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Patterns of major photosynthetic pigments in freshwater algae. 1. Cyanoprokaryota, Rhodophyta and Cryptophyta

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This study investigated major pigment patterns of 8 cyanoprokaryota, 2 rhodophytes and 2 cryptomonads isolated from freshwater ecosystems. Analysis was done by means of HPLC. The method, historically adapted to marine phytoplankton, was modified to accommodate limnic algae. Quantitative results obtained in this study can be used for phytoplankton quantification techniques based on pigment patterns. Compared to marine strains, the studied freshwater cyanoprokaryote strains reveal a more complex pigment pattern, including myxoxanthophyll, canthaxanthin and echinenone. Cryptophyta possess the two acetylenic class-specific marker compounds allo- and monadoxanthin, crocoxanthin was not detectable. Rhodophytes show a simple pigment pattern similar to marine species. Previous reports as to the existence of chlorophyll-d could not be confirmed (historical reports probably refer to an artefact of preparation). Besides methodological considerations, the phenomenon of complementary chromatic adaptation is discussed briefly.

Keywords : HPLC, freshwater algae, pigments, carotenoids, cyanoprokaryota, cyanobacteria, rhodophyta, cryptophyta.

Introduction

Besides ultrastructural and biochemical characteristics, thallus color, which is due to antenna pigments of the photosynthetic apparatus, is one of the major distinguishing criteria in the classification of algae, finding its reflection in the likes of cyanoprocaryota, rhodophyta, chlorophyta, xanthophyceae, chrysophyceae, or phaeophyceae. The majority of past studies on algal pigments, however, have dealt almost exclusively with abundant marine taxa of the coccolithophorids (prymnesiophyceae - haptophyta), bacillariophyceae (heterokontophyta), dinophyta or benthic macroalgae (eg. Gieskes & Kraay 1983, Mantoura & Llewellyn 1983, Wright & Shearer 1984, Gieskes & Kraay 1986a, b, Klein & Sournia 1987, Roy 1989, Veldhuis & Kraay 1990, Kohata & Watanabe 1991, Wright et al 1991, Tester et al. 1995, Jeffrey et al. 1997, Hiraoka et al. 1998, Meyer-Harms & Pollehne 1998, Schofield et al. 1998). As a consequence, detailed information about typical limnic taxa is still scarce, to date (e.g. Millie et al. 1990, Senge & Senger 1991, Wilhelm et al. 1991, Woitke et al. 1996, Nicklisch & Woitke 1999).

Some twenty years ago, pigment analysis relied on paper- and thin layer chromatography, (e.g. Hager & Stransky 1970a, b, Vesk & Jeffrey 1977, Hallegraeff 1981, Hallegraeff & Jeffrey 1985, Suzuki & Fujita 1986, Jeffrey & Wright 1987), whereas high performance liquid chromatography (HPLC) has become the major tool during the past 15 years. Its high resolution performance, accuracy and reproducibility of results, as well as short analyzing times (approx. 30 min.) and the need of only small sample volumes (in the µl-range) all speak in favour of this method. Shortly after becoming available, reversed-phase (RP) colums with excellent separating properties were employed for pigment analysis. Due to their apolarity, they proofed to be ideally suited for analysis of fatty substances. Another advantage of RP-HPLC is the inert column environment, as stationary phase silanol groups, to a great extent, are bound by silanisation with octyl- and octadecylsilanes. Chlorophylls (chl-s) and carotenoids thus

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remain stable during separation and detection, there is no destruction of reactive conjugated double bindings by acid, oxygen or light. The studies by Mantoura & Llewellyn (1983), Wright & Shearer (1984) and Wright et al. (1991) are milestones in pigment analysis (review in Jeffrey et al. 1997).

In addition to the lipophilic pigments investigated in this study, cyanoprocaryota, rhodo- and cryptophyta also contain hydrophilic phycobiliproteins (not entered in this study). In the cyanoprocaryota and rhodophytes these water-soluble pigments are concentrated in phycobilisomes, acting as highly efficient extrinsic light harvesting complexes to the photosystem II (Glazer 1982, 1984, Cogdell 1988, Siefermann-Harms 1985, Hiller et al. 1991). Among the three groups, intrinsic light harvesting systems, so far, have been studied in the cryptophyta only, where they were found to consist of chls-a/c_2 and alloxanthin.

In the last decade, class-specific pigment markers have been established for phytoplankton quantification to an increasing degree (eg. Wilhelm et al. 1991, Descy & MÈtens 1996, Mackey et al. 1996, Roy et al. 1996, Jeffrey et al. 1997, Latasa et al. 1997, Goericke & Montoya 1998, Schmid et al. 1998, Steinman et al. 1998). This method could be employed with high prospects of success particularly for monitoring lake systems. Besides direct relation of specific carotenoids to algal biomass, pigment/chl-a ratios on basis of total chl-a are commonly used for estimating phytoplankton composition. From a physiological point of view the latter option sounds more promising because acclimatisation to different light and nutrient supply is relativized by division by chl-a. However, some studies have shown that a direct conversion from carotenoids to algal biomass might be more accurate (Strom & Welschmeyer 1991, Descy & Métens 1996, Schmid et al. 1998). New attempts on algal quantification have also been made using multivariate statistics like multiple linear regression (eg. Woitke et al. 1996) or factor analysis (Mackey et al. 1996, Wright et al. 1996). For these approaches, basic data sets of carotenoid contents are needed, which are obtained from pure cultures. Some quantitative pigment data are contributed in the present study.

