978; Marker et al. 1980) all have significant effects of Chl *a* estimates. Another problem is that in routine monitoring, only Chl *a* is estimated even though other chlorophylls, especially Chl *b* and Chl *c*, are present in various proportions in natural algal communities. Therefore, estimates of Chl *a* alone may be inadequate where there is variation in species mix and taxonomic composition of the phytoplankton.

A number of hypotheses can be invoked to explain the sigmoidal relationship between TP and Chl *a*, only some of which have a biological or ecological basis. In this paper, we evaluate the applicability of five hypotheses. The first two are based on the assumption that the nonlinearity is actually an artifact resulting from measurement biases and an uncoupling between PHYTO and Chl *a*; the remaining three assume that the nonlinearity is real and are based on underlying ecological mechanisms. By testing these hypotheses, we intend to provide a clearer understanding of the underlying reason(s) for a nonlinear response between nutrient and phytoplankton concentrations in lakes.

Hypotheses to Explain Sigmoidality

(1) Our first hypothesis is that the sigmoidality may simply reflect measurement error associated with the different analytical techniques that have been used to measure Chl *a*. If such is the case, then, calibrating Chl *a* measurements to some standard protocol may eliminate the nonlinearity.

(2) Our second hypothesis is that there is a systematic uncoupling between Chl *a* and PHYTO that depends on a shift in the taxonomic makeup of the algal communities such that the phytoplankton at both ends of the productivity gradient is associated with ratios of Chl *a*:total Chl that are lower than those in the intermediate range.

(3) Our third hypothesis is that the initial nonlinearity in the curve is attributed to the presence of a disproportionately large fraction of phosphorus that is not available for algal growth. If this is true, then plotting Chl *a* against total biologically active phosphorus (TBAP) (as indicated by laboratory algal assay) rather than TP may produce a linear response.

(4) Our fourth hypothesis is that the lower Chl *a* concentrations associated with oligotrophic lakes is attributed to disproportionately high grazing impact of the herbivore community, whose "top-down" (after McQueen et al. 1986) effects decrease systematically as lakes in productivity due to the corresponding increase in the proportion of interfering filamentous algae (e.g., Chow-Fraser and Sprules 1986).

(5) Our fifth hypothesis is the departure from linearity at extremely high phosphorus concentrations is indicative of nitrogen limitation. This expectation is based on observations that in lakes with extremely high TP concentrations, total nitrogen (TN):TP ratios tend to be low (<14; Downing and McCauley 1992), and in these lakes, nitrogen limitation appears to be the rule.

TABLE 1. Summary of the interactive effects of extractant used and type of algae in samples. Numbers refer to studies in which the chemicals named were more efficient than 90% acetone for extracting Chl *a* from samples dominated by the respective algal groups. Methanol refers to 100% methanol as well as chloroform–methanol. 1, Shoaf and Lium (1976); 2, Holm-Hansen and Riemann (1978); 3, Stauffer et al. (1979); 4, Burnison (1980); 5, Marker et al. (1980); 6, Nusch (1980); 7, Wood (1985); 8, Speziale et al. (1985).

	Chemical extractant			
Dominant group	DMSO	DMF	EtOH	MeOH
Blue-greens	3, 8	3, 8	5	2, 3, 7
Greens	1, 3, 4, 8	8	5, 6	6, 7
Chrysophytes				7
Diatoms	3			2, 7

Methods to Test Hypotheses

Hypothesis 1

To test the first hypothesis, we assembled a database from published and unpublished sources that had contemporaneous measurements of TP and Chl *a* (Table 2). To ensure that the database would be sufficiently homogeneous to permit a valid comparison, we used only lakes that were sampled at least 5 times during the ice-free season (generally between May and October, except for coastal lakes in British Columbia where the ice-free season sometimes extended from March to November; Stockner et al. 1980; Stockner and Shortreed 1985). Hanna and Peters (1991) found that in cross-sectional studies, the strongest within-lake differences could be attributed to sampling date rather than to sampling method or sampling sites within a lake, and this indicated that comparisons should only be made for lakes that are sampled with roughly the same sampling frequency and intervals. Since we were interested in testing the variable effects of different extraction procedures on Chl *a* measurements, we included only those studies for which extraction techniques have been adequately documented to permit calibration. Finally, we only included north-temperate lakes to avoid any confounding effects of large climatic differences. Using these criteria, we were able to assemble data for 119 lakes (152 lake-years) from published sources as well as several unpublished sources within Canadian provincial and federal agencies.

Not all of the waterbodies included in this database can be described as "typical" lakes, although they are all lake-like. A notable example is Cootes Paradise Marsh (Table 2; "Y" in Fig. 1), which is a large degraded urban wetland located in Hamilton, Ontario. In its present state, it acts very much like a shallow hypereutrophic lake, with less than 10% cover of emergent or submergent aquatic vegetation. Inclusion of this marsh in the present analysis broadened our range of trophic states and offered us a heuristic if not predictive model of how Chl *a* behaves at extremely high TP concentrations.

In order to calibrate existing measurements to that of a standardized protocol, we conducted an investigation to determine the extent to which the different methods from documented studies affect Chl *a* measurements. Specifically, we wanted to compare the extraction efficiency of

TABLE 2. Description of study lakes included to test the sigmoidal shape of the TP–Chl *a* curve. See text for explanation of abbreviations for protocols. Chl *a* values are in μg/L.

Location	Sources	Number of lakes	Extraction protocol	Correction for phaeophytin	Range of Chl values	Years sampled
Alberta	Chow-Fraser and Trew, 1990; Naquadat files of Alberta Environment (D. Trew, unpublished data)	57	ACET-96-F	Yes	1.1–86.6	1981–88
	Atlas of Alberta lakes (Mitchell and Prepas 1990)	5	EtOH-S	No	1.7–10.2	1981–86
British Columbia	Stockner et al. 1980; Shortreed and Stockner 1981; Stockner and Shortreed 1985	24	ACET-24-F	No	0.6–3.3	1978–80
Experimental Lakes Area, northwestern Ontario	Freshwater Institute (D. Findlay, B. Hecky, M. Stainton, unpublished data)	8	ACET-24-F ACET-24-S	No	0.8–15.2	1974–83
South-central Ontario	Zimmerman et al. 1983	24	ACET-24-S	Yes	0.8–7.5	1980
Cootes Paradise Marsh (western end of L. Ontario)	Painter et al. 1991	1[a]	ACET-24-S	No	22.2–684	1973–90

[a]Two stations were sampled in Cootes Paradise.

different chemicals for the same sample, as well as compare the relative efficiency of a given chemical for samples with different taxonomic compositions from different lakes. The chemicals we chose were intended to represent many of the commonly used solvents besides 90% acetone that are known to give higher yields of Chl *a* for samples dominated by filamentous green and blue-green algae (see Table 1). These included *N*,*N*-dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), and methanol. We did not include ethanol in our comparison because Marker et al. (1980) indicated that of the two alcohols, methanol was used more widely. Additionally, Prepas and Trew (1983) found no significant differences in performance when ethanol was compared with 90% acetone. We also compared the performance of the spectrophotometer versus the fluorometer in calculating Chl *a* values for the same samples to quantify differences between photometric and fluorometric data.

For this comparison, three waterbodies from the large database were sampled to obtain a range of Chl values from 1 to 90 μg/L. These included an oligotrophic reservoir (Gleniffer Reservoir (GL); TP = 9 μg/L), the south and north basins of a eutrophic lake (Baptiste South (BS) and Baptiste North (BN); TP = 55 and 65 μg/L, respectively), and a hypereutrophic shallow lake (Dried Meat (DM); TP = 450 μg/L). To avoid confounding effects of season, we visited all the lakes over a 48-h period in July 1992. Logistical considerations prevented us from sampling a larger number of lakes. Triplicate samples were collected in amber polyethylene bottles and kept cool until filtration (within 12 h of sampling). Appropriate aliquots of composite samples from each lake (200–1000 mL) were filtered through Gelman GF/C, wrapped in aluminum foil, and kept frozen until different extraction protocols were carried out. We used four suites of chemicals to extract the Chl lawns: 90% alkaline acetone, 100% methanol, DMSO with acetone, and DMF with acetone.

Filters were placed in 10 mL of 90% acetone or 100% methanol and extracted for 24 h at below 0°C (ACET-24 and MeOH), while one set was placed in 90% acetone and extracted for 96 h at below 0°C (ACET-96). These samples were subsequently homogenized and centrifuged at 4000 rpm for 5 min. The solvent was decanted into a cuvette and analyzed with a spectrophotometer (Milton Roy 1201). Absorbances were read at 665 and 750 nm. Each extract was then acidified with two drops of 0.3 N HCl and allowed to mix for 3 min. Absorbance was read at 750 and 665 nm after acidification. We used Eq. 1 from Speziale et al. (1984) to calculate Chl *a* concentrations in the samples. All samples were subsequently analyzed fluorometrically with a Turner model 111 fluorometer according to the procedures of Yentsch and Menzel (1963) as modified by Holm-Hansen et al. (1965). To facilitate comparison, both corrected and uncorrected Chl *a* values were calculated in this study.

