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RESEARCH ARTICLE

Thresholds of terrestrial nutrient loading for the development of eutrophication episodes in a coastal embayment in the Aegean Sea

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Abstract

1 - Thresholds of terrestrial nutrient loading (inorganic N and P) for the development of eutrophication episodes were estimated in an enclosed embayment, the gulf of Kalloni, in the Aegean, Eastern Mediterranean. Terrestrial loading was quantified by a watershed runoff model taking into account land use, geomorphology, sewerage, industrial and animal farming by-products. The eutrophication episodes were assessed by an existing scale for the Aegean coastal waters based on chl a, whereas the necessary nutrient concentrations (N and P) for the development of such episodes were defined using a probabilistic procedure. Finally, for the linking between nutrient loading arriving at the gulf and the resulting nutrient enrichment of the marine ecosystem, three loading factors were applied, developed by Vollenweider for lake and marine ecosystems. The first assumes no exchange between the embayment and the open sea, whereas the two others take into account water renewal time. Only the threshold for inorganic nitrogen estimated by the first factor was exceeded in the study area during February after a strong rainfall event coinciding with a eutrophication episode observed in the interior of the gulf, implying that the waters of the gulf are rather confined and the receiving body operates as a lake. The degree of confinement was further examined by studying the temperature, salinity, and density distributions inside the gulf and across the channel connecting the gulf to the open sea. It was found that the incoming freshwater from the watershed during winter results to the formation of a dilute surface layer of low salinity and density, clearly isolated from the open sea. The nutrients from the river inputs are diluted into this isolated water mass and the eutrophication threshold for nitrogen is exceeded. Although phosphorus loading was also high during winter, the corresponding limits were never exceeded. The proposed methodology sets a quantitative relationship between terrestrial nutrient loading and the development of eutrophication episodes in coastal embayments, assuming that information on the physical setting of the system is available. These cause-and-effect relationships can be invaluable tools for managers and decision makers in the framework of Integrated Coastal Zone Management.

Keywords: terrestrial loading, nutrient thresholds, eutrophication, Vollenweider factors, watershed model, Aegean Sea.

Introduction

Research on the effects of anthropogenic activities on inland and coastal waters has intensified during the last decades leading to the development of new environmental protection policies. The Water Framework Directive (EC, 2000) is an important policy tool aiming to provide a water quality classification scheme and to support the achievement of good ecological status for European waters by the year 2015, based upon physicochemical, hydromorphological, and biological criteria. It seems however, that many aspects related to the definition of “good water quality” still remain unresolved, and in order to address the WFD objectives, further research is essential towards the assessment of changes in the ecological status of water bodies (Mouillot *et al*., 2006).

Eutrophication, defined as the nutrient enrichment of the marine environment that stimulates primary production and algal growth (Vollenweider, 1992), is one of the major factors leading to the degradation of water quality. Coastal ecosystems in particular, are susceptible to eutrophication episodes, mainly due to the loading from terrestrial sources (Justic *et al*., 1995a). Increased primary production in these ecosystems is often fuelled by large quantities of inorganic nutrients, and particularly nitrogen (N) in the freshwater inputs, as a result of increased fertilization and use of detergents in the watershed (Justic *et al*., 1995b, Nixon, 1995, Anderson *et al*., 2002, Beman *et al*., 2005). Although Mediterranean is one of the most oligotrophic seas in the world and most of its biological productivity takes place in the euphotic zone (UNEP, 1989), eutrophication phenomena along the coastline are reported by an increasing number of studies that draw attention to specific areas, susceptible to eutrophication and its adverse effects including Harmful Algal Blooms (Ignatiades *et al*., 1992, UNEP, 1999, Arhonditsis *et al*., 2000, Druon *et al*., 2004, Spatharis *et al*., 2007a). Therefore, the understanding and quantification of the relationship between terrestrial inputs and ecosystem response are essential for the evaluation and prediction of coastal marine eutrophication in regional seas, directly related to the objectives of WFD.

