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Testing of the CHEMTAX program in contrasting Neotropical lakes, lagoons, and swamps

Cástor Guisande[1], Aldo Barreiro[1], Alberto Acuña[1], Lili J. Marciales[2], Esnedy Hernández[3], Angélica M. Torres[2], Nelson Aranguren[4], Willian López[4], Santiago R. Duque[2], Lina J. Gallo[3], Néstor Aguirre[3], Marta Mogollón[5], Jaime Palacio[3], and Guillermo Rueda-Delgado[6]*

[1]Facultad de Ciencias, Universidad de Vigo, Campus Lagoas-Marcosende, 36310-Vigo, Spain
[2]Instituto Amazónico de Investigaciones-IMANI, Universidad Nacional de Colombia, 215, Leticia, Colombia
[3]Grupo de Investigación en Gestión y Modelación Ambiental – GAIA, Universidad de Antioquia, Medellín, Colombia
[4]Unidad de Ecología en Sistemas Acuáticos-UDESA, Universidad Pedagógica y Tecnológica de Colombia, Carretera Central del Norte, Tunja, Boyacá, Colombia
[5]Universidad de Córdoba, Programa de Biología, Facultad de Ciencias Básicas e Ingenierías, Carrera 6 N 76_ 103 Montería, Córdoba, Colombia
[6]Laboratorio de Limnología, Universidad Jorge Tadeo Lozano, Carrera 4 No 22-61. Modulo 5 Piso 8, Bogotá, Colombia

Abstract

The problem using the CHEMTAX program in freshwater environments is that the few matrices of pigment ratios available have not been tested in a wide range of environments. Sixteen Amazonian, Andean, and Caribbean lakes, lagoons, and swamps were sampled over a year. The aim was to determine if it was possible to obtain a reliable matrix of input pigment ratios that may be used in freshwater habitats with different environmental conditions. There were no clear differences among regions for most of the ratios of marker pigments to Chlorophyll *a* (Chl *a*) in most of the phytoplankton groups. Only the zeaxanthin/Chl *a* ratio showed clear variations among areas. The estimates for the mean relative contribution of each phytoplankton group calculated for the pigment ratios obtained in each separate habitat and season were very similar to the estimates calculated using the average pigment ratio obtained for all habitats and seasons. Our study suggests that the matrix of the average pigment ratio obtained in this study can be used to estimate phytoplankton class abundances with the CHEMTAX program in freshwater habitats with different limnological conditions.

Introduction

The application of the CHEMTAX program to pigment data from phytoplankton samples has been shown to be a useful tool in marine environments for several purposes, including medium-large scale analysis of phytoplankton class distribution in the open ocean (Schlüter et al. 2000; Wright and Van der Enden 2000; Vidussi et al. 2000; Higgins and Mackey 2000; Gibb et al. 2001; Latasa et al. 2005) and zooplankton feeding studies (Irigoien et al. 2000; Guisande et al. 2002). However, the application of CHEMTAX in freshwater environments has received little attention (Buchaca et al. 2004; Fietz and Nicklisch 2004; Descy et al. 2005; Schlüter et al. 2006).

A critical point when using the CHEMTAX program in freshwater environments is the lack of suitable matrices of pigment ratios that could be used in a wide range of environments. Schlüter et al. (2006) demonstrated that, under controlled conditions, different temperature and light treatments had a relatively insignificant impact on the absolute values for the diagnostic pigment/Chl *a* ratios in 20 freshwater phytoplankton species, with the exception of a considerable variation in the zeaxanthin/Chl *a* and alloxanthin/Chl *a* ratios for cyanobacteria and cryptophytes. This may indicate that CHEMTAX could also be applicable to freshwater phytoplankton. However, it has not been tested whether or not pigment/Chl *a* ratios are also constant in the field under different environmental conditions.

The problem of testing different pigment ratios in distinct environments is that it is necessary to corroborate the validity

*Corresponding author: E-mail: Castor@uvigo.es

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of the prediction of the CHEMTAX program by estimating phytoplankton class abundances under the microscope. The recent advances in the application of CHEMTAX, which greatly improve the fitting of the input pigment ratio matrix to the actual ratios (Latasa 2007), could overcome this problem. The study of Latasa with synthetic sets of samples showed that after 6-10 runs, when using the output matrix as a new input matrix in successive runs of CHEMTAX, in most cases, the estimated ratios significantly matched the true ones, with the exception of haptophytes.

In our study, the phytoplankton pigments and physicochemical characteristics were analyzed of samples taken over the year in 16 Neotropical lakes, lagoons, and swamps, accounting for considerable environmental variation. The aims were to compare the limnological characteristics, pigment profiles, and phytoplankton groups among areas, and to determine whether it was possible to obtain a reliable matrix of input pigment ratios, which may be used in freshwater habitats with different environmental conditions.

Materials and procedures

Field sampling—The location of the lakes, lagoons, and swamps sampled in this study is shown in Fig. 1. Several sampling stations were used for each lake, with samples taken 1-4 times over the year. This sampling design, which covers a high spatial variation and a small temporal variation, made it possible to obtain phytoplankton communities under a wide range of environmental conditions.

