

Noctiluca scintillans MACARTNEY in the Northern Adriatic Sea: long-term dynamics, relationships with temperature and eutrophication, and role in the food web

S. FONDA UMANI^{1,2*}, A. BERAN¹, S. PARLATO¹, D. VIRGILIO¹, T. ZOLLET¹, A. DE OLAZABAL¹, B. LAZZARINI¹ AND M. CABRINI¹

¹LABORATORY OF MARINE BIOLOGY, VIA PICCARD 54, 34010 TRIESTE S. CROCE AND ²DEPARTMENT OF BIOLOGY, UNIVERSITY OF TRIESTE, VIA GIORGERI 10, 34127 TRIESTE, ITALY

*CORRESPONDING AUTHOR: labbioma@units.it

*The first 'bloom' of *Noctiluca scintillans* in the Northern Adriatic Sea was recorded in 1977. The organism caused several red tides in the whole basin during the late 1970s, a period characterized by increasing nutrient loads. During the 1980s and early 1990s, there was no 'red tide', but the species was an almost constant summer presence, associated with high temperatures. *Noctiluca scintillans* was almost completely absent from 1994 until May 1997, concurrent with a general plankton decrease. From summer 1997, *N. scintillans* was recorded again in the whole basin, although there was no other signal of increasing eutrophication. In contrast to all previous observations, during winter 2002-2003, *N. scintillans* was continuously sampled in the Gulf of Trieste. We estimated experimentally growth and grazing rates of the dinoflagellate at 9–10°C in culture and consuming the natural assemblage. *Noctiluca scintillans* was able to reproduce actively at low temperatures, showing similar growth rates in both experiments ($k = 0.2 \text{ day}^{-1}$). The values found were close to those reported in the literature for higher temperatures. The natural diet was mainly composed of phytoplankton (ingestion = $0.008 \mu\text{g C. Noctiluca}^{-1} \text{ day}^{-1}$), microzooplankton (ingestion = $0.008 \mu\text{g C. Noctiluca}^{-1} \text{ day}^{-1}$) and bacteria (ingestion = $0.005 \mu\text{g C. Noctiluca}^{-1} \text{ day}^{-1}$) with an average carbon content of $0.138 \pm 0.020 \mu\text{g C. Noctiluca cell}^{-1}$.*

INTRODUCTION

Noctiluca scintillans is an unarmoured, marine, planktonic dinoflagellate. It is a non-photosynthetic heterotrophic and phagotrophic species; chloroplasts are absent and the cytoplasm is mostly colourless. Cells range from 200 to 2000 μm in diameter, most commonly from 500 to 600 μm . A number of food vacuoles are present within the cytoplasm. A large nucleus is located near the ventral groove with cytoplasmic strands extending from here to the surface of the cell. It is strongly buoyant due to its large cell vacuole, filled with ammonium ions (Elbrächter and Qi, 1998). *Noctiluca scintillans* is mainly a passive ambushing predator. Its distribution along the water column usually seems quite homogeneous. Some authors (Uhlig and Sahling, 1990) think that the formation of

patches at the surface might actually be an early indication of starvation.

This large cosmopolitan species is phagotrophic, feeding on phytoplankton (mainly diatoms and other dinoflagellates) detritus, protozoans, and copepod and fish eggs [Elbrächter and Qi (Elbrächter and Qi, 1998) and references therein; (Nakamura, 1998a; Quevedo *et al.*, 1999; Strom, 2001)]. It is distributed worldwide in cold and warm waters, being common in neritic and coastal regions. *Noctiluca scintillans* is a well-known red-tide organism. Spectacular blooms result from an interaction of biological features (vertical position regulation) and physical concentration mechanisms (currents, upwelling, fronts). Blooms are mainly formed by an accumulation of the buoyant cells on the sea surface

and may be transported by prevailing winds, tides and oceanic currents (Le Fevre and Grall, 1970; Huang and Qi, 1997). *Noctiluca scintillans* red tides frequently form in spring and summer in many parts of the world, often resulting in a bright pinkish red or orange discolouration of the water [(Elbrächter and Qi, 1998) and references therein]. Even if *N. scintillans* forms blooms mostly in spring–summer, in nature it has been reported from temperatures below 0°C up to ~30°C, and in the North Sea off Sylt, Germany, this species is found throughout winter even at temperatures below 0°C (Elbrächter and Qi, 1998). Maximum growth, however, was detected at 24°C, and maximal population densities never occurred if water temperatures were <15°C in the German Bight (Uhlig and Sahling, 1995).

Aggregations causing discolouration of the water (hereafter termed 'bloom') have been reported from Australia (Hallegraeff, 1991), Japan (Montani *et al.*, 1998), Hong Kong (Tang *et al.*, 2003), China (Huang and Qi, 1997) and several European areas: the German Bight (Uhlig and Sahling, 1990; Fock and Greve, 2002), the Cantabrian coast (Quevedo *et al.*, 1999), the Black Sea (Porumb, 1992; Kamburska *et al.*, 2003) and the Adriatic Sea (Sellner and Fonda Umani, 1999) where the water is discoloured red. Recent blooms in New Zealand were reported as being pink with cell concentrations as high as 1.9×10^6 cells L⁻¹ (Chang, 2000). In Australia, blooms were noticed in the coastal waters off Sydney (Dela-Cruz *et al.*, 2002, 2003). In Indonesia, Malaysia and Thailand, however, the water colour is green due to the presence of green prasinophyte endosymbionts (Sweeney, 1978). Toxic blooms of *N. scintillans* have been linked to massive fish and marine invertebrate kills. Although the species does not produce toxins, it has been found to accumulate toxic levels of ammonia, which is then excreted into the surrounding waters, possibly acting as the killing agent in blooms (Okachi and Nishio, 1976; Montani *et al.*, 1998). Deoxygenation of the water column associated with bloom decay is another factor.

In the Gulf of Trieste, there is only one historical record of *N. scintillans*, for November 1902 (Steuer, 1903). In spite of the frequent plankton surveys carried out at the beginning of the 20th century by local researchers (Fonda Umani *et al.*, 1983), there is no plankton record available for the interval between 1903 and 1970. During the 1970s, *N. scintillans* appeared only sporadically; the first red tide was observed in June 1977 (Cassinari *et al.*, 1979; Fonda Umani, 1985).

In the rest of the Adriatic Sea, *N. scintillans* has formed red tides since March 1978 along the western coast, south of the Po river mouth (Emilia Romagna) (Boni, 1983). The most widespread event took place in the

whole Northern Adriatic in the summer of 1980 (Bianchi *et al.*, 1982; Fonda Umani *et al.*, 1983; Malej, 1983). After that, the presence of *N. scintillans* decreased, although it remained a constant component of the heterotrophic local plankton, beside in the period 1993–1997. Since 1997, summer blooms of *N. scintillans* have appeared again, although never reaching a 'red tide' level in the whole Northern Adriatic basin.

In this paper, we report the unusual winter presence of *N. scintillans* during the winter of 2002–2003 in the Gulf of Trieste, and compare all the available historical data of *N. scintillans* presence with temperature, Po river outflow, microphytoplankton abundance and netzooplankton biomass. Furthermore, we present experimental data on growth and grazing efficiency, obtained in the winter of 2002–2003, in an attempt to estimate the theoretical impact on the food web of *N. scintillans*.

METHOD

Sampling area

The Northern Adriatic basin has been recognized as one of the few regions of high permanent production in the Mediterranean Sea (Buljan, 1964; Franco, 1973; Fonda Umani *et al.*, 1992). Biological characteristics of the ecosystem are influenced by the bathymetry, meteorology and hydrodynamics of the area, and by the great river run-off (~20% of the total Mediterranean river run-off) (Fonda Umani *et al.*, 1992; Franco and Michelato, 1992; Russo and Artegiani, 1996). The waters from the Po river, the frequency of North and Northeastern winds (e.g. Bora wind), and the water exchange with the Southern Adriatic have a strong influence on the composition and activity of pelagic communities in the Northern Adriatic (Gilmartin and Revelante, 1981).

The Gulf of Trieste is the most northern and shallowest part of the Adriatic Sea with maximum depths at ~23 m in the southern part. The surface area of the Gulf is ~600 km² with 10% average bottom depth <10 m (Malej and Malacic, 1995), with a volume of 9.5 km³ (Olivotti *et al.*, 1986). The main freshwater input is through the Isonzo River from the north-west coast. The river inputs show a high temporal variability that affects salinity (ranging from 32.7 ± 0.48 to 37.6 ± 0.34 , as average surface values) (Fonda Umani, 1991; Celio *et al.*, 2002). The highest river discharges are generally observed in late spring and autumn, whereas the lowest discharge occurs during winter and summer. However, during these seasons a high inter-annual variability has been observed (Malej *et al.*, 1995). The concentration of inorganic nutrients, mainly due to the river inputs, can be highly variable (e.g. at the surface, between 0.5 and

>30 μM for NO_3) (Cantoni *et al.*, 2003). Allochthonous inputs of phosphates, which range between 0.05 and >3 μM at the surface, are mainly due to the sewage supply, since the discharge from the karstic system is very low (Burba *et al.*, 1994). The temperature shows a regular annual pattern from winter minima as low as 6°C in February to summer maxima at >25°C (Cardin and Celio, 1997).

Sampling

Temperature (and salinity) profiles were acquired by an Idronaut 316 probe. The long-term temperature data set was derived from the literature (Stravisi, 2000). Po river flow rates were kindly provided by the Ruder Boskovic Institute (Center for Marine Research; CMR) of Rovinj.

Noctiluca scintillans and netzooplankton samples were collected biweekly monthly at a station (C1) situated in the Gulf of Trieste (Northern Adriatic Sea), from October 1972 to June 2003 (data for the periods August 1974–April 1976 and 1981–1985 are not available). The station is located at about 200 m offshore at 45°40.06'N, 13°42.60'E (Figure 1B).

Samples were collected by vertical tows with a 0.25 m² sampling area, 200- μm -mesh plankton WP2 net, which covers a total water volume of 3.25–3.75 m³. Samples were preserved with 4% buffered formaldehyde until laboratory analysis. Taxonomic analyses were performed on the total sample or on a significant subsample.

Samples from 1970 to 1996 were dried at 60–110°C until constant weight was obtained (minimum 24 h)

(Lovegrove, 1966; Benovic *et al.*, 1984). A Mettler electrobalance was used to obtain dry weight (DW). The biomass was calculated in mg of DW m⁻³. From 1996 onwards, DW was calculated as part of an elemental analysis performed using a CHN Perkin Elmer analyser, using precombusted GF/F Whatman filters (25 mm).

