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GROWTH AND ECOLOGICAL ROLE OF THE SELECTED CLASSES OF MARINE PHYTOPLANKTON

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ABSTRACT

Growth and some biochemical characteristics were followed in six monocultures from four classes of marine phytoplankton. The growth and size parameters, as well as pigment composition of selected species were examined. The approach of chemotaxonomic pigment biomarkers was used to interpret the pigment fingerprints of natural phytoplankton from the Gulf of Trieste. The presence and concentrations of pigments analysed by HPLC method were supported by microscopic observations of the same samples.

Key words: phytoplankton, monocultures, growth, biomarkers, HPLC, Gulf of Trieste
Ključne besede: fitoplankton, monokulture, rast, biomarkerji, HPLC, Tržaški zaliv

INTRODUCTION

Phytoplankton monocultures have been largely used to determine the species/group characteristics and dynamics. Controlled conditions are usually the only possible way to study the morphology, ecophysiology, biochemical composition and therefore the taxonomic position of algae. For example, a large number of small or rare organisms, as well as toxic or potentially toxic species have been identified and their characteristics have become known only in monocultures.

The past and current efforts to identify phytoplankton from natural waters rely largely on microscopic evaluation. This requires a high level of taxonomic skill, but it can be also significantly variable among researchers, and time consuming work. However, by using a light microscope it is not always possible to identify an organism not only at the species but also at a higher, class level. This is the case of the non-taxonomic phytoplankton group, commonly denominated as a group of microflagellates. Microflagellates are small (approx. 10 µm) naked flagellates, which belong to different algal classes. Because of their small size, fragile structure and use of aggressive fixatives (for example formaldehyde) for storage and counting procedures, microflagellates can be often overlooked, wrongly identified and their number underestimated.

Besides classical microscopic techniques, either optical or electronic, new methods using different biomarkers as a tool to assess phytoplankton diversity have emerged lately. Among several biomarkers, photosynthetic pigments have proved to be effective for providing information about the phytoplankton chemotaxonomic composition, physiological status, primary production and trophic state (Millie *et al.*, 1993). High-performance liquid chromatography (HPLC) was successfully applied to the determination of chlorophylls *(a, b, c, d, e)*, phycobilins of cyanobacteria and the red al-

PIGMENTS	PHYLOGENETIC GROUPS
Chlorophylls	
a	all groups (the only chlorophyll in Cyanophyta and Eustigmatophyta)
b	Chlorophyta, Euglenophyta, Prasinophyta, ProChlorophyta
c_1	Bacillariophyta, Chrysophyta, Prymnesiophyta, Raphidophyta, Xanthophyta
c_2	Bacillariophyta, Cryptophyta, Dynophyta, Prymnesiophyta, Raphidophyta, Xanthophyta
c_3	Bacillariophyta, Chrysophyta, Dynophyta, Prymnesiophyta
8-desethyl, 8-vinyl *a*	ProChlorophyta
8-desethyl, 8-vinyl *b*	ProChlorophyta
Mg 2,4-divinilpheoporphyrin a_5 monomethyl ester	Prasinophyta
Carotenoids	
alloxanthin	Cryptophyta
19' butanoyloxyfucoxanthin	Dynophyta, Prymnesiophyta, Raphidophyta
crokoxanthin	Cryptophyta
dinoxanthin	Dynophyta, Prymnesiophyta
echinenone	Cyanophyta, ProChlorophyta
fucoxanthin	Bacillariophyta, Chrysophyta, Dynophyta, Prymnesiophyta
19'-hexanoyloxyfucoxanthin	Dynophyta, Prymnesiophyta
lutein	Chlorophyta, Prasinophyta
monadoxanthin	Cryptophyta
myxoxanthophyll	Cyanophyta
oscillaxanthin	Cyanophyta
peridinin	Dynophyta
prasinoxanthin	Prasinophyta
pyrrhoxanthin	Dynophyta
siphonaxanthin	Chlorophyta, Euglenophyta, Prasinophyta
vaucheriaxanthin	Eustigmatophyta, Xanthophyta
zeaxanthin	Cryptophyta, Cyanophyta, Prasinophyta, Prochlorophyta
Phycobilins	
allophycocyanin	Cyanophyta
phycocyanin	Cyanophyta, Cryptophyta
phycoerythrin	Cyanophyta, Cryptophyta, Rhodophyta

Table 1: Photosynthetic pigments present in phylogenetic algal groups (after Millie et al., 1993).
Tabela 1: Zastopanost posameznih fotosintetskih pigmentov pri filogenetskih skupinah alg (povzeto po Millie et al., 1993).

gae, and a wide range of the oxidised carotenoids - the xanthophylls. To date, more than 400 compounds are known, and many of them are highly specific only for one taxonomic group (Table 1).