In each sampling station, samples were taken at the surface, at the depth of Secchi disk and at the depth of three times Secchi disk with a 2.5 L Van Dorn bottle. For the quantification of pigments, two replicates of 25-100 mL, depending on phytoplankton biomass, were filtered on the boat through 13 mm Whatman GF/C filters (around 1.2 μm pore size) and then stored in dark ultracentrifuge plastic tubes. The samples were stored at –20°C within 4 h of collection. A total of 558 samples of pigment were analyzed.

At the three depths mentioned above, pH, conductivity, temperature, nitrate (NO^-_3), silicate (SiO^-_2), and phosphate (SiO^-_2) were analyzed. Filtered water (0.45 μm) was used for analyzing nutrients with an autoanalyzer BRAN + LUEBBE AAIII (Norderstedt, Germany).

Pigment analysis by high-performance liquid chromatography (HPLC)—Filters with pigment samples were lyophilized inside eppendorff tubes in a Telstar lioalfa–6 device. Pigment extraction was undertaken by adding 2 mL of 95% methanol to the lyophilized material, and then, the sample was homogenized using a pipette tip adapted to fit the shape of the vial. Marker pigments were analyzed following Zapata et al. (2000). Pigment profiles were analyzed by HPLC using a Waters Alliance System 2696, a 996 Waters photodiode array detector, and a Waters Symmetry C_8 column (150 × 4.6 mm, 3.5 μm particle size, 100 Å pore size). The results were processed with Empower software (®2002 Waters Corp.).

CHEMTAX runs—The CHEMTAX program works with an initial matrix of marker pigment/Chl *a* ratios, containing all values specific for each phytoplankton group (*F* matrix). There is also a sample matrix with the ratios of the same pigments measured in field samples (*F*). From these two matrices, the program calculates the proportion of each phytoplankton group (in terms of Chl *a*) present in the samples. The program also changes the initial values of *F* in a more coherent estimate according to the ratios of the samples.

Sample matrices containing ratios of marker pigment/Chl *a* were created for each habitat and each season separately. Inside each lake, samples of all depths (when several depths were sampled) were analyzed together when running the CHEMTAX program, which was set with all the default options (Mackey et al. 1996). The initial matrix of marker pigment/Chl *a* ratios for each phytoplankton group was the same in all the cases analyzed (Table 1). The values of this matrix are the average value of each ratio considering all the matrices from Schlüter et al. (2006), which is the most complete set of values reported in the literature for the application of CHEMTAX to freshwater environments. The phytoplankton groups included in the matrix were previously known from both the microscopic counts (data not shown) and the marker pigments present in the samples. The xantophytes are not included in Schlüter et al. (2006) matrices, therefore pigment content was taken from the following web page http://www.jochemnet.de/fiu/bot4404/BOT4404_21.html (F. J. Jochem, Florida International University). However, as the ratios for this group were unknown, they were set as an average of the ratios for all other groups. The output matrix of pigment ratios from each run was used as input in the next run, as suggested by Latasa (2007), who showed that after 6-10 runs, most of the ratios of matrices agreed significantly with the true ratios. Following this procedure, to be more confident about the reliability of the matrices employed to yield the final solution, we performed 6 runs of CHEMTAX.

Statistical analyses—A discriminant analysis (a multivariate variable statistical method) was applied to the data. Discriminant analysis is a pattern-recognition method that helps to separate 2 or more groups from data provided for several variables (Guisande et al. 2006). Discriminant analysis has been successfully used in the identification of phytoplankton groups using pigment markets (Guisande et al. 2002).

Assessment

Limnological characteristics—Fig. 2 shows that there were important differences in the limnological characteristics among areas, corroborating that the study covered a wide range of environmental conditions.

Phosphate concentrations were usually low and did not show important differences among areas, with the exception of the relatively low concentrations observed in the swamps of the Sinú basin. Nitrate concentrations were below or around 1 μM, which can be considered moderately low, although the