The response of lakes or semi-enclosed marine ecosystems to terrestrial loading is always being considered in the framework of Coastal Zone Management (Vollenweider, 1987). Simple dynamic models, designed to provide an overview of the trophic status of water bodies based on few related parameters, were initially applied for freshwater ecosystems (Vollenweider, 1968, Vollenweider, 1974, Jones-Lee and Lee, 1993), and were later expanded to include water bodies of restricted exchange with the open sea (Tett *et al*., 2003). The theoretical concept for the development of a relationship between nutrient inputs and ecosystem response was based on the normalization of phosphorus and nitrogen loads, according to the morphological and hydrological characteristics of the receiving water bodies. Earlier studies aiming to establish eutrophication limits were based on loading per unit area or loading per unit volume (Nixon, 1983, Nixon *et al*., 1986). In order to consider the role of flushing rate, or alternatively the fact that better flushed systems can tolerate greater pollutant inputs, loading formulas have been proposed including renewal time. This approach was taken by Vollenweider (1976, 1985) who related the response of freshwater systems (in terms of chlorophyll concentration) with phosphorus loading, turnover time, and the volume of the system. For coastal marine ecosystems, the development of limits and the role of renewal time is a matter under consideration (Boynton and Garber, 1988, Costa *et al*., 1999).

In the present work, an attempt is made to establish a quantitative relationship between terrestrial nutrient loading and eutrophication development in the Kalloni gulf, Island of Lesvos, Greece in Eastern Mediterranean. The gulf is characterized by high residence time and is surrounded by a watershed of 413 Km^2. The main anthropogenic activities in the watershed include agriculture, animal farming and tourism. A detailed description of the study area has been given in a previous paper (Spatharis *et al*., 2007b). The availability of data, for both the marine ecosystem and the intermittent rivers draining the surrounding watershed, enabled the investigation of direct associations between terrestrial nutrient loads and stock nutrient concentrations, as well as the establishment of nutrient loading thresholds above which eutrophication episodes are developed. The role of the physical setting of the system is also discussed by studying the salinity, temperature, and density distributions.

Methods

Outline of the method

Nitrate and phosphate concentration limits in seawater, above which eutrophication is developed, were set following the methodology proposed for the Aegean Sea by Stefanou *et al*. (2000). These concentrations were used in the loading scale formulas proposed by Costa *et al*. (1999) in order to estimate the corresponding nutrient terrestrial loads able to trigger eutrophication episodes. These threshold values were further compared with the actual terrestrial loads originating from the surrounding basin. These loads from non-point and other sources (agricultural runoff, animal farming wastes, sewerage and industrial by-products), were estimated for the Kalloni basin using a watershed runoff model (Arhonditsis *et al*., 2002), calibrated with field data. Finally the definition of eutrophication episodes was based on a phytoplankton biomass scale (in chl a terms) developed by Simboura *et al*. (2005). This five-level coastal water quality scale (High, Good, Moderate, Poor and Bad) has been developed for the implementation of the WFD in the Aegean sea, taking into account the oligotrophic character of the Eastern Mediterranean waters (Krom *et al*., 1991).

Data used

Information on chemical and biological parameters was collected on a monthly basis (August '04-July '05) from a network of stations in the Kalloni gulf (Fig. 1), most of them (K5-K8) located along the plume of river Tsiknias, the major river draining the Kalloni basin. This information was used to study the temporal variation of nutrients and chl a in seawater and for the assessment of the eutrophication level. Additionally, vertical CTD profiles for temperature, salinity, and density were available, for stations K5-K8, the reference station K1 in the open sea, and K2 in the channel connecting the gulf to the open sea. This oceanographic information was used to investigate the level of confinement of the receiving body and its effect on eutrophication. Nutrient concentrations (nitrate, nitrite, ammonium and phosphate) measured at stations K9-K12 in the mouth of river Tsiknias (Fig. 1), were used to calibrate the watershed runoff model.

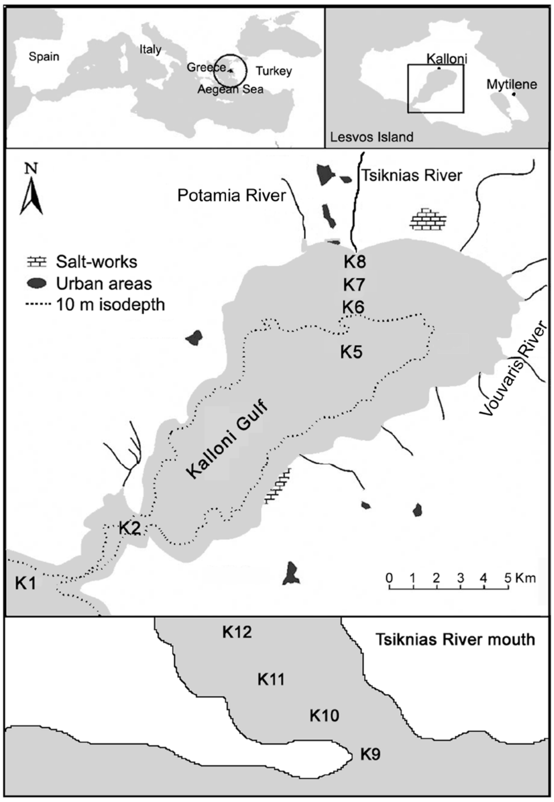

Figure 1. Map of the Kalloni gulf showing the location of stations: K5 to K8 are located in the inner part of the gulf, K9 - K12 inside the mouth of river Tsiknias and K1, K2, in the open sea and the channel, respectively.

