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V. PITACCO[1], M. ORLANDO-BONACA[1], B. MAVRIČ[1], A. POPOVIĆ[2] and L. LIPEJ[1]

[1] Marine Biology Station, National Institute of Biology, Fornače 41, 6330 Piran, Slovenia
[2] FAMNIT, University of Primorska, Glagoljaska 8, 6000 Koper, Slovenia

Corresponding author: valentina.pitacco@mbss.org

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Abstract

Mollusc assemblages associated with *Cystoseira* associations were sampled in the southern part of the Gulf of Trieste during the summers of 2008 and 2012. Samples were collected by SCUBA diving in the infralittoral belt (from 1 to 4 m depth). The surface within frames of 20 x 20 cm was scraped off and the samples were collected by hand or using an air-lift sampler. Four erect algal species were found: *Cystoseira barbata, C. compressa, C. corniculata* and *Halopithys incurva*. A total of 69 species of molluscs were identified in those associations. Gastropoda were dominant, with the highest species richness and abundance, followed by Bivalvia and Polyplacophora. A large number of juveniles were found, proving the importance of *Cystoseira* associations for mollusc recruitment. Differences in composition, structure and abundance of mollusc assemblages were found for sites dominated by different algal species, and correspond to different morphology and degree of development of canopy-forming species. This study confirms that the dominant algal species within *Cystoseira* associations strongly influence, although at different levels, abundance and distribution of mollusc assemblages in the Northern Adriatic Sea.

Keywords: molluscs, biodiversity, *Cystoseira* associations, infralittoral, Northern Adriatic Sea.

Introduction

Marine macroalgae and seagrasses are the main benthic primary producers in the marine realm (Mann, 1973). In the Mediterranean Sea, environmental conditions (e.g. oligotrophic waters, availability of hard substrata, relatively high salinity and high mean water temperature) seem to be favourable for species of the genus *Cystoseira* (Fucales, Ochrophyta), following five-million-year-long diversification (Roberts, 1978). *Cystoseira* species are widespread in the Mediterranean infralittoral belts, and represent the final stage of the succession of photophilic algal communities on hard bottoms (Pérès & Picard, 1964). These communities also play a role of habitat former (Giaccone & Bruni, 1971) since they display a three-dimensional structure that provides habitat and shelter for a large number of algae and invertebrates (Mann, 1973; Antit *et al.*, 2013). The importance of these associations for fish (Lipej *et al.*, 2009; Orlando-Bonaca & Lipej, 2005; Fiorin *et al.*, 2008; Orlando-Bonaca *et al.*, 2008a; Vergés *et al.*, 2009; Cheminée *et al.*, 2013) and invertebrate communities (Milazzo *et al.*, 2000; Costa & Ávila, 2001; Chemello & Milazzo, 2002; Gozler *et al.*, 2010; Urra *et al.*, 2013) has already been the subject of investigation in different areas of the Mediterranean Sea. Nevertheless, many questions regarding relationships between macroalgae and benthic invertebrates still remain unanswered.

Cystoseira spp. are long-living, complex macroalgae, and two associations with *Cystoseira* species are today present in the southern part of the Gulf of Trieste: *Cystoseiretum barbatae* Pignatti 1962 and *Cystoseiretum crinitae* Molinier 1958. The latter is present with two sub-associations: *Halopithetosum incurvae* Boudouresque 1971 and *Cystoseiretum compressae* Molinier 1958 (Orlando-Bonaca *et al.*, 2008b), since under particular ecological conditions other species than *C. crinita* become very abundant in this association (Giaccone *et al.*, 1994). The red macroalga *Halopythis incurva* usually becomes dominant in high hydrodynamic conditions (Orlando-Bonaca *et al.*, 2008a), while *C. compressa* was defined as dominant at sites with mild pollution (Giaccone *et al.*, 1994). *Cystoseira corniculata* does not form a recognisable subassociation, but becomes vicariant of *C. crinita* in environments with a high sedimentation rate (Giaccone *et al.*, 1994). The *C. barbatae* association remains dominant in Slovenian waters, since it is typical of areas with high sedimentation rates and low organic pollution (Vukovič, 1980). The fish assemblage inhabiting *Cystoseira* associations proved to be very rich in terms of species richness (Orlando-Bonaca *et al.*, 2008a). Moreover, *Cystoseira* spp. associations showed the highest fish species diversity compared to other habitat types (Orlando-Bonaca & Lipej, 2005).