As previously mentioned, to date, most studies of algal pigments have concentrated on marine taxa. In contrast, this study focused on pigment patterns of common freshwater species, in an attempt to help find an answer to the question of whether, or not, major pigment differences exist between limnic and marine species. This study will also provide a basis for systematic, physiological and ecological investigations.

Materials and methods

Organisms (see Table 1) were obtained from the culture collection of algae at the Institute of Ecology and Conservation Biology at the University of Vienna (Kusel-Fetzmann & Schagerl 1992). The unialgal, non-axenic batch-clones were cultured at 20 °C. Growth was established using a light:dark regime of 14 h white light (30 µmol photons m^{-2} s^{-1}) and 10 h darkness. Several clones were grown in parallel cultures. Rhodophytes were procured directly from the Fischa river or from aquaria after careful microscopical examination which excluded attached algae in higher numbers. To test the variability of carotenoids per unit chl-a, data sets of cyanoprokaryotic continuous cultures with varying nutrient supply (original Chu_{10} according to Kusel-Fetzmann & Schagerl 1992 and modifications like 0.5 N, 5 N + 0.5 P and 10 N on a weight basis) at different light conditions were added (150 µE m^{-2} s^{-1} and 60 µE m^{-2} s^{-1}, respectively). Details are given in Donabaum (1992).

Prior to extraction, algal suspensions were harvested on Whatman GF/C filters and stored at -30 °C at least overnight for breaking the cell walls. Extraction was carried out by using 90 % cold acetone. Filters were immersed in 5 ml 90 % acetone and ground for 30 seconds using a Potter-homogenizer (Elfechiem). The suspensions were transferred to centrifuge tubes, and the pigments stored at 2 °C for 12 hours to achieve optimal extraction (Gätz 1990, Jeffrey et al. 1997). After centrifugation for 10 min at 3000 rpm (Sigma-centrifuge) the supernatant was injectetd into the HPLC system. Extraction took place in dim light to minimize formation of artificial cis-trans-isomeres.

The HPLC-system (Merck-Hitachi) employed consisted of an autosampler (AS-4000), a gradient-pump (L-6200) and an UV-VIS-detector (L-4250). 10 minutes prior to injection, the extract was diluted with ionpairing reagent TBAA = 3 + 1 (volume/volume; Table 2). Chromatography was carried out on a RP-column (Merck Superspher rp-18 250/4 ; precolumn : Merck Lichrospher rp-18 endcapped). Solvents used were HPLC grade (Table 2). Pigment extracts were separated by use of a ternary low pressure gradient program (Table 2).

Peaks were detected at 440 nm and identified by cochromatography with authentic standards (DHI Bioproducts, Denmark), their specific absorption maxima, and by comparison with values from the literature (Foppen 1971 ; Mantoura and Llewellyn 1983, Wright and Shearer 1984, Wright et al. 1991, Jeffrey et al. 1997). D-6000 HPLC-manager software (Merck) was

employed for analyses, nomenclature of pigments followed trivial names. Peak quantification of major pigments with calibration curves (DHI Bioproducts, Denmark) : chl's-a and -c, alloxanthin, echinenone, myxoxanthophyll, α- and ß-carotene. For lutein, zea- and monadoxanthin the calibration curve of ß-carotene was used and for canthaxanthin that of echinenone, respectively.

Linear regression analyses and analyses of variance (ANOVA) were performed by SPSS 9.0.1. (SPSS inc.).

Results

Cyanoprokaryota

Detected pigments : chl-a and derivatives, «cyano-peaks» I and II, a compound similar to myxoxanthophyll, myxoxanthophyll, nostoxanthin, caloxanthin, zeaxanthin, canthaxanthin, echinenone, ß-carotene, cis-ß-carotene.

Besides chl-a, all investigated cyanoprocaryotic strains revealed larger quantities of myxoxanthophyll, zeaxanthin, echinenone, and ß-carotene (Figs. 1, 2,

Table 1. Cultures used in this study (media composition according to Kusel-Fetzmann & Schagerl 1992).