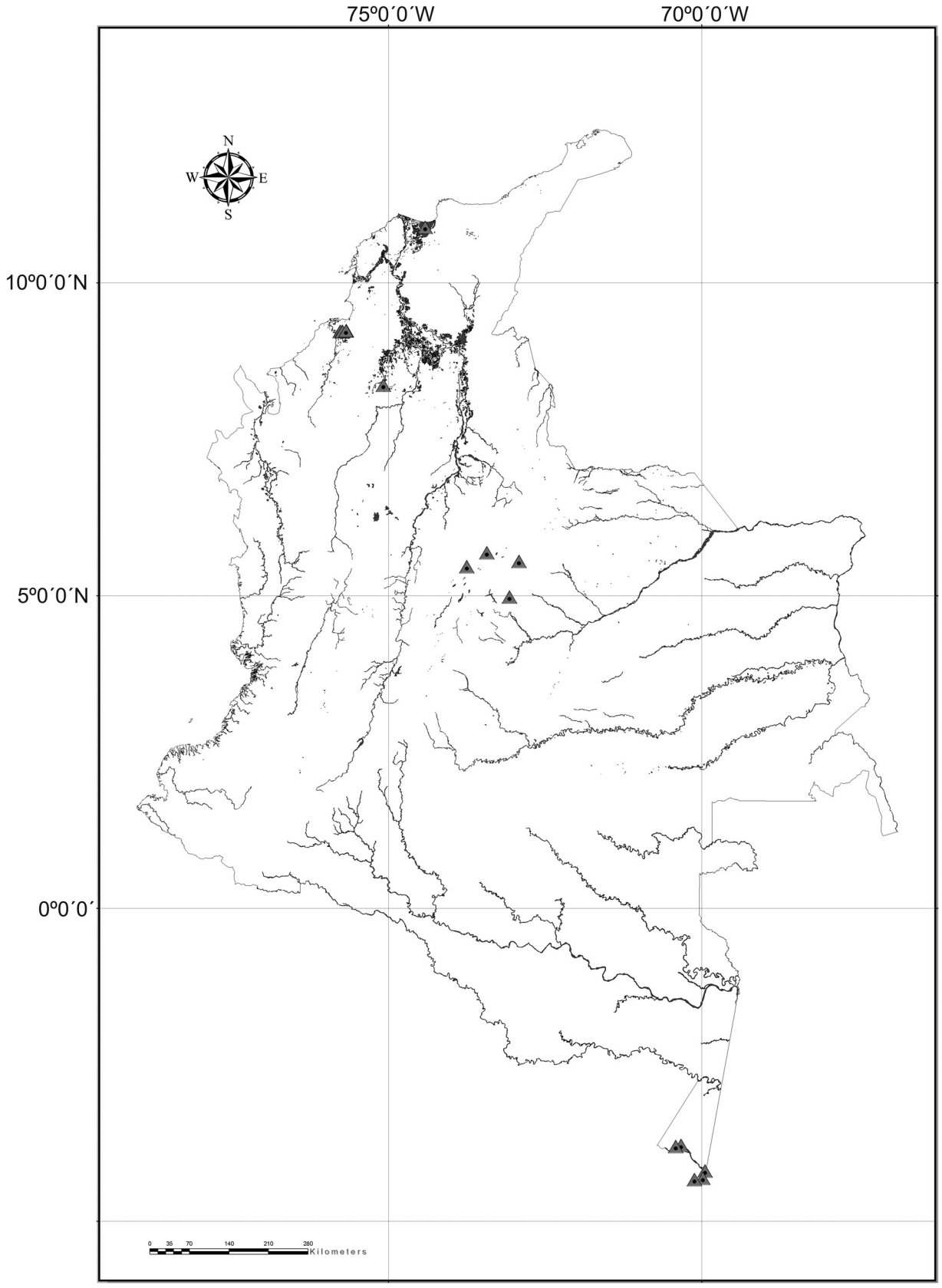

Fig. 1. Map showing the habitats sampled in the study

Table 1. Initial input matrix of marker pigment/Chlorophyll *a* ratios belonging to each phytoplankton group

Phytoplankton groups	Marker pigment/Chl *a* ratio*									
	Chl *c*	Per	Fuc	Neo	Vio	Diad	Allo	Zea	Lut	Chl *b*
Cyanophytes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.208	0.0	0.0
Euglenophytes	0.034	0.0	0.0	0.0	0.0	0.327	0.0	0.0	0.0	0.198
Chlorophytes	0.0	0.0	0.0	0.038	0.026	0.0	0.0	0.035	0.147	0.362
Bacilliariophytes	0.102	0.0	0.515	0.0	0.003	0.342	0.0	0.007	0.0	0.0
Chrysophytes	0.031	0.0	0.283	0.0	0.063	0.016	0.0	0.016	0.0	0.0
Cryptophytes	0.091	0.0	0.0	0.0	0.0	0.0	0.405	0.0	0.0	0.0
Dinophytes	0.155	0.508	0.0	0.0	0.0	0.246	0.0	0.0	0.0	0.0
Xantophytes	0.082	0.0	0.399	0.0	0.0	0.233	0.0	0.0	0.0	0.0

*Chl *c* (chlorophyll *c*1 + *c*2), Per (peridinin), Fuc (fucoxanthin), Neo (neoxanthin), Vio (violoxanthin), Diad (didinoxanthin), Allo (alloxanthin), Zea (zeaxanthin), Lut (lutein), Chl *b* (chlorophyll *b*)

Ayapel swamp showed higher values. Silicate was high in most areas, with the exception of the Andean aquatic habitats and the Zacambú and Javarí lakes in the Amazon.

Conductivity was high in the Caribbean swamps (the Magdalena basin), particularly in Pajarales and Isla San Antonio, but with the variations within an area showing different degrees of interaction with marine and freshwater systems. At the lower end were lake Guatavita and the lagoon Iguaque, which had the lowest conductivity values.

The pH values tended to be acidic in most of the systems, but the Magdalena basin showed more alkaline values, possibly due to a greater marine influence. Temperatures were quite similar in all lakes, but lower in Andean systems, due to the higher altitude.

The Secchi disk was similar among areas, with the exception of Tota Lake and Iguaque in the Andes, with higher transparency than in other habitats (Fig. 2).

Pigment profiles—There were significant differences in pigment profiles among areas (Fig. 3a). A discriminant analysis performed on the raw data (pigment profiles, pigment to Chl *a* ratios), considering the different geographical areas as different groups, showed that all discriminant functions were significant (<0.001), and the percentage of cases correctly classified by cross-validation was 80.8%. The first discriminant function seems to separate the phytoplankton communities of Amazonia and the Andes on one side and the Caribbean on the other (with the Magdalena basin in the most extreme position in this latter group). However, a discriminant analysis performed on the phytoplankton groups estimated after running CHEMTAX, considering also the different geographical areas as different groups, showed that all discriminant functions were significant (<0.001), but the percentage of cases correctly classified by cross-validation was lower than with the raw data, only 62.7%.