In Slovenian coastal waters macroalgae are widely used as bioindicators for the assessment of ecological sta-

tus (ES), according to the Water Framework Directive (Orlando-Bonaca *et al.*, 2008b). Currently, they are considered as a relevant benthic element also within the preparation of the monitoring program according to the Marine Strategy Framework Directive (Orlando-Bonaca *et al.*, 2012a). Both Directives require that European Member States develop monitoring programs for the assessment of Good Environmental Status (GEnS) at least every six years.

Since macroalgae and especially long-lived genera like those from the order Fucales follow long-term periodicity, their absence from a site should be regarded as indicative of environmental degradation. The ES of coastal waters is currently also assessed with regards to phytoplankton (Mozetič *et al.*, 2012) and soft-bottom invertebrates (Mavrič *et al.*, 2010). A recent study has proven that hard bottom macrozoobenthic communities could also be used as indicators for the assessment of hydromorphological alterations of the rocky shore (Orlando-Bonaca *et al.*, 2012b). However, until now, invertebrate assemblages associated with macroalgal associations in Slovenian waters have not been accurately characterized.

The objective of the present work was to investigate the importance of *Cystoseira* species associations in terms of mollusc diversity, comparing different sampling sites and different *Cystoseira* associations in the Gulf of Trieste.

Materials and Methods

Study area

The Gulf of Trieste is a shallow semi-enclosed embayment located in the northernmost part of the Adriatic Sea (Mediterranean Sea). It extends from Cape Savudrija (Croatia) to Grado (Italy) and includes the entire Slovenian coast. The maximum depth (approximately 33 m) is found in waters off Piran. The area is characterized by the lowest winter temperatures in the Mediterranean Sea and can fall below 10°C (Boicourt *et al.*, 1999). Salinity is about 37 psu on average, but it is influenced near the coast by fresh water inputs, mainly from the Isonzo River (Mozetič *et al.*, 1998). During the summer, a typical thermal stratification of the water column develops, due to surface heating and fresh water inflow (Boicourt *et al.*, 1999). In winter, the water column is characterized by considerable vertical homogeneity due to autumnal cooling processes and wind mixing (Mozetič *et al.*, 1998). The embayed situation of the Gulf, together with dominant winds blowing in an offshore direction (from the North-East) and very shallow waters create a quite sheltered condition (Boicourt *et al.*, 1999).

The coastal morphology of the Gulf varies from steep rocky cliffs to gradual sloping beaches consisting of gravel and pebbles (Ogorelec *et al.*, 1991). The rocky substratum of the Slovenian coast consists mainly of Eocene flysch layers, with alternating solid sandstone and soft marl (Ogorelec *et al.*, 1997).

Sampling sites and techniques

Five sampling sites were selected along the Slovenian coast (Fig.1, sites: Debeli Rtič - DR, Pacug - PA, Piranček - PI, Rtič Ronek – RR and Marine Biology Station - MBP) in summer 2012, according to the previously known presence of *Cystoseiretum crinitae* and *Cysto-*
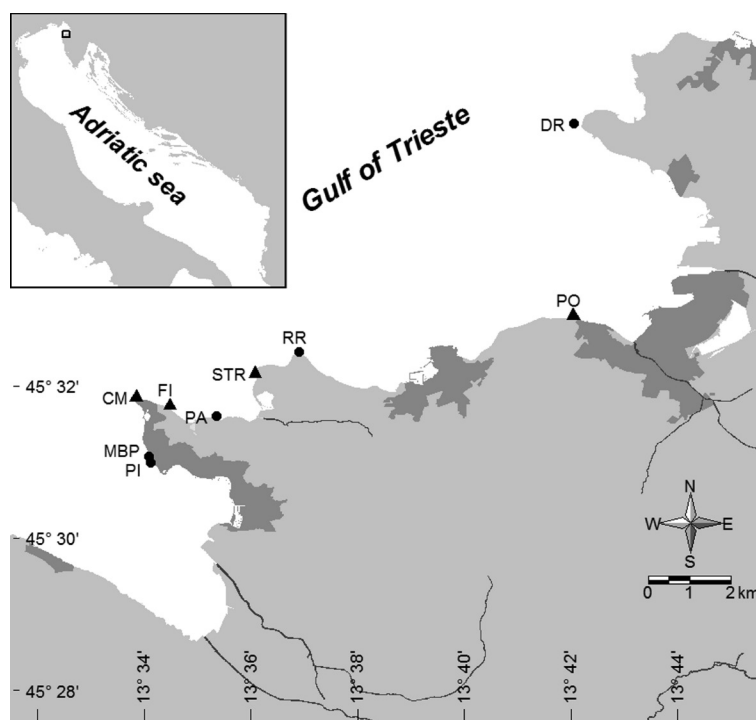

