



## Response of ecological indices to nutrient and chemical contaminant stress factors in Eastern Mediterranean coastal waters



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### ABSTRACT

Environmental data produced throughout monitoring activities in the framework of the implementation of Water Framework Directive 2000/60/EC (WFD) in Eastern Mediterranean (Greece) were used to assess the sensitivity and response of ecological indices against trace metals, eutrophication and multiple stress factors. The applied ecological indices include multi-metric eutrophication indices, a physicochemical status index applied for the first time in the Greek marine area, benthic indices, phytoplankton biomass index, and integrated status indices assessed through the application of the decision tree integration scheme. To investigate the exceedances in the eco-stoichiometric relationship between nutrients, considered a stressing factor, all physicochemical elements influenced directly or indirectly by eutrophication, such as nutrient concentrations, water transparency, oxygen saturation, particulates concentration, and sediment organic content, were related to ecological indices. Also, chemical contaminant stress factors represented by heavy metal concentrations in the water, as well as multiple stress factors represented by a pressure index, were related to ecological indices. A graphical visualization multivariate tool and statistical correlations were used to evaluate the sensitivity or explanatory power of the tested ecological indices against single and multiple stress factors. Results showed a strong response of all ecological indices to stress factors, although a diversification of sensitivity was evident. Primary production-related indices, i.e., macroalgae and chlorophyll-*a* indices, are more sensitive to particulates and nitrogen, while secondary production-related indices, i.e., benthic macroinvertebrates indices and eutrophication indices, including nutrients, are more sensitive to phosphates in the water column. The macroalgae index shows the strongest sensitivity to multiple stress factors. Among metals, mostly cadmium seems to match all indices' performance. Nutrient relationships were shown as critical to eutrophication and ecological status.

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### 1. Introduction

Coastal marine ecosystems represent systems of high value and multiple uses that result in environmental effects that often interact in complex ways (Trush, 2015). The working group IMPRESS (2002) established the “Driving forces-Pressures-States-Impacts,” widely known as the DPSIR approach, which is the main analytical framework for determining pressures and impacts under the WFD. Borja et al. (2006), based upon this approach, grouped the main estuarine and marine pressures into pollution, including urban, industrial, agricultural and aquaculture discharges, hydro-morphological changes, and biology and its uses (alien species

introduction, etc.). Among the pollution stressors, excessive nutrient discharges can lead to accelerated eutrophication of coastal environments and adverse symptoms of over enrichment (Ferreira et al., 2011). On the other hand, chemical contaminants from urban and industrial activities can be potentially toxic for organisms living both within the sediments and the water column (Dafforn et al., 2012 and references therein).

Aquatic ecosystems are increasingly stressed not only by increased nutrient loads (eutrophication) but also by changing forms and proportions of nutrients. Within this context, ecological stoichiometry recognizes that at the base of the food web, the elemental composition of the primary producers is affected by nutrient composition, whether nutrients are limiting or not (Glibert, 2012). Ecological stoichiometry (eco-stoichiometry) is based on stoichiometric theory and the metabolic theory of ecology, which involves the balance of energy and multiple chemical

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elements in ecological interactions at the subcellular to ecosystem scale (Sinsabaugh et al., 2009). The exceedances in the eco-stoichiometric relationship between nutrients form a stressing factor that impacts the delicate balance governing ecological interactions.

A multitude of direct human influences have significantly altered the environmental conditions, composition, and diversity of marine biological communities (Whiteley, 2016). However, understanding and predicting the combined impacts of single and multiple stressors are particularly challenging because observed ecological feedbacks are underpinned by a number of physiological and behavioral responses that reflect stressor type, severity, and timing. Furthermore, integration between the traditional domains of physiology and ecology tends to be fragmented and focused towards the effects of a specific stressor or set of circumstances.

Given this gap between physiology and ecology, causal relationships among ecological indicators and stressors are rarely explored, and statistical correlations and probability analysis are used to validate pressure effects on biota (Nöges et al., 2016; Benyi et al., 2009). The European policies for water and environmental quality (European Water Framework Directive—WFD, EC, 2000; Marine Strategy Framework Directive—MSFD, EC, 2008) use ecological indicators as tools that reflect the overall stress on the environment. Most ecological indicators provide the overall picture of the cumulative and synergistic impacts of a variety of environmental stresses, and raise awareness of the consequences of ecosystem degradation. However, each biological or environmental element may be sensitive or responsive to different stressors, or even to a magnitude of stressors.

When acting simultaneously, pressures may have effects that are additive, but often they have cumulative, synergistic or antagonistic effects, either strengthening or weakening each other (Uusitalo et al., 2016). The synthetic or integrated assessment is expected to reflect the whole range or variety of stressors that affect the ecosystem in an adequate way. Thus, information on the sensitivity and responsiveness of single or synthetic ecological indices to single and multiple stress factors is needed in order to evaluate and understand the individual indices' and synthetic status assessment. Also, the synthetic assessment follows different integration methods in case the various metrics or elements are sensitive to the same or different pressures (Borja et al., 2006, 2014; Prins et al., 2013).