Methods used to measure TP, PHYTO, and Chl *a* in this large database are documented elsewhere as noted in Table 2. Since we are only concerned here with calibrating Chl *a* measurements, we outline below the four different protocols that have been used to obtain Chl *a* values in the large database: (1) extraction with acetone for 96 h followed by fluorometric analysis (ACET-96-F), (2) extraction with acetone for 24 h followed by fluorometric analysis (ACET-24-F), (3) extraction with ethanol followed by spectrophotometric analysis (EtoH-S, and (4) extraction with acetone for 24 h followed by spectrophotometric analysis (ACET-24-S).

Hypothesis 2

To test Hypothesis 2, we required contemporaneously measured PHYTO data in addition to the Chl *a* and TP values. This reduced our dataset to only 89 lake-years. Since another component of this hypothesis is that the taxonomic makeup of phytoplankton varies predictably with lake trophic status, we also required information on the species composition of algal communities. This procedure further reduced our working database to 33 lake-years. Raw counts and cell dimensions corresponding to each sampling date (at least five times over the sampling season) for each of the 33 lakes

were obtained from Alberta Environment (D. Trew) and the Freshwater Institute (D. Findlay). Biomass values were subsequently calculated and sorted into seven major taxonomic groups: cyanophytes, chlorophytes, euglenophytes, chrysophytes, diatoms, cryptophytes, and dinoflagellates (see Appendix).

Hypothesis 3

To test the third hypothesis, we used concurrent measurements of TP, TBAP, and Chl *a* concentrations from 31 of the study lakes in Alberta. To calculate TBAP, a relationship between orthophosphate and algal biomass was derived in the laboratory and was subsequently used to estimate the amount of phosphorus utilized from test water by a given stock culture of *Selenastrum capricornutum* (supplied by USEPA, Oregon). The relationship was developed which included a range of phosphorus concentrations between 3 and 186 μg/L ($r = 0.98$; $P < 0.01$). Algal biomass was measured by Coulter Counter (model ZBI) following a preliminary calibration with direct microscopic counts and a fluorometer.

The algal assay involved autoclaving flasks of the test lake water for 20 min at 15 psi (1 psi = 6.895 kPa) and 121°C. All flasks were weighed before and after autoclaving and the appropriate quantity of sterile double-distilled water was added to compensate for differences created by evaporation. One millilitre of stock *Selenastrum* was added to 29 mL of autoclaved test water and this was allowed to grow for 96 h at 24°C in continuous illumination (2100 lx). The *Selenastrum* inoculum was prepared from actively growing stock cultures (1–2 wk old). A subsample was dispensed into a sterile tube and centrifuged at 1000*g* for 10 min. The supernatant was subsequently replaced by sterile double-distilled water and the final density was adjusted to 1.8×10^5 cells/mL with a fluorometer. Growth of the test cultures was monitored using in vivo chlorophyll fluorescence until the stationary growth phase was identified (at least 96 h after inoculation). At that time, 0.5 mL of the sample was preserved with Lugol's iodine and later enumerated with the Coulter particle counter.

Hypothesis 4

As an alternative, investigators in the past have assigned algae to functional rather than taxonomic groups (e.g., Sprules and Knoechel 1984) to study the ecological relationships between adjacent trophic levels. However, the majority of these studies have focused on separating algae into two main groups: "edible" and "inedible" based only on a size criterion (e.g., Chow-Fraser and Knoechel 1985; Carpenter et al. 1991; Watson et al. 1992). This approach ignores the fact that other factors such as algal shape, taste, and colony morphology can also affect the edibility of algae (DeMott 1986; Knisely and Geller 1986; Vanderploeg 1990; Chow-Fraser and Maly 1992) and that the "inedible" category is an umbrella group that contains large noningestable phytoplankton as well as filamentous algae, which in addition to being inedible are also known to interfere with the grazing activities of zooplankton (Chow-Fraser and Sprules 1986; Hawkins and Lampert 1989). In this study, algae have been grouped according to size, shape, and colonial morphology as follows: (1) cells <10 μm, (2) cells or colonies 10–30 μm, (3) cells >30 μm, (4) colonies >30 μm, and (5) filamentous algae (see Appendix).

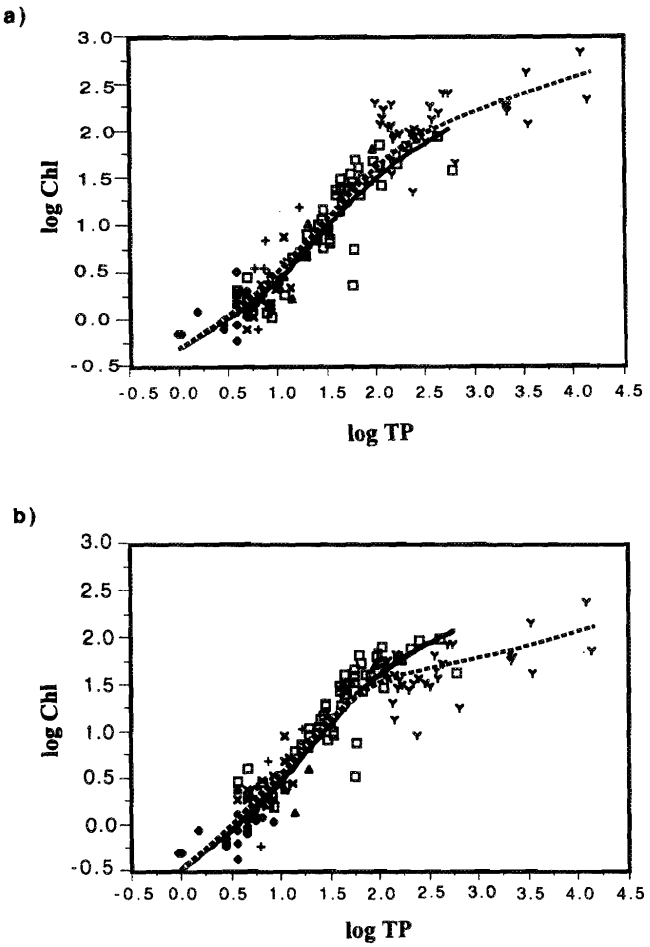

FIG. 1. Plot of $\log_{10}$ Chl (μg/L) versus $\log_{10}$ TP (μg/L) for (a) unstandardized data and (b) standardized data. Lines were obtained by applying a smoothing spline procedure to the data (SAS JMP, SAS Institute, North Carolina; lambda = 1). Data are for 119 waterbodies and include 152 lake-years. Symbols: X, from Zimmerman et al. (1983); Y, from Painter et al. (1991); +, from Freshwater Institute (Experimental Lakes Area); □, from Alberta Environment; △, from Mitchell and Prepas (1990); ◇, from Stockner et al. (1980) and Stockner and Shortreed (1985); broken line, all data; solid line, relationship excluding Cootes Paradise Marsh (Y).

Hypothesis 5

In most cases, contemporaneous measurements of TN were available for lakes in the large database and these were used to test the last hypothesis that TN rather than TP is a better predictor of Chl *a* across the entire range of trophic conditions.

In this paper, data were appropriately log-transformed or arcsin-transformed before they were subjected to statistical analyses. The base for all log-transformations was 10. For convenience, the base will be omitted in the remainder of the text.

Hypotheses and Tests

To test the first hypothesis, we first used a smoothing spline procedure (SAS JMP, SAS Institute, North Carolina; lambda = 1) to determine the shape of the best-fit curve through the log-normalized TP-Chl *a* data of the large database

TABLE 3. Summary of mean Chl *a* values (μg/L; $n = 3$), corrected for phaeopigments. Numbers in parentheses are 1 SE of the mean. See text for explanation of abbreviations.

Lake	Machine	ACET-24	ACET-96	DMF-A	DMSO-A	MeOH
GL	F	0.96	1.35	2.19	1.68	1.05
		(0.106)	(0.078)	(0.538)	(0.171)	(0.253)
	S	1.54	2.05	1.78	1.86	2.25
		(0.365)	(0.295)	(0.289)	(0.399)	(0.685)
BS	F	49.69	57.82	58.90	60.89	49.46
		(3.812)	(1.506)	(0.163)	(3.122)	(5.273)
	S	60.65	63.06	70.41	83.13	91.12
		(2.571)	(1.676)	(0.750)	(1.464)	(7.637)
BN	F	63.15	67.98	70.63	66.26	53.95
		(0.732)	(0.614)	(4.125)	(1.464)	(8.888)
	S	81.65	73.66	86.98	81.21	103.54
		(3.766)	(1.202)	(5.831)	(1.569)	(12.739)
DM	F	83.63	90.91	84.66	84.89	73.85
		(3.807)	(2.438)	(0.162)	(7.840)	(0.845)
	S	122.04[a]	103.94	110.05	109.16	136.08
			(1.506)	(0.754)	(11.055)	(2.719)

[a]No SE calculated because $n = 2$.