Fig. 1: The study area with sampling sites. Five sites were sampled in 2012 [DR Debeli rtič], RR (Cape Ronek), PA (Pacug), MBP (Marine Biology Station), and PI (Piranček)). Four sites were sampled in 2008 (STR (Natural Reserve Strunjan), FI (Fiesa), CM (Cape Madona Natural Monument) and PO Izola)].

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seiretum barbatae associations. In order to reduce the bias related to the sampling season, the sampling period was chosen to correspond with the moment of maximum development of *Cystoseira* species (Falace *et al.*, 2005). Samples (Table 1) were randomly taken at each site within an area of 10 x 10 m located in the upper infralittoral belt, mostly between 1 and 2 m, with the only exception of site RR, where no *Cystoseira* association was present above 3 m of depth and samples were taken down to 4 m depth. Sampling was carried out by SCUBA diving. At each site, five samples were taken by scraping off the substratum within a 20 x 20 cm frame (Boudouresque & Belsher, 1979; Bianchi *et al.*, 2003), collecting all macroalgae and associated sessile and vagile animals by hand and preserving them in a plastic bag. Algal species were determined before fixation and then preserved in 70% ethanol. Four erect algal species forming the canopy layer of associations were determined: *Cystoseira barbata, C. compressa, C. corniculata* and *Halopithys incurva* (Table 1). The number of thalli and the minimum and maximum height of the collected algae were assessed in the laboratory.

Moreover, in order to increase the pool of data related to mollusc species inhabiting *Cystoseira* spp. associations, the dataset was completed with data from a previous sampling survey performed in 2008 with SCUBA diving, scraping off the substrate with scrapers and collecting it using an air-lift pump. Samples were collected at four sites (Fig. 1, sites: Strunjan - STR, Fiesa - FI, Cape Madona - CM, Coastal road between Koper and Izola - PO), between 1 and 2 m depth, using the same frame (20 cm x 20 cm). At each site, five samples were also randomly collected, but for the present work only 12 samples with high coverage of *Cystoseira* species (>30%) were considered. Two erect algal species were determined from these samples: *C. barbata* and *C. compressa* (Table 1).

Samples were sieved through a 0.5 mm mesh and after a sorting process, live molluscs were determined to the lowest possible taxonomic level, according to Tebble (1966), Ghisotti & Sabelli (1970), Parenzan (1970-1976), Torelli (1982) and Doneddu & Trainito (2005), and counted. Individuals with less than half the known adult size of the species were considered juveniles and were counted separately. Juvenile gastropods were easily recognized due to a sharp, incompletely developed outer lip. Most juveniles were determined only to the family or genus level. The nomenclature follows WoRMS (WoRMS Editorial Board, 2013).

Each species was assigned to one of the following trophic groups: carnivores feeding on other motile organisms (C); scavengers feeding on remains of dead organisms (SC); deposit feeders feeding on organic particles contained in the sediment (D); ectoparasites and specialized carnivores feeding on much larger organisms on which they live during their life cycle (E); filter feeders capturing seston particles with their gills or with mucous strings (F); micrograzers feeding on microalgae, cyanobacteria or detritus attached to algal fronds (MG).

Feeding guilds were assessed according to Bachelet (1981), Solis-Weiss *et al.* (2004) and Rueda *et al.* (2009).

Data analysis

Number of species (S) and number of individuals (N) per 400 cm^2 were counted, and the Margalef index of richness (d), Shannon & Wiener diversity index (H'), Pielou index of equitability (J'), Simpson index of dominance (D$_\lambda$) (Clarke & Warwick, 2001) were calculated for all sampling sites (from 2008 and 2012). Percentage of dominance (%D) and frequency (%F) was calculated for each species at the different sampling sites.

Differences between sites investigated in 2012 were also checked against k-dominance curves (Clarke & Warwick, 2001) and statistically tested using the one way

Table 1. Description of sampling sites (sampling depth and date, dominant canopy forming algal species, and sampling method) (SC= scraper; SO= sorbona, air-lift pump).