Species	Strain	Isolated from	Medium
Cyanoprokaryota			
Anabaenopsis elekini Mill.	01 027	Lake Neusiedler See	F
Aphanizomenon flexuosum Kom. et Kovacik	01 033	backwater in Vienna	FE
Chroococcus minutus (Kütz.) Näg.	01 017	Lake Neusiedler See	F
Cylindrospermum sp.	01 016	Lake Neusiedler See	DN
Microcystis aeruginosa Kütz.	01 001	Lake Neusiedler See	F
Microcystis flos-aquae (Wittr.) Kirchn.	01 004	Lake Neusiedler See	F
Microcystis wesenbergii Komarek	01 024	Lake Neusiedler See	F
Nostoc sp.	01 010	Lake Neusiedler See	D
Rhodophyta			
Audouinella sp.	-	fresh material, aquarium	-
Batrachospermum moniliforme (Roth.) Sirodot	-	fresh material, river Fischa	-
Cryptophyta			
Cryptomonas sp.	09 001	Museumsteich near Neusiedl	MK
Cryptomonas rostratiformis Skuja	09 004	backwater in Vienna	MK

Table 2. Ternary low pressure gradient program applied in this investigation. Solvent A : 100 % Acetonitrile (HPLC-grade, MERCK) ; Solvent B : distilled water : TBAA = 4 + 1 (vol/vol) ; Solvent C : 100 % acetone : 100 % acetonitrile = 2 + 1 (vol/vol ; HPLC-grade, MERCK). TBAA preparation : dissolve 77 g ammonia-acetate and 30 ml 0.5 M tetrabutylammonia-hydroxyde in 1000 ml distilled water.

Time (min)	% A	% B	%C	Flow (ml min^{-1})
0	50	50	0	1
5	70	30	0	1.5
18	20	10	70	1.5
23	0	0	100	1.5
33	0	0	100	1
35	50	50	0	1