Nutrient scaling for eutrophication assessment

Nutrient limits above which eutrophication is developed in the Aegean coastal waters were set according to the methodology proposed by Stefanou *et al.* (2000). This work was based on the development of a standard normal distribution, named physical normal distribution, which can be used as a potential probabilistic tool for coastal water quality assessment in the Aegean. It can also serve as a useful source of information for statistical evaluation of polluted sites using simulated data and an advantageous method of built-in standardization, allowing parametric multivariate procedures to be applied. Based on the advantages of this approach, the methodology proposed by Stefanou *et al.* (2000) was applied to establish nutrient limits for eutrophication development in the current study. For nitrate the already developed scaling (Stefanou *et al.*, 2000) was used, whereas for phosphate the scaling was developed following the same methodology. The critical concentration in seawater above which eutrophication is developed was set at 0.38 μM for nitrate and 0.37 μM for phosphate.

Loading scales and eutrophication thresholds

The eutrophication limits in seawater for nitrate and phosphate calculated above, were further used to estimate the corresponding loading limits or thresholds of terrestrial input resulting to the development of eutrophication episodes. Three formulas were applied in the current study. The first (LS1) was proposed by Nixon (1983) and scales the loading by dividing its value by the volume of the receiving water body, considering no exchange of water with the open sea. The second formula (LS2), proposed by Vollenweider (1976, 1985), is more optimistic, taking into account a reduction in the effects of terrestrial loading, therefore an increase in the eutrophication threshold value, due to the water renewal of the receiving body. It is calculated by dividing the loading estimated by the first formula to the water renewal time τw (expressed as a fraction of a month). Finally, the third formula (LS3), also proposed by Vollenweider (1976, 1985), is calculated by multiplying the previous formula by the so called Vollenweider adjustment term, that is $1+\tau w^{1/2}$, where τw is expressed in years. This adjustment term becomes important in rapidly flushed systems; for instance in an embayment with a flushing time of 3 days, the relative reduction of loading is about 9%. The mathematical formulas of the three loading scales are given below:

The watershed model

$$\mathrm{LS1} = \frac{\text{terrestrial loading (in Kg)}}{\text{volume of embayment (in m}^3\text{)}}$$

$$\mathrm{LS2} = \frac{\text{terrestrial loading (in Kg)}}{\text{volume of embayment (in m}^3\text{)}} \times \frac{1}{\tau_w}$$

$$\mathrm{LS3} = \frac{\text{terrestrial loading (in Kg)}}{\text{volume of embayment (in m}^3\text{)}} \times \frac{1+\sqrt{\tau_w}}{\tau_w}$$

A watershed model was applied to calculate the input of dissolved nutrients to the sea from the surrounding basin, taking into account both non-point and other sources (Arhonditsis *et al.*, 2002). Surface runoff was estimated according to the Curve Number Equation (Haith and Tubbs, 1981) based on the land use and the geomorphology. The amounts of nutrients and organic matter transported into the gulf due to surface runoff were determined using a special function of the 'loading functions' category. These functions are models in which the calculated surface runoff is multiplied by the transferred concentrations of nutrients or pollutants in dissolved phase. In the current application, the concentrations of nitrate, ammonium, and phosphate were estimated.

The loading from other sources was also estimated. The sources taken into account were sewerage, by-products of animal farming, and industrial activities (olive-oil refineries). Transfer coefficients from the literature were used to estimate loading from each source, whereas a retention factor was applied to consider decomposition processes occurring along the route connecting the various sources to the sea. These factors were calibrated using field data collected at the mouth of Tsiknias River.