In this work, the response of some ecological indices (eutrophication indices, benthic indices, physicochemical status indices and integrated status indices) to the environmental stress factors (eutrophication and metals) is studied by using statistical correlations and a graphical visualization tool associating status classification to stress factors. Indices agreement was also explored as a measure of affinity among indices results. Moreover, the physicochemical quality index (PCQI) method developed by Bald et al. (2005) for the assessment of the physicochemical status has been applied for the first time in Greek waters.

The innovative nature of this work is to investigate the response of indices of ecological indicator concepts that are based on deterministic approaches under conditions of increasing the concentration of nutrients and, potentially, of the toxic trace elements, and attempt to quantify this response.

## 2. Material and methods

### 2.1. Dataset and indices

Analytical biological and environmental data, derived from the Greek WFD coastal waters monitoring network (Fig. 1; Table 1), were used. The location of stations was selected on the basis of the 'risk-based approach' and the use of assessment areas following

guidance on defining spatial scales in assessment and monitoring (Prins et al., 2013). The monitoring network is comprised of 50 surveillance monitoring stations, which are stations considered unlikely to achieve good ecological status, and 30 operational monitoring stations, which are stations considered likely to achieve good ecological status based on the baseline assessment of Greek water bodies (HCMR, 2008). All the stations are located within a distance of 1 nm from the baseline of the coast (EC, 2003a), which, due to the irregular coastline, means going further along the heads of the bays. Surveillance monitoring stations were sampled during a single year of monitoring covering 1–3 sampling periods, and operational stations were sampled every year covering 4–7 sampling periods during the three years of monitoring. Sampling periods ranged between spring (March or April), late summer (September), and late autumn or early winter (November, December). In total, 292 sampling observations, 193 from operational stations and 99 from surveillance ones, were used. All data were treated on a matrix, including yearly average values. Table 1 gives the mean values of all parameters included in the analysis over the whole sampling period (2012–2014).

A number of ecological indices were applied and grouped into eutrophication indices, benthic indices, phytoplankton biomass index, physicochemical status indices and integrated status indices that were tested in response to stress factors.

To investigate the exceedances in the eco-stoichiometric relationship between nutrients, considered as a stressing factor, all physicochemical elements that can be influenced directly or indirectly by eutrophication were related to ecological indices, namely: total organic carbon (TOC) and nitrogen (TON) percentage in sediment, dissolved oxygen (DO) and % oxygen saturation (Osat), Secchi disk disappearance depth (SD), phosphates (PO<sub>4</sub>), nitrates (NO<sub>3</sub>), nitrites (NO<sub>2</sub>), ammonium (NH<sub>4</sub>), dissolved inorganic nitrogen (DIN; DIN stands for nitrate + nitrite + ammonium), total nitrogen (TN) and total phosphorus (TP), particulate organic phosphorus (POP) and particulate organic carbon (POC) in the water column. Chemical contaminant stress factors include trace or heavy metal measurements in water: cadmium – Cd, cobalt-Co, copper-Cu, nickel-Ni, lead-Pb, zinc-Zn, and chromium-Cr.

The multiple stresses or multi-stressors were assessed by applying a pressure index (PI) (Aubry and Elliott, 2006; Borja et al., 2010, 2011a; Pavlidou et al., 2015) that quantifies all the pressures exerted in the water bodies, including eutrophication, sewage discharge, organic enrichment-agriculture, industrial discharge-chemical pollution, physical and hydro-morphological alteration (the latter including natural hypoxia cases), dredging and sediment disposal-spoil wastes, aquaculture, and harbor-marina-port featuring the main anthropogenic stressors as reported in the Water Information System for Europe (WISE-SoE) for the coastal and marine waters (EEA, 2015). The pressure types and values of PI are shown in Table 2.

The four level-intensity scores (0–3) were estimated using expert judgment, based on our knowledge of the study areas. The pressure intensity scale of Borja et al. (2011a,b) was modified (Pavlidou et al., 2015) to include five levels of evaluation, ranging from 0 (no pressure) to 2 (heavy pressure), providing intensity scores for each pressure type within the corresponding area. The PI was calculated as the average intensity of all pressure types (Table 2) and ranges—0: no or minor pressures; 0.1–0.44: slight pressure; 0.56–1: moderate pressure; 1.11–1.44: high pressure; 1.56–2: heavy pressure.

Eutrophication indices include single metric indices and multi-metric indices as the eutrophication index (EI) (Primpas et al., 2010), and the TRIX index (Primpas and Karydis, 2011).

Benthic indices tested include single metric indices such as the Bentix index (Simboura and Zenetos, 2002) for the ben-

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