($n = 119$ lakes; 152 lake-years). This produced a sigmoidal relationship which accounted for almost 92% of the variation in the data (broken line, Fig. 1a; $r^2 = 0.92$). By comparison, a linear regression analysis performed on the same dataset yielded a relationship that only described 87% ($r^2 = 0.87$; $P < 0.0001$) of the variation. These results, which are consistent with those of McCauley et al. (1989) and Prairie et al. (1989), confirm that the relationship between reported TP and Chl *a* is best described by a sigmoidal rather than a linear equation.

Our first hypothesis concerns the differential effects of various analytical procedures on the extraction efficiency of Chl *a* in samples collected from lakes with a wide range of trophic states. To address this, we conducted a three-factor experiment to determine the effects of instrument type (spectrophotometer versus fluorometer), chemical extractant/extraction duration (ACET-24, ACET-96, DMSO-A, DMF-A, and MeOH; see Methods to Test Hypotheses), and lake origin on Chl *a* values (Table 3). A nested analysis of variance indicated that each variable explained a significant amount of variation in the data. Lake origin alone, however, explained close to 81% of the variation ($P < 0.0001$). Inclusion of the nested effect of instrument type in the model explained 6% of the variation ($P < 0.0001$), while the effect of extraction protocol explained an additional 6% ($P = 0.0015$).

When the effects of lake origin and chemical extractants were factored out, photometric data were consistently higher than fluorometric data except for one treatment (GL samples extracted with DMF-A; Table 3). The magnitude of this disparity ranged from 47% (GL-MeOH treatment) to 92% (BS-ACET-96) with majority of the discrepancies between 75 and 85%. Schanz and Rai (1988) also reported similar discrepancies between fluorometric and photometric data and attributed these differences to interference from carotenoids which produced overestimates when a spectrophotometer was used and underestimates when a fluorometer was used. Other investigators had also noted that Chl *b* causes overestimation of phaeopigments by fluorometry, thus producing lower acid ratios and consequently lower Chl *a* values (e.g., Yentsch 1965; Stauffer et al. 1979).

There was least agreement between fluorometric and photometric data when MeOH was used, probably because the acidification step causes the absorption maximum of phaeophytin *a* a shift from 665 nm in MeOH and thereby leads to underestimates of phaeopigments on the one hand and overestimates of Chl *a* on the other (Nusch 1980). The problem was not apparent with the other extraction mixtures because phaeophytin is more stable in acetone. Thus, the almost twofold higher photometric measurements corresponding to MeOH extraction are largely because we excluded a neutralization step in our protocol (Holm-Hansen and Riemann 1978). Another reason may be because Chl *b* fluoresces close to the same wavelength as Chl *a* in MeOH but not in acetone (Nusch 1980), and this may also have inadvertently led to comparatively low Chl values in the methanolic extracts when the fluorometer was used.

Fluorometric and photometric techniques also differed with respect to the ratio of uncorrected to corrected Chl *a* values (UNCORR:CORR; Fig. 2a and 2b, respectively). Regardless of instrument type used, the ratios corresponding to the MeOH treatment tended to be low, probably because of the problem with acidification as discussed previously. With the exception of the methanolic extracts, uncorrected Chl *a* concentrations were consistently twice those of corrected values when samples were analyzed with the fluorometer. By comparison, samples analyzed with the spectrophotometer were a great deal more variable, with ratios ranging from less than 2 to greater than 4.

Cross-study comparisons using different measurement protocols are inappropriate because differences in analytical techniques produced significantly different Chl *a* values in parallel samples (Table 3). Studies also differed in the treatment of phaeophytin interference, and since only some of the investigators in Table 2 reported corrected Chl *a* values, this may have contributed another source of error to the cross-study comparison. Therefore, we sought to calibrate the published data to a common unit by standardizing all reported measurements to phaeophytin-corrected Chl values, equivalent to those that had been obtained photometrically following DMSO-A extraction (Table 4). We chose this because

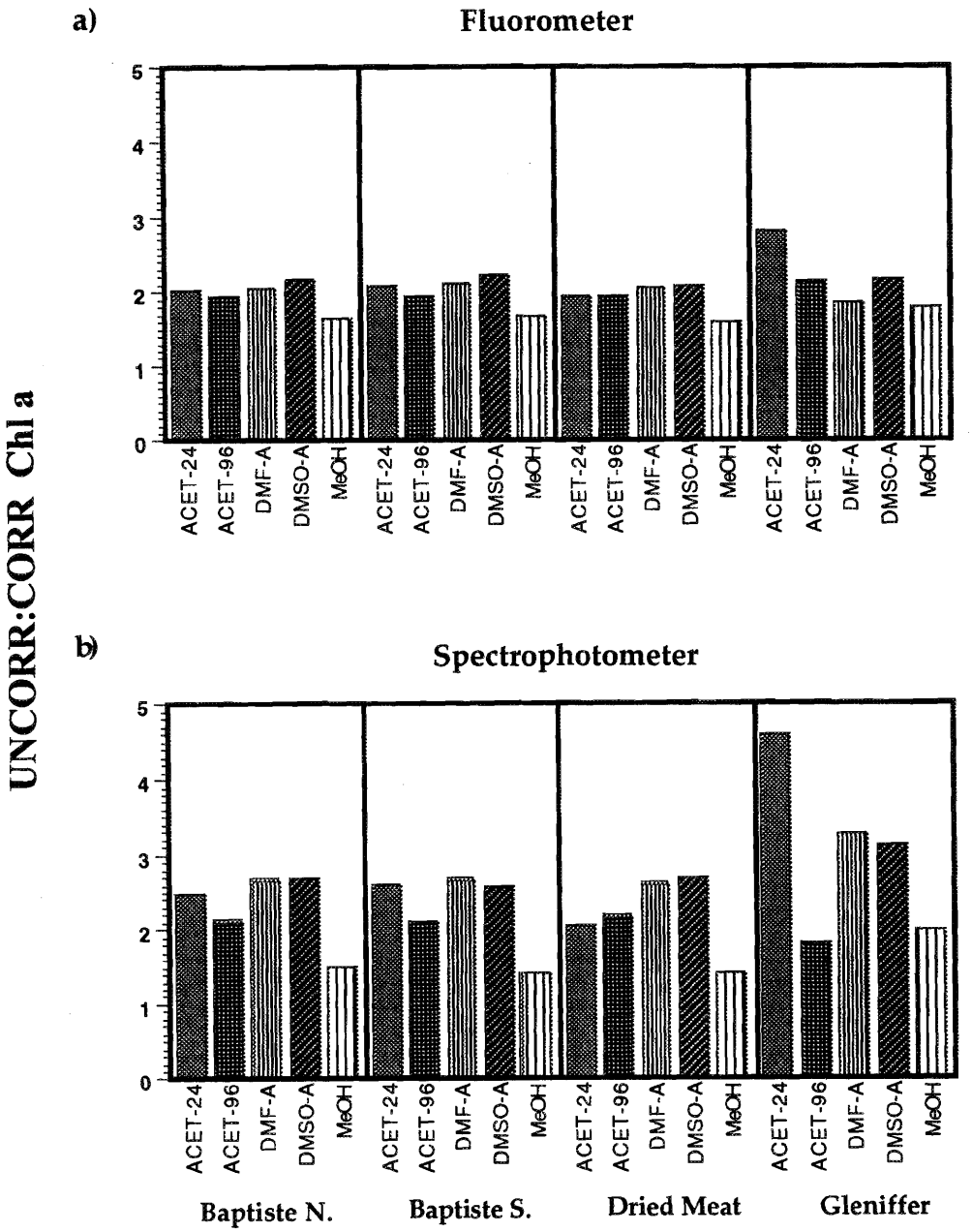

FIG. 2. Ratio of uncorrected (UNCORR; for phaeophytin) to corrected (CORR) Chl *a* values for different extraction protocols that had been measured with (a) a fluorometer and (b) a spectrophotometer. See Table 1 for explanation of abbreviations for protocols.

we had not included the EtOH-S method (used by Mitchell and Prepas 1990) in our earlier comparison of extraction protocols, but Webb et al. (1992) performed a direct comparison of data obtained with the EtOH-S method and a photometric protocol involving DMSO-A extractions. We used information from Webb et al.'s (1992) study to calculate a conversion factor of 0.78 that relates uncorrected data obtained by the EtOH-S method to corrected Chl concentration obtained photometrically by a method that is similar to our DMSO-A method.

We then used information from our own experiments (Table 3; Fig. 2) to derive the remainder of the conversion factors to standardize the other published Chl values (Table 4). In deriving these conversion factors, we considered both the extraction protocol as well as the trophic status of the lakes in question. This meant that we used experimental data (Table 3) corresponding to GL, BN and BS, and DM, respectively, to calculate conversion factors for lakes with low (TP < 10 μg/L), medium (TP between 10 and 100 μg/L), and high (TP > 100 μg/L) productivity. For example, in the low productivity category, we used data corresponding to GL samples and determined that photometric data extracted with DMSO-A were on average 1.38 times higher than corresponding fluorometric data that had been extracted in acetone for 96 h (corrected for phaeophytin).