HPLC determination of biomarker pigments is a highly sensitive and accurate method which sometimes enables the identification and detection of groups that have been overlooked by the standard microscopic method (Gieskes & Kraay, 1983). The greater part of the pigment composition studies have been done in the oceanic waters (Wright & Jeffrey, 1987; Bidigare *et al.*, 1990; Buma *et al.*, 1990; Barlow *et al.*, 1993), while coastal areas and estuaries have received less attention (Denant *et al.*, 1991; Malej *et al.*, 1995; Terzić, 1996).

Besides the taxonomic identification it is essential, from the ecological point of view, to characterise the flow of organic matter through the pelagic ecosystem (Verity *et al.*, 1992). The basic parameter of living organic matter is the biomass of organisms in terms of organic carbon. Estimation of organic or cell carbon (C) from the chlorophyll *a* (Chl *a*) concentration is commonly used. However, the C:Chl *a* ratio can vary greatly among different phytoplankton groups and seasons (Booth *et al.*, 1988; Sieracki *et al.*, 1992), therefore the estimates of carbon are not very accurate. For example, the C:Chl *a* ratio of 50 (Strickland & Parsons, 1972) is frequently used. A common but time consuming alternative is the estimation of cell carbon from the cell volume using appropriate conversion factors or formulas as shown in Table 2.

The aim of this work was to determine the growth and some biochemical characteristics (pigment composition, cell carbon) of six monocultures. The species were chosen from the most important - dominant groups of the phytoplankton community in the Gulf of Trieste. The predominant groups in this shallow bay are diatoms and microflagellates, the latter being the most abundant group in the greater part of the year (Fanuko, 1981). The other important groups are dinoflagellates, coccolithophores, and silicoflagellates. Phytoplankton succession is strongly influenced by the riverine and urban freshwater inputs and the seasonal stratification of the water column (Smetacek, 1991). Diatom peaks occur in early spring and autumn, which are characterised by the freshwater inputs and mixing of the water column, and occasionally during the summer following episodic storms and consequently nutrients' input (Malej *et al.*, in prep.).

MATERIALS AND METHODS

Culturing and sampling procedure

The isolated species were: *Isochrysis galbana* (class Prymnesiophyceae =Haptophyceae), *Emiliania huxleyi* (class Prymnesiophyceae), *Nitzschia closterium* (class Bacillariophyceae), *Phaeodactylum tricornutum* (class Bacillariophyceae), *Prorocentrum micans* (class Dinophyceae) and *Tetraselmis suecica* (class Prasinophyceae). These species originated from the Culture collection of Plymouth Marine Laboratory, except for the spe-

cies *N. closterium* and *E. huxleyi*, which were isolated from the Gulf of Trieste. The cultures were grown in 20-litre polycarbonate containers at a temperature between 16 and 17°C. Light was provided at a 12 hour light/dark interval by neon "cool white" bulbs, with the intensity varying from 20 to 50 μE $m^{-2}s^{-1}$. Guillard's f/2 medium (Guillard, 1975) was used for most of the cultured species, with the addition of silicate only for the diatoms. For the species *E. huxleyi*, which requires lower nitrogen content, Keller's medium was used (Keller *et al.*, 1987). All media were prepared with filter-sterilised natural sea water from the Gulf of Trieste.

Immediately after the inoculation (60-120 ml culture in the stationary phase was added to 10 l medium) subsamples for cell counts, pigments' analyses and Chl *a* concentration were taken. Cell number and volume were determined in neutralised formaldehyde preserved subsamples (1.5% final concentration). The growth of monocultures based on the cell counts was followed daily, while the cell volume measurements and biochemical analyses were performed again during exponential and stationary phase.

Natural sea water samples for phytoplankton and pigment composition were taken monthly from January till December 1993 at a station in the southern part of the Gulf of Trieste. A 5-litter Niskin bottle was used for sampling at five depths: 0, 5, 10, 15 and 21m (bottom). 800 ml phytoplankton samples were preserved with 1.5% neutralised formaldehyde.