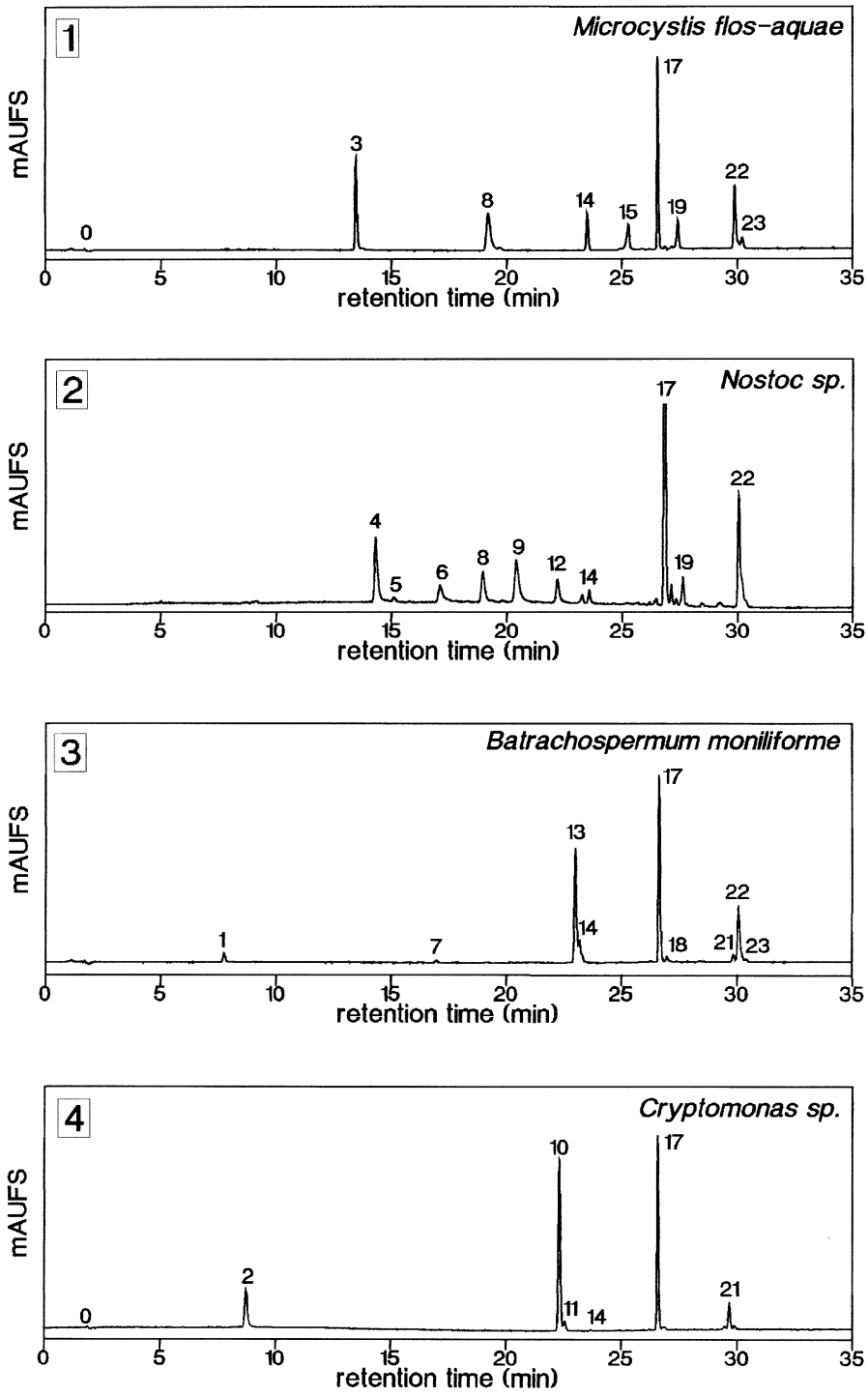

Fig. 1. 1. Chromatograms of the cyanobacteria *Microcystis flos-aquae* ; 2. *Nostoc* sp.; 3. The rhodophyte *Batrachospermum moniliforme* ; 4. The cryptophyte *Cryptomonas* sp.. Peak numbers according to table 3.

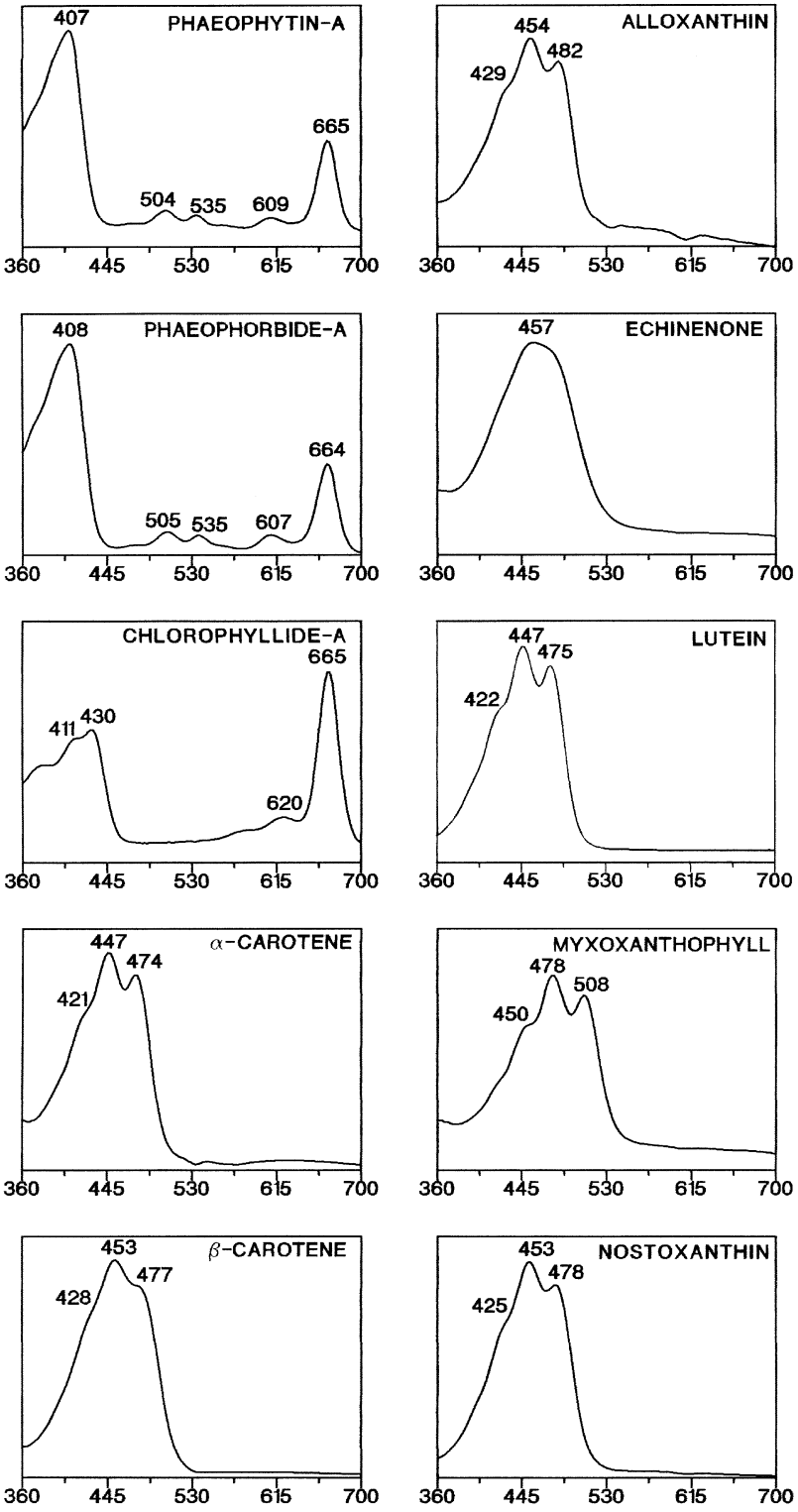

Fig. 2. Absorption spectra of chl-a degradation products and major carotenoids.