Phytoplankton samples were simultaneously collected at the same station from March 1986 to April 2003. Samples of surface water were taken using Niskin bottles and fixed with formaldehyde (1% final concentration buffered with CaHCO_3). Samples were processed using sedimentation chambers following the Utermöhl method (Utermöhl, 1958) and observed under a Leitz-Diavert inverted microscope (at $\times 320$). Cells were measured and linear dimensions were converted to cell volumes using standard geometric formulae. Cell volumes were converted to carbon (C) content using standard conversion formulae (Strathmann, 1967; Smayda, 1978). The average C content of *N. scintillans* was determined by CHN analysis as $0.138 \pm 0.020 \mu\text{g C cell}^{-1}$ (Beran *et al.*, 2003).

Phytoplankton productivity was measured by the ¹⁴C technique (Steeman-Nielsen, 1952). Water samples were poured into light and dark 70 mL polycarbonate bottles and 6 μCi of $\text{NaH}^{14}\text{CO}_3$ per bottle were added. The samples were incubated *in situ* for 4 h around noon. At the end of incubation, the samples were filtered on 0.2 μm polycarbonate Whatman Nuclepore filters and the filters were placed in scintillation vials and acidified with a few drops of 5 M HCl to remove residual [¹⁴C]bicarbonate. Scintillation cocktail (5 or 10 mL) was added and

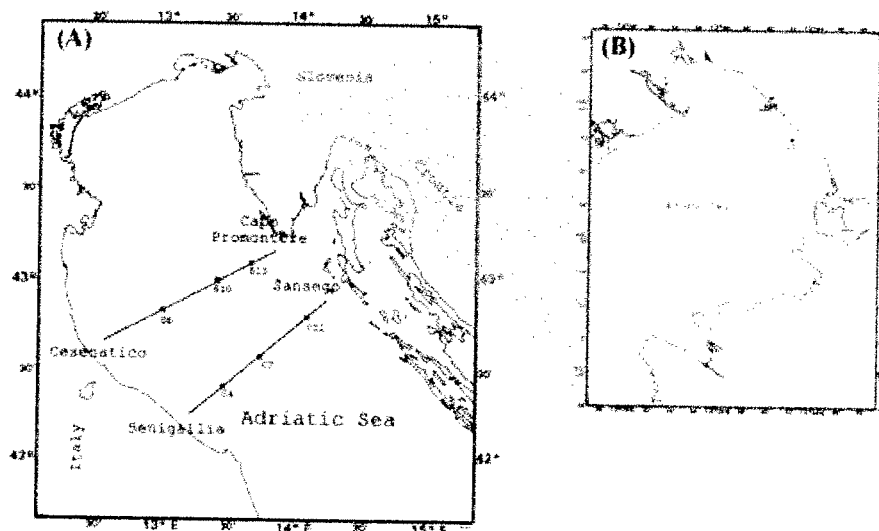


Fig. 1. (A) Position of stations B6, B10, B13 along the transect from Cesenatico to Capo Promontore and C4, C7, C12 along the transect from Senigallia to Sansego in the Northern Adriatic Sea sampled on a monthly base from June 1999 to July 2002 during the MAT Project. (B) Position of station C1 in the Gulf of Trieste (Northern Adriatic Sea).

the activity of the samples was measured on a Canberra TriCarb 2500 scintillation counter. Assimilation of carbon was calculated as described by Gargas, assuming 5% isotope discrimination (Gargas, 1975). The activity of added $\text{NaH}^{14}\text{CO}_3$ and the inorganic carbon concentration (tCO_2) were calculated on the basis of total alkalinity measured in the same samples.

Within the framework of the Progetto Mucillagini Adriatico-Tirreno (MAT Project), netzooplankton (including *N. scintillans*) were collected by vertical tows from the bottom to the surface with the same WP2 net on a monthly basis from June 1999 to July 2002 at six stations located along two transects: from Cesenatico (Italy) to Capo Promontore (Croatia) (transect B, Figure 1A) and from Senigallia (Italy) to Sansego (Croatia) (transect C, Figure 1A).

Grazing experiments

Dilution protocols

Incubations to estimate grazing on the natural assemblage were prepared according to the dilution method (Landry and Hassett, 1982; Landry, 1993). The water for the experiments was collected at site C1 at a depth of 0.5 m and at a temperature of 9°C on 11 March 2003. To eliminate any netzooplanktonic grazers, the samples were transferred into 25 L polypropylene carboys by passing them through a nylon sieve with a 200 µm mesh on board ship. This passage eliminated almost all the cells of *N. scintillans* present in the natural assemblage. The samples were immediately transferred to the laboratory.

The predator- and prey-free water for the dilutions was immediately prepared from the sample water. For the dilution experiments regarding microzooplankton grazing, it was filtered using a filtration ramp (Ø 142 mm; Sartorius) and Durapore HVLP 0.22 µm pore size filters made of hydrophilic fluorocopolymer. All the parts of the ramp in contact with the water are made of teflon. This filtration step eliminates all but some very small bacteria, mostly vibrios. Initial and final samples were taken to check for any possible presence and growth of bacteria in the filtered water, and bacterial numbers were corrected for the error.

Five target concentrations of 20, 40, 60, 80 and 100% (whole water) were prepared and transferred in triplicates of 2.3 L polycarbonate bottles for incubation. During transfer, the water was gently mixed to prevent the formation of clusters of planktonic organisms. Reagent-grade nutrient additions of 5 µM NaNO_3 and 1 µM KH_2PO_4 were added to all the bottles, which were incubated for 24 h in a flowing seawater incubator at corresponding conditions of temperature (9°C) and light. Three bottles of whole water were added without the addition of nutrients

to be able to correct for the influence of nutrients on phytoplankton growth.

Special care was taken to control the success of the dilutions. For all dilutions, two initial samples for any of the investigated parameters (from picoplankton to microzooplankton) were taken. Regression analysis for any of the single parameters measured at the various dilutions against the dilutions had to give significant results for further consideration. Only parameters that passed this test were used for the final elaboration.

The experiments with *N. scintillans* as grazer were run in a further set of three undiluted bottles without nutrients. Cells of *N. scintillans* had been collected using a phytoplankton net (25 µm pore size) at site C1. Immediately on arrival in the laboratory, they were isolated using a Pasteur pipette, counted and transferred to the incubation bottles at a final concentration of 200 cells L^{-1} .

Samples of microzooplankton, phytoplankton, nanoplankton and picoplankton were taken from each bottle after 24 h. This allowed the distinction of different growth and grazing rates between phytoplanktonic groups with the same pigments, and the determination of growth and grazing of non-pigmented heterotrophic species.

Sample analysis

Picoplankton samples were preserved in formaldehyde (2% final concentration) and stained in the dark with DAPI (5 µg mL^{-1} final concentration) following a modification of the method of Porter and Feig (Porter and Feig, 1980). Picoplankton enumeration was carried out under a $\times 100$ oil immersion objective using an Olympus BX50 epifluorescence microscope equipped with a 100 W high-pressure mercury burner.

Nanoplankton were fixed with glutaraldehyde (1% final concentration), stained on the filter with DAPI (5 µg mL^{-1} final concentration) and processed as described by Verity *et al.* (Verity *et al.*, 1993). Nanoplankton were enumerated using an Olympus BX 50 fluorescence microscope with the $\times 100$ oil immersion objective using the corresponding filter cubes and counting at least 100 cells for autotrophs and 100 cells for heterotrophs.

Microphytoplankton samples were processed using sedimentation chambers (Utermöhl, 1958). Where necessary, several chambers were screened. The method of enumeration was adapted to the abundance of the various groups. Abundant cells were counted in defined random fields, rarer species were counted on the whole chamber. At least 100 cells in each dilution of each observed group had to be enumerated for the counts to be considered significant. Cell volumes were converted to carbon content using standard conversion formulae (Strathmann, 1967; Smayda, 1978).

Microzooplankton were analysed in two steps. To be able to distinguish heterotrophic and autotrophic dinoflagellates, 100 mL samples were fixed and processed as described for nanoplankton. Dinoflagellates that were heterotrophic were counted together with the remaining microzooplankton as follows. Samples of 1 L were fixed with formaldehyde (1% final concentration buffered with CaHCO_3) to determine cell abundance (Verity *et al.*, 1996) and pre-concentrated to 100 mL by sedimentation. At least 50 mL of these pre-concentrated samples were used for microscopic analysis using sedimentation chambers and an inverted microscope. At least 100 cells of each observed group or species had to be enumerated for the counts to be considered significant. Samples were processed immediately, and enumeration and determination were concluded within 1 month. Cells were assigned to taxonomic categories, measured and grouped according to size and standardized geometric forms. Cell volume was transformed into carbon content using the formulae of Putt and Stoecker (Putt and Stoecker, 1989).

Culture experiment

Noctiluca scintillans was isolated from net samples at 9°C and cultures were established with *Dunaliella tertiolecta* at 10°C. Cultures had to be acclimatized to the new food for 1 month, as at first there was no growth with the new food source but only survival. The grazing experiment was performed keeping the culture conditions constant to avoid any possible acclimatization effects. Three batch cultures of 50 mL with 100 cells of *N. scintillans* and the equivalent of $\sim 500 \mu\text{g C L}^{-1}$ of food in the form of *D. tertiolecta* were incubated for 4 days. Cells of living *N. scintillans* were counted daily using a Pasteur pipette and resuspended in the cultures, while 5 mL of *D. tertiolecta* were fixed daily for cell counts with an inverted microscope.