Analyses

The cell number was counted on a Fuchs-Rosenthal haemocytometer using a light microscope at a 100x and 400x magnification. The growth rate (k in division day^{-1}) was calculated daily from the cell number using the equation (Guillard, 1973):

$k = \ln(N_1/N_0) / (0.6931 \times (t_1-t_0))$

where N_1 and N_0 are cell numbers at times t_1 and t_0, respectively, and t_1-t_0 is the time difference in days.

Phytoplankton from natural samples was identified and counted on an inverted microscope using the technique of Utermöhl (1958), where 50 or 100 fields of the bottom chamber were examined at 200x and 400x magnification.

The cell volume was determined as an average volume of 15 cells using cells dimensions and the best fitting geometric formula based on the cell shape (Edler, 1979). The carbon content was calculated from the cell volume using conversion factor 0.13 pg $(\mu m)^{-3}$ for *P. micans*, and 0.11 pg $(\mu m)^{-3}$ for other species. The carbon to chlorophyll *a* ratio (C:Chl *a*) was based on the cell carbon calculation and Chl *a* concentration.

Chlorophyll *a* concentration was determined fluorometrically on a Turner 112 Fluorimeter (Holm-Hansen *et al.*, 1965). 15-25 ml subsamples were filtered onto 0.22 μm Millipore filters, extracted in 90% acetone and the fluorescence of extracts measured.

conversion factor/formula (pg C $(\mu m)^{-3}$)	group/size class	reference
0.13	armoured dinoflagellates	Smetacek, 1975
0.11	eukaryotic autotrophs except armoured dinofl.	Strathman, 1967
0.35	10-100 $(\mu m)^3$	Verity *et al.*, 1992
0.24	100-1000 $(\mu m)^3$	"
0.16	>1000 $(\mu m)^3$	"
0.075	flagellates	Hegmeier, 1961
log C=-0.864+0.857 x logV	diatoms	"
log C=(-0.24 x logV)-0.29	general	Mullin *et al.*, 1966
$C=aV^b$	general	Montagnes *et al.*, 1994

Table 2: Conversion factors and formulas for calculating the cell carbon (C) based on cell volumes (V) for different classes and size groups of phytoplankton.
Tabela 2: Pretvorbni faktorji in enačbe za preračunavanje celičnega ogljika (C) iz celičnih volumnov (V) za posamezne skupine in velikostne razrede fitoplanktona.

The qualitative and quantitative analyses of pigments in the monocultures as well as in the natural samples were determined using a reverse-phase HPLC method (Mantoura & Llewellyn, 1983; Barlow *et al.*, 1993). Water samples (30 ml to 1 l) were filtered through 47 mm Whatman GF/F filters and immediately frozen until analysed. Frozen samples were extracted in 4 to 10 ml of 90% acetone using sonication and centrifuged to remove cellular debris. Chlorophylls and carotenoids were detected by absorbance at 440 nm in the UV/Vis spectrophotometric detector. Data collection and processing utilised Spectra Physics PC1000 software.

The phytoplankton abundance and concentration of pigments from discrete natural samples were depth-integrated over the whole water column.

RESULTS

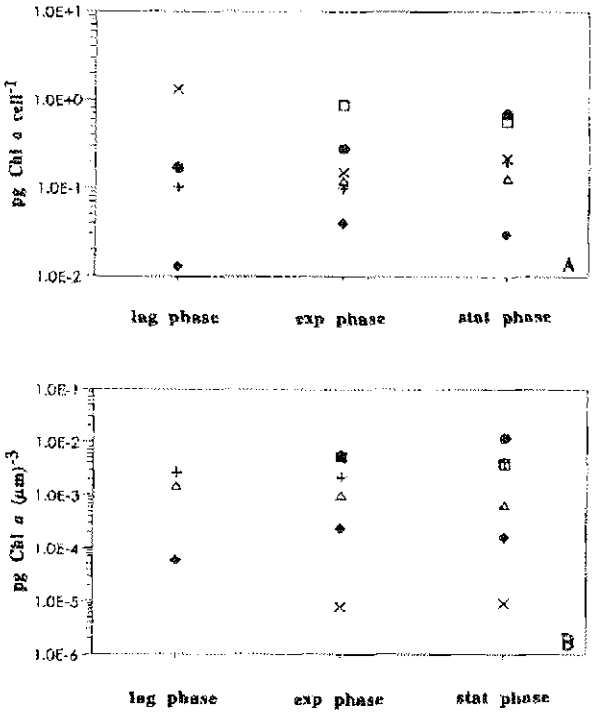
Monocultures

The growth and size parameters of the monocultures determined in the exponential and stationary phase are presented in Table 3.

