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Any signs of replacement of canopy-forming algae by turf-forming algae in the northern Adriatic Sea?

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ABSTRACT

Canopy-forming algal species, like those of the genus *Cystoseira,* are known to play an important role as ecosystems-engineers. Under key anthropogenic stressors (*e.g.* nutrient enrichment and urbanization), these species are replaced by persistent and smaller taxa, defined as turf-forming algae. The scope of this work was to search for shifts from canopy-forming to turf-forming taxa, changes in macrophytes spatial and seasonal diversity, and trends in macrophytes biodiversity on the infralittoral rocky bottom of the northern Adriatic Sea, during the period from 2006 to 2016. At many sampling sites a decline in the proportion of canopy-forming algae (*Cystoseira* spp. and *Halopithys incurva)* was observed until 2012, followed by a slight recovery in 2016. Moreover, the Shannon diversity index calculated for all upper-infralittoral macrophytes increased from 2006 to 2012, then decreased in the following two years, with a new increase from 2015. Despite the assessed fluctuations in the status of macroalgae, we can conclude that there are no visible signs (yet) of degradation of the benthic vegetation.

1. Introduction

There are 41 taxa of long-living brown macroalgae of the genus *Cystoseira* currently reported for the Mediterranean Sea (Taşkin et al., 2012; Cormaci et al., 2012). These canopy-forming species are widespread in the Mediterranean mediolittoral and infralittoral belts, and represent the final stage of the succession of photophilic algal communities on hard substrata (Pérès and Picard 1964). *Cystoseira* species play an important role as ecosystem-engineers (Cormaci, 1995; Gianni et al., 2013) since their benthic communities display a three-dimensional structure that provides habitat and shelter for smaller algae, invertebrates and fish (Lipej et al., 2003, 2009; Orlando-Bonaca and Lipej, 2005, 2007; Orlando-Bonaca et al., 2008a; Ballesteros et al., 2009; Vergés et al., 2009; Cheminée et al., 2013; Antit et al., 2013; Urra et al., 2013; Mačić and Svirčev, 2014; Pitacco et al., 2014). Assemblages dominated by *Cystoseira* species are considered among the most productive in the Mediterranean Sea (Ballesteros, 1989). Moreover, species of the genus *Cystoseira* (except *C. compressa*) are included in Annex II (List of endangered or threatened marine species in the Mediterranean) of the Barcelona Protocol concerning Specially Protected Areas and Biological Diversity (UNEP, Decision IG.21/6; entry into force: 30 March 2014). Since those species are also known for their significant morphological plasticity, further studies would be necessary to improve their taxonomy (Bouafif et al., 2014).

Many authors have observed that coastal ecosystems are subjected to multiple local stressors (mainly from anthropogenic sources), which can result in shifts between alternative habitats (Airoldi and Bulleri, 2011; Orlando-Bonaca et al., 2012; Parravicini et al., 2013; Strain et al., 2014; Connell et al., 2014; Orfanidis et al., 2014; Devescovi, 2015). Since long-living genera of the order Fucales follow long-term periodicity, their disappearance from a coastal rocky-bottom site should be considered as indicative of environmental degradation, if related to important local pressures (Gibson et al., 2000; Falace and Bressan, 2003; Thibaut et al., 2005, 2015; Mangialajo et al., 2008; Iveša et al., 2016; Bulleri et al., 2016). Under key anthropogenic stressors (like nutrient enrichment and urbanization), *Cystoseira* and *Sargassum* species are often replaced by persistent, smaller and less complex algae that are usually defined as turf-forming or mat-forming taxa (Airoldi et al., 2008; Perkol-Finkel and Airoldi, 2010; Connell et al., 2014). These low-lying algae then form an alternative stable state that is able to inhibit recolonization by canopy-forming species (Connell, 2005; Gorman et al., 2009). A recent review paper concluded that in order to prevent shifts from canopy to turf-forming algae, priority should be given to the management of nutrients levels, especially in enclosed bays and estuaries (Strain et al., 2014). After declines in nutrient and organic loads in various Mediterranean areas (Mozetič et al., 2010), a general increase in macroalgal biodiversity and a decrease in the abundance of nitrophilous algae were detected, since Fucoids proved to be stronger