Pigment ratios—A discriminant analysis was performed for each phytoplankton group comparing the ratios of marker pigments between lakes (average ratios considering all depths for the different seasons were used as replicates within each lake). Table 2 shows the results of this discriminant analysis. The first discriminant function was only significant for chlorophytes and xantophytes. In the case of chlorophytes, the first discriminant function was significant due to the pigment lutein and in xantophytes due to the pigment fucoxanthin. The percentage of cases correctly classified by cross-validation was always low, even in chlorophytes and xantophytes (Table 2). Therefore, these results show no clear differences among regions in the ratios of marker pigments to Chl *a* for most of the phytoplankton groups, because it was not possible to discriminate areas according to their pigment ratios for any of the phytoplankton groups.

Generating an average pigment ratio matrix—Considering the interest of obtaining an input ratio matrix that can be used in freshwater habitats with a broad range of environmental conditions, we calculated an average matrix with the values of the output matrices (after the 6 runs) from all lakes during all the seasons sampled. Table 3 shows this average output matrix, which was used as input matrix in the CHEMTAX program with the whole set of samples (all habitats and all seasons). Zeaxanthin and alloxanthin were the pigments that show a higher variation (Fig. 4), whereas for the rest of the main pigments the variation was low.

Fig. 5 shows that the estimates of the mean relative contributions of each phytoplankton group in each habitat were similar when they were calculated with the pigment ratios obtained in each habitat and season separately than when the estimates were calculated with the average pigment ratio obtained considering all habitats and seasons. As each phytoplankton group pair was not independent of the others, a bootstrap method was used to evaluate the statistical significance of this relationship (Davison and Hinkley 1997). Regression was recalculated 1,000 times using random series in which only 50% of the abundance data were used to calculate the correlation. In all cases, the slope of the regression was both positive and significantly different from zero.

The pigment ratio used in our study as input matrix (Table 1) is, as mentioned in material and methods, generally the matrix obtained under experimental conditions by Schlüter et al. (2006). Although there are some differences, particularly for