At the beginning of the experiment, the highest cell number was counted in *I. galbana* culture ($9.3x10^4$ cells ml^{-1}), which reached also the highest cell density in the whole experimental period ($1.0x10^7$ cells ml^{-1}). Only $2.0x10^3$ cells ml^{-1} were counted in *P. micans* culture at

species	growth phase	cell volume ((μm)^3)	k (divisions day^{-1})	Chl a (pg cell^{-1})	cell carbon (pg cell^{-1})	C:Chl a
E. huxleyi	exp	137.9	0.54	0.04	15.7	392
	stat	183.1		0.03	20.2	673
I. galbana	exp	44.5	0.33	0.10	4.9	49
	stat	46.3		0.20	5.1	25
N. closterium	exp	50.9	0.49	0.28	5.6	19
	stat	58.3		0.70	6.4	9
P. tricornutum	exp	118.9	0.49	0.12	13.1	109
	stat	192.1		0.13	21.1	162
P. micans	exp	19122	0.16	0.15	2486.0	16573
	stat	23903		0.22	3107.0	14122
T. suecica	exp	170.2	0.26	0.88	18.7	21
	stat	153.0		0.58	16.8	29

Table 3: The size and growth parameters chlorophyll a (Chl a) and cell carbon (C) content and the C:Chl a ratio of the monocultures in the exponential (exp) and stationary (stat) growth phase.
Tabela 3: Parametri velikosti in rasti, vsebnost klorofila a (Chl a) in celičnega ogljika (C) ter razmerje C:Chl a v monokulturah v eksponentni (exp) in stacionarni fazi rasti (stat).



□ *T. suecica*, ● *N. closterium*, Δ *P. tricornutum*, + *I. galbana*, ◆ *E. huxleyi*, X *P. micans*

Fig. 1: Chlorophyll a content (log_{10} pg Chl a) calculated per (a) cell and (b) volume unit in the lag, exponential (exp) and stationary (stat) phase of the monocultures. (Note the different units on y axes.)
Slika 1: Vsebnost klorofila a (log_{10} pg Chl a), preračunana na (a) celico in (b) volumsko enoto v lag, eksponentni (exp) in stacionarni (stat) fazi rasti monokultur. (Upoštevaj različne enote na oseh y.)

the beginning and 4.1×10^4 cells ml^{-1} at the end of the experiment. This species had also the longest lag phase (10 days), while diatoms and *E. huxleyi* passed over a 4 days lag phase. The growth rates differed substantially between the monocultures. The fastest growing species were diatoms *N. closterium* and *P. tricornutum*, the latter reaching the highest growth rate of 1.1 divisions day^{-1} in the exponential phase. Other species had a growth rate about 1 division day^{-1} in the exponential phase, whereas *P. micans* did not exceed 0.73 division day^{-1}. Except for *T. suecica*, the cell volumes increased with ageing of the monocultures. The largest cell volume was measured in *P. micans* (23903 (μm)^3), while other species had a cell volume below 200 (μm)^3.
During the stationary phase, the highest Chl *a* concentration was measured in *I. galbana* (1678.4 μg l^{-1}) and *N. closterium* culture (787.9 μg l^{-1}), while the lowest in *P. micans* (8.9 μg l^{-1}) and *E. huxleyi* culture (35.1μg l^{-1}). Chl *a* biomass was expressed as Chl *a* concentration per cell (pg cell^{-1}) and per unit of cell volume (pg (μm)$^{-3}$; Fig. 1) and in all cultures the latest concentrations were lower than the former. In both exponential and stationary phases the highest Chl *a* concentration per cell and per (μm)^3 was found for *N. closterium* and