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competitors for space than algal turfs in oligotrophic water conditions (Benedetti-Cecchi et al., 2012; Tsiamis et al., 2013; Iveša et al., 2016). However, some variability in macroalgal assemblages is also ascribed to the synergy between anthropogenic and natural environmental factors (Rinne et al., 2011), such us overgrazing by sea urchins that in some Mediterranean areas is one of the major threats to algal forests (Nikolić et al., 2013).

In the 1970s, the macroalgal cover along the Slovenian rocky infralittoral belt almost disappeared due to overgrazing by the sea urchin *Paracentrotus lividus* (Vukovič, 1976). Macroalgal communities started to reappear in the coastal area from 1992 (Turk and Vukovič, 1994). Species of the genus *Cystoseira* recovered quite well, while *Sargassum* species didn't recover at all (Orlando-Bonaca and Mavrič, 2014). The presence of grazers was identified as an important stressor that can promote the replacement of canopy-forming algae with turf-forming algae (Strain et al., 2014).

In accordance with the Water Framework Directive (WFD, 2000/60/EC) requirements, from 2006, macroalgae are used as a relevant biological element for the assessment of the ecological status (ES) of Slovenian coastal waters. Initially, their status was evaluated on the basis of the Ecological Evaluation Index (EEI) (Orfanidis et al., 2001, 2003) and, in more recent years, using the new Ecological Evaluation Index continuous formula (EEI-c) (Orfanidis et al., 2011). According to this methodology, macrophyte species are divided into 2 Ecological State Groups (ESG). ESG I comprises thick perennial (IA), thick plastic (IB) and shade-adapted plastic (IC) coastal water species, and angiosperm plastic (IA), thick plastic (IB) and shade-adapted plastic (IC) transitional water species. ESG II comprises fleshy opportunistic (IIB) and filamentous sheet-like opportunistic (IIA) species, both in coastal and transitional waters (Orfanidis et al., 2011). The results of the first assessment of the ES of benthic macrophytes in Slovenian coastal waters (Orlando-Bonaca et al., 2008b) indicated that the ES was High/Good, and led to a selection of sampling sites on km-scale for the national surveillance monitoring programme. From 2007 to date, macroalgal samples are almost regularly collected in this coastal area twice per year in the upper-infralittoral belt. Recently, macrophytes were also included in the monitoring programme prepared according to the requirements of the Marine Strategy Framework Directive (MSFD, 2008/56/EC) (Orlando-Bonaca et al., 2013). Both Directives require EU Member States to develop monitoring programmes for the assessment of Good Ecological Status (GES) and Good Environmental Status (GEnS) at least every six years.

The scope of this work was an in-depth investigation of the infralittoral macrophyte cover on the rocky bottom in the Gulf of Trieste, during the period from 2006 to 2016. The goals of the study were to search for: i) insights in macrophyte cover structure and abundance, ii) changes in the cover of canopy-forming taxa, iii) shifts from ESG I to ESG II taxa, iv) changes in macrophyte spatial and seasonal diversity, and v) trends in macrophytes biodiversity in this shallow Mediterranean area.

2. Material and methods

2.1. Study area

The Gulf of Trieste is a shallow semi-enclosed embayment situated in the northernmost part of the Adriatic and Mediterranean Seas. The Gulf extends from Cape Savudrija (Croatia) to Grado (Italy) and includes the entire Slovenian coast, with an average depth of around 21 m. The area is characterized by the lowest winter temperatures (usually below 10 °C) in the Mediterranean Sea (Boicourt et al., 1999). Average salinity is about 37, influenced by fresh water inputs near the coast, mainly from the Isonzo River (Mozetič et al., 1998). The embayed situation of the Gulf, with dominant winds blowing in an offshore direction (mainly from the North-East) and very shallow waters, creates quite sheltered conditions (Boicourt et al., 1999). The general water circulation pattern is predominantly counter clockwise in the lower layer and clockwise in the surface layer (Stravisi, 1983).