The standardized Chl *a* values were then plotted against log TP. The same smoothing spline procedure that had yielded an obviously sigmoidal curve for the unstandardized data (broken line, Fig. 1a) produced a line with a reduced curvature in the initial portion of the curve and a more flattened line

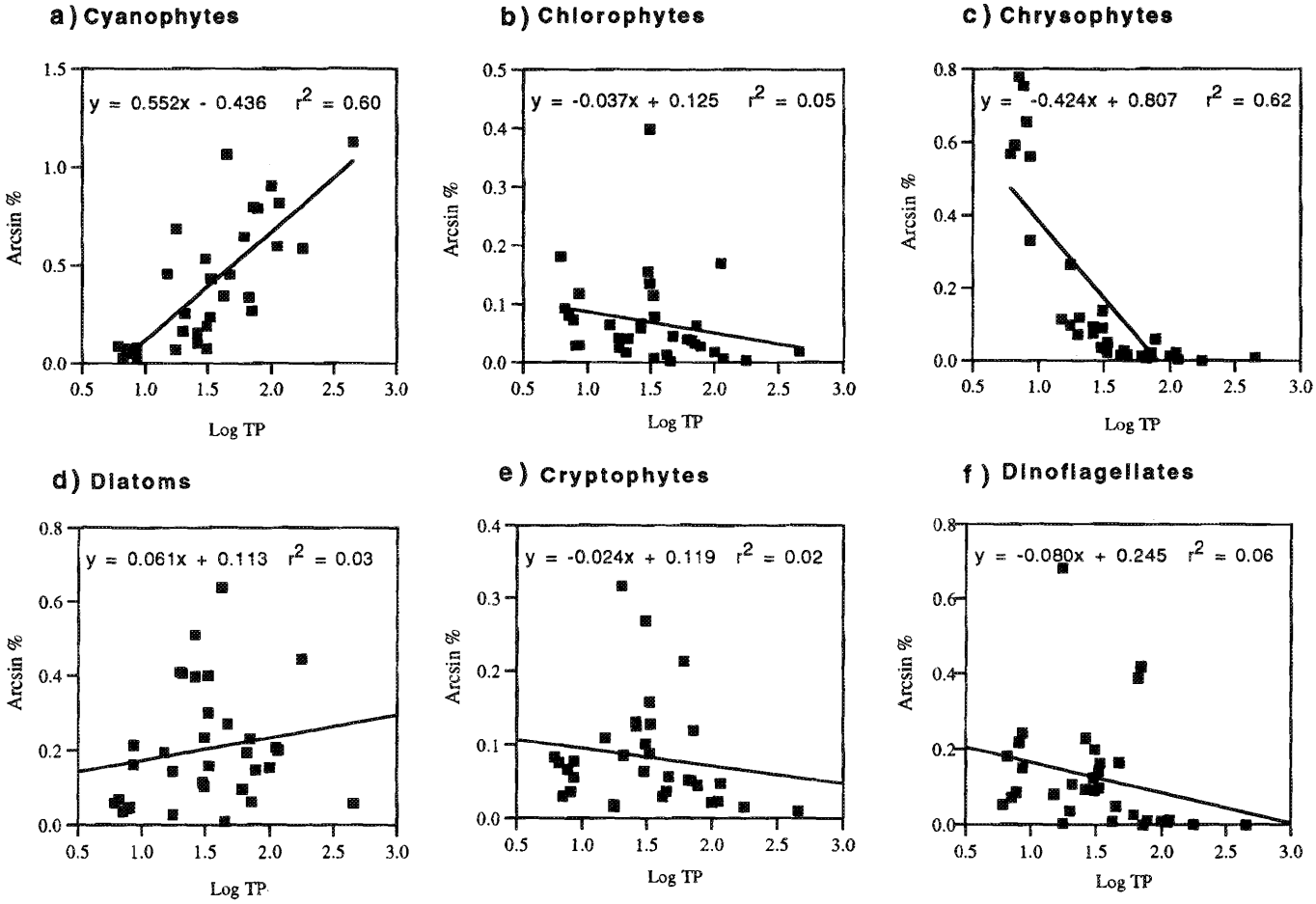

FIG. 3. Proportion of total phytoplankton biomass (arcsin-transformed) plotted as a function of $\log_{10}$ TP (μg/L) for (a) cyanophytes, (b) chlorophytes, (c) chrysophytes, (d) diatoms, (e) cryptophytes, and (f) dinoflagellates. Data are seasonal mean values calculated for 33 lakes in Ontario (Experimental Lakes Area) and Alberta.

TABLE 4. Correction factors used to calculate a standardized Chl value for valid comparisons. Productivity categories were designated as follows: low, lakes with Chl < 10 μg/L; medium, Chl between 10 and 60 μg/L; high, Chl >60 μg/L. See text for explanation of how correction factors were calculated. n/a, not applicable.

Source	Factors used for each category		
	Low	Medium	High
Alberta Environment (D. Trew, unpublished data)	1.381	1.318	1.125
Atlas of Alberta Lakes (Mitchell and Prepas 1990)	0.780	n/a	n/a
Stockner et al. 1980; Shortreed and Stockner 1981; Stockner and Shortreed 1985	0.693	n/a	n/a
Freshwater Institute (M. Stainton, unpublished data)	0.693	n/a	n/a
Zimmerman et al. 1983	1.212	1.155	n/a
Painter et al. 1991	n/a	0.399	0.348

in the latter portion for the standardized data (broken line, Fig. 1b). Overall, the standardized relationship explained only marginally less variation (r^2 = 0.90) compared with the unstandardized data (r^2 = 0.92), but consistent with earlier analyses, a least-squares linear regression procedure resulted in a statistically poorer fit (r^2 = 0.79). Thus, accounting for errors due to differential extraction efficiencies, different analytical technique, and different treatment of phaeophytin content in samples did not appear to alter the nonlinear relationship between TP and Chl *a*.

Since all of the extremely high values of TP (>300 μg/L) and Chl (>100 μg/L) were associated with only one source, and this source was not a typical lake (Cootes Paradise Marsh; denoted by "Y"; see discussion in Methods to Test Hypotheses), we excluded the marsh data and fit the curve through the remainder of the data to determine the extent to which our original results had been biased. In the case of unstandardized data, the curve through the reduced dataset was essentially the same (solid line, Fig. 1a), although the overall truncated presentation does not give as strong an impression of sigmoidality as does the curve through the entire dataset (broken line, Fig. 1a). In the case of standardized data, however, the curve through the reduced dataset (solid line, Fig. 1b) did not level off at the same point as did the curve through the larger dataset (broken line, Fig. 1b), suggesting that application of the conversion factors to calibrate data for Cootes Paradise Marsh may have been inappropriate. Nevertheless, the strong sigmoidal shape of the TP–Chl *a* curve was retained whether or not we included data from the marsh.