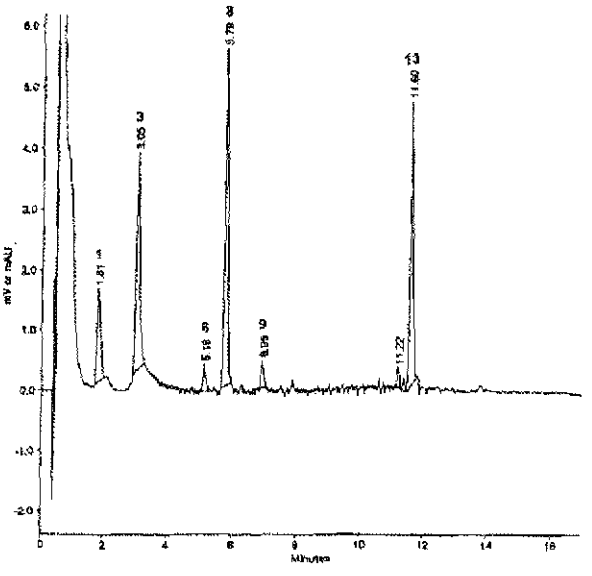


Fig. 2: A HPLC chromatogram showing characteristic pigment pattern of the species E. huxleyi grown in the monoculture (exponential phase). Legend of the absorbance peaks: (1)=chlorophyll c_3, (3)=chlorophyll c_1+ c_2, (6)=fucoxanthin, (8)=19'-hexanoyloxyfucoxanthin, (9)=diadinoxanthin, (13)= chlorophyll a.
Slika 2: HPLC kromatogram in značilni pigmenti vrste E. huxleyi, gojene v monokulturi v eksponentni fazi. Legenda absorbcijskih viškov: (1)=klorofil c_3, (3)= klorofil c_1+c_2, (6)=fukoksantin, (8)=19'-heksanoiloksifukoksantin, (9)=diadinoksantin, (13)= klorofil a.

T. suecica. The largest differences between pg Chl *a* cell^{-1} and pg Chl *a* (µm)$^{-3}$ were observed in *P. micans* culture, due to very low biomass as compared to cell number and large cell volume. The discrepancy between high cell number and low biomass was observed also for *E. huxleyi* with the lowest Chl *a* concentrations per cell. With the ageing, the Chl *a* concentration decreased in some species, while in others increased.

The highest carbon content, based on the cell volume, was calculated for the largest species - *P. micans*. The cell carbon increased in all species during the experiment, except for *T. suecica* in the stationary phase. Here, the carbon content decreased following the change of cell volume.

The pigment composition was determined by the HPLC method. Besides Chl *a*, many accessory pigments were present in monocultures. Different biomarkers were selected according to the presence and concentration of the dominant accessory pigments. A typical chromatogram of a monoculture is shown in Fig. 2. Besides Chl *a*, the diatom monocultures were characterised by the presence of Chl c_1+c_2, diadinoxanthin, ß-carotene, and a characteristic biomarker fucoxanthin. *P. micans* was characterised by the presence of Chl c_1+c_2, diadinoxanthin, and peridinin as the biomarker for dinoflagellates. 19'-hexanoyloxyfucoxanthin (19'hex), Chl c_1+c_2, Chl c_3, and diadinoxanthin were found in *E. huxleyi* culture, and 19'-hex was defined as a biomarker for this species as well as for the group of prymnesiophytes. However, the other prymnesiophyte *I. galbana* showed untypical pigment composition which resembled that of the diatoms. The chromatogram of *T. suecica* revaled the presence of Chl *b*, zeaxanthin/lutein and ß-carotene, the typical prasinophycean as well as green algae pigments. In some monocultures, the concentration of biomarkers incrased in such proportions in the stationary phase that exceeded Chl *a* content.