Tables 4, 5). ß-carotene showed largest amounts per chl-a (21 %), followed by myxoxanthophyll (17 %). Highest relation at different light and nutrient conditions were calculated between ß-carotene and chl-a ($r^2 = 0.895$). In all cultures, canthaxanthin eluted prior to chl-a, however, concentrations varied considerably ($r^2 = 0.459$, n = 66, Table 5). Nostoxanthin and caloxanthin were found exclusively in *Nostoc* sp. (Fig. 1), whilst other strains of the Nostocales apparently lack these pigments (Table 4). Myxoxanthophyll-like compounds were detected in *Nostoc* sp. and *Aphanizomenon flexuosum*, absorption maxima were similar to that of myxoxanthophyll (Table 3). In some of the blue-greens, two unidentified peaks were detected (numbers 3 and 4, Table 3 ; *Anabaenopsis elenkinii, Aphanizomenon flexuosum, Chroococcus minutus, Microcystis flos-aquae, Nostoc* sp.). These pigments exhibit typical carotenoid absorption spectra (maxima at 447nm and 466nm), early elution hints at hydrophilic substituents.

Rhodophyta

Detected pigments were chl-a and derivatives, (neoxanthin), lutein, zeaxanthin, α-carotene, ß-carote-

Table 3. Retention times (Rt) and absorption maxima of pigments in the eluent; shoulders in brackets.

Peak	Pigment	Rt	Absorption maxima
0	solvent front	1,61	
1	chlorophyllide-*a*	8,01	(411), 430, 620, 665
2	chls *c1+c2*	8,31	441, 581, 631
3	„cyano-peak I“	13,60	447, 466
4	„cyano-peak II“	14,07	464, (481)
5	phaeophorbide-*a*	15,10	408, 505, 535, 607, 664
6	myxoxanthophyll-like	16,78	448, 482, 506
7	neoxanthin	16,86	(411), 437, 466
8	myxoxanthophyll	18,46	(450), 478, 508
9	nostoxanthin	20,22	(425), 453, 478
10	alloxanthin	21,57	(429), 454, 482
11	monadoxanthin	21,68	(428), 448, 480
12	caloxanthin	21,92	(425), 453, 478
13	lutein	22,98	(422), 447, 475
14	zeaxanthin	23,30	(423), 454, 478
15	canthaxanthin	24,48	456
16	chl-*a* allomer	26,28	(414), 428, 615, 662
17	chl-*a*	26,67	(408), 428, 617, 663
18	chl-*a* epimer	26,96	(408), 429, 617, 662
19	echinenone	27,37	457
20	phaeophytin-*a*	28,83	407, 504, 535, 609, 665
21	α-carotene	29,86	421, 447, 474
22	β-carotene	30,08	428, 453, 477
23	*cis*-β-carotene	30,38	448, 475

Table 4. Major pigments of strains analyzed in this study.

		Chlorophyll c_2	Neoxanthin	Myxoxanthophyll	Nostoxanthin	Alloxanthin	Monadoxanthin	Caloxanthin	Lutein	Zeaxanthin	Canthaxanthin	Chlorophyll-a	Echinenone	α- Carotene	ß-Carotene
01 033	*Aphanizomenon flexuosum*			●							●	●	●		●
01 027	*Anabaenopsis elekini*			●						●	●	●	●		●
01 017	*Chroococcus minutus*			●						●	●	●	●		●
01 016	*Cylindrospermum* sp.			●						●	●	●	●		●
01 001	*Microcystis aeruginosa*			●						●	●	●	●		●
01 004	*Microcystis flos-aquae*			●						●	●	●	●		●
01 024	*Microcystis wesenbergii*			●						●	●	●	●		●
01 010	*Nostoc* sp.			●	●			●		●	●	●	●		●
09 001	*Cryptomonas* sp.	●				●	●			●		●		●	●
09 004	*Cryptomonas rostratiformis*	●				●	●					●		●	●
Fresh	*Audouinella* sp.									●		●		●	●
Fresh	*Batrachospermum moniliforme*		●						●	●		●		●	●

Table 5. Major pigments on basis of chl-a (% pig ; weight/weight) obtained from linear regression and ANOVA, CD...coefficient of determination, SE...standard error, df...degrees of freedom, sign...level of significance.

	% pig	CD	SE	df	F-value	sign
Cyanoprokaryota						
Myxoxanthophyll	16.7	0.745	0.012	66	190	< 0.001
Zeaxanthin	8.5	0.688	0.007	66	143	< 0.001
Canthaxanthin	2.6	0.459	0.003	66	55	< 0.001
Echinenone	6.2	0.792	0.004	66	248	< 0.001
ß-carotene	20.8	0.895	0.009	66	553	< 0.001
Cryptophyta						
Chl c_2	7.3	0.980	0.004	8	346	< 0.001
Alloxanthin	25.7	0.869	0.038	8	47	< 0.001
Monadoxanthin	0.8	0.848	0.001	8	39	< 0.001
α-carotene	5.3	0.953	0.004	8	142	< 0.001
ß-carotene	1.1	0.912	0.001	8	73	< 0.001
Rhodophyta						
Lutein	22.9					
Zeaxanthin	2.5					
α-carotene	1.8					
ß-carotene	11.3					

ne, cis-ß-carotene. Analyzed strains contained chl-a only, chl-d was absent (Fig. 1). Apart from small quantities of neoxanthin, the xanthophylls lutein and zeaxanthin, as well as α, ß- and cis-ß-carotene were found. Neoxanthin is probably an impurity of *Batrachospermum moniliforme*, which was collected from the field (Bjornland & Aguilar-Martinez 1976, Liaaen-Jensen 1978).