Data analysis

Growth and grazing of *N. scintillans* were calculated following Frost in both experiments (Frost, 1972). In the following, these values are called the 'uncorrected values' for the field experiment. *Noctiluca scintillans* may graze on microzooplankton and therefore influence the grazing rates of microzooplankton on phytoplankton. This problem has been considered earlier (Nejstgaard *et al.*, 2001) for grazing experiments with copepods. To be able to correct these values for *N. scintillans*, the growth and grazing of the microzooplankton have to be determined first. Following the general method of Nejstgaard *et al.* (Nejstgaard *et al.*, 2001), the corrected grazing coefficient of *N. scintillans* is:

$$g_{\text{corr. p}} = g_{\text{noc. p}} + k_p$$

where $g_{\text{corr. p}}$ is the corrected grazing coefficient of *N. scintillans* (day^{-1}) for prey p and $g_{\text{noc. p}}$ is the uncorrected grazing coefficient of *N. scintillans* for prey p. k_p is the correction for the loss of microzooplankton grazing on prey p in the bottle with *N. scintillans*. k_p is calculated according to the equation:

$$k_p = g_{\text{mic. p}} [(c - c^*)/c]$$

with

$$c = (c_t - c_0) \ln (c_t/c_0)^{-1}$$

and

$$c^* = (c_t^* - c_0) \ln (c_t^*/c_0)^{-1}$$

$g_{\text{mic. p}}$ is the microzooplankton grazing coefficient for prey p (day^{-1}), obtained from the simultaneous dilution experiment. c is the average carbon concentration of all microzooplankton in the control without nutrients and c^* is the carbon concentration in the bottle with *N. scintillans*, while c_0 is the microzooplankton concentration at the start of the experiment, c_t is the concentration at the end in the control and c_t^* is the concentration at the end with *N. scintillans*. For a detailed discussion, see the original publication (Nejstgaard *et al.*, 2001).

Growth rates of picoplankton, nanoplankton and microphytoplankton, and microzooplankton grazing rates were calculated from Model I regressions of apparent growth against dilution factor (Landry and Hassett, 1982; Landry, 1993). The growth of the prey is described by:

$$C_t = C_0 e^{(k-g)t}$$

where C_0 is the carbon concentration of the prey at the beginning, C_t is the carbon concentration at the end of the experiment (time t), k is the growth coefficient and g is the grazing coefficient. Production (P) and ingestion (I) were calculated as $P = k \times C_m$, and $I = g \times C_m$, where C_m is the average carbon concentration during the incubation:

$$C_m = [P_0 e^{(k-g)t} - P_0]/(k - g)$$

according to Frost (Frost, 1972; Strom and Strom, 1996).

RESULTS

Long- and short-term dynamics

Environmental parameters

The Po river is the main forcing factor of the whole Northern Adriatic system (Franco and Michelato, 1992). River

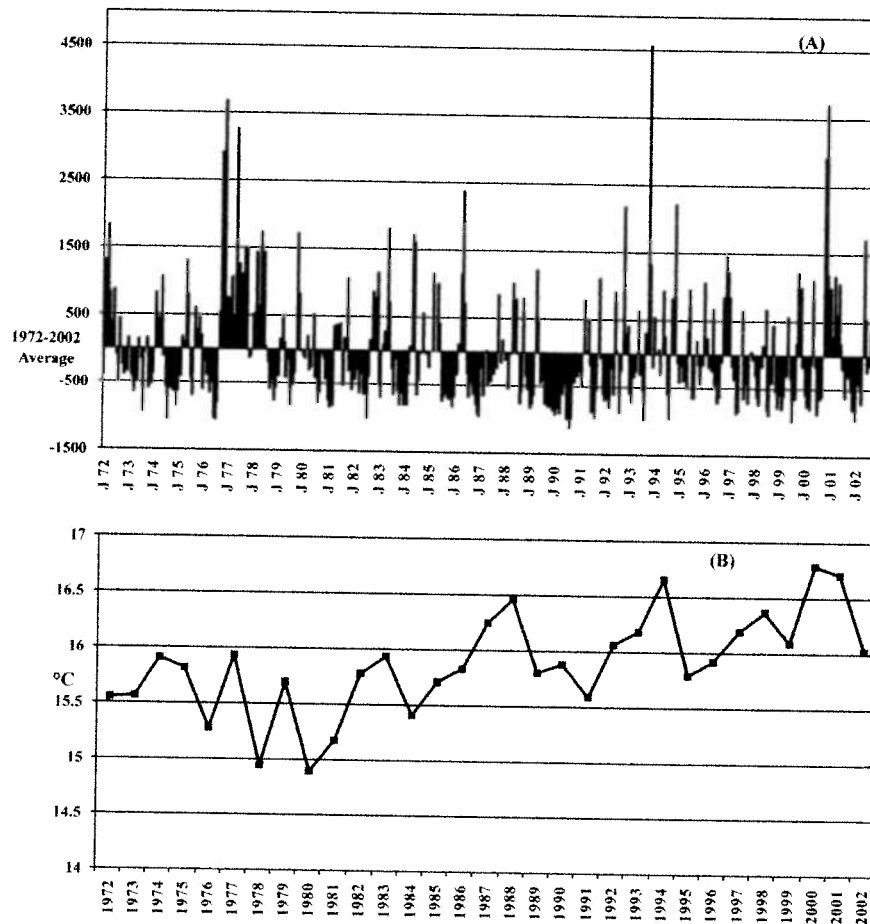


Fig. 2. (A) Bars indicate the monthly deviation for the multiyear (1972–2002) average flow rates of the Po river ($1600 \text{ m}^3 \text{ s}^{-1}$) (kindly provided by R. Precali, CMR, Rovinj). (B) Mean annual surface (2 m) temperature in the Gulf of Trieste from Stravisi (Stravisi, 2000).

outflow rates can be used as a proxy for the hydrodynamics of the whole basin. Figure 2A shows the monthly deviation from the multiyear (1972–2002) average ($1600 \text{ m}^3 \text{ s}^{-1}$). It is evident that a long period of high rates in the late 1970s was followed by a very long period of prevalent low discharge until 1992. The years from 1992 to 1997 are characterized by high and wide month-to-month variations of the river flow rate. The following period, until 2000, is again characterized by a prevalent low discharge and a more homogeneous behaviour. High discharge rates of 2000 were followed by a long period of drought in 2001.

Multiyear monthly mean surface (2 m) temperature in the Gulf of Trieste (Stravisi, 2000) is reported in Figure 2B. The increase by $0.5 \pm 0.26^\circ\text{C}$ in the last 22 years is the most evident pattern of the temporal sequence. Within this general trend, the years from 1988 to 1991 were signed by an inverse pattern and another sharp decrease was evident from 1994 to 1995.

Time series of *N. scintillans*

Temporal dynamics of *N. scintillans* at station C1 in the Gulf of Trieste are shown in Figure 3. In the 1976–2003 data set, excluding red tides, cell numbers ranged from 0 to $58\,880 \text{ individuals (ind.) m}^{-3}$, and revealed high seasonal and inter-annual variability. Until 2002, in winter time, when the seawater surface temperature reaches the lowest annual values (Figure 3A), very low presence (maximum of 300 ind. m^{-3} in 1988) or complete absence of *N. scintillans* was the norm. In spring and summer, a numerical increase took place concomitantly with surface heating. *Noctiluca scintillans* red tides occurred in the Gulf of Trieste in June 1977 and June 1980 ($>10^6 \text{ ind. m}^{-3}$), water masses became slightly red to brown because of the presence of oil in the vacuoles, and the seawater had a gelatinous aspect. Relatively high abundance persisted throughout the spring/summer in 1978 ($>4 \times 10^4 \text{ ind. m}^{-3}$). After the period without data (1980–1986), when, in fact, no red tide was noticed by

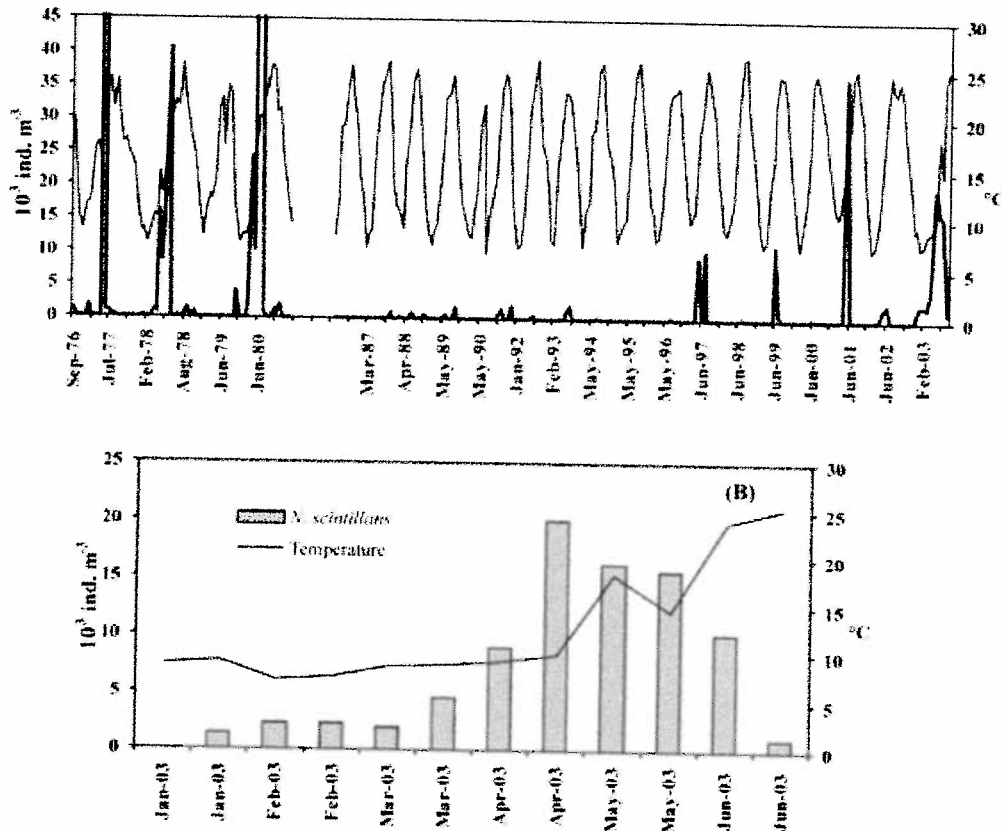


Fig. 3. (A) Temporal dynamics of *N. scintillans* at station C1 in the Gulf of Trieste (raw data); the dashed line represents temperature simultaneously registered at the surface. (B) *Noctiluca scintillans* abundance from January to June 2003.

casual inspection, numbers remained very low until May 1997. The presence of *N. scintillans* was recorded in spring-summer (June 1988, 1989, 1991; July 1992, 1993) and autumn (November 1991); the numbers never exceeded $2 \times 10^3 \text{ ind. m}^{-3}$. *Noctiluca scintillans* was again almost completely absent from summer 1993 to summer 1997. Since May 1997, *N. scintillans* again attained spring-summer maxima $>10 \times 10^3 \text{ ind. m}^{-3}$ in 1997, 1999 and 2001. In summer 2002, the maximum did not exceed $2.2 \times 10^3 \text{ ind. m}^{-3}$. Usually, maxima $>10 \times 10^3 \text{ ind. m}^{-3}$ were recorded at temperatures $>20^{\circ}\text{C}$, with the only exception of May 1999 (temperature = 15.7°C). An increase in abundance followed the late spring temperature rise (Figure 3A). *Noctiluca scintillans* abundance is notably related to temperature ($n = 131$, $r = 0.275$, $P < 0.01$) excluding the winter 2002–2003 data. A highly anomalous presence of *N. scintillans* was recorded from January to June 2003 (Figure 3B), reaching $>2 \times 10^3 \text{ ind. m}^{-3}$ in February 2003, at a temperature of 7.3°C , and causing several patches of discoloured waters in the harbour area of Trieste in December 2002 and February 2003, which alarmed the

local population. The increasing trend was continuous and attained a maximum in April ($19978 \text{ ind. m}^{-3}$) at a temperature of 10.0°C , after which numbers remained high until the end of June, when *N. scintillans* disappeared.

