Pheopigment dynamics, zooplankton grazing rates and the autumnal ammonium peak in a Mediterranean lagoon

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Abstract: The dilution technique was used to estimate chlorophyll and pheopigment, net and gross production as well as zooplankton grazing over a 12-month period in a coastal lagoon in Southern France. Chlorophyll a (Cha) based gross growth rates of phytoplankton ranged from undetectable in February to 2.6 day−1 in June, corresponding to 3.8 divisions per day. Cha-based grazing rates ranged from undetectable in February to 1.1 d−1 in June. The seasonal growth pattern of picoplankton was similar to that of the whole community, with a peak in July, corresponding to four divisions per day. Grazing processes represented from 20 to 150% of the phytoplankton daily growth, and the grazing pressure was stronger on small phytoplankton cells than on larger cells. Gross growth rates of phytoplankton were related to zooplankton grazing rates, and both were related to water temperature. Mesozooplankton which escaped sampling or oysters had to be also invoked as additional sinks for the primary production. In the fall, pheopigment concentrations greater than chlorophyll concentrations coincided with high ammonium levels in the water column. Pheopigment a production rates were highly correlated to chlorophyll -based microzooplankton grazing rates. The pheopigment a to chlorophyll a ratio was correlated with ammonium concentrations and could be used an index of the balance between ammonium supply (degradation) and demand (uptake by phytoplankton). In addition, pheopigment degradation rates in absence of grazing could be related to irradiance, indicating photo-degradation of these compounds.

Keywords: phytoplankton - growth - degradation - pheopigments - ammonium - zooplankton

Introduction

The application of the so-called "dilution method" (Landry & Hassett, 1982) has yielded simultaneous data on phytoplankton gross and net growth rates and zooplankton grazing rates over the last 20 years, and has been extremely helpful in furthering our understanding of the balance between phytoplankton source and loss terms. This technique usually involves chlorophyll <u>a</u> as a phytoplankton biomass index, and growth and grazing rates are derived from changes in this variable as a function of dilution (Andersen et al., 1991; Dagg, 1995; Landry et al., 1995; Dupuy et al., 2000; Murrell et al., 2002; Calbet & Landry, 2004). However, this technique has not been applied to pheopigments so far. As pigment degradation products, pheopigments could give useful indications dealing with the phytoplankton mortality through grazing by zooplankton (Yentsch, 1965; Therriault & Platt, 1978; Welschmeyer & Lorenzen, 1985). Still, the use of pheopigments in such studies has been limited by lack of accuracy due to the use of filter fluorometers (Neveux & deBilly, 1986; Vernet & Lorenzen, 1987). More recent instruments such as spectrofluorometers or HPLC allow much better estimates of those degradation products which are important in highly productive coastal environments (Neveux et al., 1990). As these pigments are thought to result from both grazing and photo-oxidation processes (Yentsch, 1965; Lorenzen, 1967; Welschmeyer & Lorenzen, 1985), we applied the dilution technique in order to separate both effects and therefore assess their respective influence on pheopigment concentrations.

Here we report data from an annual cycle which allows a wide variety of environmental conditions to be encountered and tested for their influence on phytoplankton growth and zooplankton grazing rates. In particular, we will focus on the relationship between phytoplankton mortality and the peak in ammonium concentrations occurring in the fall every year in the Thau lagoon (Casellas et al., 1990; Mazouni et al., 1996; Collos et al., 1997;

Souchu et al., 2001). This work is part of a long term program which aims to describe and understand the functioning of the Etang de Thau lagoon.

Materials and Methods

The Etang de Thau (Fig. 1) is a large (75 km^2) and shallow (4 m mean depth, 10 m maximal depth) Mediterranean coastal lagoon located in Southern France (43°24'N, 3°36'E). It is exploited mainly for oyster farming which makes it an important economic area. The sampling station (ZA) was located near oyster pens (see Souchu et al., 2001 for more details).

Physical and chemical variables

Photosynthetic available radiation (PAR) was measured at the water surface with a LI-COR (Model LI-190SA) quantum sensor and a LI-1000 data logger. Underwater irradiance was measured every 50 cm with a LI-COR (Model 193SA) spherical quantum sensor. The visible light extinction coefficient was calculated from linear regressions of irradiance vs. depth.

Samples for ammonium (NH$_4$) determination were immediately fixed and measured in the laboratory using the method of Koroleff (1976). Additional samples for nutrient analyses samples were brought to the field laboratory and frozen. Soluble Reactive Phosphorus (SRP), nitrate (NO$_3^-$) and nitrite (NO$_2^-$) and dissolved reactive silica were analysed with a segmented flow analyser (Tréguer & Le Corre 1975).

Sampler effect

Following the results of Price et al. (1986), Chavez & Barber (1987) and Williams & Robertson (1989), we modified the samplers used in our laboratory by replacing the toxic

central rubber spring with a silicone tubing. Surface samples thus collected were incubated

and compared to samples collected directly by immersing the polycarbonate (PC) incubation

bottle below the surface. While the results were satisfactory for the Niskin bottle, this was not

the case for the Van Dorn type bottle which clearly appeared to remain toxic for the

organisms under study (Fig. 2). Note that these were surface samples and that the contact time

between water samples and the bottle were kept to a minimum (2-3 minutes at most). To our

knowledge, this is the first time such a persistent problem is reported for sampling bottles

following the change of the central rubber spring.