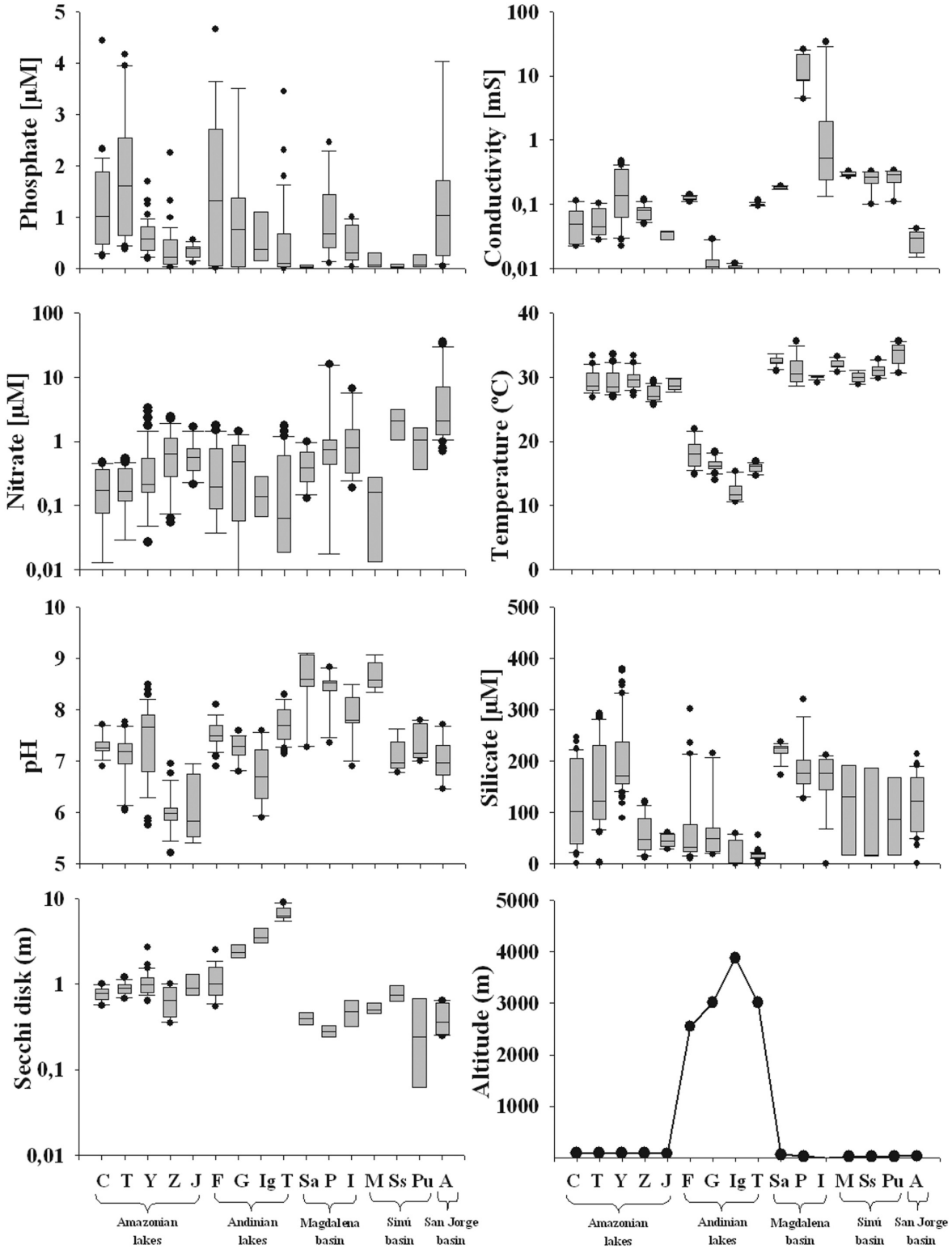

Fig. 2. Limnological parameters measured in the study. Lake abbreviations: C (Correo), T (Tarapoto), Y (Yahuarcaca), Z (Zacambú), J (Javarí), F (Fúquene), G (Guatavita), Ig (Iguaque), T (Tota), Sa (San Antonio), P (Pajarales), I (Isla Salamanca), M (Momil), Ss (San Sebastián), P (Purísima), and A (Ayapel).

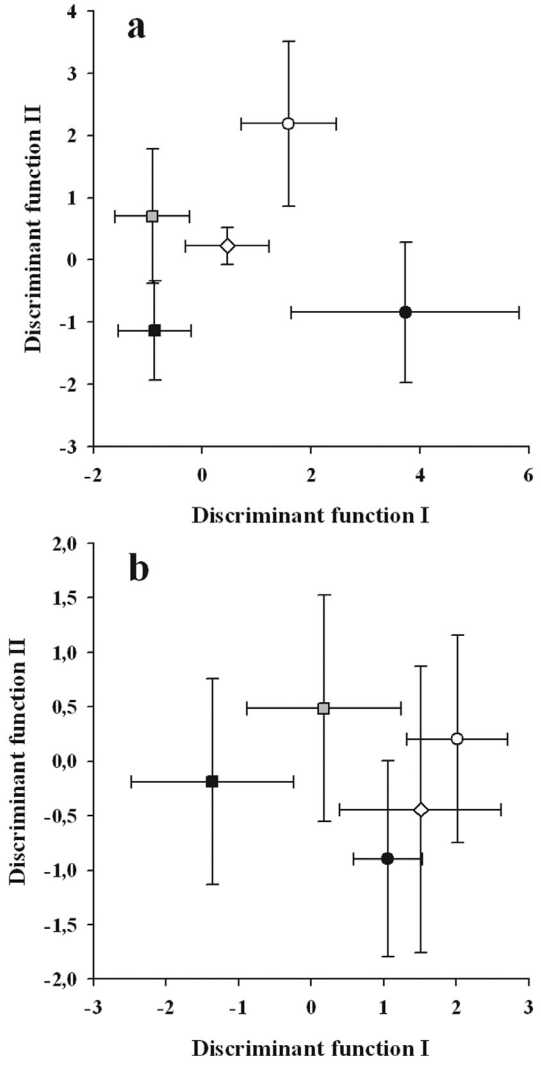

Fig. 3. Plots of the mean ±SD of the scores for the first two discriminant analyses on raw pigment profiles (a) and on the phytoplankton groups estimated after running the CHEMTAX program (b) in the areas. Black square (Amazonia), gray square (Andes), open circle (the San Jorge basin), black circle (the Magdalena basin), open diamond (the Sinú basin).

Table 2. Discriminant analysis performed with marker pigment ratios, comparing each phytoplankton group between zones (as in Fig. 3)

Phytoplankton groups	p^*	%†
Cyanophytes	0.78	17.9
Euglenophytes	0.54	38.5
Chlorophytes	0.003	51.3
Bacilliariophytes	0.51	30.8
Chrysophytes	0.88	23.1
Cryptophytes	0.26	25.6
Dinophytes	0.22	30.8
Xantophytes	0.01	28.2

*p, significance of first discriminant function
†%, percentage of individuals correctly classified by cross validation

lutein (only present in chlorophytes) and for zeaxanthin in cyanophytes, Fig. 6 shows that this input matrix is similar to our final average matrix (Table 3). These differences between the input matrix (Table 1) and the average pigment ratio matrix (Table 3) lead to differences in the estimates for the phytoplankton class abundances (Fig. 7), particularly in chlorophytes, bacilliariophytes, chrysophytes, and xantophytes.

Discussion

We aimed to cover a wide range of environmental conditions in freshwater habitats and to measure an equally wide range of physico-chemical variables (Fig. 2), such as temperature (low temperatures in Andean habitats, high temperatures in Amazonian and Caribbean habitats), pH (Andean, Amazonian, and Caribbean acidic habitats, alkaline lakes in the Magdalena basin), Secchi disk (high transparency in some Andean habitats as compared to lower transparency in other habitats), and conductivity (low conductivity in all habitats except for the almost brackish water of the Magdalena basin). For other parameters, variations within each area (mainly due to temporal variation) overlap the variations observed between areas. Hence, there was no clear pattern in terms of areas with no visibly lower or higher values for any of the nutrients.