In the 3 year (1999–2002) data set on the whole Northern Adriatic Sea (MAT project), *N. scintillans* varied from 0 to $142\,477 \text{ ind. m}^{-3}$. The highest abundances were always observed in the most coastal stations in April, June–July 2001 and in June–July 2002 (Figure 4). Few specimens were also collected from October to December 2000, never exceeding 300 ind. m^{-3} .

Phytoplankton time series ✓

Total phytoplankton as well as diatoms presented a very high year-to-year variability in the period 1986–2003 (Figures 5 and 6). Mean monthly abundances ranged from 80 cells L^{-1} (June 1994) to $5.3 \times 10^6 \text{ cells L}^{-1}$ (October 2000) and from 900 cells L^{-1} (October 2002) to $7.2 \times 10^6 \text{ cells L}^{-1}$ (January 1994) for diatoms and total phytoplankton, respectively. In the first period (from March 1986 to December 1993), annual phytoplankton

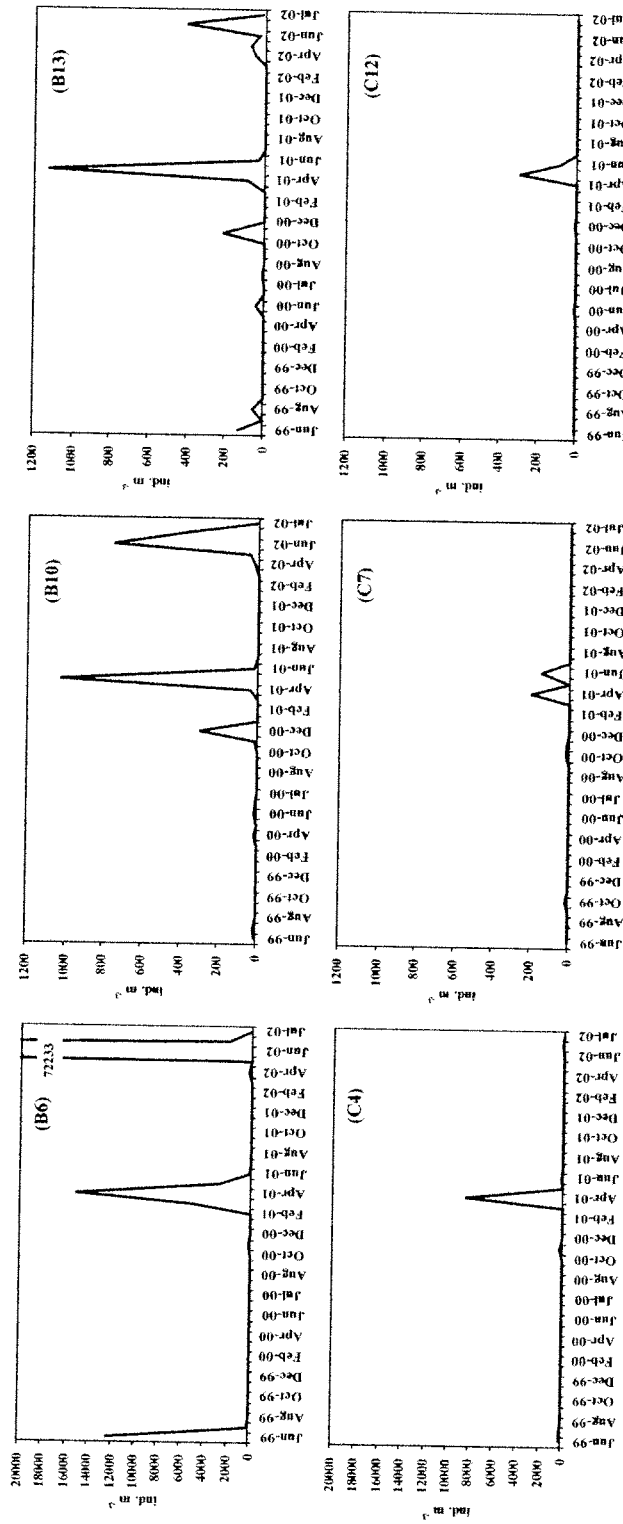


Fig. 4. Abundance of *N. viridula* in samples collected at six stations along the two transects of the M&T Project in the Northern Adriatic Sea on a monthly base from June 1999 to July 2002.

dynamics were characterized by two maxima: the first, more relevant maximum, was registered in spring (February–March), and the second, less intense, one in autumn (October–November). Since 1994, the peaks have been lower; the first ones tended to move to January–February, and in spring of 1996, 1999, 2001, 2002 and 2003 we did not detect any relevant bloom. Autumn maxima were generally even lower, with the only exception of October 2000, when we observed the absolute maximum of the period. Since 1993, a continuous decrease of total phytoplankton was evident due to the decline of both diatoms and other groups. After 1997, a slight increase in diatoms was observed (Figure 6), whereas the other groups (mainly small phytoflagellates and larger dinoflagellates) never attained the same values of the first years of observations. There was no significant relationship of *N. scintillans*

abundance either with total phytoplankton or with diatoms alone.

Netzooplankton time series

The netzooplankton data set (Figure 7) also includes the period from October 1972 to July 1974. The mean monthly biomass (expressed as DW) ranged between 1 mg m^{-3} (January 1977) and 94.78 mg m^{-3} (March 1990). Maxima were generally detected from late spring to summer, with some spring exceptions. The period from 1972 to 1980 was characterized by a relatively low biomass; from 1986 to 1992 we detected higher values, followed by a period until 1998 again signed by lower values with the last years characterized by a new slight increase.

We found an inverse relationship between *N. scintillans* abundance and netzooplankton biomass that was

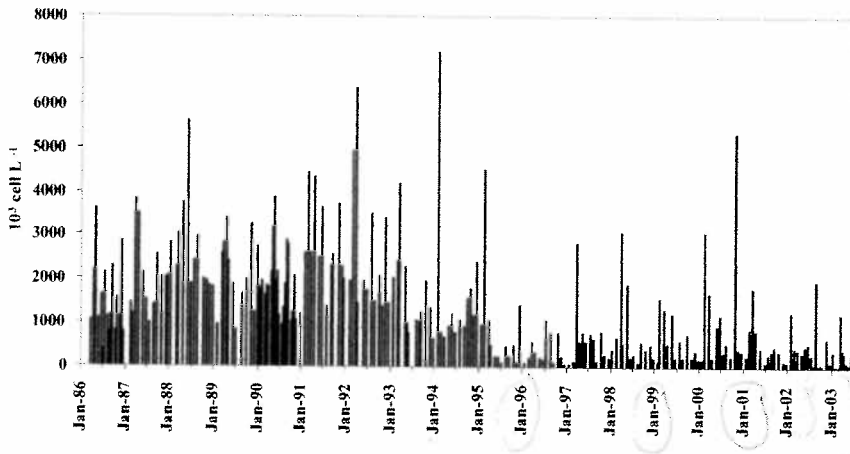


Fig. 5. Temporal dynamics of the monthly average of the microphytoplankton total abundance collected at station C1 in the Gulf of Trieste from 1986 to 2003.

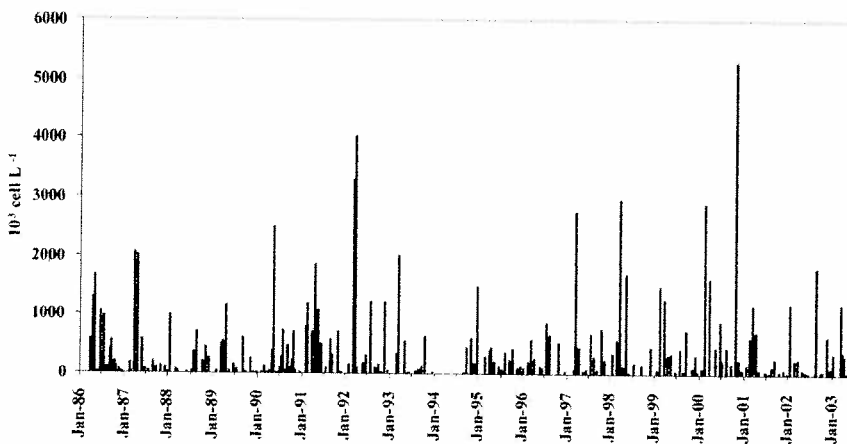


Fig. 6. Temporal dynamics of the diatom monthly average at station C1 in the Gulf of Trieste from 1986 to 16 December 2003.

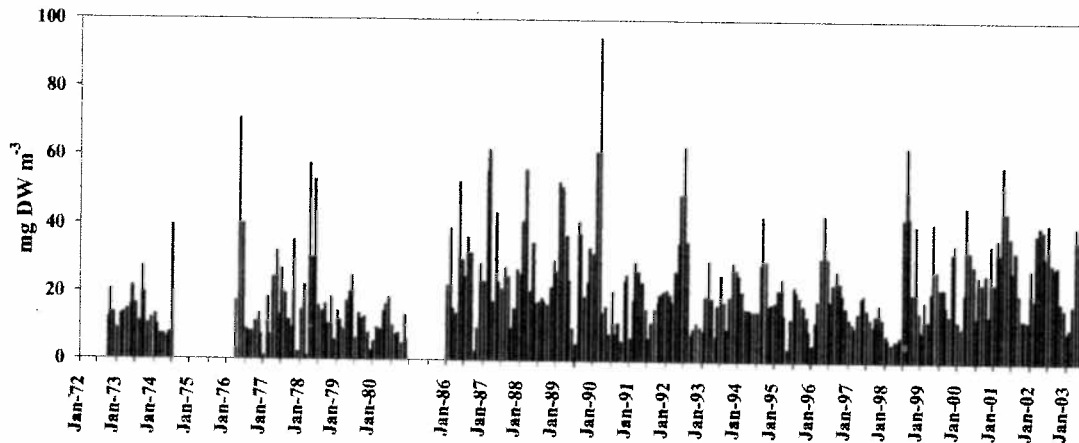


Fig. 7. Temporal dynamics of the monthly average of netzooplankton DW (as mg m^{-3}) collected at station C1 in the Gulf of Trieste in the periods 1972-1974, 1976-1980 and 1986-2003.

significant, although not very high ($n = 102$, $r = 0.198$, $P < 0.05$).