Results

The phytoplankton biomass (expressed in chl a) in the inner part of the Kalloni gulf (stations K5-K8) during 2004-2005 varied between 0.3 and 1.5 µg L^{-1} (Fig. 2), characteristic values of mesotrophy for Aegean coastal waters (Simboura *et al*., 2005). An exception was only observed in February '05, when the chl a concentration was 3.21 µg L^{-1}, therefore exceeding the eutrophication limit of 2.21 µg L^{-1} specified by Simboura *et al*. (2005). The inorganic nutrient concentrations in the water column (DIN and DIP) have also shown maximal values in the beginning of February (Fig. 2), these maxima coinciding with the chl a peak. Positive correlations were observed between chl a and DIN, and chl a and DIP in the water column (Table 1). In the wet period (November to April), when terrestrial nutrient enrichment mostly occurs, chl a was correlated only with nitrogen, whereas during the dry period (May-October), chl a was weakly correlated with phosphorus.

The temporal variation of Dissolved Inorganic

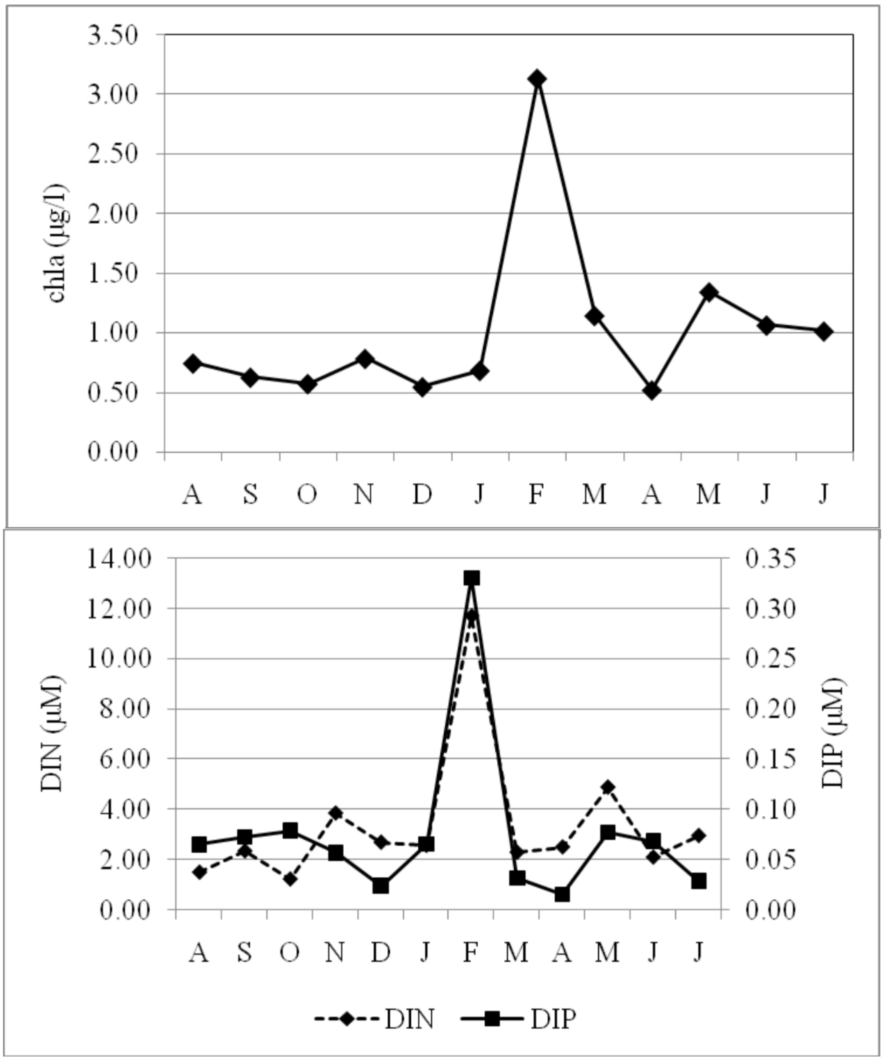

Figure 2. Temporal variability of phytoplankton biomass (expressed in chl a), Dissolved Inorganic Nitrogen (DIN), and Dissolved Inorganic Phosphorus (DIP) in the Kalloni gulf from August '04 to July '05.

Table 1. Spearman rank correlation coefficients between inorganic nutrients and chl a, in the water column of the Kalloni gulf during the wet (November-April) and dry (May-October) period.

	Spearman correlation coefficient		
	All (N =113)	Dry period (N=48)	Wet period (N=65)
chl a-DIN	0.405**	0.266	0.394**
chl a-DIP	0.220*	0.327*	0.239

*Significant correlation at the 0.05 level (2-tailed).
**Significant correlation at the 0.01 level (2-tailed).