Site	Code	Depth (m)	Date	Species	Method
MBP	MBP	1.0-2.0	12. 6. 2012	*C. compressa, C. barbata*	SC
Cape Ronek	RR	3.1-4.0	9. 7. 2012	*C. corniculata*	SC
Piranček	PI	1.4-1.8	10. 7. 2012	*C. compressa, C. barbata*	SC
Pacug	PA	1.4-2.0	11. 7. 2012	*C. barbata*	SC
Debeli rtič	DR	1.4-2.1	20. 7. 2012	*H. incurva*	SC
NR Strunjan	STR	1.0-2.0	10. 6. 2008	*C. barbata*	SO
Fiesa	FI	1.0-2.0	10. 6. 2008	*C. barbata*	SO
Cape Madona	CM	1.0-2.0	18. 6. 2008	*C. compressa*	SO
Coastline between Koper and Izola	PO	1.0-2.0	26.6. 2008	*C. compressa*	SO

ANOSIM (analysis of similarity) test (Clarke, 1993). These statistical analyses were carried out using the Primer 6.1.5. software package, developed by the Plymouth Marine Laboratory.

One-way ANOVA was performed to test differences in the values of diversity indices and macroalgae parameters between sites. Data that did not display a normal distribution (Shapiro-Wilks test) were transformed with arcsine (J' and D_λ) and natural logarithm (H', density and minimum height of algae) prior to ANOVA analyses. A *post-hoc* Tukey test (P<0.05) was used to identify the sites responsible for the detected differences. These analyses were performed using R version 2.4.0 (de Mendiburu, 2012; R core team, 2012).

Since the number of thalli of *Cystoseira* species differed between sampling sites, the possible correlation between the number of thalli and species abundance and richness of the mollusc assemblage was tested using the Pearson (1895) correlation coefficient. Moreover, a possible correlation between the mean and maximum height of thalli and species richness and abundance was also assessed.

ANOSIM tests on abundance data from 2012 were conducted to test whether mollusc assemblages varied in different algal associations and at different sampling depths.

An ANOSIM test on abundance data from 2008 and 2012 was performed in order to check whether there were significant differences between mollusc assemblages collected by scraping in 2012 and those sampled using the air-lift sampler in 2008. The same test was performed for the differences between the different sampling sites.

A complete-linkage sorting classification (Cluster) analysis was performed using mollusc abundances from samples associated with studied *Cystoseira* associations both in 2008 and 2012. Mollusc abundances were log-transformed and analyzed based on the Bray Curtis similarity index with the Primer software. Groups were created with a cut at 20% of similarity.

Results

Description of the mollusc assemblage

Considering results obtained from sampling surveys in 2008 and 2012, molluscs inhabiting the two sub-associations of *Cystoseiretum crinitae* and the association *Cystoseiretum barbatae* amounted to a total of 2649 individuals, belonging to 69 different taxa (3 Polyplacophora, 42 Gastropoda and 24 Bivalvia) (Table 2). Gastropods were the most abundant group, with 2244 individuals (85%), followed by Bivalvia with 401 individuals (15%) and Polyplacophora with 4 (0.15%). The molluscs belonged to 35 different families, of which Trochidae, Rissoidae and Mytilidae displayed the highest number of species.

The most frequent and abundant species was *Bittium reticulatum* (86.5% of frequency, 22% of total abundances). *Jujubinus exasperatus* (70.3%), *Rissoa guerinii* (70.3%), *Columbella rustica* (56.8%) and *Modiolus barbatus* (54.1%) were frequent. *Jujubinus exasperatus* and *R. guerinii* were also very abundant (10.7% and 12% of total abundances respectively), together with *Alvania cimex* (16.6%). All these species were present in both juvenile and adult form and were collected with both methods (in 2008 and 2012).

Nearly half (46%) of the individuals were juveniles, and 10 taxa (*Chiton olivaceus*, *Tricolia* sp., *Cerithium* sp., Gastropoda indet., *Fusinus* sp., *Bulla striata*, *Mytilus galloprovincialis*, Muricidae indet., *Anomia ephippum,* Cardiidae indet.) were only found as juveniles.

The identified mollusc species belonged to five feeding guilds: carnivores, scavengers, filter feeders, micrograzers and ectoparasites. Even if some micrograzers can also feed on sediment deposited among microalgae, deposit feeders *sensu stricto* were absent (Table 2).

