



## Research papers

# Will nutrient and light limitation prevent eutrophication in an anthropogenically-impacted coastal lagoon?



Rita B. Domingues<sup>a,b,\*</sup>, Cátia C. Guerra<sup>a</sup>, Ana B. Barbosa<sup>a</sup>, Helena M. Galvão<sup>a</sup>

<sup>a</sup> CIMA – Centre for Marine and Environmental Research, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

<sup>b</sup> MARE – Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

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## ABSTRACT

The Ria Formosa coastal lagoon (southern Portugal) is a highly productive and valuable temperate ecosystem, subjected to strong anthropogenic impacts and highly vulnerable to climate change. The main goal of this work is to understand ecosystem susceptibility to eutrophication, by evaluating the isolated and combined effects of nutrient (N, P and Si) and light enrichments on phytoplankton growth and community composition during autumn, winter and spring. Microcosms of natural phytoplankton collected in the lagoon were subjected to different nutrient and light treatments and incubated in situ for 48 h. Nutrient consumption, and phytoplankton growth and community structure were evaluated using spectrophotometric methods, and inverted and epifluorescence microscopy. Diatoms were the only group potentially limited by nitrogen, and only during spring. Increased nutrient consumptions were observed for all nutrient additions in all seasons, not associated with phytoplankton growth, suggesting that luxury consumption was used to build up intracellular nutrient pools. Responses to light enrichment were inconsistent among phytoplankton groups, probably due to a high taxonomic seasonal variability. Positive responses to light enrichment were mostly observed during winter. Negative synergistic interactions between nutrients and light were also observed. We conclude that eutrophication is currently not a problem in the Ria Formosa coastal lagoon, but future nutrient enrichments may lead to accelerated growth of specific functional groups and species, if light is not limiting.

## 1. Introduction

Nutrient over-enrichment of surface waters has been widely addressed in the last five decades, but eutrophication still remains one of the foremost problems in freshwater and coastal ecosystems (Schindler, 2006). The effects of nutrient availability on phytoplankton growth, community composition and succession, and the role of nutrients, particularly nitrogen (N) and phosphorus (P), as growth limiting resources, represent an important tool for ecosystem management and eutrophication control (Paerl, 2009). Indeed, nutrient availability is probably the most studied phytoplankton driver across different ecosystems, typically through nutrient enrichment experiments (e.g., Domingues et al., 2011a; Fouillaron et al., 2007; Morris and Nicholson, 2015; Varona-Cordero et al., 2014), where higher growth rates following nutrient additions are indicative of nutrient limitation.

Considerably less attention has been given to light as a growth-limiting resource. However, light is a major phytoplankton driver, especially in turbid, nutrient-rich and/or confined coastal ecosystems

(Bowes et al., 2012; Domingues et al., 2011b; Gameiro et al., 2011; Kocum et al., 2002a; Pan et al., 2016). Given that optimum light intensities for photosynthesis and acclimation capabilities vary across phytoplankton groups and species, light availability is a paramount resource that controls phytoplankton growth, community structure, competition and succession (Huisman et al., 1999; Litchman, 1998). The susceptibility of aquatic ecosystems to eutrophication and to the occurrence of harmful algal blooms is also a function of light availability; for instance, turbid systems with low/moderate light regimes are less likely to respond to nutrient over-enrichment (Cloern, 2001; Painting et al., 2007).

Due to a restricted water exchange with the adjacent coastal waters, semi-enclosed coastal ecosystems, such as estuaries and coastal lagoons are extremely vulnerable to eutrophication that results from agricultural practices and urbanization in the watershed (Newton et al., 2014). The Ria Formosa coastal lagoon, in southern Portugal, is subjected to multiple anthropogenic stressors (Newton et al., 2003), and to discharges of untreated or partially treated domestic and industrial sewage, and runoffs from golf courses and agriculture,

\* Corresponding author at: CIMA – Centre for Marine and Environmental Research, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.  
E-mail address: [rbdomingues@ualg.pt](mailto:rbdomingues@ualg.pt) (R.B. Domingues).

associated with increasing nutrient concentrations (Cabaço et al., 2008; Cravo et al., 2015; Mudge et al., 2007; Newton et al., 2014; Newton and Mudge, 2005). Due to its biological and economic importance, water quality and eutrophication in the Ria Formosa coastal lagoon have long been addressed and evaluated, particularly in the context of the implementation of European Union policies, such as the Water and Marine Strategy Framework Directives. Results, however, have not been consistent. Comparison of nutrient concentrations placed the Ria Formosa in a “poor” to “bad” condition, whilst a symptom-based approach that included chlorophyll *a* concentration indicated that the Ria Formosa is near pristine (Newton et al., 2003). A recent assessment of eutrophication symptoms in the Ria Formosa coastal lagoon referred, among others, large inputs of N and P, unbalanced N:P and N:Si, problems with oxygen, anoxic sediments, phytoplankton blooms and HABs, opportunistic green algae, loss of seagrasses, decreased benthos and fish biodiversity, and fish kills (Newton et al., 2014).

Nutrient enrichment, particularly N and P, has long been considered the most common single factor causing eutrophication of coastal marine ecosystems (Nixon, 1995). Due to phytoplankton quick responses to nutrient enrichment and other environmental changes, a comprehensive knowledge of how nutrients affect phytoplankton composition, abundance and biomass is critical to properly assess eutrophication occurrence and potential impacts on the ecosystem. Contrasting with descriptive monitoring studies, experimental studies are particularly appropriate to quantify the effects of nutrient enrichment on phytoplankton dynamics. This will allow the establishment of cause-effect relationships that will aid managers to draw up appropriate nutrient loading budgets and respond to perturbations on an informed basis (Beardall et al., 2001), and to properly evaluate the efficiency of subsequent nutrient reduction strategies (Gobler et al., 2006).