Nitrogen (DIN) and Dissolved Inorganic Phosphorus (DIP) in the mouth of Tsiknias River showed an increasing trend from the start of the rainfall period (October) until December (Fig. 3). This trend continues for DIN until the end of February when the highest mean monthly rainfall height was recorded (max 16 cm per week) and episodic events occurred. The concentration of DIP was almost constant from mid-December until March and started to decrease gradually afterwards. The same decreasing trend after March was observed for DIN. Considering that the river flow was also maximum during the period when the highest nutrient concentrations were measured, the loading of the receiving water body with DIN and DIP mainly occurred from mid-January until the end of February.

The inorganic nutrient concentrations measured in the mouth of Tsiknias River were used for the calibration of the watershed runoff model. The model was then run for a typical year and the DIN and DIP loadings of the Kalloni gulf from non-point and other sources were estimated. The terrestrial loading thresholds, above which eutrophication is developed, calculated according to the method previously described in detail, are shown in Table 2. The water residence time for the Kalloni gulf was estimated at about 20 days (Millet and Lamy, 2002), and the volume of the inner part of the gulf was estimated at 85x10^7 m^3. The actual values of terrestrial loading on a monthly basis, estimated by the model, and the eutrophication threshold values for nitrate and phosphate are presented in Figure 4. The nutrient loading is considerable during the rainfall period (November to March), nitrogen being always higher than phosphate loading. The maximum nutrient loading is observed during February, when both the river flow rate and the nutrient concentrations in river water were maximal. There is no loading during the dry period of the year (May to September), when the intermittent rivers are dry and the low nutrient concentrations measured in the river mouth are due to the intrusion of seawater upstream. The threshold values for nitrate, taking into account the water renewal time (LS2 and LS3), are always higher than the actual terrestrial loads. Only, the nitrate loading of February was greater than the threshold LS1, implying that the observed eutrophication episode in February was probably triggered by the nitrate terrestrial inputs. For phosphate, terrestrial loading was always lower than the three thresholds, supporting the view that phosphate input is not a possible driver for primary production in the area under consideration. The distributions of temperature, salinity, and density present

Table 2. Thresholds of nitrate and phosphate terrestrial loading above which eutrophication is developed.

Threshold	Nitrate (Kg month^{-1})	Phosphate (Kg month^{-1})
LS1	4522	9749
LS2	6783	14624
LS3	8371	18049

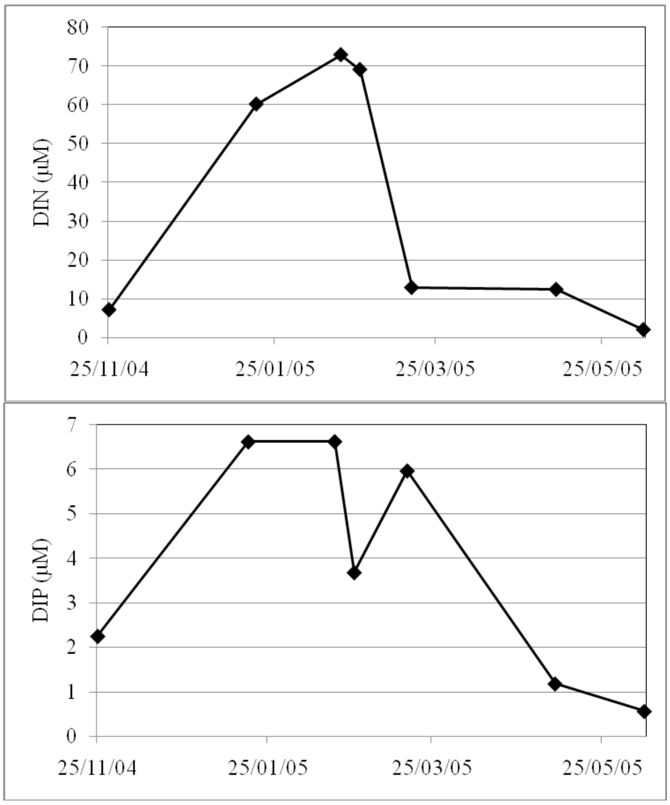

Figure 3. Temporal variation of Dissolved Inorganic Nitrogen (DIN) and Dissolved Inorganic Phosphorus (DIP) in the mouth of Tsiknias River during the rainfall period of 2004-2005.