Cryptophyta

Detected pigments were chls-c^2, -a, alloxanthin, monadoxanthin, zeaxanthin, α- and ß-carotene. In pigment extracts, chls-a and - c_2 were identified. Alloxanthin was the most abundant carotenoid (around 26 % per chl-a), but cultures also contained small quantities of monadoxanthin. Zeaxanthin, present in many algal groups, was detected only in traces (Fig. 1) ; α-carotene dominated over ß-carotene (Table 5).

Discussion

Accommodation of the method

Pigment analysis starts with sample concentration (Otsuki et al. 1987) and selection of an appropriate extracting agent (for important criteria see Jeffrey et al. 1997). In this study acetone, a common and efficient extraction solvent, was employed (eg. Mantoura & Llewellyn 1983, Shioi et al. 1984, Bidigare et al. 1985, Kohata & Watanabe 1988, 1989, Kohata & Watanabe 1991, Wright et al. 1991, Descy & Métens 1996, Latasa et al. 1997, Nicklisch & Woitke 1999). Acetone is rated as being non-toxical, whereas highly efficient extracting agents, such as dimethyl sulfoxide or dimethyl formamide, are classified as being carcinogenic. Occasionally, alcohols like 2-propanol (Scholz & Ballschmiter 1981), methanol (Kraay et al. 1992, Wright & Shearer 1984), ethanol (Nusch 1980, Sartory 1985), or mixtures (Abaychi & Riley 1979) are prefered to acetone. However, the potential extraction efficiency of individual solvents remains to be controversial. Comparing hot ethanol, cold methanol, and acetone, Jacobsen & Rai (1990) observed inefficient extraction by acetone. Zapata & Garrido (1991) found no differences in extraction efficiency between acetone and methanol. Jeffrey et al. (1997) recommended sonication in dimethyl formamide, but they did not favor this procedure for routine work because of the dimethyl formamide`s toxicity.

Another factor to be considered, are chemical reactions that might take place between the extracting agent and the sample compounds. Alcohols enhance the formation of chl derivatives (Hynninen & Assandri 1973, Otsuki et al. 1987). Thus, methanol and ethanol, apparently, are involved in allomere formation.

The gradient program employed in this investigation had to aim at a high resolution, moderate use of solvents and short analyzing times. As in the case of extracting agents, solvents should stay as inert as possible considering the sample. For preparation of the mobile phase, chiefly methanol, acetonitrile, acetone, or ethylacetate, all diluted with distilled water, are in use. This study finally opted for a acetonitrile/acetone/water mixture, after pilot tests had revealed numerous decomposition products caused by the commonly used methanol gradient program (compare Scholz & Ballschmiter 1981, Shioi et al. 1983). Also, resolution of the acetonitrile gradient is significantly higher, due to the lower viscosity of this mixture (Unger 1989, Kohata & Watanabe 1991, Meyer 1999).

Cyanoprokaryota

The monocyclic carotenoid-glycoside myxoxanthophyll, typical of many freshwater cyanoprokaryota, apparently is a class specific pigment, since it has not been detected in eucaryotic algae to date (Weber & Wettern 1980, Rowan 1989, Young & Britton 1993, Jeffrey et al. 1997, Lee 1999). Another particularity of freshwater cyanobacteria are the primary cetocarotenoids canthaxanthine and echinenone (Healey 1968, Stransky & Hager 1970, Hertzberg et al. 1971, Fiksdahl et al. 1983). The same cetocarotenoids have also been encountered in seasoned cultures of some chlorellales, however, exclusively functioning as secondary carotenoids concentrated either in droplets outside of the plastids, or within the cell wall in combination with sporopollenine (Britton 1988). During xanthophyll synthesis, cyanobacteria are not capable of ε-ring synthesis (Goodwin & Britton 1988), thus lacking α-carotene and lutein, whereas zeaxanthin, α-carotene and cis-ß-carotene are always present in this group. There are no light induced xanthophyll transformations in cyanoprocaryota (Stransky & Hager 1970). Due to the lack of a violaxanthin cycle (synthesis of the epoxycarotenoids viola- and antheraxanthin has not yet been observed) cyanoprocaryota adjust quite slowly to intense illumination. Supersaturating light energy is probably dissipated by zeaxanthin, which has also been found in higher plants (Demmig-Adams 1989, 1990).