Published information on the effects of nutrient enrichment on phytoplankton in the Ria Formosa coastal lagoon is limited to late spring and summer periods (Domingues et al., 2015; Loureiro et al., 2005). The role of light as a limiting variable was only addressed during summer (Domingues et al., 2015). Although phytoplankton inside the Ria Formosa follows a unimodal cycle with summer maxima (Barbosa, 2010), “phytoplankton do not rest in winter” (Zingone et al., 2009) nor during “less” productive periods. In addition, the evaluation of eutrophication requires regular monitoring, at least monthly for phytoplankton abundance, biomass and composition in transitional and inshore coastal waters (Ferreira et al., 2007), although this sampling frequency can also be too low to detect some algal blooms (Domingues et al., 2008). Therefore, a thorough knowledge on the effects of nutrient and light availability on phytoplankton and their seasonal variability in the Ria Formosa coastal lagoon is still missing. The goal of this study is, thus, to evaluate the isolated and combined effects of nutrient (N, P and Si) and light enrichment on phytoplankton nutrient consumption, growth and composition in the Ria Formosa during autumn, winter and spring. Based on previous assessments of eutrophication and monitoring data in this shallow ecosystem (Barbosa, 2010; Domingues et al., 2015), we hypothesize that light is probably limiting to phytoplankton growth during winter and nutrient concentrations are limiting throughout the year. Due to a continuous nutrient limitation, eutrophication should not be a current problem in the Ria Formosa coastal lagoon, but nutrient enrichments will have the potential to enhance algae growth in this ecosystem, particularly during spring and summer, when light is plentiful.

## 2. Materials and methods

### 2.1. Study site and sampling strategy

The Ria Formosa coastal lagoon (southern Portugal) is an euryhaline, shallow (mean depth = 2 m), mesotidal multi-inlet barrier island system that extends approx. 55 km E-W and 6 km N-S at its widest

point. Tides are semidiurnal with mean amplitude of 2.1 m. This ecosystem is located in a highly vulnerable area to climate change (IPCC, 2014), and is subjected to Mediterranean climate with hot, dry summers and moderate winters. The coastal region adjoining the lagoon is affected by regular upwelling events, more frequent from March to October (see review by Relvas et al., 2007), that impact the outer section of the lagoon (Barbosa, 2010; Loureiro et al., 2006) and may extend at least ca. 6 km upstream the lagoon inlets (Cravo et al., 2014). The Ria Formosa and its hinterland, with a total humid area of 84 km<sup>2</sup> (Andrade, 1990) and a total area of 185 km<sup>2</sup>, constitute a national and internationally recognized wetland area, being protected by multiple policies, e.g., a National Natural Park, a Special Bird Protection Area under the Directive on the Conservation of Wild Birds, a Natura 2000 site and a Ramsar site (Barbosa, 2010).

Microcosm experiments with nutrient and light enrichments were integrated in PHYTORIA research project, that included a fortnight sampling program conducted in several sampling stations in the Ria Formosa to evaluate spatial and seasonal dynamics of phytoplankton and their environmental drivers. Four experiments were conducted during representative seasons for phytoplankton growth in the Ria Formosa coastal lagoon: autumn (early December 2011), winter (February 2012), spring (late March 2012) and summer (June–July 2012). Given that summer is the most relevant season in terms of phytoplankton biomass and occurrence of HABs (Barbosa, 2010; Brito et al., 2012), results from the summer experiments were presented and discussed elsewhere (Domingues et al., 2015). For each experiment, water samples were collected at a representative location in the inner lagoon, with a mean depth of 2 m, representing lagoon landward boundaries (Fig. 1).

### 2.2. Experimental setup

For each experiment, 2 L aliquots of sub-surface water were collected during ebb tide into transparent 2 L polycarbonate bottles (Nalgene). Samples were not pre-filtered to eliminate zooplankton, given that this procedure also eliminates phytoplankton, leading to significant alterations in the structure of the initial phytoplankton community, and thus increasing problems associated to the extrapolation of results to natural communities (Nogueira et al., 2014). Two distinct light treatments were set up:  $I_m$  (exposure to the mean light intensity in the mixed layer) and  $I_{plus}$  (exposure to light intensity higher than  $I_m$ , corresponding to approx. 150–300% of  $I_m$ , depending on light extinction coefficients). Experimental bottles were covered with different layers of net to achieve the desired light intensity.

For each light treatment ( $I_m$  and  $I_{plus}$ ), nine nutrient treatments were prepared, in duplicate (Table 1). Nutrients were added alone and in combinations at day 0 in a single, saturating pulse. Nutrient concentrations added were higher than values previously referred for the Ria Formosa (Barbosa, 2010), in order to suppress potential nutrient limitations that would have inhibit phytoplankton responses to nutrient additions. Nitrogen was added as potassium nitrate (KNO<sub>3</sub>) and ammonium chloride (NH<sub>4</sub>Cl), phosphorus as potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) and silicium as sodium hexafluorosilicate (Na<sub>2</sub>SiF<sub>6</sub>). The experimental treatments and nutrient concentrations added can be found in the supplementary material. The bottles (36 bottles per experiment) were incubated in situ, at the water surface, fixed to a buoy, for 48 h. Based on previous experiments (Barbosa, 2006; Domingues et al., 2015), this incubation time is suitable to observe significant phytoplankton responses, whilst minimizing potential “bottle effects” that may arise from longer incubations (see Nogueira et al., 2014).

During incubation, experimental bottles were exposed to in situ temperature and natural light: dark cycle, and were continuously shaken by tidal currents and wind. Samples for determination of dissolved inorganic macronutrient concentrations, chlorophyll *a* concentration, and phytoplankton composition and abundance were

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