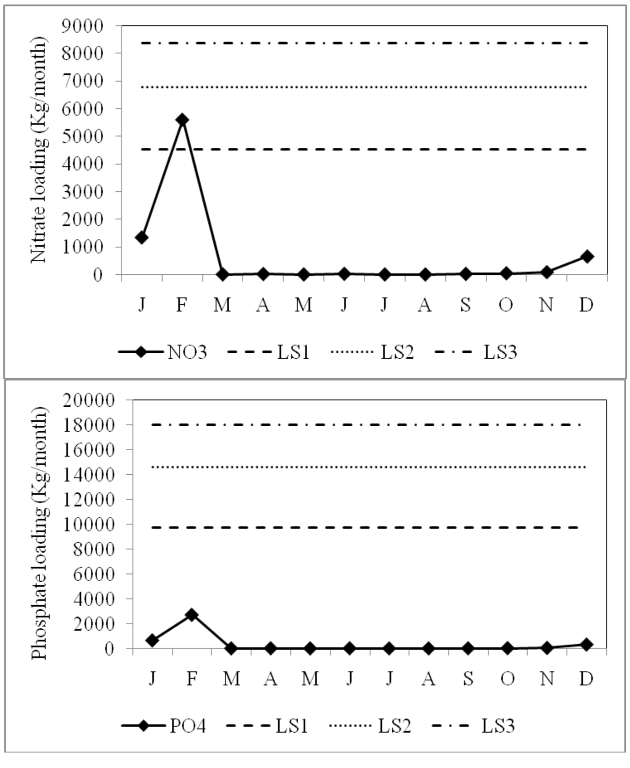

Figure 4. Terrestrial nitrate and phosphate loading on a monthly basis and the corresponding threshold values for the development of eutrophication episodes.

considerable temporal and spatial variability in the Kalloni gulf. This variability is determined by the water exchange with the open sea throughout the year and particularly the freshwater inputs during winter. Differences in the density of the water masses between the gulf and the open sea directly affect the hydrodynamic regime (Millet and Lamy, 2002), controlling the water exchange and the renewal time, which in turn may have a major ecological impact, by triggering indirectly the development of eutrophication episodes. During winter, low temperature values are recorded inside the gulf (below 10° C), whereas the corresponding surface temperature values in the open sea are above 14° C (Fig. 5a). This cooling, characteristic of semi-enclosed shallow systems, is due to their low heat capacity as well as to the cold freshwater inputs from the surrounding land. This abrupt change in temperature along the channel connecting the gulf to the open sea, and the corresponding change in density, results to the isolation of its water mass and therefore directly affects the water residence time. The reverse trend is observed in summer (Fig. 5b). The water mass of the gulf is rapidly heated during spring, resulting to a temperature difference of about 4° C, between the gulf and the open sea in summer. This abrupt temperature change is observed along the channel, therefore isolating the inner gulf water masses from the open sea. The cross-sections of temperature, salinity and density during February ‘05, soon after the major episodic rainfall event leading to the development of the eutrophication episode under consideration, are shown in Figure 6. The increased freshwater flow especially in the north (right side of the cross-section), greatly affected the vertical salinity, temperature, and density distributions and a dilute, cold surface layer was formed in the inner part of the Kalloni gulf. The formation of this layer further intensified the confinement of the gulf water masses observed during winter and the limited exchange of water with the open sea. Moreover this surface layer is nutrient-rich due to the terrestrial inputs, therefore increasing the risk for the development of algal blooms, including HABs.