Natural samples

Over the whole sampling period (January - Decem-

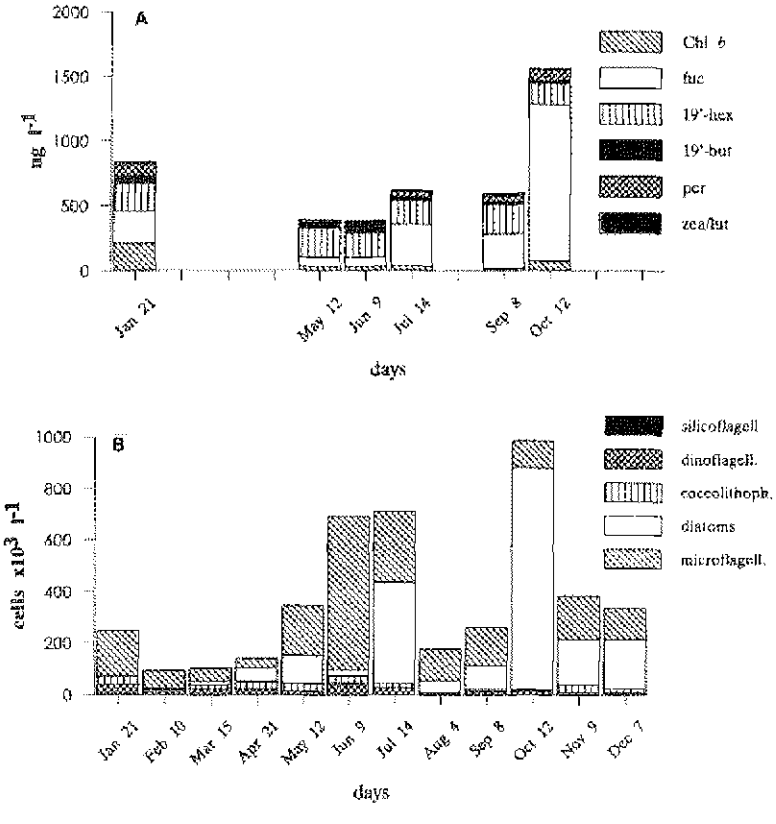


Fig. 3: Seasonal variations in (a) phytoplankton pigment composition and (b) phytoplankton groups during 1993 in the southern part of the Gulf of Trieste (integrated values). Legend: Chl b=chlorophyll b, fuc=fucoxanthin, 19'-hex=19'-hexanoyloxyfucoxanthin, 19'-but=19'-butanoyloxyfucoxanthin, per=peridinin, zea/lut=zea-xanthin/lutein. Slika 3: Sezonske spremembe v (a) pigmentni sestavi fitoplanktona in (b) fitoplanktonskih skupin 1993. leta v južnem delu Tržaškega zaliva (integrirane vrednosti). Legenda: Chl b=klorofil b, fuc=fukoksantin, 19'-hex=19'-heksanoiloksifukoksantin, 19'-but=19'-butanoiloksifukoksantin, per=peridinin, zea/lut=zeaksantin/lutein.

ber 1993), six months were chosen in order to show the distribution of typical biomarkers and the chemotaxonomic composition of the phytoplankton community (Fig. 3a). The seasons chosen are: winter (January), spring (May, June), summer (July, September) and autumn (October). Chl *a* is not included, since it was present in all samples. In January there was a strong prevalence of 19'-hex, Chl *b* and fucoxanthin, which showed the presence of prymnesiophytes, green algae and diatoms or *I. galbana*-like prymnesiophytes. Peridinin and 19'-butanoyloxyfucoxanthin (19'-but) were also present revealing the presence of dinoflagellates and silicoflagellates. The concentration of pigments was rather low in this period. The prymnesiophycean biomarker 19'-hex dominated the spring period, when the zeaxanthin/lutein was also detected showing the presence of green algae and/or cyanobacteria. Another characteristic of this period was the lowest concentration of fucoxanthin which significantly increased in July. In September, a very complex pigment composition was found, with 19'-hex, fucoxanthin, peridinin, and zeaxanthin/lutein being the most abundant. The highest concentration of fucoxanthin occurred in October.

The results of microscopic examinations of the same samples are presented as integrated values in Fig. 3b. Two largest phytoplankton abundance peaks were distinctive in the June-July period and in October. The minimum abundance was detected in spring (from February to April). In January, microflagellates prevailed over coccolithophores and dinoflagellates, while diatoms were almost undetectable. After the early-spring minimum, diatom and microflagellate abundance increased in May. Microflagellates peaked in June, prevailing over diatoms and dinoflagellates which reached their annual maximum. A shift towards diatom-dominated community occurred in July, following by a significant decrease of diatom and total abundance in August. After a slight increase in September, the community reached the annual abundance maximum in October with a large predominance of diatoms (up to 86% of total abundance).

DISCUSSION

Growth and biochemical characteristics of the monocultures

Much research work concerning growth rates in nature and/or cultures has been done. In general, diatoms proved to be the fastest growing group, dinoflagellates the slowest, and the others somewhere in between (Glover *et al.*, 1987; Furnas, 1990). These differences can arise from physiological, ecological, morphological and also phylogenetic factors (Banse, 1976; Tang, 1996).

This general outline was observed also in our experiment. The maximal growth rate (1.1 divisions day^{-1}) was lower from those found in literature that vary from 1.2 to 3.3 divisions day^{-1} for the same species used in this work (Brand & Guillard, 1981; Glover *et al.*, 1987). Studies on the effects of different light intensities (Saunders, 1991) have shown that among 27 species grown at low and normal light intensities, 11 grew better at normal light (*E. huxleyi* and *P. tricornutum* among them) and 14 at low light conditions (e.g., *I. galbana*). Two species did not show any preference (e.g., *T. suecica*). The light conditions in our experiment (20-50 $\mu E\ m^{-2}s^{-1}$) resembled more the low light conditions, therefore our results are comparable better with the growth rates from such conditions.