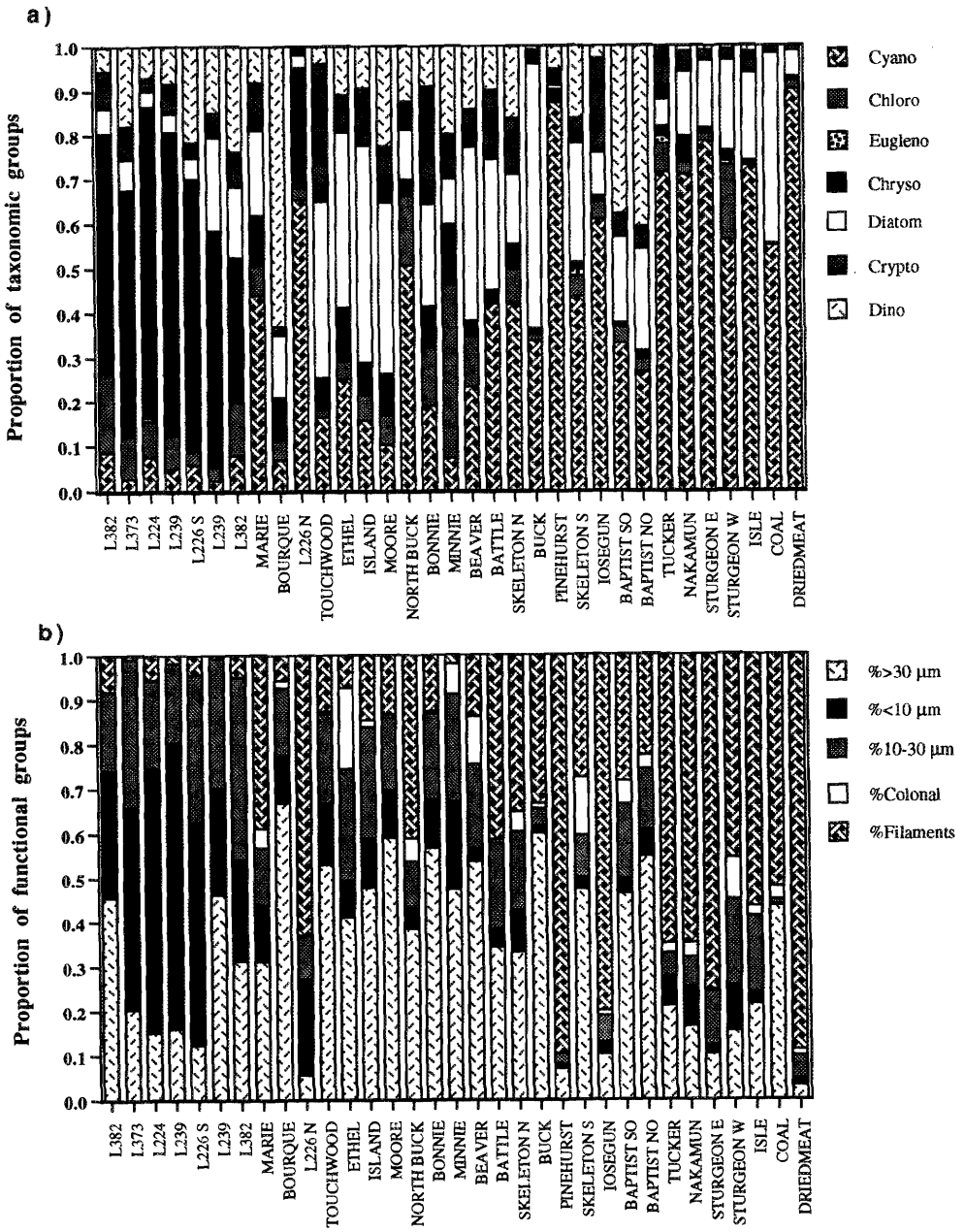

FIG. 4. Distribution of (a) seven taxonomic groups and (b) five functional groups for 33 lakes in Ontario (Experimental Lakes Area) and Alberta. Data are means of at least five sampling trips during the ice-free season. Lakes are sorted by ascending TP concentrations.

In addressing our second hypothesis, we first tested the assumption that the taxonomic makeup of phytoplankton changed predictably across the trophic gradient. Owing to availability of limited data, algal biomass from only 33 of the 199 lakes could be used to test this hypothesis (raw data in Appendix). The seasonal mean percent distributions of six major taxonomic groups in each of the 33 lakes were arcsin-transformed and plotted against log TP (Fig. 3). The proportion of blue-greens increased significantly with TP concentration, while those of chrysophytes decreased significantly ($r^2 = 0.60$ and 0.62, respectively; $P < 0.05$). There was no apparent pattern in the distribution of the remaining taxonomic groups although diatoms and cryptophytes seemed to have been more abundant at intermediate TP concentrations.

The distribution pattern that emerges from this analysis is a shift in dominance of chrysophytes in oligotrophic communities to one of diatoms, green algae, and cryptophytes in mesotrophic communities to a largely blue-green community in eutrophic and hypereutrophic lakes. This gradient of change in phytoplankton community structure with increasing lake trophic status is better visualized when all of the taxonomic groups are presented together in stacked histograms (Fig. 4a). Canfield et al. (1989) and Duarte et al. (1992) have reported a similar shift in algal community structure for a large number of shallow Florida lakes. They noted that there was a gradual shift from dominance by green algae in oligotrophic lakes to dominance by blue-green algae in eutrophic and hypereutrophic lakes, with a peak in diatom abundance in mesotrophic lakes. The main difference between results of our study and theirs is that chrysophytes were

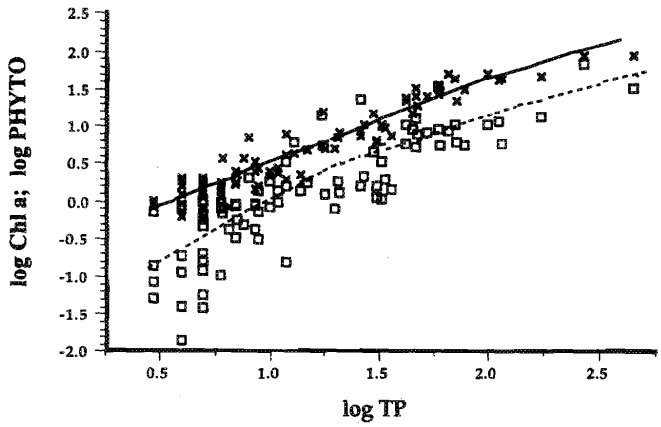

FIG. 5. Log_{10} standardized Chl *a* (X; μg/L) and log_{10} PHYTO (□, mg/L) plotted as a function of log_{10} TP (μg/L). Lines were obtained by applying a smoothing spline procedure to the data (see Fig. 1 legend).

more prominent in unproductive lakes in Canada whereas green algae were more prominent in the corresponding lakes in Florida. That there is such striking similarity in the overall pattern of algal community in these lake sets is remarkable considering the geographical and climatic disparities between the two.

To determine if Chl *a* and PHYTO uncouple along this trophic gradient, we regressed contemporaneous measurements of Chl *a* and PHYTO against TP (n = 89 lake-years; Fig. 5). A spline-fit resulted in a curve through the TP–Chl *a* data that appeared linear, with a corresponding r^2 value of 0.93, which was virtually identical to that of a linear regression analysis (0.92). Similar regression analyses performed on the corresponding TP–PHYTO data also produced more or less linear relations (r^2 = 0.71 and 0.69, respectively) although the residual variation about the lines was much larger. The disappearance of the previously noted sigmoidal TP–Chl *a* curve was obviously the result of truncating the range of TP values (reduced from 152 to 89 lake-years) when we restricted our analysis to only lakes with parallel measurements of Chl and PHYTO. Therefore, merely comparing the shape of the TP–Chl *a* and TP–PHYTO curves was not sufficient to test the second hypothesis.

Although the slopes of the TP–Chl (1.23 ± 0.040) and TP–PHYTO (1.22 ± 0.087) linear regression equations were statistically similar (ANCOVA; $P < 0.05$), there was an obvious uncoupling of Chl and PHYTO at low TP concentrations that is more clearly demonstrated by a sharp decrease in the Chl:PHYTO ratio with TP concentrations above 10 μg/L (Fig. 6a). In contrast with the findings of Watson et al. (1992), we found inconsistencies in the relationship between log Chl *a* and log PHYTO (Fig. 6b; breakpoint at approximately 1 mg/L of phytoplankton biomass). Therefore, although the overall trends in the TP–Chl *a* and TP–PHYTO regressions are similar, there is an uncoupling between Chl *a* and PHYTO at low TP concentrations that may explain why there is a departure from nonlinearity in the initial portion of the TP–Chl *a* curve.

One hypothesis for the uncoupling between PHYTO and Chl *a* is that the ratio of Chl *a* to total Chl increases as lakes become more productive. This may be because cyanophytes, which contain only Chl *a* (as opposed to Chl *b* or *c*), dominate in productive lakes, while chlorophytes and chrysophytes,

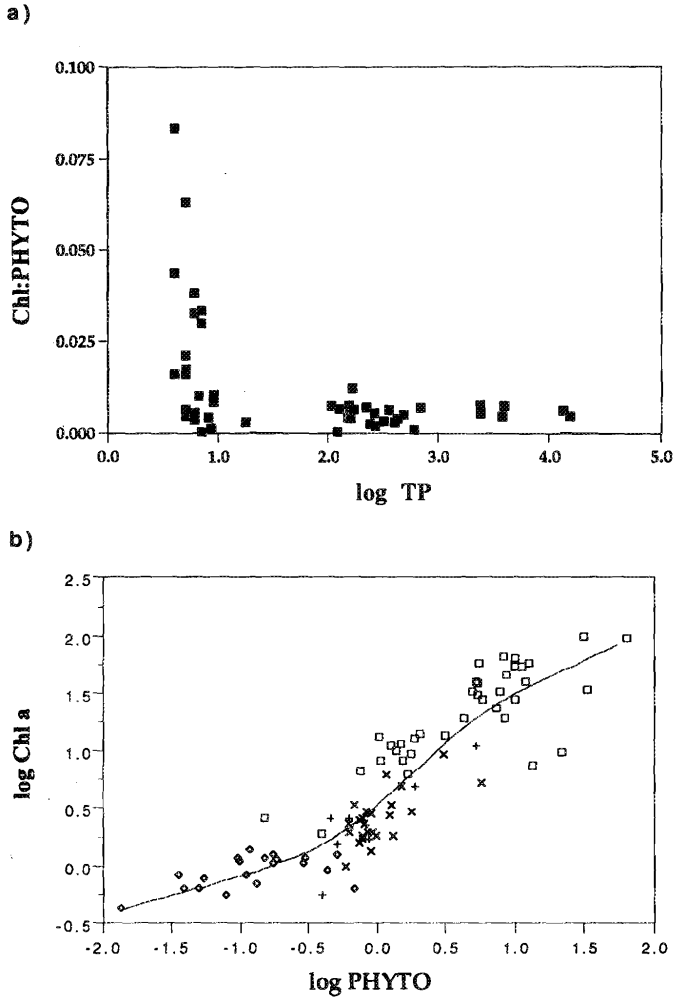

FIG. 6. (a) Ratio of standardized Chl *a* to PHYTO plotted as a function of log_{10} TP (μg/L). (b) Log_{10} standardized Chl *a* (μg/L) plotted as a function of log_{10} PHYTO biovolume (mg/L); the line was obtained by applying a smoothing spline procedure to the data (see Fig. 1 legend).

which usually contain quantities of Chl *b* and *c* (which are known to interfere with the measurement of Chl *a* by producing overestimates of phaeopigments), dominate in unproductive lakes. These two phenomena in concert would likely result in a nonlinear increase in Chl *a* with TP concentrations, even if there is a linear increase in algal biomass within the same range. Future studies should therefore be carried out to determine the relative distributions of the various chlorophyll pigments as lakes increase in trophic status.