In order to avoid such potential problems, seawater samples dedicated to growth

dynamics/grazing measurements were then collected by directly immersing 20 liter PC

containers 10 cm below the surface. Seawater was then passed through a 1000 μm mesh to

remove larger debris without removing large diatoms (Price et al., 1985; Collos & Slawyk,

1986; Dupuy et al., 2000). The PC containers were emptied into a 60 liter polyethylene vat

(tested for non toxicity) in order to homogenize the sampled water. Aliquots were then taken

to fill duplicate series of one liter PC experimental bottles.

Serial dilution experiments (Landry & Hassett, 1982) were conducted monthly over a

12 month period. Part of the seawater was gravity filtered via silicone tubing and an in-line,

0.2 μm Suporcap (Pall-Gelman) filter (Landry et al., 1995). Each filter capsule was prepared

by rinsing with one liter of deionized water and one liter of seawater before use. Final

plankton densities were about 14, 24, 48, 71 and 100% of ambient levels. Nutrient enrichment

was made by adding 100 μl of K medium (Keller et al., 1987) at 100 fold concentration. This

resulted in a final nitrate concentration of about 9 μmol N l^{-1}, which was sufficient to fulfill

the phytoplankton nutrient requirements over 24 hours in summer (Fouilland et al., 2002). *In

situ* incubations were carried out over 24 hours at 50 cm below the surface with

unfractionated natural populations under full nutrient sufficient conditions (enriched samples)

and with a duplicate control without nutrient addition (unenriched samples). Pigment contents (chlorophylls a, b, c and pheopigments a, b and c) were measured spectrofluorimetrically in acetone extracts as described by Neveux & Lantoine (1993). The term pheopigments includes both pheophytin and pheophorbide here.

Temporal changes in chlorophyll a (Cha) were used to compute the apparent growth rate m(x) for each dilution (x=1 for the undiluted sample, and x=0 at infinite dilution):

$$m(x,t) = 1/t \ln (C_{x,t}/C_{x,0}) \qquad (1)$$

Where C(x;0) and C(x,t) are initial and final concentrations of Cha.

Linear regressions between apparent growth rate and dilution factor (Landry and Hassett, 1982) allow to determine simultaneously the instantaneous growth rate μ (Y intercept) and the phytoplankton mortality rate by microzooplankton grazing (slope of the line) :

$$m(x) = \mu - gx \qquad (2)$$

where μ is independent from dilution and is determined in enriched samples.

Post incubation fractionation on Nuclepore filters allowed to isolate the less than 2 μm fraction, which is characteristic of *Ostreococcus tauri* (Courties et al., 1994) and estimate its growth and mortality rates by the dilution technique mentioned above.

Besides being a degradation product of Cha, pheopigment a (Pa) is also an integral part of photosystem (PS) II and is present in proportions of about 4% (Pa/Cha) in non degraded plant systems (Ort, 1986). The calculation of pheopigment gross rate of change must therefore be corrected for any production due to synthesis of PS II components coming from phytoplankton growth during incubation. Such a correction was introduced by using "relative pheopigment values" (Pa/Cha) and calculating a "change in relative Pa" (KrelPa) according to the equation:

$$KrelPa = [\ln ((fPa/fCha)/(iPa/iCha))]/T \qquad (3)$$

where fPa = final pheopigment a

fCha: final chlorophyll a

iPa = initial pheopigment a

iCha = initial chlorophyll a

T = incubation time.

Then the usual computations were applied in order to obtain an apparent rate of change which was plotted against the dilution factor. The Y intercept was interpreted as the change in pheopigments in absence of grazing.

Zooplankton composition and biomass were analysed before starting the dilution experiment. The sampling was operated by pump from –1 to –0.5 m at the same station. The organisms which were collected through a filtering collector of 40 μm mesh size, did not suffer any damage, but obviously, individuals less than 40 μm (flagellates and aloricate oligotrichs), were not retained in the samples. The plankton material was then fixed in a buffered 4% formaldehyde solution and slightly coloured with Rose Bengal to obtain the best contrasted image for sorting and image analysis. Monotype sorted individuals were handled with an image analysis routine for counts and measures (Lam-Hoai 1991, Lam-Hoai *et al*., 1997). According to Mullin (1969) and Omori & Ikeda (1984), if the specific weight of an animal is close to 1, it is reasonable to establish the equivalence of its volume to its wet weight ($1cm^3 \approx 1g$). Therefore abundance and wet weight were evaluated per litre, respectively for density (ind l^{-1}) and biomass (μg wet weight l^{-1}), from 10 litres of pumped water.

Results

Physical and chemical conditions

Water temperature ranged more than 3 fold over the year (Table 1), with minimum in January (6.5°C) and maximum in August (25°C). Mean surface irradiance during the incubation period ranged more than four fold. Salinity ranged from 31.3 in November to 38.8 in April. Inorganic nitrogenous nutrients were generally low excepted for ammonium which attained a peak in October (8.7 μmol N l^{-1}), and relatively high values in September (4.9 μmol N l^{-1}) and November (4.8 μmol N l^{-1}). Cha ranged more than 10 fold with minimum in November (0.4 µg l^{-1}) and maximum in May (5.4 µg l^{-1}). Pa also ranged more than 10 fold, with minimum in February (0.13 µg l^{-1}) and maximum in October (2.2 µg l^{-1}) when it was almost twice as high as that of Cha. In December, all incubation bottles were lost. Among the nano and microphytoplankton assemblages, the numerically dominant genus was *Chaetoceros* sp. in April (94.5%) and May (99.2%), and *Skeletonema costatum* in June (85.7%).