Table 3. Final average ratios of marker pigments to Chl *a* calculated from the whole subsets of output ratios derived after 6 CHEMTAX runs in each habitat and season*

Phytoplankton groups	Final average ratios									
	Chl *c*	Per	Fuc	Neo	Vio	Diad	Allo	Zea	Lut	Chl *b*
Cyanophytes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.617	0.0	0.000
Euglenophytes	0.036	0.0	0.0	0.0	0.0	0.325	0.0	0.0	0.0	0.241
Chlorophytes	0.0	0.0	0.0	0.144	0.127	0.0	0.0	0.097	0.426	0.358
Bacilliariophytes	0.132	0.0	0.491	0.0	0.003	0.441	0.0	0.008	0.0	0.0
Chrysophytes	0.042	0.0	0.261	0.0	0.116	0.020	0.0	0.021	0.0	0.0
Cryptophytes	0.186	0.0	0.0	0.0	0.0	0.0	0.544	0.0	0.0	0.0
Dinophytes	0.187	0.528	0.0	0.0	0.0	0.241	0.0	0.0	0.0	0.0
Xantophytes	0.123	0.0	0.392	0.0	0.0	0.186	0.0	0.0	0.0	0.0

*Pigment abbreviations as in Table 1.

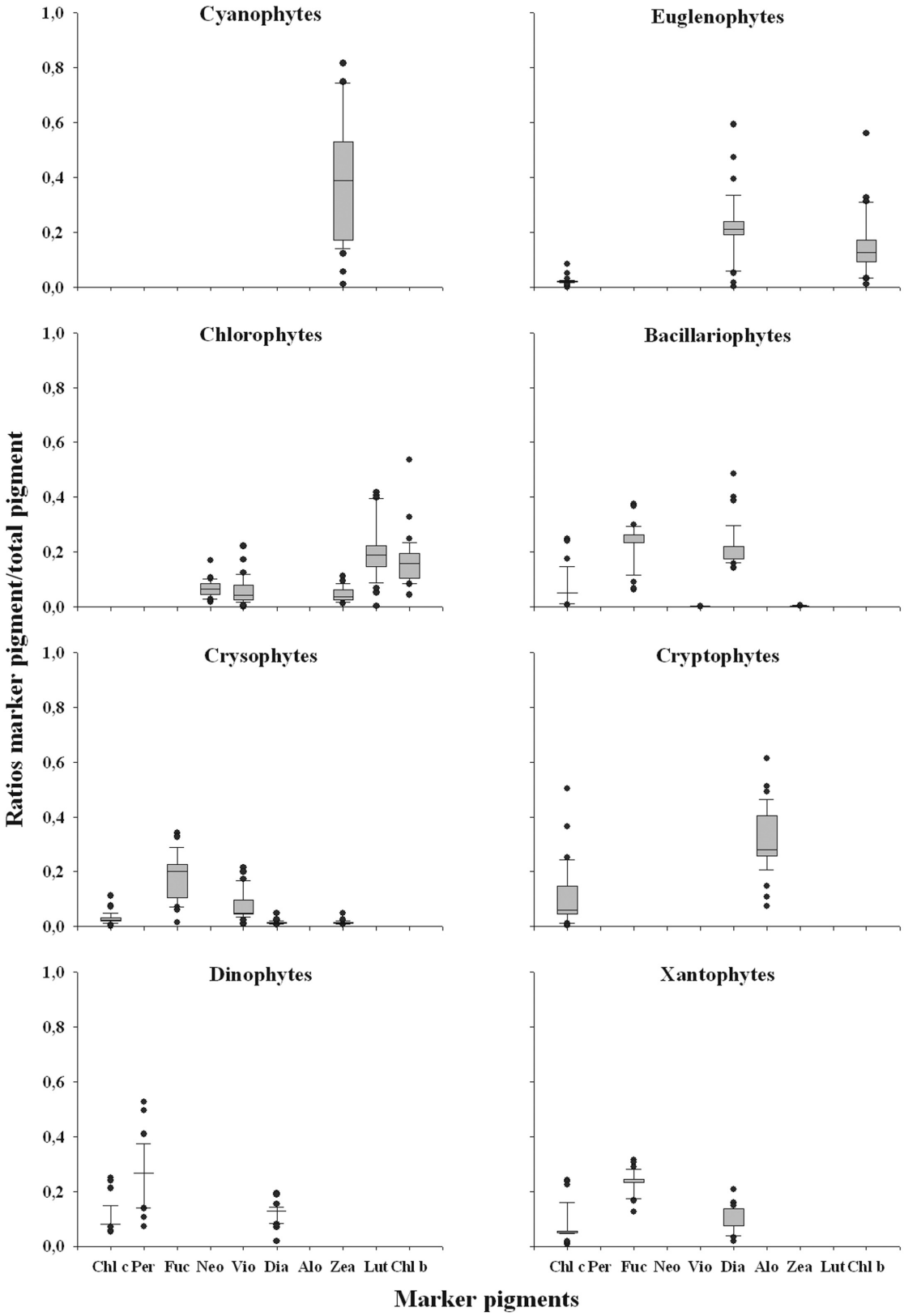

Fig. 4. Pigment ratios of the different phytoplankton groups considering all habitats, depths, and seasons. Pigment abbreviations as in Table 1.