Growth and grazing experiments

Growth rates

The intrinsic growth rate of *N. scintillans* (k) in cultures (Table I) maintained at 10°C was $0.20 \pm 0.04 \text{ day}^{-1}$. In the grazing experiment on the natural assemblage at 9°C , the *N. scintillans* growth rate ($0.17 \pm 0.02 \text{ day}^{-1}$) was very close to that encountered in the culture experiment.

Uncorrected grazing rates

Ingestion obtained in cultures was equal to $0.043 \pm 0.018 \mu\text{g C } Noctiluca^{-1} \text{ day}^{-1}$, which corresponded to a clearance rate of $0.11 \pm 0.04 \text{ mL } Noctiluca^{-1} \text{ day}^{-1}$. Among the natural assemblage, *N. scintillans* exerted a significant grazing on microzooplankton, which was abundant ($12.04 \mu\text{g C L}^{-1}$) (Table II). Average ingestion was equal to $0.008 \pm 0.0003 \mu\text{g C } Noctiluca^{-1} \text{ day}^{-1}$, when larvae of metazoans were excluded (Table II). The clearance was high ($0.87 \pm 0.03 \text{ mL } Noctiluca^{-1} \text{ day}^{-1}$). The ingestion and clearance of metazoans were high but not significant (Table III). The grazing on subgroups of ciliates was usually significant for groups of organisms that occurred in high numbers, e.g. small oligotrichs and the tintinnids *Stenosemella nivalis* and *Tintinnidium mucicola* (Table III). Phytoplankton ($20.89 \mu\text{g C L}^{-1}$) (Table II) were mainly composed of diatoms, namely small *Chaetoceros* spp. ($15.18 \mu\text{g C L}^{-1}$) (Table III) and phytoflagellates ($5.02 \mu\text{g C L}^{-1}$). When the grazing impact of *N. scintillans* was not corrected for the effect of the diminished grazing of the microzooplankton on phytoplankton, caused by the grazing of *N. scintillans* on microzooplankton, ingestion of phytoplankton was low

($0.003 \pm 0.003 \mu\text{g C } Noctiluca^{-1} \text{ day}^{-1}$) and not significant. This would correspond to a clearance of $0.16 \pm 0.15 \text{ mL } Noctiluca^{-1} \text{ day}^{-1}$.

Microzooplankton grazing rates

The removal of microzooplanktonic grazers by *N. scintillans* predation influenced their grazing impact on smaller phytoplankton. To be able to calculate this influence,

Table I: Grazing of *N. scintillans* on *D. tertiolecta*

	1 ^o day	2 ^o day	3 ^o day
Clearance	(mL <i>Noctiluca</i>⁻¹ day⁻¹)		
Experiment A	0.09	0.06	0.16
Experiment B	0.13	0.17	0.05
Experiment C	0.12	0.14	0.10
Average	0.11		
SD	0.04		
Ingestion	($\mu\text{g C } Noctiluca^{-1} \text{ day}^{-1}$)		
Experiment A	0.052	0.028	0.054
Experiment B	0.069	0.065	0.020
Experiment C	0.063	0.056	0.036
Average	0.043		
SD	0.018		
Growth coefficient <i>k</i>			
Experiment A	0.13	0.24	0.21
Experiment B	0.20	0.20	0.27
Experiment C	0.19	0.22	0.16
Average	0.20		
SD	0.04		

SD, standard deviation.

Table II: Grazing of *N. scintillans* on the natural assemblage at station C1, 11 March 2003

	initial C ($\mu\text{g C L}^{-1}$)	SD	Clearance (mL Noc^{-1} day^{-1})	SD	Ingestion ($\mu\text{g C Noc}^{-1}$ day^{-1})	SD	Corrected for grazing on microzooplankton			
							Clearance (mL Noc^{-1} day^{-1})	SD	Ingestion ($\mu\text{g C Noc}^{-1}$ day^{-1})	SD
Total microzooplankton	12.04	1.44	0.92	0.22	0.010	0.003	0.92	0.22	0.010	0.003
Total microzooplankton without Metazoa	9.58	1.10	0.87	0.03	0.008	0.0003	0.87	0.03	0.008	0.0003
Bacillariophyceae	15.87	0.94	0.08	0.20	0.001	0.003	0.39	0.16	0.005	0.003
Phytoflagellates 2–20 μm	5.02	0.02	0.52	0.32	0.002	0.001	0.78	0.26	0.003	0.001
Total phytoplankton	20.89	0.96	0.16	0.15	0.003	0.003	0.47	0.12	0.008	0.003
HNAN	1.18	0.06	0.34	0.37	0.0003	0.0004	0.69	0.31	0.0007	0.0004
Heterotrophic picoplankton	4.29	0.04	0.82	0.29	0.003	0.001	1.37	0.24	0.005	0.001
Total ingestion	35.93	2.16	0.53	0.23	0.014	0.004	0.78	0.19	0.022	0.004

SD, standard deviation; Noc, cell of *N. scintillans*; HNAN, heterotrophic nanoflagellates.

Table III: Grazing of *N. scintillans* on some planktonic subgroups and species at station C1, 11 March 2003

	Initial C ($\mu\text{g C L}^{-1}$)	SD	Clearance (mL Noc^{-1} day^{-1})	SD	Ingestion	SD ($\mu\text{g C Noc}^{-1}$ day^{-1})
<i>Chaetoceros</i> spp. <10 μm	15.18	1.14	-0.03	0.16	-0.0003	0.002
<i>Chaetoceros</i> spp. <10 μm^a	15.18	1.14	0.30	0.13	0.004	0.002
<i>Chaetoceros curvisetus</i>	0.69	0.20	2.12	1.09	0.001	0.0006
Protozoa non-Ciliata	0.82	0.10	1.26	1.04	0.001	0.001
Ciliophora non-Tintinnida	7.23	1.10	0.88	0.29	0.006	0.002
Oligotrichida indet. 20 μm	0.64	0.05	1.65	0.06	0.0008	0.0001
Tintinnida	1.53	0.18	0.58	0.08	0.0009	0.0002
<i>Stenosemella nivalis</i>	1.08	0.12	0.58	0.24	0.0006	0.0003
<i>Tintinnidium mucicola</i>	0.37	0.05	0.59	0.04	0.0002	0.00004
Metazoa	2.46	0.58	1.13	1.44	0.002	0.003

Noc, cell of *N. scintillans*.

^aCorrected for grazing of *N. scintillans* on microzooplankton.

the grazing impact and abundances of microzooplankton on the various phytoplanktonic subgroups need to be known. The results of the dilution experiment to determine the microzooplankton grazing are shown in Table IV. Microzooplankton grazed on all available prey apart from *Chaetoceros* cf. *curvisetus*.

Corrected grazing rates

The correction of ingestion values, following the general method of Njstgaard *et al.* (Njstgaard *et al.*, 2001),

resulted in a more than doubled ingestion ($0.008 \pm 0.003 \mu\text{g C Noctiluca}^{-1} \text{day}^{-1}$) of phytoplankton, which was basically caused by the ingestion of the predominant small *Chaetoceros* spp., which was negative in the uncorrected data set (Table III). Small heterotrophic nanoflagellates were grazed on by *N. scintillans*, but also by microzooplankton (Tables II and IV). The corrected values for ingestion and clearance are $0.0007 \pm 0.0004 \mu\text{g C Noctiluca}^{-1} \text{day}^{-1}$ and $0.69 \pm 0.31 \text{mL Noctiluca}^{-1} \text{day}^{-1}$, respectively. Heterotrophic picoplankton were

Table IV: Grazing of microzooplankton on the natural assemblage, dilution method, 11 March 2003

	k_n	SD	k	SE	g	SE	r^2	initial C ($\mu\text{g C L}^{-1}$)	r^2	Production ($\mu\text{g C L}^{-1}$ day^{-1})	Ingestion ($\mu\text{g C}$ $\text{Mic}^{-1} \text{L}^{-1}$ day^{-1})	%SS _g (% day^{-1})
<i>Chaetoceros</i> cf <i>curvisetus</i>	-0.08	0.17	-0.05	0.12	0.08	0.21	0.01	0.74***	0.77	ns.	ns.	ns.
<i>Chaetoceros</i> spp. < 10 μm	0.35	0.08	0.37	0.06	0.74	0.11***	0.81	15.05***	0.93	4.69	9.29	74
Bacillariophyceae	0.33	0.09	0.35	0.06	0.71	0.11***	0.81	15.79***	0.94	4.71	9.39	71
Phytoplankton 2-20 μm	-0.07	0.05	-0.08	0.08	0.57	0.14***	0.62	4.96***	0.95	-0.31	2.08	57
Total phytoplankton	0.20	0.07	0.22	0.05	0.69	0.09***	0.86	23.38***	0.95	4.07	12.80	69
HNAN	0.43	0.10	0.36	0.09	0.80	0.15***	0.73	1.31***	0.76	0.38	0.85	80
Heterotrophic picoplankton	1.25	0.09	1.27	0.07	1.24	0.12***	0.91	3.66***	0.82	4.70	4.59	124

HNAN, heterotrophic nanoflagellates; k_n , net growth rate; SD, standard deviation; k , growth coefficient; SE, standard error; g , grazing coefficient; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant; Mic, total microzooplankton; %SS_g, % of standing stock grazed.

another important food source for microzooplankton and *N. scintillans*. The values of ingestion were $0.003 \pm 0.001 \mu\text{g C Noctiluca}^{-1} \text{day}^{-1}$ without and $0.005 \pm 0.001 \mu\text{g C Noctiluca}^{-1} \text{day}^{-1}$ with correction for the lost grazing of microzooplanktonic grazers on bacteria. Based on the corrected values, the total ingestion of *N. scintillans* was relatively close to the value obtained in the culture experiment with an average of $0.022 \pm 0.004 \mu\text{g C Noctiluca}^{-1} \text{day}^{-1}$, which equates to $\sim 0.16 \text{ g prey-C g}^{-1} \text{ predator-C day}^{-1}$ given a predator biomass of $0.138 \mu\text{g C}$ per *Noctiluca* cell. This value is slightly less than that required, assuming 100% efficiency, to support the growth rate of 0.17 day^{-1} estimated in the same experiment. This suggests either an underestimate of predation rate and/or an overestimate of predator growth rate; the latter is perhaps more likely.