The taxonomic richness and mean abundances of zooplankton individuals larger than 40 µm were lower in winter (5 taxa, 4 ind l^{-1} or 3 µg l^{-1} in February), than in the rest of the year (14 to 23 taxa and 9 to 70 ind l^{-1} or 11 to 39 µg l^{-1}). The seasonal succession was clearly marked by dominant populations, notably copepod nauplii in February, *Tintinnopsis beroidea* and *Acartia margalefi* copepodites in March, copepod nauplii and bivalve veligers in April, *Oikopleura dioica* and *Favella serrata* in May and June, *Tintinnopsis corniger* and copepod nauplii in July, copepod nauplii and bivalve veligers in September and October. With the exception of copepods (*Acartia* and *Oithona*), *Oikopleura* and meroplankton such as ascidian and polychaete larvae, most of the zooplankton belonged to the microzooplankton class size.

Examples of dilution plots are given in Fig. 3. Excepted for February and September, all regressions between the unfiltered fraction and phytoplankton growth were significant and

they all exhibited a linear relationship. This was also valid for the August 1998 data taken from Dupuy et al. (2000).

Gross growth rates of phytoplankton, as well as net growth rates with and without nutrient enrichment, and grazing rates showed more than 10 fold seasonal variations over the study period (Fig. 4). During winter, and up to early spring, all rates were low and similar (negative to 0.5 d^{-1}). In May, differences were clear between a gross phytoplankton growth rate of about 2.1 d^{-1}, lower net rates in enriched samples (1.7 d^{-1}) and net rates without enrichment which were near zero. In summer, differences between rates were greatest, with peaks in gross growth rates around 2.5 to 2.7 d^{-1}, corresponding to 3.6 – 3.9 divisions per day, while net growth in enriched samples remained at the level found in late spring (1.5 – 1.7 d^{-1}). In June, net growth without enrichment was still extremely low (about 0.1 d^{-1}) but increased regularly through July (0.8 – 0.9 d^{-1}) and peaked in September (1.5 – 1.7 d^{-1}) when all rates became similar again. In October, the situation was back to low rates (0.1 – 0.4 d^{-1}) with the exception of gross growth rates which presented a secondary peak in November (0.8 d^{-1}). Gross growth rates of the whole fraction and microzooplankton grazing rates are shown in Table 2, with descriptive statistics.

Gross growth rates for the fraction lower than 2 μm exhibited a seasonal pattern similar to that of the whole community (Table 2), with a minimum (zero) in February and a peak in July (2.75 d^{-1}, corresponding to 4 divisions.d^{-1}).

In spite of the similarity in shape between seasonal trends in gross growth rates and irradiance during incubation, there was no significant correlation between both variables, but the correlation between gross growth rate and water temperature was significant (Fig. 5, r = 0.834, P = 0.0014). In a somewhat similar pattern, zooplankton grazing rates were not related to chlorophyll-a, but were correlated to water temperature (r = 0.673, P = 0.0233), and to phytoplankton gross growth rates. The slope of the regression between growth and grazing

indicated that micrograzers consumed about 41% of the phytoplankton daily growth, and grazing was not statistically related to ascidian larvae wet biomass (r = 0.696, n = 8, P>0.05), but to no other zooplankton group > 40 μm. Finally, ammonium concentrations were not related to grazing rates, but were significantly related to the Pa/Cha ratio (r = 0.844, n = 11; P = 0.0042), with large values of ammonium (Table 1) and Pa/Cha ratios (Table 3) in the fall.

Initial values of Pa/Cha for the whole community ranged from 0.17 in May to 1.86 in October (Table 3), thus representing various stages of phytoplankton degradation, with large values of Pa/Ca for both the total and picoplankton fractions in the fall. In the enriched samples of the total fraction, those ratios generally decreased during incubation, except in February, June and November (Table 2), and final Pa/Cha ranged from 0.06 in June to 0.80 in October (100% bottles). In the controls (data not shown), the ratios generally decreased except in May and June, and final Pa/Cha ranged from 0.10 in January to 0.72 in October.

Initial values of Pa/Cha of picophytoplankton ranged from 0.16 in April to 2.1 in October. In the enriched samples (Table 3), Pa/Cha ratios always decreased during incubation attaining final Pa/Cha values from 0.10 in January to 0.96 in October. Estimates of grazing on picophytoplankton were significantly correlated only to ascidian larvae biomass (r = 0.757, n = 8, p = 0.0296).

In absence of grazing (Y intercept values of the relationships between relative Pa changes and dilution), relative Pa changes for the whole phytoplankton community were mostly negative or near zero, indicating degradation of pheopigments during incubation. Maximal negative values were reached in summer, and minima in winter. The relative Pa changes were related to mean PAR and indicated that photodegradation of Pa occurred during the incubations (Fig. 6). The photodegradation followed a first-order decay in the range of PAR encountered.

Discussion

The maximal phytoplankton growth rate (2.7 d^{-1}) observed in June was close to the highest values recorded with the dilution technique (Murrell et al., 2002). At temperatures above 20°C, gross growth rates were much higher (up to 70%) than those predicted by the model of Eppley (1972), corrected for day length. This discrepancy could be due to unbalanced growth and cell division presenting a time lag relative to pigment synthesis so that cell-based growth estimates are much lower than Cha-based growth estimates (Collos, 1986). The maximum grazing rate of 1.98 d^{-1} is also in the high range of values recorded by this technique, and is close to the value of 2.11 d^{-1} observed by Dagg (1995) in an oyster farming site.