The rocky bottom along the Slovenian coast (approximately 46.7 km) consists mainly of Eocene Flysch layers, with alternating solid sandstone and soft marl (Ogorelec et al., 1997). The coastal morphology varies from steep rocky cliffs to gradual sloping beaches (Ogorelec et al., 1991). In past decades, the coastal area has suffered from many anthropogenic impacts such as new infrastructures, intensive fishing, sewage outfalls and mariculture (Francé and Mozetič, 2006; Mozetič et al., 2008; Grego et al., 2009).

According to the WFD requirements, in 2005, the Slovenian sea was divided into six water bodies (WBs), four of them being assigned as Coastal Waters (Fig. 1). Two WBs in Coastal Waters have been characterized as "rocky shallow moderately exposed" (Sl5VT2, Sl5VT4), one as "sedimentary shallow moderately exposed" (Sl5VT5), and one as "heavily modified water body" (SI5VT3) due to the presence and activities of the Port of Koper and related maritime traffic.

2.2. Fieldwork and laboratory work

For an initial assessment of the status, benthic macrophytes of three EUNIS habitat types (European Environmental Agency, 2004) were sampled during the summer-autumn period of 2006, at 51 sampling sites along the Slovenian coastline, at a depth range from 2 m to 5 m (Appendix A in Table A1). Those habitat types are: A3.2 Infralittoral rock moderately exposed to wave action and/or currents and tidal streams; A3.3 Infralittoral rock sheltered from wave action and currents and tidal streams; and A5.5–A5.53 Sublittoral seagrass beds with macrophyte-dominated rocks. Thus, a national monitoring programme started, with 8 sampling sites sampled in 2007, 2008, 2010 and 2012, 3 in 2009, 2015 and 2016, 5 in 2011, and 9 in 2013 (Fig. 1). Monitoring samples were collected twice per year (in spring and autumn), except in 2009, when macrophytes samples were collected only in the summer (Appendix A in Table A1).

At each sampling site (an area of 10 m × 10 m), three samples were randomly scraped from the bottom (20 cm × 20 cm). Such a surface (400 cm^2) is considered to be the minimum sampling area for Mediterranean infralittoral communities (Boudouresque and Belsher, 1979). Each sample was collected in a plastic bag and all the material was transported to the laboratory of the Marine Biology Station of the National Institute of Biology. Samples were then fixed in formalin (4–5%) or ethanol (70%). Macrophyte species identification was carried out in accordance with Ribera et al. (1992), Gallardo et al. (1993), Gómez Garreta et al. (2001), and Bressan and Babbini (2003). Samples were carefully sorted and the surface covered by each species (the orthogonal projection) was quantified in cm^2 (4 cm^2 = 1% of the sampling surface). Only species covering at least 1% of the sampling area were considered. In cases where the coverage (as a measure of abundance) of morphologically similar species could not be measured precisely, those species were grouped together (as spp.).

2.3. Data analysis

We used a background dataset that included, for every species that was present in at least one sample in a particular year, the species Genus, Family and Order, the percentage cover and whether the species is considered as ESG I or ESG II.

In order to test the hypothesis of shifts from ESG I to ESG II taxa, and also shifts in the coverage of canopy-forming taxa, sampling sites that were monitored seasonally in at least five years were considered. Using this criterion, data from five sites (RR1, Por2, Se1, Iz4 and PP4) were analyzed. At each site, we tested whether the proportion of the percentage coverage of ESG I versus ESG II taxa, and the proportion of the percentage coverage of canopy-forming versus all other taxa changed over the years. χ^2 statistics was used to test for the equality of the proportions. All the species from the genus *Cystoseira* and *Halopithys*