Micrograzers (mainly Trochidae and Rissoidae) were the dominant group, both in terms of species richness (25 taxa) and abundance (2028 individuals), followed by filter feeders (23 taxa and 399 individuals) represented mainly by species of the family Mytilidae. Carnivores, such as Muricidae (9 taxa, 53 individuals), scavengers (mainly Nassaridae) (5 taxa, 125 individuals) and specialized carnivores (34 individuals), represented by 4 species (*Marshallora adversa*, *Calliostoma laugieri*, *C. conulus* and *Odostomia acuta*) feeding on sponges, cnidarians, bivalves and polychaetes, were less represented.

The most frequent micrograzers were *B. reticulatum* (86.5%), *J. exasperatus* (70%) and *R. guerinii* (70%). The most frequent filter-feeders were *Modiolus barbatus* (54%), *Musculus costulatus* (43%) and *Arca noae* (32%). *Nassarius incrassatus* was the most frequent scavenger (40.5%), while carnivores and specialized carnivores were infrequent (<25%).

Comparison of sampling sites investigated in 2012

Four erect algal species forming the canopy layer of associations were found: *Cystoseira barbata, C. compressa, C. corniculata* and *Halopithys incurva* (Table 1). *C. barbata* is representative of the association *Cystoseiretum barbatae*, whereas the other three belong to association *Cystoseiretum crinitae*. A weak negative relationship was found between thalli density and their dimensions (minimum (R^2= 0.22; *df*= 23; P=0.018) and maximum height (R^2= 0.23; *df*= 23; P=0.014)). Density and height of thalli showed significant differences between sites (Table 3). The highest mean density of macroalgae was observed at site PI, where the thalli of *C. compressa* were short, showing the lowest minimum and maximum height values (Table 4). At site MBP, macroalgal associations were more developed in height, since the thalli of *C. barbata* and *C. compressa* reached the highest length

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Table 2. List of determined molluscs, their taxonomic position, feeding mode (MG=micrograzer; C=carnivore; SC= scavenger; E=ectoparasite; F=filter feeders; ND=no data available), life stage (J=juvenile; A=adult), percentage of dominance (%D), percentage of frequency (%F) and sampling method (1= scraper; 2= air-lift pump).