In *Nostoc* sp. and *Aphanizomenon flexuosum* a peak with absorption maxima similar to myxoxanthophyll was observed which is probably an isomere formation.

In some strains two additional unidentifiable compounds were detected. These pigments exhibit typical carotenoid absorption spectra (maxima at 447nm and 466nm), early elution hints at hydrophilic substituents. Absorption maxima and the short retention time exclude the acyclic car otenoid oscillaxanthin as an explanation. The compounds possibly originated from the cell walls (Resch & Gibson 1983) ; a definite identification would call for structural analysis.

Compared to marine organisms, all studied strains revealed much more complex pigment patterns. However, an exception that proves the rule is *Trichodesmium* showing small amounts of myxoxanthophyll (Carpenter et al. 1993, Mackey et al. 1996). Marine picoplanktonics (*Synechococcus* spp.), in particular, seem to exhibit a simple pattern containing zeaxanthin, ß-carotene and chl-a (Guillard et al. 1985, Kana et. al. 1988, Mackey et al. 1996, Jeffrey et al. 1997), demonstrating why zeaxanthin acts as a chemotaxonomical marker in marine ecosystems (eg. Roy et al 1996, Latasa et al. 1997, Goericke 1998). For freshwater environments, where zeaxanthin - involved in the violaxanthin-cyclus - is present in a number of classes, other tracers such as echinenone or myxoxanthophyll should be preferred. By dissipating surplus energy, zeaxanthin is an essential protective part of the photosynthetic apparatus. Depending on light intensity, varying amounts of zeaxanthin are to be expected, which is another reason why zeaxanthin is not suited as a marker pigment (Wilhelm et al. 1991). In *Planktothrix agardhii* (Gom.) Anagn. et Kom., Millie et al. (1990) showed a an increase in the relative abundances of myxoxanthophyll and zeaxanthin at higher light intensities, whilst chl-a, echinenone and ß-carotene decreased. The same pattern of acclimatisation was observed by Rücker et al. (1995), who varied growth-limiting factors in three cyanobacterian species (*Planktothrix agardhii, Limnothrix redekei* (Van Goor) Meffert, *Aphanizomenon gracile* Lemm.).

The following two representative examples of *Synechococcus* and *Limnothrix* are pointing out that there still exists the basic necessity for detailed studies in pigment patterns and quantification, which may give some hints of taxonomic classification, too. By comparison of carotenoid pattern within *Synechococcus*, one will find only zeaxanthin and ß-carotene (Kana et al. 1988, Mackey et al. 1996) or additionally echinenone (Woitke et al. 1996). In the freshwater species *Synechococcus elongatus* (Näg). Näg. three more carotenoids, namely calo-, nostoxanthin and myxoxanthophyll were observed by Stransky & Hager (1970). Guillard et al. (1985) analyzed 4 different marine clones of *Synechococcus*, each of them holding a different carotenoid pattern. In *L. redeckei*, echinenone generally was absent in investigations made by Rücker et al. (1995), but was verified by Nicklisch & Woitke (1999).

Because zeaxanthin is used for an estimation of blue-green abundances, special attention has been paid upon this carotenoid. Interestingly, on a basis of biovolume this carotenoid seems to be quite stable independend of different light and nutrient conditions (Kana et al. 1988, Nicklisch & Woitke 1999), which entices to a direct conversion (eg. Woitke et al. 1996, Schmid et al. 1998). However, one should question crucially, if a direct conversion of physiologically highly influenced components like pigments into biovolume is justified for the specific problem. In the author's opinion, for natural algal assemblages an estimation on basis of chl-a should be preferred. This pre-condition cuts down possible marker pigments to carotenoids which co-vary with chl-a.

Rhodophyta

In the rhodophyta, only chl-a was detected ; chl-d, differing from chl-a by an oxidized 3-vinyl group, was not found. As most reports of chl-d in the rhodophyta were published without references (Ettl 1980, Kohl & Nicklisch 1988, Häder 1999, Lee 1999) this might actually hint at a historical, artificial preparatory phenomenon, nowadays unobserved. Isolated reports of chl-d in the rhodophyta are by Manning & Strain (1943), Smith & Benitez (1955), French (1960) and O'hEocha (1971). Sagromsky (1964) observed within three marine taxa the formation of a chl-d-like compound caused by photooxydation. Pigment analyses of *Batrachospermum* sp. (Stransky & Hager 1970), *Porphyridium aerugineum* (Stransky & Hager 1970) and *P. cruentum* (Brody & Emerson 1959, Stransky & Hager 1970, Wright et al 1991, Jeffrey et al. 1997) did not yield chl-d.

Red algae are characterized by a simple carotenoid pattern (O'Heocha 1971, Bjornland & Aguilar-Martinez 1976, Palermo et al 1991, Young & Britton 1993). The dominating of zeaxanthin over lutein, and of ß-carotene over ß-carotene commonly observed in marine rhodophyta (Goodwin & Britton 1988) was not found in *Batrachospermum moniliforme* (Table 5).