To test our third hypothesis, we investigated whether or not the proportion of TBAP in the TP pool changed as lakes increased in productivity. To eliminate any confounding effects due to differences in analytical and measurement protocol, we selected a dataset in which samples had been collected over a 3-wk period during July 1983 and where all Chl *a* had been measured with the ACET-96-F method (31 lakes). The proportion of biologically active phosphorus in the TP pool clearly increased along the trophic gradient (Fig. 7a). The percent TBAP increased from a low of 5% at TP concentrations <10 μg/L to over 85% at TP concentrations in excess of 100 μg/L. At concentrations above 200 μg/L, the proportion of TBAP seemed to decline, but this should be verified with a larger number of hypereutrophic lakes.

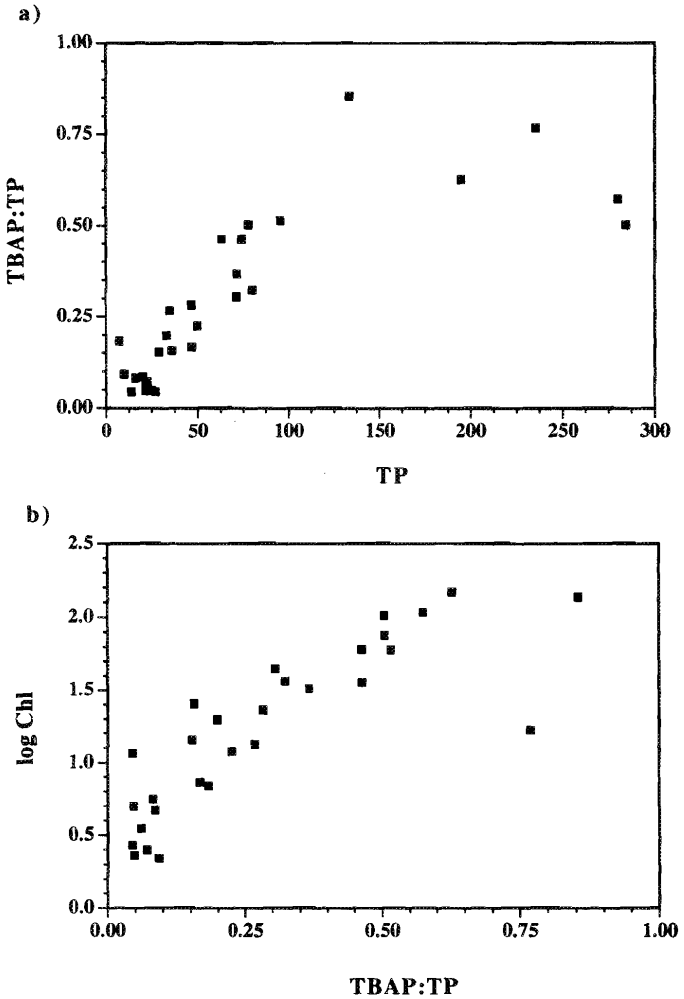

FIG. 7. (a) Plot of the ratio TBAP:TP versus TP (μg/L). (b) Plot of $\log_{10}$ Chl (μg/L) versus the ratio of TBAP:TP for 31 lakes in Alberta.

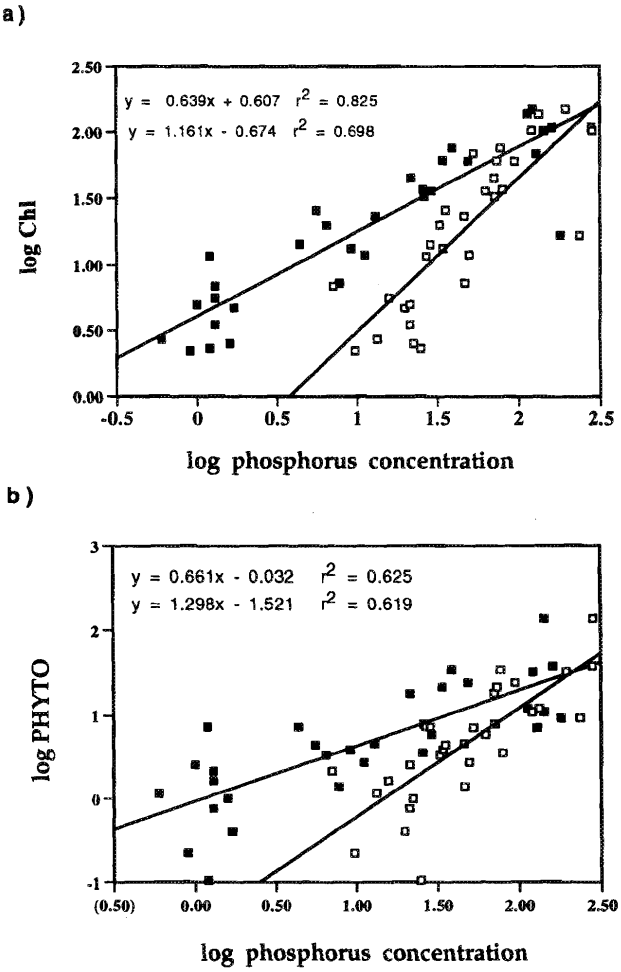

FIG. 8 Plot of (a) $\log_{10}$ Chl *a* (μg/L) and (b) $\log_{10}$ PHYTO (mg/L) versus $\log_{10}$ TP (□; μg/L) and $\log_{10}$ TBAP (■; μg/L) concentrations for 31 lakes in Alberta. Lines through the data are the least-squares linear regression equations (all $P < 0.001$).

Corresponding Chl *a* values varied linearly with the proportion of TBAP up to 0.70, and thereafter seemed to plateau (Fig. 7b).

Not surprisingly, there was no obvious indication of sigmoidality in either the TP–Chl or TBAP–Chl relationships (Fig. 8a), since there was only a limited range of phosphorus concentrations. It is worth noting, however, that although TBAP was a much better predictor of Chl *a* than TP (r^2 = 0.83 versus 0.70, respectively), there was no such improvement in the TBAP–PHYTO relationship when compared with that of TP–PHYTO (r^2 = 0.63 versus 0.62, respectively; Fig. 7b). Consistent with earlier analyses (Fig. 6b), a plot of Chl *a* against PHYTO clearly showed a departure from linearity at low phytoplankton concentrations (Fig. 9). A comparison of results from the spline-fit procedure (solid line) and a linear regression analysis (broken line) indicated that the data were better described by a curvilinear than a linear fit (r^2 = 0.85 versus 0.79, respectively). These observations thus provide further evidence that there is an uncoupling between Chl *a* and PHYTO in unproductive systems, and we caution against using them interchangeably throughout the entire productivity gradient without additional study.