Biomass expressed as the cell carbon was based directly on the cell volume; the same was for the C:Chl *a* ratio. The ratios for *T. suecica*, *I. galbana* and both diatoms are comparable with those found in literature (Geider, 1987; Faganeli *et al.*, 1989; Cloern *et al.*, 1995). However, it should be stressed that ratios from literature were obtained by direct measurements of organic carbon (CHN analyser), which remains one of the most accurate method of the biomass assessment. Our calculations are only approximations based on the cell dimensions, geometric formulas and experimentally defined conversion factors. On the other hand, the C:Chl *a* ratios of *E. huxleyi* and especially *P. micans* are extremely high and in our opinion should not be taken as representative for these species and applied in natural conditions. We suspect that these high values originate from low Chl *a* concentrations in both monocultures. Although the organic carbon was calculated from the biovolume, the latter as well as the carbon content (pg C cell^{-1}) of these species do not differ much from the values obtained by CHN analyses (Saunders, 1991). In the stationary phase, the Chl *a* concentrations of *P. micans* did not exceed 10 $\mu g\ l^{-1}$, which is very low value for the culture conditions. Also in *E. huxleyi* culture, Chl *a* concentrations were low (35 $\mu g\ l^{-1}$), compared to other cultures (>200 $\mu g\ l^{-1}$) with approximately the same cell numbers.

Pigment biomarkers in the monocultures and natural samples

Pigments detected in the monocultures were used as biomarkers to determine the presence of the respective phytoplankton groups in natural samples. However, uncertainties arise because some of the accessory pigments are present in many groups or, on the contrary, some of them are not typical of all the species of the specific group. For example, the diatom biomarker fucoxanthin is present also in prymnesiophyte *I. galbana* and unarmoured dinoflagellates (Jeffrey *et al.*, 1975).

Knowing the ratios between the concentration of Chl

a and typical accessory pigment, one can calculate the relative contribution of a specific group to the total Chl *a* biomass (Everitt *et al.*, 1990). Peridinin as a selective biomarker for dinoflagellates (Whittle & Casselton, 1968) is suitable for the estimation of their biomass in a natural sample. The Chl *a*: peridinin ratio obtained from the monoculture *P. micans* is 1.4 compared to 2.6 found in literature (Everitt *et al.*, 1990). The relative abundance of a phytoplankton group (X in percentage) is calculated using the formula:

$$X = K \times (C_{pig}/C_{Chla})$$

where K is the known ratio between the concentration of Chl *a* and accessory pigment of the same species/group, C_{pig} and C_{Chla} are concentrations of the accessory pigment and Chl *a* in natural sample. Using the formula and Chl *a*: peridinin ratio of 1.4, relative abundance of dinoflagellates in natural samples were calculated (Table 4). These percentages are compared with the percentages based on the microscopic counts. Some discrepancies appear when comparing the results, since peridinin concentration does not always follow dinoflagellate cell numbers. In fact, in June the highest cell number was counted, while the peridinin concentration was very low. Again, in May dinoflagellate abundance was low, but peridinin was not even detected. After a more accurate examination of the sample it showed up that in late spring a group of dinoflagellates - Gymnodiniales dominated, which contain fucoxanthin as the main carotenoid (Jeffrey *et al.*, 1975), and the genus *Gyrodinium* that contains 19'-hex (Tangen & Björnland, 1981) was also present. In October, however, a very high concentration of peridinin was detected and the amount of the pigment per cell was 22-times higher than in June (6.35 pg cell^{-1} in October and 0.29 pg cell^{-1} in June). In this period armoured dinoflagellates were more abundant and the ones containing fucoxanthin or 19'-hex were almost missing. The amount of peridinin calculated per cell reached a maximum of 0.0614 pg cell^{-1} in *P. micans* monoculture, which is in an astonishing disagreement with the estimate made in field conditions. Since the concentration of pigment per cell varies significantly not only seasonally but also during the lifetime of a population and in relation to the abiotic factors in the environment, the quantitative estimations of the cell abundance using biomarker pigment concentrations should be performed with a great care.