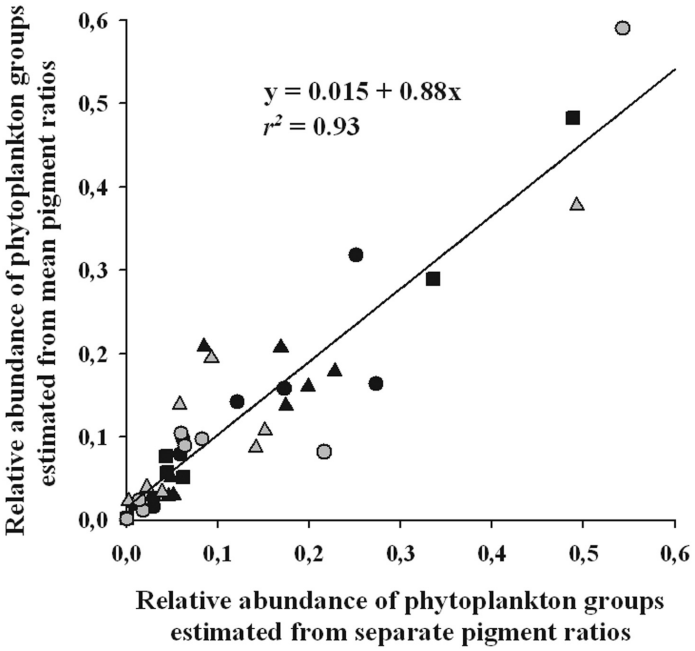

Fig. 5. Relationship between the mean relative contribution of each phytoplankton group in each lake, pond, and swamp considering all stations, depths, and seasons, estimated with the pigment ratios obtained separately for each habitat and season, and the average pigment ratio obtained considering all the pigment ratios obtained in each habitat and season. Maximum SD is 0.28. Black triangle (Amazonia), black circle (Andes), black square (the San Jorge basin), gray triangle (the Magdalena basin), gray circle (the Sinú basin).

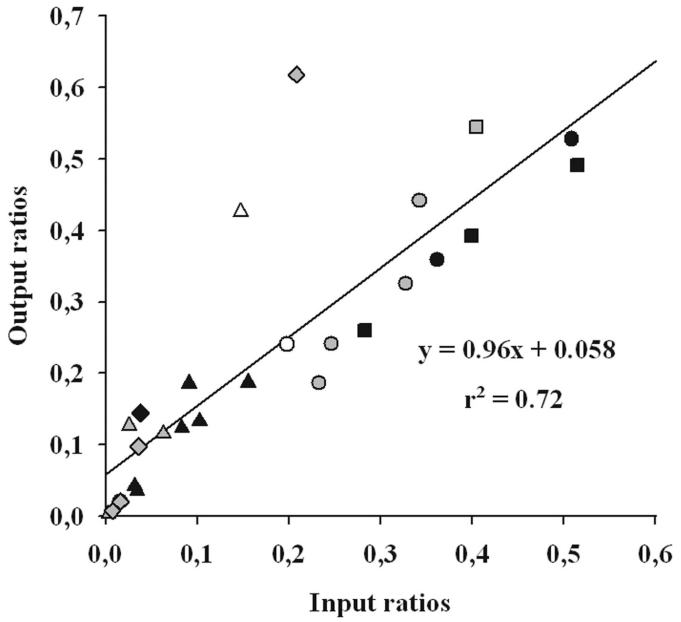

Fig. 6. Comparison between the input ratios (Table 1) and the final average output ratios of marker pigments (Table 3). Symbols: black triangle (chlorophyll *c*), black circle (peridinin), black square (fucoxanthin), black diamond (neoxanthin), gray triangle (violaxanthin), gray circle (diadinoxanthin), gray square (alloxanthin), gray diamond (zeaxanthin), open triangle (lutein), open circle (chlorophyll *b*).

As pigment profiles varied between regions (Figs. 3 and 4), there were also differences in the phytoplankton composition among habitats. In Caribbean lakes, the cyanophytes are dominant, while the chlorophytes are the second most important group. In terms of the contribution of all phytoplankton groups, there appears to be more balanced proportions in the Amazonian and Andean lakes than in the Caribbean regions. In the Andean lakes, the phytoplankton community is mainly dominated by the chlorophytes and to a lesser extent by the cyanophytes. In Amazonia, cryptophytes dominate the community, with cyanophytes, euglenophytes, and chlorophytes also well represented in this region.

Although our study covered a wide range of habitats with different limnological characteristics and phytoplankton communities, for most pigments, there were no important differences in pigment ratios among areas (Fig. 4). It is probably for this reason that the mean phytoplankton class abundances, estimated in each aquatic habitat using the average pigment ratio, were similar to those estimated using the individual pigment ratios for each habitat and season (Fig. 5).

The exceptions to this relatively low variation were the pigment zeaxanthin and also, to a lesser extent, alloxanthin (Fig. 4). Interestingly, Schlüter et al. (2006) also observed that different light and temperature treatments had a relatively insignificant impact on pigment ratios, with the exception of those for zeaxanthin/Chl *a* and alloxanthin/Chl *a*, which varied considerably.