For healthy phytoplankton, values of the Pa/Cha ratio usually range from 0.05 to 0.10 (Shuman & Lorenzen, 1975; Barlow et al. 1993; Fundel et al. 1998). The initial values we observed (0.17 to 1.86) were greater and indicated a range of physiological state, with heavily degraded populations in October. The general decrease in the Pa/Cha ratio during incubation indicates an improvement of the cell status, which was not due to nutrient enrichment because it was also observed in the unenriched controls (Table 3). The only exception was in June when the ratio increased in the 3 least diluted samples. This was probably an artefact already observed by Andersen et al. (1991), which was due to the presence of *S. costatum* in the samples (Suzuki & Fujita 1986).

Our results concerning photodegradation of pheopigments (Fig. 6) are similar to those reported by Welshmeyer & Lorenzen (1985), although in the latter study some grazing activity was present as well as the water was filtered throughout a 10 μm mesh "to remove as many grazers as possible" but it must have included some of the microzooplankton community (Taguchi et al., 1993). The first order rate constants (0 to 0.15 h^{-1}) we found for

photooxidation (Fig. 6) were within the range of values previously reported (SooHoo &

Kiefer, 1982).

The lack of correlation between ammonium and grazing rates indicate that ammonium

is not a direct result of grazers present in the incubation bottles. Other sources of ammonium

include anoxia events in summer (Souchu et al., 1998), but no such event occurred during our

study. Grazing by large filter feeders such as mesozooplankton, oysters and mussels could

certainly release ammonium to the water column at any time of the year. Significant

correlations are sometimes found between mesozooplankton biomass and phytoplankton

abundance estimates as Cha (Cotano et al., 1998). In our case, the only zooplankton group

that exhibited such a relationship was the ascidian larvae ($r = 0.941$, $n = 8$, $p = 0.0005$). The

importance of those organisms is probably due to the presence of oyster cultures, where

epibionts such as ascidians are abundant. It also suggests that microzooplankton is not the

only group responsible for grazing, as often assumed in dilution studies. A recent review of

the global impact of microzooplankton grazers on marine phytoplankton indicates that

micrograzers consume about 60% of phytoplankton daily growth in coastal environments

(Calbet & Landry, 2004). In our case, microzooplankton grazing consumes only 41% of

phytoplankton daily growth, so that 59% must be due to other organisms.

The lack of relationships between the biomass of various groups of mesozooplankton

and Pa may indicate that the Pa we measured could be the "suspended Pa" produced by

microzooplankton which do not form fast sinking fecal pellets and can be sampled accurately

by our sampling procedure (Welschmeyer & Lorenzen, 1985), although this has been disputed

(Conover et al., 1988; Taguchi et al., 1993). This "suspended Pa" is subjected to

photodegradation because of its low sinking rate. This possibility is supported by the

decreasing trends in Pa/Cha during incubation (Table 2) that are independent of nutrient

status. They may be an indication of release of grazing pressure from oysters and

mesozooplankton which are excluded from the bottles during incubation, thereby leading to reduced Pa production.

Ammonium was not correlated to Pa concentrations, as sometimes observed in other environments. For example, Therriault & Platt (1978) found significant correlations between ammonium and Pa concentrations in a temperate coastal embayment and suggested a link with grazing activity. In the present study, ammonium concentrations were correlated to the Pa/Cha ratios, in a way similar to findings by McCarthy & Kamykowski (1972) in California coastal waters and Litaker et al. (1988) in the Newport River estuary. Lorenzen (1967) found that the Pa/Cha ratio was related to the productivity index as well as the copepod/Ca ratio, and suggested that the Pa/Cha ratio is a measure of nutrient turnover in the euphotic zone. Although his assertion was not really supported by his data because the chosen variables were not independent and there was probably a lot of noise due to the methodology used (Neveux & deBilly, 1986; Vernet & Lorenzen, 1987), his conclusion could be correct. In our study, the fact that ammonium was related to the Pa/Cha ratio rather than Pa alone suggests that Pa/Cha is an indicator of the balance between ammonium source (phytoplankton degradation) and sink (phytoplankton growth). Data from other coastal environments support this assertion (McCarthy & Kamykowski, 1972; Litaker et al. 1988). Now that reliable estimates of Pa can be obtained (Neveux et al., 1990), the Pa/Cha ratio could be a useful and easily measured indicator of phytoplankton nutritional status in relation to the nitrogen cycle. At least in our case, this ratio indicates a general degradation of the phytoplankton community in the fall.

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Table 1. Physical, chemical and biological variables at station ZA. August data are taken from

Dupuy et al. (2000). N.A.: not available; U: undetectable

Date	mean PAR $(\mu mol.m^{-2}.s^{-1})$	Temperature (°C)	Cha $(\mu g.l^{-1})$	Pa $(\mu g.l^{-1})$	NO$_3$	NO$_2$ (μmol N.l^{-1})	NH$_4$
11 February 1999	616	12.2	0.48	0.13	0.12	0.24	0.60
25 March 1999	199	12.1	0.80	0.31	0.38	0.06	0.56
22 April 1999	470	13.9	1.29	0.24	0.06	0.31	0.57
20 May 1999	405	19.7	5.38	0.95	U	U	0.31
8 June 1999	752	21.7	3.59	0.80	U	U	0.61
8 July 1999	852	23.3	2.04	1.09	0.16	0.05	2.26
17 August 1998	724	25.0	2.65	0.68	0.09	0.07	0.30
16 September 1999	915	23.3	0.62	0.27	0.42	1.03	4.89
21 October 1999	444	17.5	1.19	2.20	NA	NA	8.70
18 November 1999	244	10.5	0.39	0.14	NA	NA	4.80
16 December 1999	247	7.4	NA	NA	NA	NA	0.60
20 January 2000	363	6.5	2.22	0.43	NA	NA	0.06

Table 2. Gross growth rates ($\mu \pm$ SE, d^{-1}) and microzooplankton grazing rates (g $\pm$ SE, d^{-1}) based on Cha in enriched samples; r^2: coefficient of determination; n: number of observations. Grazing is expressed as a loss term (negative values). The significance level of the regression (i.e. slope, g, was significantly different from zero, $p < 0.05$) is indicated by p: ns: non significant, * p<0.01, ** p<0.001, *** p<0.0001. Data for August are taken from Dupuy et al. (2000). Data for enriched samples only.