Fucoxanthin is present also in other algal classes. Consequently, without a support of microscopic observations the HPLC fingerprint can be misunderstood. In January, in contrast to October and July, the high concentration of fucoxanthin cannot be related to a diatom bloom, since this group was almost missing as revealed from the microscopic observations. An explanation could be that some unidentified species of prymnesiophytes with a pigment composition similar to *I. galbana* or other algal classes from the group of microflagellates are responsible for the high fucoxanthin level. On the other hand, it was shown that classic microscopy is sometimes insufficient especially in the case of microflagellates. A January the 19'-hex peak coincides with the elevated number of coccolithophores, while in May, June and September other unidentified prymnesiophytes or some dinoflagellates contribute to the high concentrations of this pigment. A minor zeaxanthin/lutein peak is detected in June and it might again represent unidentified green algae or even cyanobacteria.

In conclusion, the pigment composition roughly followed the taxonomic composition determined with microscope. The greatest discrepancies were present in the case of fucoxanthin and peridinin, but even with other pigments it was impossible to assess the exact contribution of a certain group, since many pigments are not highly specific. HPLC method on the other side contributes largely to the identification of the taxa that are commonly placed within the microflagellates, which is phylogenetically a very heterogenic group. Also, to improve the knowledge of the pigment composition in relation to the physiology of the phytoplankton and abiotic factors in the environment, more experiments on monocultures have to be done.

date	% dinoflagell. (Chl a biomass)	% dinoflagell. (total cell No.)
Jan 21	12	14
May 12	0	4
Jun 9	4	6
Jul 14	13	3
Sept 8	10	5
Oct 12	8	1

Table 4: The relative abundances (%) of dinoflagellates of the total Chl a biomass and total cell numbers in natural samples, based on peridinin and Chl a concentration and microscopic cell counts respectively.
Tabela 4: Relativni deleži (%) dinoflagelatov pri skupni klorofilni biomasi in skupnem številu celic v naravnih vzorcih, izračunani iz koncentracije peridinina in klorofila a ter iz števila celic.

POVZETEK

Avtorice so raziskovale rast in nekatere biokemične značilnosti monokultur šestih vrst štirih najpogostejših razredov morskega fitoplanktona iz Tržaškega zaliva. Predstavljeni so bili rastna hitrost, celični volumen, vsebnost celičnega ogljika, preračunana iz celičnega volumna, koncentracija klorofila a in pigmentna sestava.

Vrste so se med seboj razlikovale v hitrostih rasti in trajanju začetne lag faze. Najhitreje rastoče monokulture so bile diatomeji P. tricornutum *in* N. closterium *ter kokolitoforida* E. huxleyi. *Njihova lag faza je trajala 4 dni, medtem ko je bila pri drugih monokulturah 8-10 dni. Največje število celic in največjo biomaso, izraženo kot koncentracijo klorofila a, je dosegla* I. galbana *v stacionarni fazi. Največji celični volumen je imela vrsta* P. micans, *pa tudi tudi vsebnost celičnega ogljika, preračunana iz volumna ter razmerje C:Chl a, sta bila pri tej vrsti največja.*

Analiza pigmentne sestave posameznih monokultur je bila narejena s tekočinsko kromatografijo visoke ločljivosti (HPLC metoda). Diatomejski biomarker fukoksantin je bil najden tudi pri netipični primnezioficeji I. galbana, *peridinin pa je bil najden le pri dinoflagelatu* P. micans. *19'-heksanoiloksifukoksantin, značilen pomožni pigment primnezioficej, je bil najden v kulturi* E. huxleyi, *pri zeleni algi* T. suecica *pa biomarkerja klorofil b in zeaksantin/lutein. Značilni pomožni pigmenti - biomarkerji so bili uporabljeni pri razlagi pigmentnih spektrov naravnih vzorcev iz Tržaškega zaliva. Na osnovi koncentracije biomarkerjev so avtorice določile pojavljanje in zastopanost posameznih razredov fitoplanktona. Kemotaksonomska sestava je bila potrjena z mikroskopskimi pregledi istih vzorcev. Analiza pigmentnih spektrov se je v večini primerov ujemala z rezultati mikroskopskih pregledov, največja neskladja pa so bila pri fukoksantinu in peridininu. Raziskava je pokazala, da je metoda biomarkerjev zelo uporabna za določevanje taksonomske sestave naravnih vzorcev. Vendar je zaradi nekaterih pomanjkljivosti, na katere so avtorice naletele pri obeh metodah, za taksonomsko analizo fitoplanktona priporočljiva uporaba obeh.*

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