Taxonomic position	Species	Feeding mode	Life stage	%D	%F	Sampling method
Polyplacophora						
Ischnochitonidae	*Ischnochiton rissoi*	MG	A	0.0	2.7	2
Chitonidae	*Chiton olivaceus*	MG	J	0.1	5.4	1
Acanthochitonidae	*Acanthochitona fascicularis*	MG	A	0.0	2.7	2
Gastropoda						
Fissurellidae	*Diodora gibberula*	MG	A	0.2	13.5	1
Turbinidae	*Tricolia* sp.	MG	J	0.1	5.4	1
Trochidae	*Clanculus cruciatus*	MG	A	1.0	18.9	1
Trochidae	*Clanculus jussieui*	MG	A	0.3	16.2	1
Trochidae	*Gibbula adansonii*	MG	J,A	1.1	16.2	2
Trochidae	*Gibbula adriatica*	MG	A	0.0	2.7	2
Trochidae	*Gibbula ardens*	MG	A	1.3	18.9	1
Trochidae	*Gibbula umbilicaris*	MG	A	1.1	35.1	1, 2
Trochidae	*Gibbula varia*	MG	J,A	1.6	24.3	1
Trochidae	*Jujubinus exasperatus*	MG	J,A	10.7	70.3	1, 2
Trochidae	*Jujubinus striatus*	MG	A	0.3	16.2	1
Trochidae	*Calliostoma conulus*	E	J,A	0.3	16.2	1
Trochidae	*Calliostoma laugieri*	E	A	0.2	13.5	2
Rissoidae	*Rissoa guerinii*	MG	J, A	12.0	70.3	1, 2
Rissoidae	*Rissoa splendida*	MG	A	0.1	2.7	1
Rissoidae	*Rissoa variabilis*	MG	A	0.1	2.7	2
Rissoidae	*Alvania cimex*	MG	J,A	14.6	40.5	1, 2
Rissoidae	*Alvania discors*	MG	A	3.2	27.0	1, 2
Rissoidae	*Alvania lineata*	MG	A	4.4	2.7	1
Cerithiidae	*Cerithium* sp.	MG	J	0.1	5.4	2
Cerithiidae	*Bittium latreillii*	MG	A	0.6	21.6	1
Cerithiidae	*Bittium reticulatum*	MG	J,A	22.0	86.5	1, 2
	Gastropoda indet.	ND	J	0.1	2.7	1
Triphoridae	*Marshallora adversa*	E	J,A	0.6	24.3	1, 2
Muricidae	*Ocinebrina edwardsii*	C	J, A	0.5	24.3	1, 2
Muricidae	Muricidae indet.	C	J	0.0	2.7	1
Buccinidae	*Enginella leucozona*	SC	A	0.2	8.1	1
Buccinidae	*Pisania striata*	SC	A	0.5	24.3	1, 2
Columbellidae	*Colombella rustica*	MG	J,A	1.6	56.8	1, 2
Columbellidae	*Mitrella scripta*	MG	A	0.1	2.7	1
Nassariidae	*Nassarius corniculum*	SC	J, A	2.8	18.9	1, 2
Nassariidae	*Nassarius incrassatus*	SC	J, A	1.2	40.5	1, 2
Nassariidae	*Nassarius pygmaeus*	SC	A	0.0	2.7	1, 2
	Nudibranchia indet.	ND	A	0.2	5.4	2
Fasciolariidae	*Fusinus* sp.	C	J	0.1	5.4	1
Costellariidae	*Vexillum ebenus*	C	A	0.1	5.4	2
Mangeliidae	*Mangelia attenuata*	C	A	0.2	5.4	2
Mangeliidae	*Bela* sp.	C	J,A	0.8	8.1	1
Raphitomidae	*Raphitoma bicolor*	C	A	0.2	8.1	2
Conidae	*Conus mediterraneus*	C	A	0.1	2.7	2
Pyramidellidae	*Odostomia acuta*	E	A	0.2	5.4	2
Bullidae	*Bulla* sp.	C	J	0.1	2.7	2
Bivalvia						
Arcidae	*Arca noae*	F	J, A	0.6	32.4	1, 2
Arcidae	*Barbatia barbata*	F	A	0.0	2.7	1, 2
Noetiidae	*Striarca lactea*	F	J, A	0.3	10.8	1, 2
Mytilidae	*Mytilus galloprovincialis*	F	J	2.2	24.3	1, 2
Mytilidae	*Modiolus barbatus*	F	J, A	1.7	54.1	1, 2
Mytilidae	*Modiolarca subpicta*	F	J, A	1.2	32.4	1, 2
Mytilidae	*Gregariella petagnae*	F	A	0.0	2.7	2
Mytilidae	*Musculus costulatus*	F	J, A	3.9	43.2	1, 2
Limidae	*Limaria hians*	F	A	0.3	8.1	1
Ostreidae	*Ostrea edulis*	F	A	0.1	2.7	2
Ostreidae	*Crassostrea gigas*	F	A	0.1	5.4	1
Ostreidae	*Ostrea stentina*	F	A	0.0	2.7	2
Pectinidae	*Mimachlamys varia*	F	A	0.1	8.1	1, 2
Anomiidae	*Anomia ephippum*	F	J	0.8	16.2	1, 2
Montacutidae	*Kurtiella bidentata*	F	A	0.2	8.1	2
Cardiidae	Cardiidae indet.	F	J	0.2	8.1	1
Galeommatidae	*Galeomma turtoni*	ND	A	0.1	5.4	1
Trapeziidae	*Coralliophaga lithophagella*	F	A	0.0	2.7	2
Veneridae	*Gouldia minima*	F	A	0.0	2.7	2
Veneridae	*Irus irus*	F	J, A	0.3	8.1	1, 2
Veneridae	*Petricola lithophaga*	F	A	0.1	5.4	2
Gastrochaenidae	*Gastrochaena dubia*	F	A	1.5	13.5	1, 2
Hiatellidae	*Hiatella artica*	F	J, A	0.6	24.3	1
Hiatellidae	*Hiatella rugosa*	F	A	0.6	8.1	2

values (minimum and maximum, Table 4). The lowest mean density was observed at site DR, where the algal association was dominated by *H. incurva* (Table 4).

The mean richness and diversity of molluscs (Margalef, Shannon, Pielou and Simpson indices) varied significantly between sites sampled in 2012, but not the mean number of individuals (Table 3). Major differences were found between mollusc assemblages at sites PA and MBP. The highest diversity (Margalef, Shannon and Simpson indices) was obtained for site PA, whereas the lowest diversity (Pielou, Shannon, and Simpson indices) was found for site MBP (Table 5).