To test our fourth hypothesis, algae corresponding to the smaller dataset of 33 lakes (Appendix) were sorted according

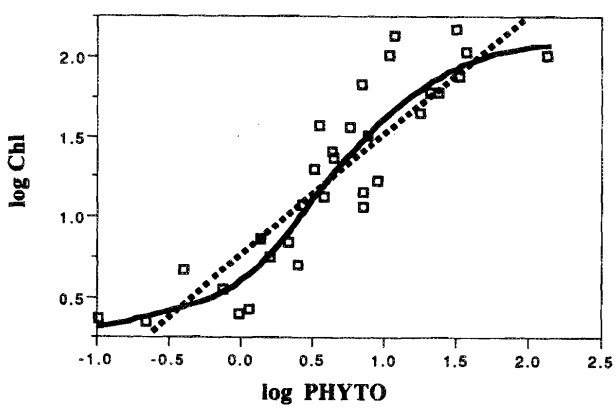

FIG. 9. Plot of $\log_{10}$ Chl (μg/L) versus $\log_{10}$ PHYTO (mg/L) for 31 lakes in Alberta. The solid line was obtained by applying a smoothing spline procedure to the data (r^2 = 0.85) (see Fig. 1 legend); the broken line is the least-squares linear fit through the data (r^2 = 0.79).

to functional categories (see Methods to Test Hypotheses). We found a statistically significant increase in the proportion of filamentous algae with increasing TP concentrations (Fig. 10a), which was accompanied by a concomitant

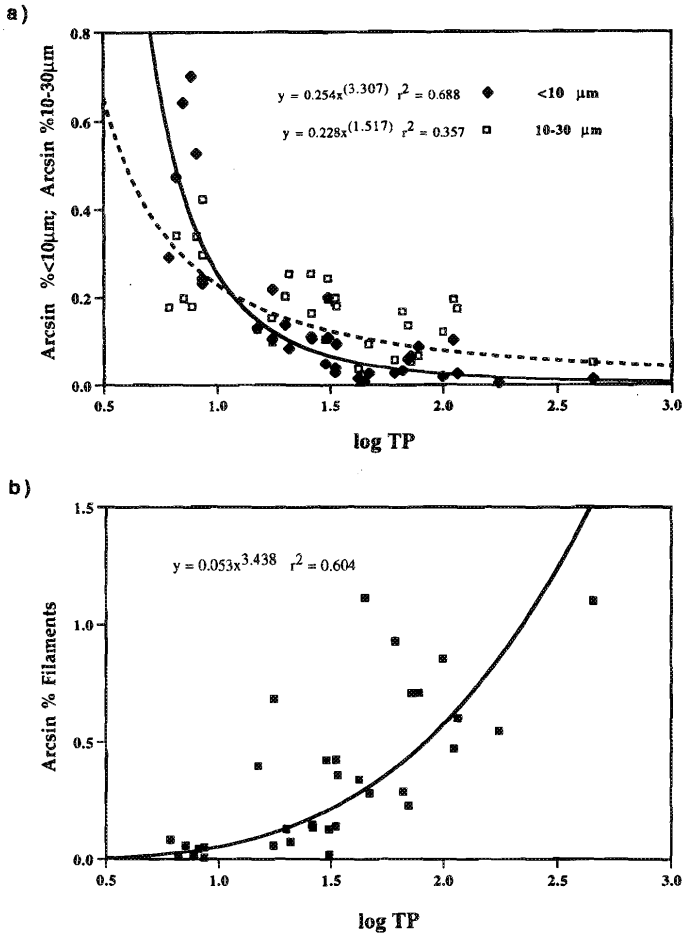

FIG. 10. Proportion of total phytoplankton biomass (arcsin-transformed) plotted as a function of $\log_{10}$ TP (µg/L) for (a) cells with maximum linear dimensions <30 µm (◆, cells <10 µm; □, cells/colonies between 10 and 30 µm) and (b) filamentous algae. Data are from 33 lakes in Ontario (Experimental Lakes Area) and Alberta.

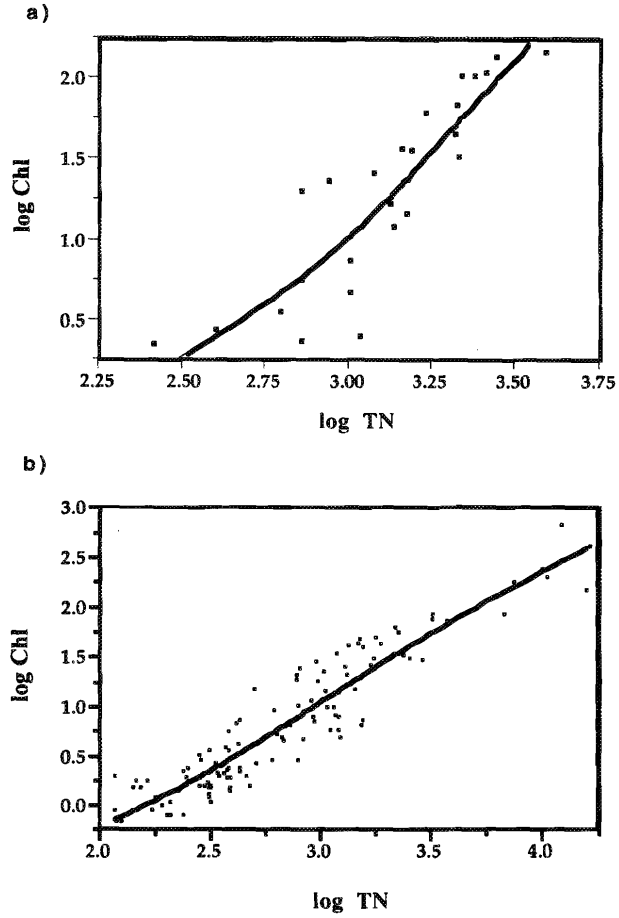

FIG. 11. Plot of $\log_{10}$ Chl *a* (µg/L) versus $\log_{10}$ TN (µg/L) for (a) 31 Alberta lakes and (b) a large database including 119 waterbodies and 152 lake-years. Lines were obtained by applying a smoothing spline procedure to the data (see Fig. 1 legend).

decrease in the proportion of cells <30 µm (including cells in both categories of <10 and 10–30 µm; Fig. 10b). Neither cells nor colonies >30 µm exhibited any systematic variation with TP levels. When the functional groups are presented together in stacked histograms (Fig. 4b), it is obvious that the filaments belonged to the cyanophytes (see Fig. 4a). These observations are therefore consistent with our fourth hypothesis that the grazing impact of herbivores decreases systematically as lakes increase in productivity due to the corresponding increase in the proportion of interfering blue-green filaments.

As a comparison, Watson et al. (1992) conducted a similar study in which algae were assigned to only two functional categories: "edible" algae whose maximum dimensions were <35 µm and "inedible" algae whose dimensions were >35–50 µm. They found that the proportion of the "inedible" fraction increased with lake trophic status, while that of the "edible" fraction decreased. Our results demonstrate that grouping all cells/colonies >35–50 µm as "inedible" may be unwarranted, since only filamentous forms were shown to vary significantly with phosphorus concentrations. The distinction between these "inedible" forms is important because in addition to being inedible, filaments also interfere with grazing activities (Chow-Fraser and Sprules 1986; Hawkins and Lampert 1989). Therefore, separating the "inedible" category into filamentous and nonfilamentous forms should make the functional categories more informative.

Our fifth hypothesis addresses the departure from linearity in the latter portion of the curve. A large body of evidence strongly implicates the limiting effects of TN and/or ambient light availability (Smith 1986; Canfield et al. 1989; Beaver and Crisman 1991; Downing and McCauley 1992) at extremely high TP concentrations (>500 µg/L). When we plotted Chl *a* values against log TN, we found no evidence of levelling off at high TN concentrations for either the smaller subset of 31 lakes (Fig. 11a) or the large dataset (Fig. 11b). It is tempting to infer from this that these hypereutrophic lakes are nitrogen limited; however, without evidence from field kinetic studies, and some information regarding the internal N:P ratios, we do not know whether or not phytoplankton are compensating for low external loading through more efficient nitrogen fixation (Findlay et al. 1994). Nevertheless, the reason that TN appears to be better than TP in predicting Chl *a* in hypereutrophic systems deserves further study.

General Discussion

We have ruled out the first hypothesis that the sigmoidal relationship between TP and Chl *a* could be an artifact of incompatible extraction protocols used in the different

published studies. Although we found that differences in analytical technique contributed significant errors to the measurement of Chl *a* across studies, the nature of these errors did not confound the strong curvilinear relationship between Chl *a* and TP, and thus our accounting for differences in measurement protocol did not change the conclusion that the Chl–TP curve is sigmoidal (Fig. 1). Our study showed that differences in extraction protocol explained as much variation as choice of analytical machine used (i.e., spectrophotometer versus fluorometer; 6% each); neither was substantial compared with the effect of lake origin (81%). Thus, in cross-lake comparisons where the range in TP concentrations spans several orders of magnitude, differences in measurement protocol may be ignored. These results thus validate the approach taken by other investigators who have combined or compared data obtained from different laboratories with different measurement protocols. However, investigators should be careful when comparing data within lakes or among lakes of similar trophic states because depending on the combination of extraction protocol and machine type used, errors may be relatively large.

Although a test of the second hypothesis was inconclusive, we found strong evidence that Chl *a* is a biased surrogate of phytoplankton biomass and that there is an uncoupling between Chl *a* and PHYTO in unproductive lakes (Fig. 6). Part of this uncoupling may be attributed to the fact that the lakes in question tend to be dominated by diatom–chrysophycean assemblages (Stockner et al. 1980; Shortreed and Stockner 1981) that contain large amounts of Chl *c* in addition to Chl *a*. Holm-Hansen et al. (1965) showed that when mixtures of Chl *a* and Chl *c* were present in samples, acid ratios tended to increase, and this resulted in overall higher estimates of Chl *a* than when no other chlorophyll pigments were present. Accordingly, Chl *a* values in these lakes may have been artificially inflated and the initial nonlinearity between TP and Chl *a* may in part reflect the inappropriateness of using Chl *a* as the sole surrogate of algal biomass across a large range of lake productivity. A detailed examination of the relative contribution of the three forms of Chl in natural communities must be undertaken before we can fully evaluate the extent to which Chl *a* and phytoplankton biomass can be used interchangeably across the trophic gradient.