Date	μ	g	r^2	p value	n
Feb. 99	-0.23 $\pm$ 0.06	0.28 $\pm$ 0.10	0.50	< 0.05	10
Mar. 99	0.60 $\pm$ 0.04	-0.17 $\pm$ 0.06	0.51	< 0.05	10
Apr. 99	0.57 $\pm$ 0.02	-0.11 $\pm$ 0.03	0.64	*	10
May. 99	2.13 $\pm$ 0.06	-0.36 $\pm$ 0.11	0.62	< 0.05	9
Jun. 99	2.63 $\pm$ 0.05	-1.13 $\pm$ 0.08	0.96	***	10
Jul. 99	2.42 $\pm$ 0.05	-0.78 $\pm$ 0.07	0.94	***	10
Aug. 98	2.68 $\pm$ 0.07	-1.46 $\pm$ 0.11	0.95	***	10
Sep. 99	1.65 $\pm$ 0.09	-0.10 $\pm$ 0.15	0.06	n. s.	10
Oct. 99	0.39 $\pm$ 0.07	-0.26 $\pm$ 0.11	0.41	< 0.05	10
Nov. 99	0.66 $\pm$ 0.04	-0.28 $\pm$ 0.06	0.74	*	9
Jan. 00	0.49 $\pm$ 0.02	-0.11 $\pm$ 0.04	0.49	< 0.05	10

Table 3. Pheopigment a/Chlorophyll a ratios in initial samples (range of triplicate values) and in final samples (range of 5 dilution levels) for the total phytoplankton community and the fraction less than 2 μm over an annual cycle in Thau lagoon. Data for August are taken from Dupuy et al. (2000). Data for enriched samples only.

| | Total | | < 2 μm | |
| | | | | |
	initial	final	initial	final
Feb. 99	0.26 - 0.28	0.17 – 0.37	0.23 – 0.45	0.12 – 0.22
Mar. 99	0.36 – 0.43	0.19 – 0.35	0.24 – 0.29	0.14 – 0.16
Apr. 99	0.18 – 0.19	0.15 – 0.17	0.16 – 0.17	0.17 - 0.20
May 99	0.17 - 0.18	0.09 – 0.12	0.34 – 0.45	0.12 – 0.14
June 99	0.21 - 0.23	0.06 – 0.72	0.25 – 0.27	0.12 – 0.17
July 99	0.52 – 0.56	0.12 – 0.21	0.30 – 0.32	0.11 – 0.25
Aug. 98	0.24 – 0.27	0.09 – 0.21	N. D.	N. D.
Sept. 99	0.39 – 0.48	0.12 – 0.19	0.76 - 0.84	0.20 – 0.42
Oct. 99	1.82 - 1.86	0.57 – 0.80	1.70 – 2.10	0.73 – 0.96
Nov. 99	0.35 – 0.37	0.24 – 0.51	0.40 – 0.41	0.26 – 0.43
Jan. 00	0.19 – 0.20	0.10 – 0.16	0.60 – 0.71	0.11 – 0.42

Figure legends

1. Location of the study site. Urban areas in dark.

2. Changes in chlorophyll a (as % of control) during *in situ* incubation of surface samples taken with a Van Dorn type sampler relative to samples taken with a polycarbonate (control) bottle. Both series were incubated in polycarbonate bottles.

3. Examples of experimental analyses for dilution experiments conducted at station ZA in Thau lagoon over a yearly cycle. Regression lines are fitted to the data for incubations with added nutrients.

4. Gross and net phytoplankton growth rates (μ) and zooplankton grazing rates over an annual cycle in Thau lagoon. TA and TB are replicate controls (no dilution and no enrichment), A and B are replicate net growth rates (no dilution but with enrichment). All rates are expressed in units of time^{-1} for comparison. Grazing is expressed as a loss term (negative values).

5. Phytoplankton gross growth rates and zooplankton grazing as a function of water temperature. Grazing is expressed as a loss term (negative values). Dashed line: 95% confidence limits.