The mollusc assemblage at site PA was the best structured, with a low initial dominance and a k-dominance curve reaching the asymptote slowly (Fig. 2). Gastropods *Rissoa guerinii* (25% of total abundance) and species of the genus *Gibbula* (24%) were dominant. *Rissoa guerinii*, together with *Bittium reticulatum*, was also the most frequent (100%). The percentage of juveniles was highly variable between replicates (from less than 40% of total abundance to more than 90%). Also, considering feeding guilds, the assemblage at this site was well structured, with dominant micrograzers (Rissoidae and Trochidae) found together with filter feeders (Mytilidae), carnivores

(Mangeliidae), scavengers (Nassariidae) and specialized carnivores feeding on sponges (*M. adversa*).

Conversely, the assemblage at site MBP was poorly structured, with an initial dominance of about 50% and a k-dominance curve reaching the asymptote quickly (Fig. 2). This site was characterized by the dominance of the gastropod *B. reticulatum* (52% of total abundance), and young specimens of the bivalve *Mytilus galloprovincialis* (17%). In this case also, the number of juveniles was highly variable between replicates (from less than 40% to 90% of total abundance). Filter feeders (mainly Mytilidae) were dominant as regards number of species, but micrograzers were dominant in terms of number of individuals (due to the dominance of the gastropod *B. reticulatum*). Molluscs with other feeding modes were absent.

Micrograzers were dominant both in terms of number of individuals (Fig. 3A) and number of taxa (Fig. 3B) at all sampling sites, except at site MBP, where filter feeders were dominant in the number of taxa, but micrograzers remain dominant for number of individuals (Fig. 3B). Carnivores, scavengers and ectoparasites were present at all sites, with the exception of PI and MBP (Fig. 3A, B).

The simplest trophic structure was found at PI, where only micrograzers (Rissoidae, Trochidae and Cerithiidae)

Table 3. Differences between sites sampled in 2012 (tested with one-way ANOVA) for mollusc assemblage variables (S = mean number of taxa for 400 cm², N = mean number of individuals for 400 cm², d = Margalef index, J = Pielou index, H = Shannon index with $\log_e$, and D_λ = Simpson index) and for macroalgal variables (Hmin= minimum height; Hmax= maximum height; Dens=thalli density). F= F ratio of mean square; p = probability; *df* = degrees of freedom. Significance:*=0.01;**=0.001; ***=0.0001; NS=not significant.

	F	p	df	Significance
Mollusc assemblages				
S	3.494	0.025	4	*
N	2.171	0.109	4	NS
d	6.557	0.00153	4	**
J'	3.733	0.02	4	*
H'	4.714	0.00764	4	**
D_λ	4.197	0.0125	4	*
Macroalgae				
	F	p	df	Significance
Hmin	3.248	0.0331	4	*
Hmax	9.81	0.000147	4	***
Dens	6.668	0.0014	4	**

Table 4. Depth range, mean number of thalli per 400 cm², mean minimum and maximum height of canopy forming algal species, for the 5 sampling sites investigated in 2012.

Sample	Depth range	Density		Minimum height		Maximum height	
		Mean	SD	Mean	SD	Mean	SD
DR	1.4 - 2.1 m	9	3.317	10.5	0.707	20.76	1.135
RR	3.1 - 4 m	21.8	7.294	9.4	0.704	18.9	1.294
MBP	1 – 2 m	11.2	2.588	12.3	4.489	30.58	3.922
PI	1.4 – 1.8 m	23.8	2.775	6.7	1.823	17.4	2.748
PA	1.3 -2 m	14.6	8.905	8.52	3.079	21.12	5.962

Table 5. Comparison of sampling sites. S = number of taxa for 400 cm², N = number of individuals for 400 cm², d = Margalef index, J = Pielou index, H = Shannon index with $\log_e$, and D_λ = Simpson index.