Cryptophyta

Chl-a and chl-c_2 were isolated from the pigment extracts. In this study, around 7 % chl-c_2 per chl-a were

found, which is in good agreement to other investigations (Mackey et al. 1996 : 8-17 % ; Goericke & Montoya 1998 : ca. 4 %). Besides chl-c_2 (Jeffrey 1976, Wright et al 1991), faint traces of chl-c_1 were detected, too *(Rhodomonas* sp.; Kraay et al. 1992).

In the investigated cryptomonads the class-specific xanthophylls alloxanthin and monadoxanthin were discovered, with alloxanthin being the main constituent of the carotenoid fraction (around 26 % per chl-a, Table 5). This result highly corresponds to amounts analyzed from the freshwater species *Cryptomonas ovata* Ehrenb. (25 %; Wilhelm et al. 1991, McManus 1995). For marine species, Goericke & Montoya (1998) found 6 - 13 % in *Chroomonas salina* (Wislouch) Butcher), Mackey et al. (1996) observed amounts between 4 and 23 %. The acetylenic xanthophyll crocoxanthin was not found. According to Goodwin & Britton (1988) also in this study only acetylenic carotenoids and zeaxanthin were detected, allenic carotenoids are missing. Zeaxanthin, so far, was detected in traces in *Cryptomonas ovata* only (Pennington et al 1985). Another particularity is the dominance of α-carotene over ß-carotene (this study, Hager & Stransky 1970b, Goodwin & Britton 1988).

Complementary chromatic adaptation

Although not studied here, the possible role of phycobiliproteins and/or carotenoids in complementary chromatic adaptation is briefly discussed, since pigments involved in this process are only of limited interest for phytoplankton quantification. At the end of the 19th century, Engelmann (1883a, b) postulated that the vertical distribution of marine macroalgae is defined by the different depths to which the individual spectral colors of the sunlight penetrate the ocean. Engelmann based this statement on his perception that assimilation is strongest when algae are exposed to light of a spectral color complementary to their own color. His student Gaidukov (1903) deliberately varied the colors of *Oscillatoria*, cultivating greenish filaments under red illumination and reddish ones by applying green light. Both, Engelmann (1883a, b, 1884) and Gaidukov (1903) established the term «complementary chromatic adaptation». In contrast to Engelmann, Oltmanns (1892), based on his own experiments, postulated, that depth distribution of marine algae is entirely controlled by light intensity. Harder (1923) united both theories, in a way, concluding from his experiments on the cyanoprokaryote *Phormidium foveolarum*, that both light intensity and spectral quality influence assimilation.

Recent studies of cyanoprokaryota confirm the ability of the blue-greens to adapt to different light qualities by means of phycobiliproteins adhering as phycobilisomes to the thylacoids (Bogorad 1975, Tandeau de Marsac 1977 in Kohl & Nicklisch 1988, van Liere & Walsby 1982, Lönneborg et al. 1985, Wood 1985 ; Ojala 1993).

To some extent, chromatic adaptation is also an adaptation to light intensity. Red and white light act as high intensities, whilst green light is interpreted as low intensity illumination (Wyman & Fay 1986, Kohl & Nicklisch 1988). Jones & Myers (1965) reported distinct reduction of the cellular chl-a content in *Synechococcus nidulans* (Pringsh.) Kom. (formerly *Anacystis*) when cultured under green illumination. Since levels of phycocyanin and of carotenoids remained almost unchanged, total pigment proportions shifted in favour of the phycobilines.

Complementary chromatic adaptation can also be expected within the rhodophyta as these organisms, contain phycobilisomes, too. In *Porphyridium cruentum* (Ag.) Näg., chromatic adaptation seems to be triggered by light intensity. At low intensities, cells adapt chromatically, whereas at high light conditions they react differently, protecting the photosynthetic apparatus by evenly supplying both photosystems (Brody & Emerson 1959). In marine macroalgae chromatic adaptation has not yet been observed (Dring 1981, Ramus & van der Meer 1983).

Cryptophyta, without phycobiliproteins concentrated in phycobilisomes, exhibit only weak chromatic adaptation (Vesk & Jeffrey 1977; Ojala 1993).

Complementary chromatic adaptation, as established by Engelmann (1883a,b, 1884) and Gaidukov (1903), makes it necessary for algae to synthesize pigments of a color complementary to the color of the prevailing light. Blue illumination thus would have to result in an increased synthesis of carotenoids (adapted to absorb the blue range of the spectrum). The results specified above indicate an involvement of water-soluble phycobilins concentrated in phycobilisomes thus not affecting carotenoid/chl-a ratios. Since in aquatic ecosystems the light quality changes with depth, this enhances the usefulness of carotenoid/chl-a ratios for algal quantification dramatically.

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