The only differences between the ratio matrix that we suggest may be used with the CHEMTAX program in freshwater habitats (Table 3) and the one obtained under experimental conditions by Schlüter et al. (2006) are for lutein and zeaxanthin (Fig. 6). There are probably no differences between the two studies for alloxanthin, because the amount of cryptophytes was low in all areas. As mentioned above, the differences for zeaxanthin may be due to the variation of the zeaxanthin/Chl *a* ratio to light and temperature. However, there is no clear explanation for the differences in lutein, as this pigment did not show visible variations either in our study (Fig. 4) or in that of Schlüter et al. (2006).

As the average pigment ratio matrix predicted similar phytoplankton class abundances to those obtained using the pigment ratios for each separate habitat and season, and we did sample freshwater habitats with different limnological conditions, it can be concluded that the average pigment ratio matrix obtained in this study (Table 3) may be used to estimate phytoplankton class abundances with the CHEMTAX program in Neotropical freshwater habitats with different environmental conditions. However, it should be pointed out that both the zeaxanthin/Chl *a* and alloxanthin/Chl *a* ratios vary according to environment, and that zeaxanthin is present in cianophytes

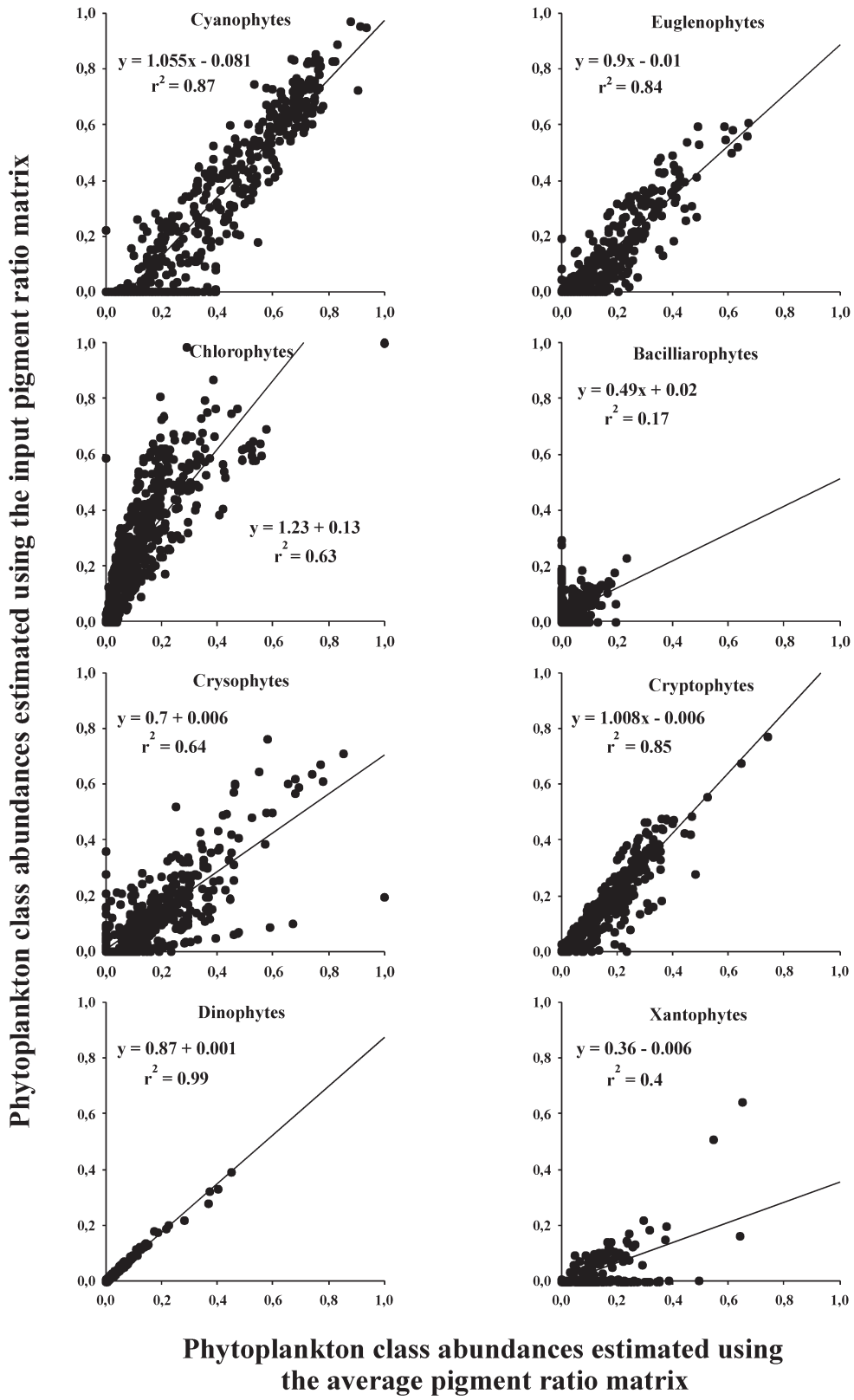

Fig. 7. Comparison between the phytoplankton class abundances predicted by CHEMTAX using the initial input pigment ratio employed in this work (Table 1) and the average output pigment ratio matrix generated (Table 3).

and alloxanthin is present in cryptophytes. Therefore, it is possible that there would be more errors when estimating these two phytoplankton groups using the CHEMTAX program than there would be for other phytoplankton groups.

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