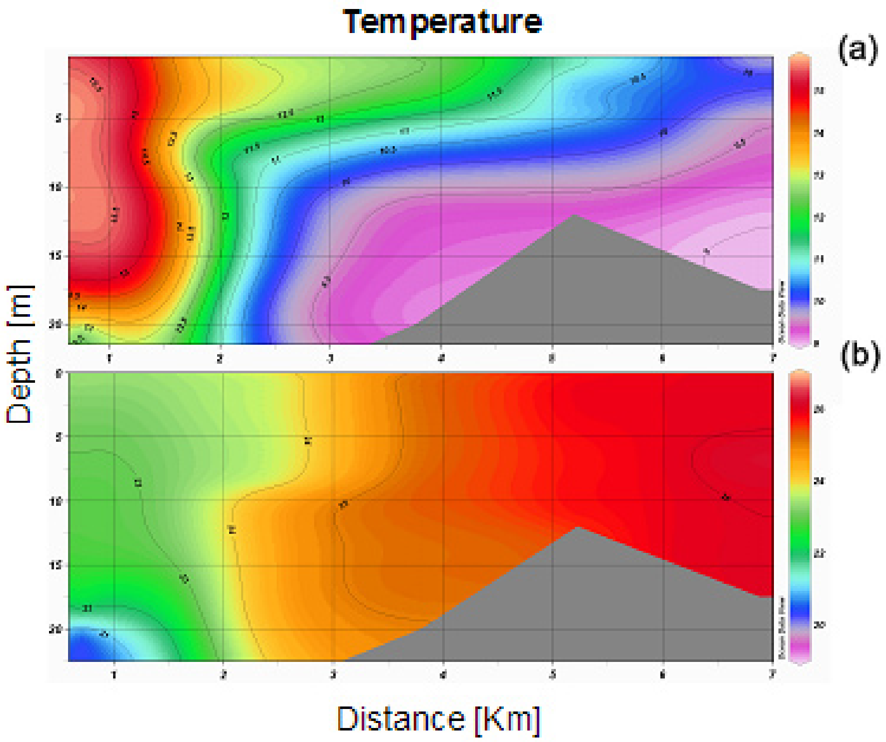

Figure 5. Temperature distribution along the channel connecting the Kalloni gulf (in the right) to the open sea (in the left), during: (a) winter and (b) summer.

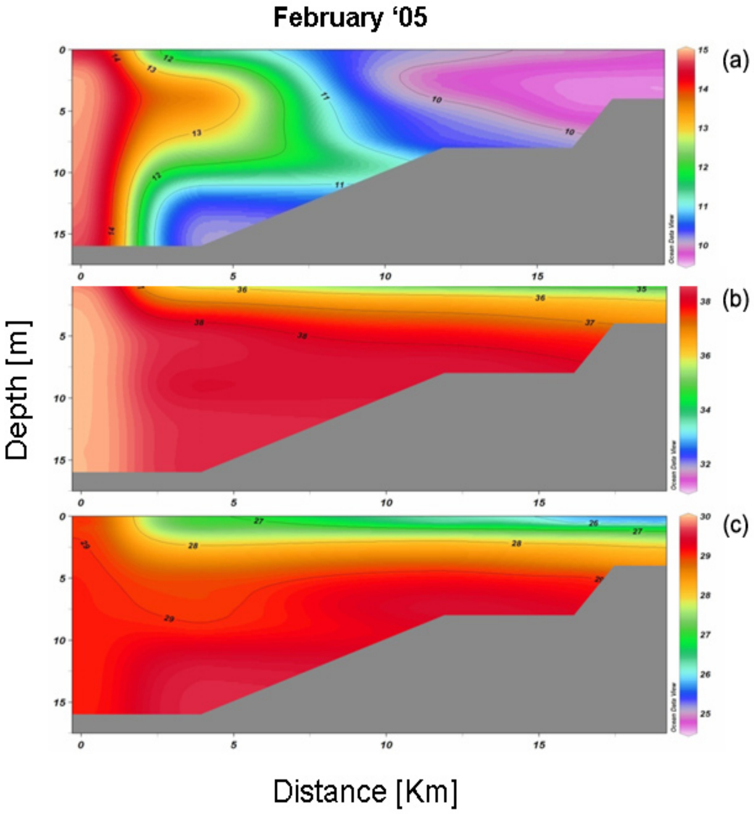

Figure 6. Cross-sections of (a) temperature in °C, (b) salinity in psu, and (c) density in sigma-t, during February '05, from the northern part of the Kalloni gulf (on the right) to the open sea (on the left).

Discussion

Among the environmental variables that control primary production in coastal marine ecosystems, nutrients and particularly nitrogen forms have been considered as crucial in triggering eutrophication episodes and bloom events (Hecky and Kilham, 1988, Justic *et al.*, 1995a, Anderson *et al.*, 2002). Nutrient concentrations in the inner part of Kalloni gulf during the studied annual cycle, were directly associated with the concentrations measured in the freshwater inputs. This association was more pronounced during February '05 soon after an episodic rainfall event, when the peak in terrestrial nutrient loading was just followed by a peak in nutrient stocks in the water column and in primary production. These observations indicate the direct interaction between the watershed (and the related anthropogenic activities) and the coastal ecosystem under investigation.