6. Pheopigments changes in absence of grazing (KrelPa) as a function of mean irradiance (PAR) during 24 h incubations and over an annual cycle in Thau lagoon. Negative values represent losses. Dashed line: 95% confidence limits.
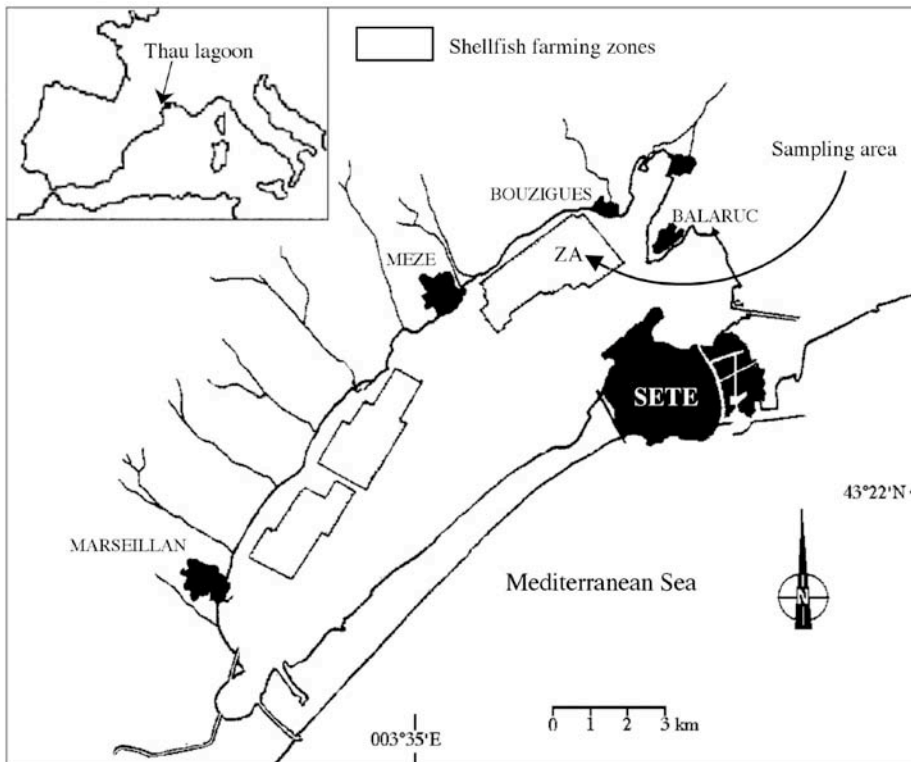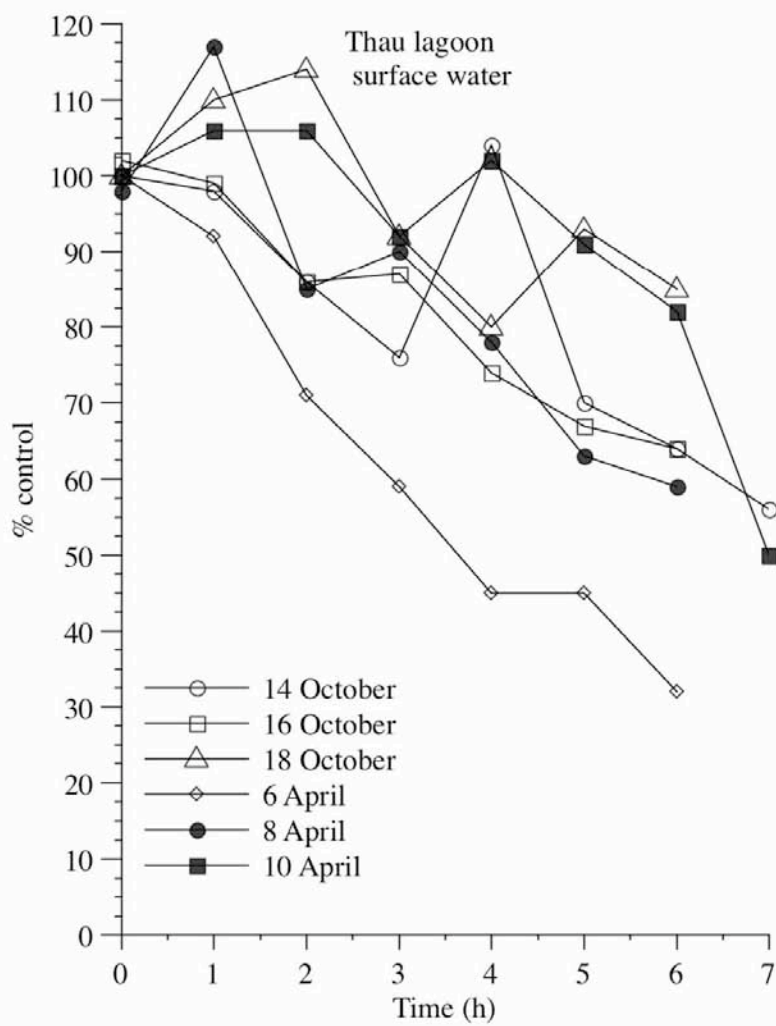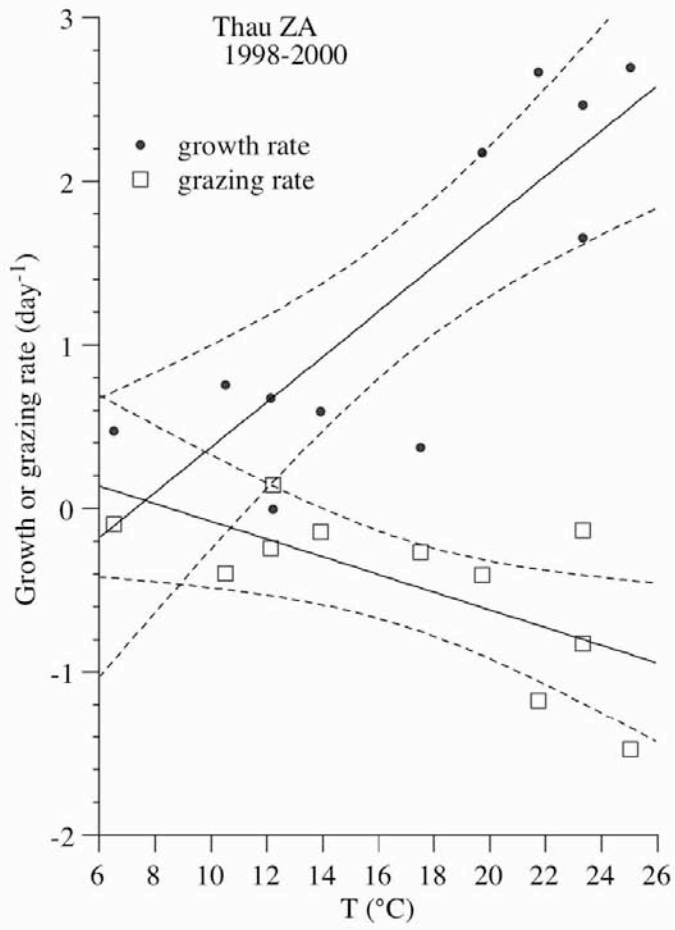