Grazing impact in the field

We use the corrected ingestion rate to calculate the theoretical grazing impact on the field, assuming that *N. scintillans* in the water column was homogeneously distributed, as reported in the literature (Uhlir and Sahling, 1990), given the fact that in the period there was no relevant accumulation at the surface at the sampling site.

Based on the numbers of *N. scintillans* derived from field observation and assuming the ingestion rate obtained in the experiments as constant, we estimated the theoretical grazing impact of *N. scintillans* on phytoplankton and on primary production (Table V) for the period January-June 2003. The theoretical total ingestion

of phytoplankton by *N. scintillans* varied from 0.096×10^{-3} up to $0.16 \mu\text{g C L}^{-1} \text{day}^{-1}$. Consequently, the theoretical grazing impact of *N. scintillans* on phytoplankton biomass can vary from insignificant values (January) up to >2% per day (May), while grazing can remove in the same month up to 1.85% of the daily primary production, which varied from an integrated value of $0.84 \mu\text{g C L}^{-1} \text{day}^{-1}$ in January up to $18.5 \mu\text{g C L}^{-1} \text{day}^{-1}$ in June.

DISCUSSION

Time series

It has frequently been discussed that long-term trends in the increased frequency of harmful algal blooms might be associated with nutrient enrichment of coastal waters (Smayda, 1990). *Noctiluca scintillans* red tides in the 1970s were related to the increased eutrophication of the Northern Adriatic Sea (Boni, 1983). At that time, almost every year along the western coast (Emilia Romagna), and less frequently in other northern Adriatic coastal areas, red tides caused by several different dinoflagellates were reported (Sellner and Fonda Umani, 1999). In the meantime, nutrient loading by the Po river increased significantly, reaching the highest concentration in the early 1980s (Harding *et al.*, 1999). In the summer of 1980, we observed the most widespread *N. scintillans* red tide, which affected almost the entire Northern Adriatic basin (Sellner and Fonda Umani, 1999).

Table V: Theoretical grazing impact of *N. scintillans* on phytoplankton and on primary production from January to June 2003

	<i>N. scintillans</i> (cells L ⁻¹)	Ingestion (μg C L ⁻¹ day ⁻¹)	Primary production (μg C L ⁻¹ day ⁻¹)	%PP _g (% day ⁻¹)	Total phytoplankton (μg C L ⁻¹)	%PC _g (% day ⁻¹)
Jan-03 I	0.012	0.00010	9.448	0.001	92.08	0.0001
Jan-03 II	1.333	0.01066	0.837	1.274	3.1	0.3403
Feb-03 I	2.336	0.01869	5.158	0.362	5.3	0.3546
Feb-03 II	2.279	0.01823	1.987	0.917	11.9	0.1532
Mar-03 I	2.008	0.01606	12.184	0.132	172.9	0.0093
Mar-03 II	4.494	0.03595	9.820	0.366	59.9	0.0600
Apr-03 I	8.933	0.07146	6.937	0.800	52.2	0.1369
Apr-03 II	19.978	0.15982	18.768	0.852	29.6	0.5399
May-03 I	16.256	0.13005	7.037	1.848	5.8	2.2422
May-03 II	15.904	0.12723	10.663	1.193	9.4	1.3535
Jun-03 I	15.552	0.12442	18.502	0.672	33.5	0.3714
Jun-03 II	10.171	0.08137	8.452	0.963	47.2	0.1725

Ingestion is the calculated ingestion of the total *N. scintillans* population in the water column, averaged at m⁻³. %PP_g, % of primary production grazed; %PC_g, % of phytoplankton carbon grazed by *N. scintillans*.

After 1988, the phosphate concentration in the seawater decreased sharply, due to the decrease and banning by Italian law of phosphorus in detergents. At the end of the 1980s, we observed a shift from red tides to the mucilage phenomena that affected the whole basin in 1988, 1989 and 1991 (Degobbi *et al.*, 1995, 1999). In this period, *N. scintillans* numbers were very low.

From 1986 until the end of 1992, phytoplankton density was quite high, although there was a significant decrease of the size spectrum from 1989 onwards (Fonda Umani *et al.*, 1995). Afterwards, phytoplankton abundance declined to reach minimal values at the beginning of 1995; a similar decrease was evident for the zooplankton too, and in this period *N. scintillans* almost completely disappeared. There is evidence that the whole plankton system changed in the 1990s throughout the Northern Adriatic: Krsinic and Precali reported the occurrence of some oceanic tintinnids in the Northern Adriatic basin in autumn 1993 due to the intrusion of high-salinity and oligotrophic waters from the south (Krsinic and Precali, 1997). This intrusion of Modified Levantine Intermediate Waters (MLIW) can be seen as a sign of a more widespread change in current intensity and direction that affected the whole eastern Mediterranean after 1987, the well known Eastern Mediterranean Transient (Roether *et al.*, 1996), which caused the shift of sources of Eastern Mediterranean deep waters from the Adriatic to the deep waters of the Aegean Sea. The recently observed salinity decrease in the inflow current from the Aegean Sea allowed intrusions to reach the northern part of the Adriatic Sea (Gulf of Trieste included) due to

the less pronounced differences in density (Demirov and Pinardi, 2002). Po river outflow was characterized by low discharges in the late 1980s and a very impulsive pattern at the beginning of the 1990s. Low discharge rates mean a decline of nutrient inputs, as well as an impulsive outflow pattern, which generates a rapid export of the river waters along the western coast inside the frontal system, not allowing any enrichment of the offshore area, hence determining an equal decrease in nutrient availability in the whole system. We can hypothesize that the observed dramatic changes in plankton dynamics in the mid-1990s are the result of both climatic changes and environmental protection increase that lowered the trophic state of the system.

Netzooplankton biomass showed an initial period of low abundance until 1980, which has already been associated with jellyfish (*Pelagia noctiluca*) and *N. scintillans* blooms (Cataletto *et al.*, 1995). *Noctiluca scintillans* indeed seems to be inversely related to netzooplankton biomass, indicating a possible competition for food resources.

In the last period (1994-2003), phytoplankton never attained the same high abundances of the former years, despite the slight increase in diatoms, due to the almost complete lack of small phytoflagellates and larger dinoflagellates that were numerically important in the early 1980s.

Surprisingly, since 1997, *N. scintillans* has started to appear again in the summer with some high numbers in the whole Northern Adriatic, causing some patches of discoloured water. In this instance, we definitely cannot relate its presence to a state of eutrophication, given the

fact that the entire Northern Adriatic was and still is following a decreasing trophic trend (Malej and Fonda Umani, 1998). *Noctiluca scintillans* was, however, more abundant in the coastal western area of the Northern Adriatic under the strong influence of the Po river, which enhances plankton productivity and particulate matter concentration.

Even more surprising was the continuous presence of *N. scintillans* during the winter of 2002–2003, which attained a maximum at a very low temperature (10°C), whereas in the past it had always been significantly associated with higher temperatures.

Growth and grazing

Growth rates obtained in culture with high food concentration as well as in the grazing experiment on natural assemblage run at 9–10°C were similar and close to values found by Nakamura (Nakamura, 1998a) (from 0.02 up to 0.28 day⁻¹, average 0.17 day⁻¹) at temperatures ranging between 24 and 25.5°C with variable natural food concentrations. They were also close to the growth rates obtained by Nakamura with monospecific phytoplankton cultures at 24°C (Nakamura, 1998b). Our growth rates were lower than those reported by Buskey for experiments at very high food concentrations (diatoms) at 20°C (Buskey, 1995). The grazing experiment on natural assemblage resulted in a total ingestion rate of 0.022 µg C *Noctiluca*⁻¹ day⁻¹. To support a growth rate of 0.2 day⁻¹ in the culture, ingestion was equal to 0.04 µg C *Noctiluca*⁻¹ day⁻¹, which means that *N. scintillans* in the field must have either exploited other resources (e.g. detritus) not included in our experimental protocol or that the natural assemblage was a richer food source than the monospecific phytoplankton offered in culture. In both experiments, *N. scintillans* was able to grow at a temperature close to the lowest limit set up by previous authors (Uhlir and Sahling, 1995) with a growth rate of 0.2 day⁻¹ and at a not particularly high concentration of picoplankton, nanoplankton and microplankton (35.93 µg C L⁻¹) in the field.

Analysing the qualitative grazing on microzooplankton, nanoplankton and picoplankton, we detected a significant predation on microzooplankton as well as on heterotrophic nanoflagellates and on bacteria. To the best of our knowledge, this is the first experimental evidence of a significant grazing impact of *N. scintillans* on natural heterotrophic assemblage <200 µm. In the Gulf of Trieste, the microzooplankton grazing impact on microphytoplankton and particularly on nanoplankton and picoplankton components is very important (Fonda Umani and Beran, 2003). *Noctiluca scintillans* can graze on both autotrophic and heterotrophic fractions, thus in a way the species can compete with netzooplankton predators by removing suitable prey items and, on the other

hand, it can decrease the grazing pressure of microzooplankton on smaller prey and lower the mortality rates of the smallest planktonic fractions.

Although we observed high ingestion of *N. scintillans* for prey of size <30 µm, as stated by various authors (Kjørboe and Titelman, 1998; Tiselius and Kjørboe, 1998), we found at the same time significant ingestion of picoplankton by *N. scintillans*. There are indications in the literature that *N. scintillans* might feed on bacteria. Gaines and Elbrächter mention ingestion of bacteria from the surface film by *N. scintillans* in cultures (Gaines and Elbrächter, 1987). Kirchner *et al.* found *N. scintillans* able to remove bacteria-sized microspheres and bacteria in culture experiments, and discuss the ingestion of bacteria in the field (Kirchner *et al.*, 1996). Smith and Staroscik found a large portion of the bacterial standing stock missing in the presence of a bloom of *N. scintillans* and the bacterial community structure altered (Smith, D. C. and Staroscik, A. M., submitted). While these publications indicate that grazing on picoplankton in the field is likely, the results of our experiment give the first quantitative estimates of the grazing impact of *N. scintillans* on bacteria in natural conditions. We want to emphasize that in this instance heterotrophic picoplankton accounted for nearly a quarter of the total ingestion (Table II).