The uncoupling of PHYTO and Chl *a* in this study differs from that of Watson et al. (1992) who found that the Chl:PHYTO ratio in their dataset varied in a nonsystematic fashion with phosphorus (their fig. 2). The primary difference in datasets that led to our respective conclusions is the high Chl:PHYTO ratios corresponding to British Columbia coastal lakes with TP concentrations <5 μg/L in our dataset (Fig. 6a), which had no counterpart in Watson et al.'s (1992) dataset. Without a corresponding breakdown of taxonomic or size categories of phytoplankton in these coastal lakes, we cannot comment on possible reasons for these differences.

We tested both our third and fourth hypotheses to explain the initial nonlinearity in the TP–Chl curve and found that both were upheld. Our third hypothesis was supported by the observation that the proportion of TBAP increased linearly with TP throughout most of the TP concentrations tested (<200 μg/L; Fig. 7a) and the concomitant observation that Chl *a* varied directly with the proportion of TBAP in the TP fraction (Fig. 7b). These observations suggest that Chl in unproductive systems is largely responding to the amount of biologically available phosphorus in the water column, and since this is a relatively small proportion of the TP pool in oligotrophic lakes, TP would tend to overestimate algal biomass. Our fourth hypothesis was supported by a significant increase in the proportion of interfering blue-green algae with increasing TP concentrations (Fig. 10a). We therefore suggest that grazers in oligotrophic lakes can exert a greater impact on the algae compared with grazers in more productive systems because phytoplankton communities associated with the former are filament-free. Unfortunately, we could not determine the extent to which either hypothesis applies or if they both contribute equally to the initial nonlinearity.

Our final hypothesis was used to explain the departure from linearity at extremely high concentrations of TP. The fact that Chl concentrations did not plateau at high TN concentrations is consistent with the hypothesis that TN is more limiting than TP in these hypereutrophic lakes (Fig. 11). However, no conclusions regarding nitrogen limitation should be made without further studies including field kinetic studies and an investigation of the internal N:P ratios.

This study has provided evidence that the sigmoidal relationship between TP and Chl *a* could be attributed to several sets of ecological interactions between adjacent trophic levels at the bottom of the aquatic foodweb. We hope that more hypotheses will be formulated in future studies to uncover the underlying mechanisms that lead to nonlinearity in the TP–Chl *a* relationship. It is important that limnologists continue to examine ecological functions over the entire productivity gradient because theories that provide a unifying framework cannot be advanced unless investigations include a global perspective.

Acknowledgements

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Appendix

TABLE A. Mean seasonal nutrient (TP, TN; μg/L), Chl *a* (μg/L), and phytoplankton biomass (mg/L) concentrations for 33 lakes in Ontario (Experimental Lakes Area) and Alberta. COL, colonies; FIL, filaments; Total, sum of all functional or taxonomic groups. Slight discrepancies are due to rounding errors. Means are calculated from data collected at least monthly between May and September inclusive. n/c, not counted; n/a, not available.

					Algal biomass										
Lake	Year	TP	TN	Chl	<10 μm	10–30 μm	>30 μm	COL	FIL	Cyanophyte	Chlorophyte	Chrysophyte	Diatoms	Cryptophyte	Dinoflagellate	Total
L382	1980	6.1	386	3.6	0.187	0.115	0.296	n/c	0.053	0.056	0.117	0.350	0.038	0.054	0.035	0.651
L373	1983	6.6	239	0.8	0.187	0.137	0.084	n/c	0.004	0.011	0.038	0.229	0.028	0.031	0.074	0.412
L224	1974	7.1	237	2.2	0.319	0.105	0.080	n/c	0.031	0.040	0.043	0.375	0.019	0.016	0.042	0.535
L239	1974	7.7	312	3.6	0.300	0.083	0.074	n/c	0.009	0.023	0.034	0.318	0.020	0.031	0.040	0.466
L226S	1974	8.1	418	6.9	0.988	0.653	0.240	n/c	0.086	0.116	0.057	1.201	0.091	0.071	0.432	1.967
L239	1982	8.6	281	3.2	0.203	0.247	0.391	n/c	0.005	0.019	0.025	0.450	0.178	0.047	0.128	0.846
L382	1983	8.6	380	2.4	0.208	0.372	0.283	n/c	0.045	0.072	0.107	0.295	0.145	0.070	0.220	0.908
Marie	1981	15.0	683	4.6	0.197	0.193	0.469	0.066	0.587	0.741	0.108	0.191	0.323	0.183	0.135	1.511
Bourque	1981	17.5	640	5.3	1.334	1.929	8.428	0.199	0.725	0.935	0.575	1.326	1.945	0.255	8.557	12.615
L226N	1974	17.6	501	15.2	1.169	0.533	0.293	n/c	3.412	3.429	0.136	1.410	0.147	0.086	0.026	5.407
Touchwood	1986	20.0	831	4.8	0.098	0.144	0.380	0.004	0.092	0.124	0.013	0.055	0.302	0.236	0.028	0.718
Ethel	1984	20.8	727	6.7	0.152	0.451	0.738	0.329	0.132	0.453	0.074	0.214	0.712	0.154	0.196	1.802
Island	1984	26.1	1160	8.3	0.168	0.381	0.728	0.024	0.221	0.238	0.089	0.113	0.741	0.198	0.143	1.522
Moore	1981	26.3	935	7.1	3.016	4.081	12.189	0.131	2.529	2.223	1.457	2.056	8.449	2.725	4.943	21.945
N. Buck	1986	30.2	1059	14.5	0.190	0.401	1.484	0.200	1.592	2.229	0.679	0.164	0.496	0.280	0.544	3.867
Bonnie	1983	31.0	1203	6.0	0.138	0.248	0.724	0.006	0.160	0.203	0.145	0.098	0.250	0.286	0.098	1.276
Minnie	1986	31.0	1117	5.9	0.276	0.336	0.663	0.096	0.029	0.113	0.596	0.212	0.158	0.155	0.304	1.401
Beaver	1986	33.2	1146	9.9	0.090	0.584	1.672	0.336	0.433	0.740	0.365	0.103	1.236	0.277	0.457	3.114
Battle	1984	33.3	611	9.4	0.042	0.205	0.357	0.006	0.431	0.438	0.008	0.023	0.308	0.164	0.103	1.041
Skeleton N.	1986	33.8	n/a	9.1	0.166	0.314	0.585	0.080	0.617	0.800	0.151	0.096	0.299	0.243	0.320	1.761
Buck	1984	42.2	772	20.8	0.159	0.385	6.143	0.127	3.412	3.463	0.131	0.158	6.080	0.306	0.090	10.227
Pinehurst	1986	44.8	1205	14.2	0.072	0.227	0.625	0.001	8.004	7.479	0.017	0.237	0.081	0.316	0.426	8.929
Skeleton S.	1986	47.0	n/a	24.7	0.127	0.444	2.233	0.606	1.306	2.202	0.227	0.086	1.346	0.284	0.892	4.716
Iosegun	1983	61.0	950	29.2	0.161	0.350	0.602	0.069	4.761	3.388	0.224	0.074	0.538	1.190	0.149	5.943
Baptiste S.	1984	66.3	1787	49.8	0.274	1.373	3.824	0.433	2.343	2.746	0.312	0.056	1.587	0.428	3.127	8.247
Baptiste N.	1984	70.0	1332	41.7	0.611	1.389	5.655	0.321	2.332	2.652	0.315	0.180	2.283	0.513	4.066	10.308
Tucker	1982	72.1	1312	21.0	0.366	0.292	1.145	0.117	3.559	4.204	0.372	0.132	0.362	0.699	0.110	5.480
Nakamun	1984	77.8	1742	30.6	0.426	0.324	0.792	0.153	3.147	3.779	0.145	0.319	0.782	0.236	0.064	4.842
Sturgeon E.	1984	99.7	1493	48.1	0.423	1.353	1.176	0.294	6.865	7.978	0.178	0.125	1.555	0.217	0.101	10.111
Sturgeon W.	1984	111.1	1546	40.9	1.111	2.089	1.651	1.022	4.906	6.402	1.920	0.256	2.345	0.266	0.185	10.779
Isle	1984	115.5	1466	43.3	0.153	1.017	1.253	0.129	3.329	4.028	0.039	0.020	1.100	0.262	0.071	5.881
Coal	1984	175.9	1856	43.8	0.054	0.125	5.557	0.386	6.646	7.032	0.039	0.007	5.483	0.191	0.017	12.768
Driedmeat	1984	452.4	3202	86.6	0.427	1.632	0.971	0.395	27.979	28.363	0.608	0.290	1.816	0.327	0.000	31.404