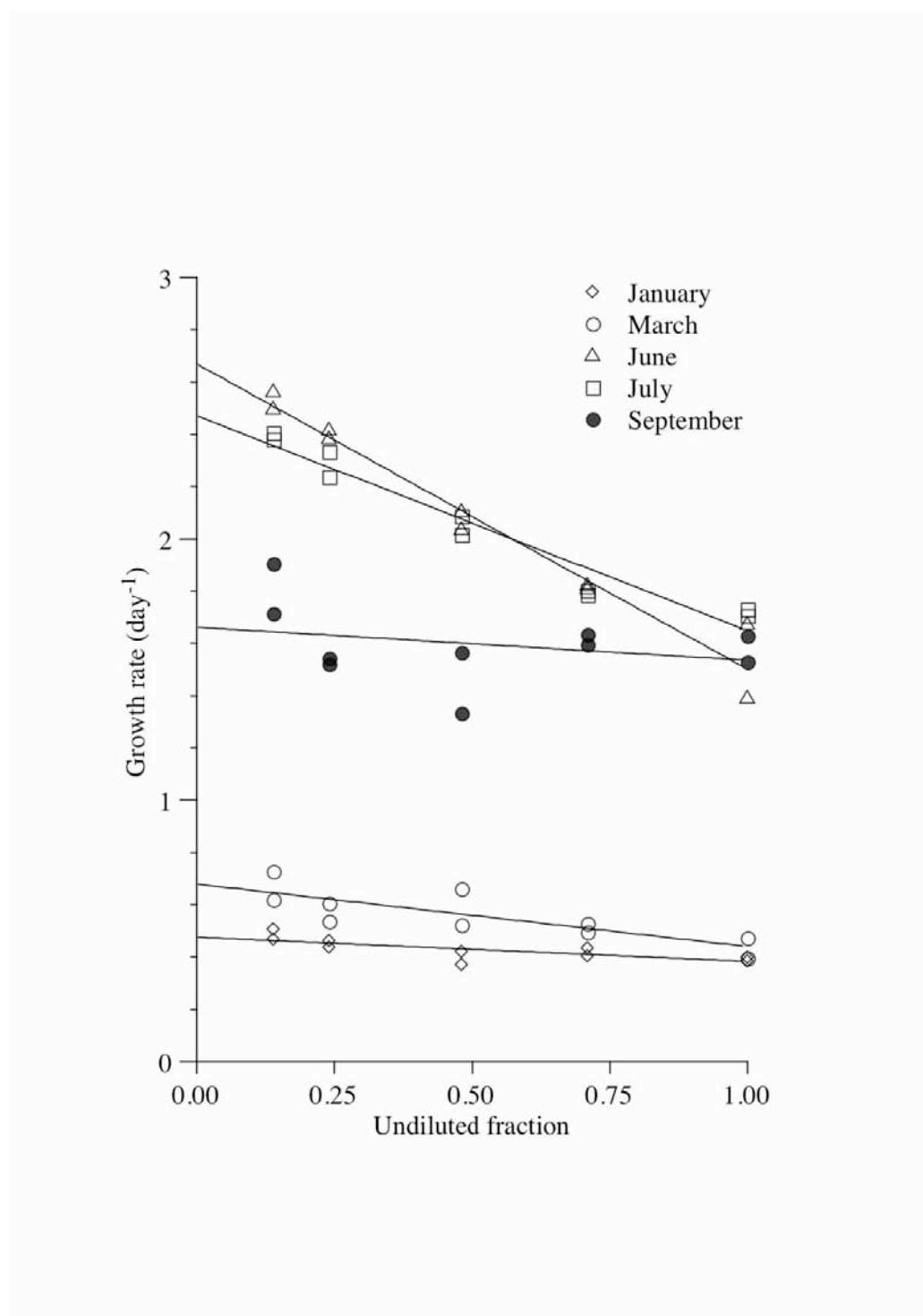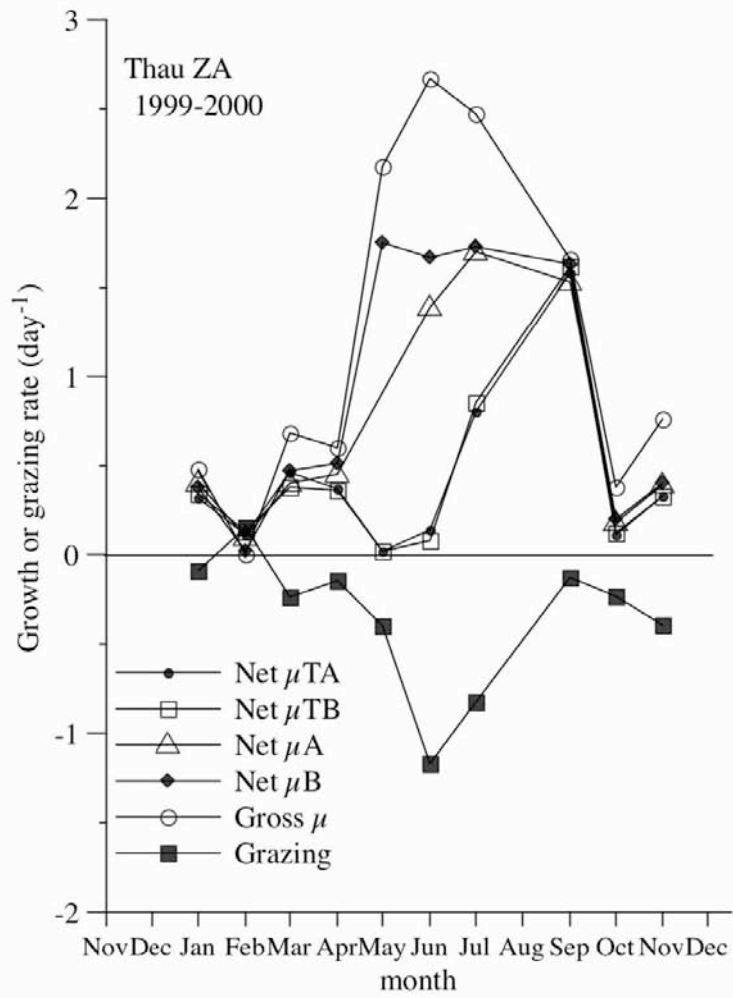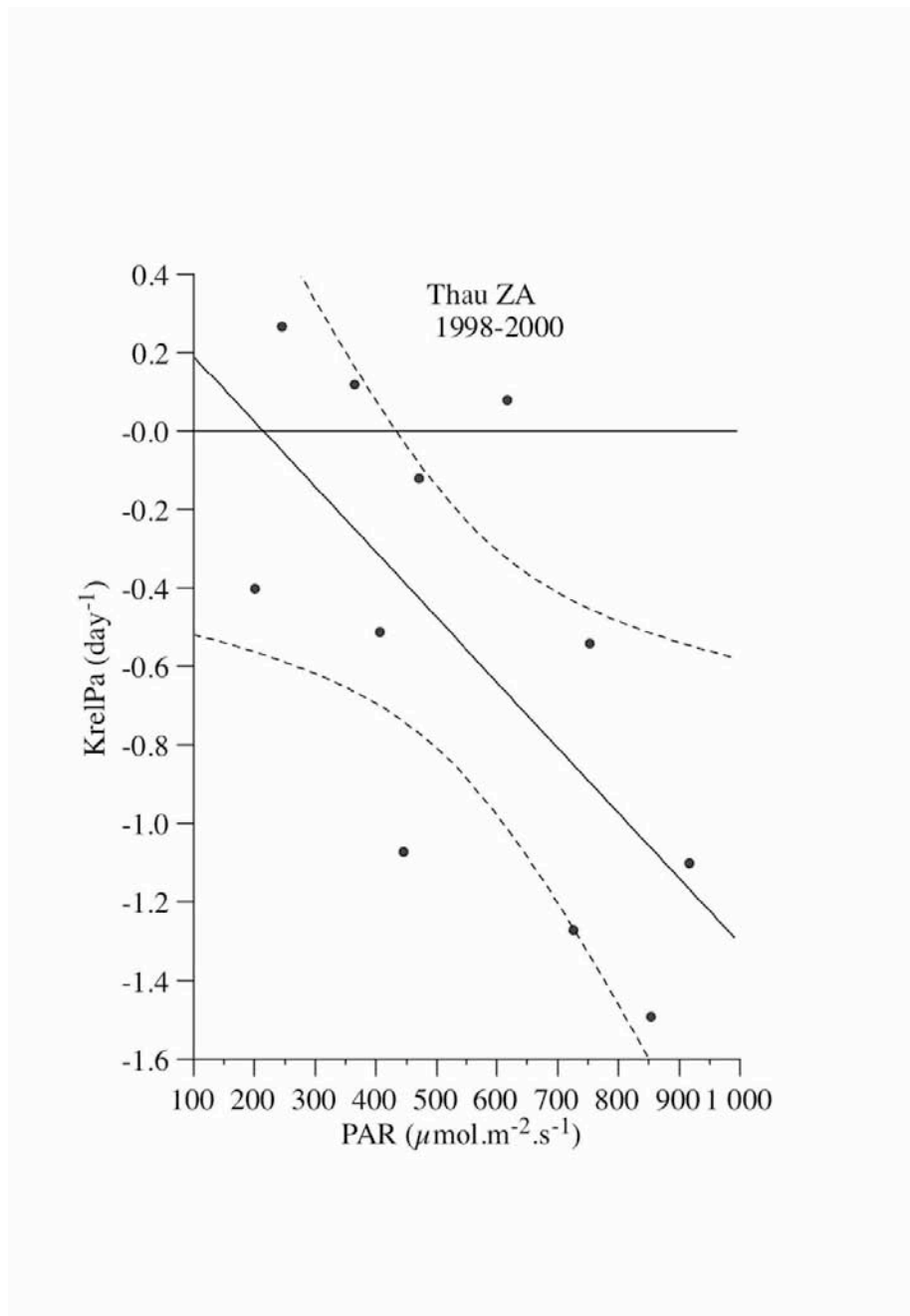
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6