To calculate the grazing impact exerted by *N. scintillans* on phytoplankton and primary production, we assumed the grazing rate obtained at 9°C as constant. This is a very conservative assumption because of the increase in metabolic needs with rising temperature. Thus, our calculations of the maximal impact in May (>2% of phytoplankton standing stock and 1.85% of daily primary production) must be considered an underestimate. In any case, the direct impact of *N. scintillans* on autotrophic fractions <200 µm does not appear to be very important. More important is the predation on microzooplankton, which can decrease the mortality induced on phytoplankton by the microzooplankton by 16%. The latter, in turn, can account for the removal of 70% of the phytoplankton standing stock (this experiment) and up to >100% of the phytoplankton potential production (e.g. May 1999) (Fonda Umani and Beran, 2003). The direct and indirect mortality induced on heterotrophic bacteria must be considered equally important.

To conclude, from our field and experimental observations we obtained three main results.

1. In the 1970s, blooms of *N. scintillans* in the Adriatic were related to a state of eutrophication. In the 1990s, characterized by decreasing phosphate levels combined with decreasing eutrophication, *N. scintillans* was present but never bloomed until 1997. The

presence of *N. scintillans* after 1997 does not seem to be related to eutrophication as the trophic level of the Northern Adriatic system continued to decrease.

- Noctiluca scintillans* in the Gulf of Trieste was able to reproduce actively at low temperatures. The winter presence of *N. scintillans* in this area may be due to the settlement of a new 'cold' strain, which was able to grow at temperatures as low as 6.5°C.
- Noctiluca scintillans* was able to live on various planktonic fractions, which range from microzooplankton to bacteria. Showing consistent growth during the atypical outbreak in winter 2001/2002, the species grazed in equal parts on microzooplankton, phytoplankton and heterotrophic picoplankton, living basically on the heterotrophic fractions. Grazing of *N. scintillans* on microzooplankton can significantly change the grazing pressure of this group on other planktonic groups such as phytoplankton or picoplankton.

ACKNOWLEDGEMENTS

We would like to thank all the technical and scientific staff at LBM for field sampling and for supplying ancillary data. Thanks are due also to some of S.F.U.'s students who analysed some subsets of samples. Two anonymous reviewers provided thoughtful comments on an earlier version of the manuscript. The study was funded by the European Community and the Friuli Venezia Giulia Region INTERREG 2 and 3 Projects for the years 1998–2003, by the Italian Ministry of Environment for the years 1990–1998 in the Gulf of Trieste and under the umbrella of the MAT Project for the years 1999–2001 in the Northern Adriatic basin.

REFERENCES

- Benovic, A., Fonda Umani, S., Malej, A. and Specchi, M. (1984) Net-zooplankton biomass of the Adriatic Sea. *Mar. Biol.*, **79**, 209–218.
- Beran, A., Guardiani, B., Tamberlich, F., Kamburska, L. and Fonda Umani, S. (2003) Carbon content and biovolume of the heterotrophic dinoflagellate *Noctiluca scintillans* from the Northern Adriatic Sea. Proceedings of the CESUM-BS 2003, Varna. Book of Abstracts, 28.
- Bianchi, F., Comaschi Scaramuzza, A., Lombardo, A. and Socal, G. (1982) Note sulla presenza di *Noctiluca scintillans* (Macartney) nel Golfo di Venezia. Aprile 1980. *Ist. Veneto Sci. Lett. Arti. Rapp. Stud.*, **8**, 121–132.
- Boni, L. (1983) Red tide of the coast of Emilia Romagna (north-western Adriatic sea) from 1975 to 1982. *Inform. Bot. Ital.*, **15**, 18–23.
- Buljan, M. (1964) An estimate of productivity of the Adriatic Sea made on the basis of its hydrographic properties. *Acta Adriat.*, **11**, 35–45.
- Burba, N., Cabrimi, M., Del Negro, P., Fonda Umani, S. and Milani, L. (1994) Variazioni stagionali del rapporto N/P nel Golfo di Trieste. *Atti X Congresso AIOL, Alassio*, 333–344.
- Buskey, E. J. (1995) Growth and bioluminescence of *Noctiluca scintillans* on varying algal diets. *J. Plankton Res.*, **17**, 29–40.
- Cantoni, C., Cozzi S., Pecchiari, I., Cabrimi, M., Mozetic, P., Catalano, G. and Fonda Umani, S. (2003) Primary production and inorganic nitrogen uptake in a shallow coastal sea (Gulf of Trieste, Northern Adriatic Sea). *Oceanol. Acta*, **26**, 565–575.
- Cardin, V. and Celio, M. (1997) Cluster analysis as a statistical method for identification of the water bodies present in the Gulf of Trieste (Northern Adriatic Sea). *Boll. Geof. Teor. Appl.*, **38** (1/2), 119–135.
- Cassinari, E., Grillo, D., Princi, M., Specchi, M., Stravisi, F. and Valli, G. (1979) Osservazioni su *Noctiluca miliaris* (Suriray) del Golfo di Trieste. *Atti Com. Sc. Naz. P. F. Oceanografia e fondi marini*. Roma marzo 1979, 1–8.
- Cataletto, B., Feoli, E., Fonda Umani, S. and Yong, S. C. (1995) Eleven years netzooplankton community in the Gulf of Trieste: time series analysis. *ICES J. Mar. Sci.*, **52**, 669–678.
- Celio, M., Comici, C. and Bussani, A. (2002) Thermohaline anomalies in the spring and early summer of 2000 in the Gulf of Trieste. *P.S. N. Mar. Ecol.*, **23**, 101–110.
- Chang, F. H. (2000) Pink blooms in the springs in Wellington Harbour. *Aquacult. Update*, **24**, 10–12.
- Degobbi, D., Fonda Umani, S., Franco, P., Malej, A., Precali, R. and Smodlaka, N. (1995) Changes in the northern Adriatic ecosystem and hypertrophic appearance of gelatinous aggregates. *Sci. Total Environ.*, **165**, 43–58.
- Degobbi, D., Smodlaka, N., Pojed, I., Skrivanic, A. and Precali, R. (1979) Increased eutrophication of the Northern Adriatic Sea. *Mar. Pollut. Bull.*, **10**, 298–301.
- Degobbi, D., Malej, A. and Fonda Umani, S. (1999) The mucilage phenomenon in the Northern Adriatic: a critical review of the present scientific hypotheses. *Ann. Ist. Super. San.*, **35**, 373–381.
- Dela-Cruz, J., Ajani, P., Lee, R., Pritchard, T. and Suthers, I. (2002) Temporal abundance patterns of the red tide dinoflagellate, *Noctiluca scintillans*, along the south-east coast of Australia. *Mar. Ecol. Prog. Ser.*, **236**, 75–88.
- Dela-Cruz, J., Middleton, J. H. and Suthers, J. M. (2003) Population growth and transport of the red tide dinoflagellate, *Noctiluca scintillans*, in the coastal waters off Sydney Australia, using cell diameter as a tracer. *Limnol. Oceanogr.*, **48**, 656–674.
- Demirov, E. and Pinardi, N. (2002) Simulation of the Mediterranean Sea circulation from 1979 to 1993: Part I. The interannual variability. *J. Mar. Syst.*, **33–34**, 23–50.
- Elbrächter, M. and Qi, Y. Z. (1998) Aspects of *Noctiluca* (Dinophyceae) population dynamics. In Anderson, D. M. et al. (eds), *Physiological Ecology of Harmful Algal Blooms. NATO ASI Series. Vol. G 41*. Springer-Verlag, Berlin, pp. 315–335.
- Fock, H. O. and Greve, W. (2002) Analysis and interpretation of current spatio-temporal patterns in zooplankton dynamics: a case study on *Noctiluca scintillans* (Dinophyceae) in German Bight (North Sea). *Mar. Biol.*, **140**, 59–73.
- Fonda Umani, S. (1985) Hydrology and 'red tides' in the Gulf of Trieste (North Adriatic). *Oebalia*, **11**, 141–147.
- Fonda Umani, S. (1991) General features of the planktonic system in the Gulf of Trieste and their variations in the last decade. *MAP Tech. Rep.*, **47**, 82–101.
- Fonda Umani, S. and Beran, A. (2003) Seasonal variations in the dynamics of microbial plankton communities: first estimates from experiments in the Gulf of Trieste, Northern Adriatic Sea. *Mar. Ecol. Prog. Ser.*, **247**, 1–16.