The comparison of the actual DIN and DIP terrestrial loads and the estimated threshold values, support the view that the development of eutrophication episodes in the Kalloni gulf is limited by nitrogen rather than phosphorus. This finding was further supported by the positive correlation between chlorophyll and nitrogen, in the inner part of the gulf during the rainfall period, when terrestrial loading occurs. Although nitrogen limitation is common in the marine environment, phosphorus has been reported as the limiting nutrient in Eastern Mediterranean (Thingstad *et al.*, 2005). Moreover, the N to P ratio in the Kalloni gulf often exceeded 100:1 (Spatharis *et al.*, 2007b), primarily due to the fact that nutrient inputs from agricultural land and farms are nitrogen rich, implying that phosphorus is the limiting nutrient instead of nitrogen. This contradiction between the observed N to P ratio and the findings from the application of thresholds may be due to a number of reasons. Dissolved phosphorus is absorbed on particles in the water column or in the sediment and the fraction available for algal growth is rather low (Harris, 1986, Reynolds, 1999, Lee, 2007), especially in turbid environments as the Kalloni gulf (Millet and Lamy, 2002). The identities of phytoplankton species thriving during the bloom event and their nutritional requirements may further explain the above paradox. The eutrophication episode observed in February was attributed to the dominance of the potentially toxic species *Pseudo-nitzschia calliantha* and *Alexandrium insuetum* which are known to form recurrent blooms in the gulf after episodic rainfall events in winter (Spatharis *et al*., 2007a). According to the literature, dissolved nitrogen compounds rather than phosphorus, trigger the blooms of both *Pseudo-nitzschia* (Lund-Hansen and Vang, 2003) and *Alexandrium* species (Collos *et al*., 2004), therefore supporting the findings of the current study regarding the critical role of nitrogen as the driver of winter algal blooms. Moreover, the role of nitrogen as the limiting nutrient in a marine environment of high N to P ratios can be supported by the fact that under certain conditions phytoplankton stoichiometry may diverge from the canonical Redfield ratio of 16:1 (Reynolds, 1999, Arrigo, 2005). This divergence is due to the ability of algal cells to store luxurious phosphorus to be used during periods of deficiency; therefore, even if phosphorus concentration in seawater seems to be limiting, cell quota concentration is enough to support cell growth for many generations without external requirement (Harris, 1986). Finally, the controversy between high N to P ratios and nitrogen limitation can be further explained by the more rapid phosphorus recycling in seawater compared to nitrogen, implying that the measurement of stock concentrations cannot lead to safe conclusions about limitation in seawater (White *et al*., 1982).

The eutrophication episode of February, which was due to a diatom bloom developed soon after a terrestrial nutrient enrichment, had a very short duration. This is in agreement with previous studies of O'Brien (1974), demonstrating that the cause for a population crash can be the large supply of nutrients in excess of that allowing a steady state, when mortality and growth are balanced. According to the same author, in this situation, the rate of nutrient replacement is not fast enough to maintain the large algal biomass arising from the consumption of the whole nutrient pool, and therefore a population crash follows the bloom (Platt, 1981).

Among the three formulas for the estimation of terrestrial loading threshold values, LS1 seems to be sensitive enough to predict eutrophication episodes in the Kalloni gulf. This formula is independent of the water residence time assuming an entirely isolated system. The insufficiency of LS2 and LS3 formulas may be associated with the geomorphological and hydrological characteristics of this shallow embayment. The inner gulf water masses were rather isolated from the open sea since water exchange, particularly during winter, is restricted. The confined inner part operates more or less as a lake and the turnover of nutrients due to the high residence time is limited. Therefore, the LS2 and LS3 formulas, which take into account the flushing rate, predict higher loading thresholds which are not appropriate to be applied in the area under consideration.

Scientific interest has been recently renewed towards the estimation of thresholds of nutrient loading that lead to excessive fertilization – eutrophication – of coastal waters (Lee, 2007). Nitrogen (N) and phosphorus (P) are among the most important nutrients stimulating algal growth and are of particular concern in coastal ecosystems where they are added through various anthropogenic activities. In this context, the development of methodologies aiming to estimate the effects of this fertilization on coastal water quality is an essential task. The methodology proposed in the current study, supports the establishment of a clear cause-and-effect relationship between the anthropogenic activities in the watershed and eutrophication episodes in the coastal marine environment. This quantitative relationship is invaluable in the framework of Integrated Coastal Zone Management since it can support the design of reliable monitoring programs for the effects of fertilizer application and therefore their sustainable use. Moreover, quantitative relations between anthropogenic activities in the surrounding basins and their influence on the receiving water bodies may support the improvement of coastal water quality and the implementation of the related policies, including the WFD.

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