- Fonda Umani, S., Princi, M. and Specchi, M. (1983) Note ecologiche su *Noctiluca miliaris* Suriray del Golfo di Trieste (Alto Adriatico). *Atti Mus. Civ. Sci. Nat. Trieste*, **35**, 259-265.
- Fonda Umani, S., Franco, P., Ghirardelli, E. and Malej, A. (1992) Outline of oceanography and the plankton the Adriatic Sea. In Colombo, G., Ferrari, L., Ceccherelli, V. U. and Rossi, R. (eds), *Marine Eutrophication and Population Dynamics*. Proceedings of the 25th EMBS, pp. 347-365.
- Fonda Umani, S., Yong, S. C., Feoli, E., Cataletto, B., Cabrini, M. and Milani, L. (1995) Is it possible to identify any plankton succession in the Gulf of Trieste (Northern Adriatic Sea)? In Eleftheriou, A., Ansell, A. D. and Smith, C. J. (eds), *Biology and Ecology of Shallow Coastal Waters*. Proceedings of 28th EMBS, pp. 59-65.
- Franco, P. (1973) L'influenza del Po sui caratteri oceanografici e sulla distribuzione della biomassa planctonica nell'Adriatico Settentrionale. *Ann. Univ. Ferrara NS*, **1**, 95-117.
- Franco, P. and Michelato, A. (1992) Northern Adriatic Sea: oceanography of the basin proper and of the western coastal zone. In Vollenweider, R. A., Marchetti, R. and Viviani, R. (eds), *Marine Coastal Eutrophication. Sci. Total Environ., Suppl.*, 35-62.
- Frost, B. W. (1972) Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.*, **17**, 805-815.
- Gaines, G. and Elbrächter, M. (1987) Heterotrophic nutrition. In Taylor, F. J. R. (ed.), *The Biology of Dinoflagellates*. Blackwell Scientific, Oxford, pp. 224-268.
- Gargas, E. (1975) A manual for phytoplankton primary production studies in the Baltic. In Gargas, E. (ed.), *The Baltic Marine Biologists*, Vol. 2, pp. 1-88.
- Gilmartin, M. and Revelante, N. (1981) Regional variations in phytoplankton standing crops in the Northern Adriatic Sea. *Rapp. Comm. Int. Mer. Médit.*, **27**, 85-87.
- Hallegraeff, G. M. (1991) *Aquaculturists Guide to Harmful Australian Microalgae*. Fishing Industry Training Board of Tasmania/CSIRO Division of Fisheries, Hobart, pp. 1-111.
- Harding, L. W., Degobbi, D. and Precaji, R. (1999) Production and fate of phytoplankton: annual cycles and interannual variability. In Malone, T., Malej, A., Harding, L. W., Smoljaka, N. and Turner, R. E. (eds), *Ecosystem at the Land-Sea Margin: Drainage Basin to Coastal Sea. Coastal Estuarine Stud.*, **55**, 131-172.
- Huang, C. and Qi, H. (1997) The abundance cycle and influence factors on red tide phenomena of *Noctiluca scintillans* (Dinophyceae) in Dapeng Bay, the South China Sea. *J. Plankton Res.*, **19**, 303-318.
- Kamburska, L., Doncheva, K. and Stefanova, K. (2003) On the recent changes of zooplankton community structure along the Bulgarian Black Sea coast - a post-invasion effect of exotic ctenophores interactions. Proceedings of the First International Conference on Environmental Research and Assessment. Bucharest, Romania, March 2003, 23-27.
- Kjørboe, T. and Fitelman, J. (1998) Feeding, prey selection and prey encounter mechanisms in the heterotrophic dinoflagellate *Noctiluca scintillans*. *J. Plankton Res.*, **20**, 1615-1636.
- Kirchner, M., Sahling, G., Uhlig, G., Gunkel, W. and Klings, K. W. (1996) Does the red tide-forming dinoflagellate *Noctiluca scintillans* feed on bacteria? *Sarsia*, **81**, 45-55.
- Krsinic, F. and Precaji, R. (1997) On the occurrence of oceanic tintinnines with particular consideration of the species *Amphorides laackmanni* (Jørgensen, 1924) (Ciliophora, Oligotrichida, Tintinnida) in the northern Adriatic Sea. *P.S.Z.N. Mar. Ecol.*, **18**, 67-81.
- Landry, M. R. (1993) Estimating rates of growth and grazing mortality of phytoplankton by the dilution method. In Kemp, P. F., Sherr, B., Sherr, E. and Cole, J. J. (eds), *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Ann Arbor, MI, pp. 715-772.
- Landry, M. R. and Hassett, R. P. (1982) Estimating the grazing impact of marine microzooplankton. *Mar. Biol.*, **67**, 283-288.
- Le Fevre, J. and Grall, J. R. (1970) On the relationships of *Noctiluca* swarming off the western coast of Brittany with hydrological features and plankton characteristics of the environment. *J. Exp. Mar. Biol. Ecol.*, **4**, 287-306.
- Lovegrove, T. (1966) The determination of the dry weight of the plankton and the effect of various factors on the values obtained. In Barnes, H. (ed.), *Some Contemporary Studies in Marine Science*. Allen and Unwin, London, pp. 429-467.
- Malej, A. (1983) *Noctiluca miliaris* Suriray red tide in the Gulf of Trieste. *Thalassia Jugosl.*, **19**, 261-269.
- Malej, A. and Fonda Umani, S. (1998) Evoluzione delle interazioni trofiche nell'ecosistema del Golfo di Trieste. Atti Convegno 'Evoluzione dello stato trofico in Adriatico: analisi degli interventi attuati e future linee di intervento' Marina di Ravenna 28-29 settembre 1995. Reg. Emilia Romagna Prov. Ravenna Autorità di bacino del fiume PO (eds), pp. 61-70.
- Malej, A. and Malacic, V. (1995) Factors affecting bottom layer oxygen depletion in the Gulf of Trieste (Adriatic Sea). *Annales*, **7**, 33-42.
- Malej, A., Mozetic, P., Malacic, P., Terzic, S. and Ahel, M. (1995) Phytoplankton responses to freshwater inputs in a small semi-enclosed gulf (Gulf of Trieste, Adriatic Sea). *Mar. Ecol. Prog. Ser.*, **120**, 111-121.
- Montani, S., Pithakpol, S. and Tada, K. (1998) Nutrient regeneration in coastal seas by *Noctiluca scintillans*, a red tide-causing dinoflagellate. *J. Mar. Biotechnol.*, **6**, 224-228.
- Nakamura, Y. (1998a) Biomass, feeding and production of *Noctiluca scintillans* in the Seto Inland Sea, Japan. *J. Plankton Res.*, **20**, 2213-2222.
- Nakamura, Y. (1998b) Growth and grazing of a large heterotrophic dinoflagellate, *Noctiluca scintillans*, in laboratory cultures. *J. Plankton Res.*, **20**, 1711-1720.
- Nejstgaard, J. C., Naustvoll, L. J. and Sazhin, A. (2001) Correcting for underestimation of microzooplankton grazing in bottle incubation experiments with mesozooplankton. *Mar. Ecol. Prog. Ser.*, **221**, 59-75.
- Okaichi, T. and Nishio, S. (1976) Identification of ammonia as the toxic principle of red tide of *Noctiluca miliaris*. *Bull. Plankton Soc. Jpn.*, **23**, 75-80.
- Olivotti, R., Faganeli, J. and Malej, A. (1986) Impact of organic pollutants on coastal waters. *Water Sci. Technol.*, **18**, 57-68.
- Porter, K. G. and Feig, Y. S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**, 943-948.
- Porumb, F. (1992) Evolution du zooplankton des eaux du plateau continental Roumain de la mer Noire au cours de trois decennies. *Rapp. Comm. Int. Mer. Médit.*, **33**, 266.
- Putt, M. and Stoecker, D. K. (1989) An experimentally determined carbon:volume ratio for marine 'oligotrichous' ciliates from estuarine and coastal waters. *Limnol. Oceanogr.*, **34**, 1097-1103.
- Quevedo, M., González-Quirós, R. and Anadón, R. (1999) Evidence of heavy predation by *Noctiluca scintillans* on *Acartia clausi* (Copepoda) eggs off the central Cantabrian coast (NW Spain). *Oceanol. Acta*, **22**, 127-131.

- Roether, W., Manca, B., Klein, B., Bregant, D., Georgopoulos, D., Beitzel, V., Kovacevic, V. and Luchetta, A. (1996) Recent changes in Eastern Mediterranean deep waters. *Science*, **271**, 333-335.
- Russo, A. and Artegiani, A. (1996) Adriatic Sea hydrography. In Palomera, I. and Rubies, P. (eds), *The European Anchovy and its Environment. Sci. Mar.*, **60**, 33-43.
- Sellner, K. G. and Fonda Umani, S. (1999) Dinoflagellate blooms and mucilage production. In Malone, T., Malej, A., Harding, L. W., Smolilaka, N. and Turner, R. E. (eds), *Ecosystem at the Land-Sea Margin: Drainage Basin to Coastal Sea. Coastal Estuarine Stud.*, **55**, 173-206.
- Smayda, T. J. (1978) From phytoplankton to biomass. In Sourin (ed.), *A Phytoplankton Manual*. UNESCO, Paris, pp. 273-279.
- Smayda, T. J. (1990) Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. In Graneli, E. et al. (eds), *Toxic Marine Phytoplankton*. Elsevier, Amsterdam, pp. 29-40.
- Smith, D. C. and Staroscik, A. M. (2004) Field evidence for the selective feeding of the heterotrophic dinoflagellate *Noctiluca scintillans* on bacteria. *Limnol. Oceanogr.*, submitted.
- Steeman-Nielsen, E. (1952) The use of radioactive carbon (^{14}C) for measuring organic production in the sea. *J. Cons. Perm. Int. Explor. Mer*, **18**, 117-140.
- Steuer, A. (1903) Beobachtungen über das Plankton des Triester Golfes im Jahre 1901. *Zool. Anz.*, **27**, 145-148.
- Strathmann, R. R. (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnol. Oceanogr.*, **12**, 411-418.
- Stravisi, F. (2000) La temperatura del mare a Trieste 1964-1999. *Hydros Inform.*, **20**, 6-7.
- Strom, S. L. (2001) Light-aided digestion, grazing and growth in herbivorous protists. *Aquat. Microb. Ecol.*, **23**, 253-261.
- Strom, S. L. and Strom, M. W. (1996) Microplankton growth, grazing, and community structure in the northern gulf of Mexico. *Mar. Ecol. Prog. Ser.*, **130**, 229-240.
- Sweeney, B. M. (1978) Ultrastructure of *Noctiluca miliaris* (Pyrrophyta) with green symbionts. *J. Phycol.*, **14**, 116-120.
- Tang, D. L., Kester, D. R., I-Hsun, N., YuZao, Q. and Kawamura, H. (2003) *In situ* and satellite observations of a harmful algal bloom and water condition at the Pearl River estuary in late autumn 1998. *Harmful Algae*, **2**, 89-99.
- Tiselius, P. and Kiorboe, T. (1998) Colonization of diatom aggregates by the dinoflagellate *Noctiluca scintillans*. *Limnol. Oceanogr.*, **43**, 154-159.
- Uhlig, G. and Sahling, G. (1990) Long-term studies on *Noctiluca scintillans* in the German Bight: population dynamics and red tide phenomena 1968-1988. *Neth. J. Sea Res.*, **25**, 101-112.
- Uhlig, G. and Sahling, G. (1995) *Noctiluca scintillans*. Zeitliche Verteilung bei Helgoland und räumliche Verbreitung in der Deutschen Bucht (Langzeitreihen 1970-1993). *Ber. Biol. Anst. Helgol.*, **9**, 1-127.
- Utermöhl, H. (1958) Zur Vervollkommnung der quantitativen Phytoplankton Methodik. *Mitt. Int. Ver. Theor.-Angew. Limnol.*, **9**, 1-38.
- Verity, P. G., Stoecker, D. K., Sieracki, M. E. and Nelson, J. R. (1993) Grazing, growth and mortality of microzooplankton during the 1989 North Atlantic spring bloom at 47°N, 18°W. *Deep-Sea Res.*, **40**, 1793-1814.
- Verity, P. G., Stoecker, D. K., Sieracki, M. E. and Nelson, J. R. (1996) Microzooplankton grazing and primary production at 140°W in the equatorial Pacific. *Deep-Sea Res. II*, **43**, 1227-1255.

Received on October 14, 2003; accepted on January 29, 2004