Site		S	N	d	J	H	D_λ
DR (2012)	mean	14	173	2.82	0.70	1.77	0.74
	(SD)	(3.91)	(206.39)	(0.50)	(0.19)	(0.43)	(0.14)
RR (2012)	mean	11	24	3.24	0.84	2.00	0.84
	(SD)	(2.77)	(8.64)	(0.60)	(0.10)	(0.24)	(0.10)
MBP (2012)	mean	11	107	2.21	0.64	1.48	0.65
	(SD)	(3.56)	(72.98)	(0.59)	(0.09)	(0.21)	(0.08)
PI (2012)	mean	8	25	2.08	0.86	1.72	0.82
	(SD)	(1.95)	(9.42)	(0.43)	(0.06)	(0.21)	(0.05)
PA (2012)	mean	13	44	3.38	0.84	2.19	0.87
	(SD)	(1.67)	(17.16)	(0.41)	(0.08)	(0.22)	(0.06)
STR (2008)	mean	15	111	3.08	0.72	1.97	0.80
	(SD)	(2.08)	(47.15)	(0.49)	(0.07)	(0.29)	(0.09)
FI (2008)	mean	13	65	2.76	0.90	2.28	0.89
	(SD)	(0.71)	(3.54)	(0.13)	(0.01)	(0.07)	(0.02)
CM (2008)	mean	9	20	2.55	0.82	1.69	0.78
	(SD)	(4.04)	(9.50)	(0.93)	(0.11)	(0.41)	(0.12)
PO (2008)	mean	15	50	3.68	0.78	2.12	0.80
	(SD)	(2.08)	(15.82)	(0.30)	(0.10)	(0.29)	(0.10)

were found. At this site also, the lowest richness (mean 8 species, and Margalef index) was recorded (Table 4), with the complete absence of bivalves and polyplacophorans. The percentage of juveniles was also low (<30%). On the other hand, the individuals were uniformly distributed among taxa (high J' and well-structured k-dominance curve), without any particularly dominant species.

Relationship between molluscs and algal associations

In the material collected in summer 2012, no significant linear correlation was found between mollusc species richness and mean macroalgal thalli height (R^2= 0.0141; *df*= 23; P=0.571), nor between richness and maximum thalli height (R^2= 0.0031; *df*= 23, P=0.791). No significant correlation was found either between mollusc abundances and minimum (R^2= 0.0364; *df*= 23; P=0.361) or maximum thalli height (R^2= 0.0179; *df*= 23; P=0.523). Eventually, no significant correlation was found between mollusc species richness and thalli density (R^2= 0.061; *df*= 23; P=0.235), while a weak negative correlation was
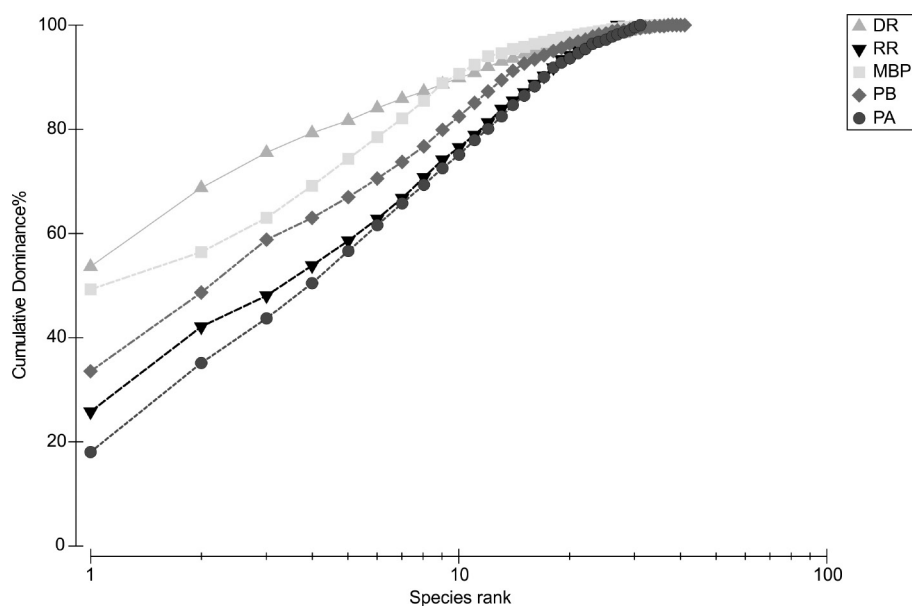

Fig. 2: K-dominance curve for sampling sites investigated in 2012. The graph shows the distribution of species abundances in the assemblage, plotted as a percentage of cumulative abundance (y axis) as a function of rank (x axis). Curves with low initial dominance reaching the asymptote slowly represent assemblages with homogeneous abundances and with high diversity.
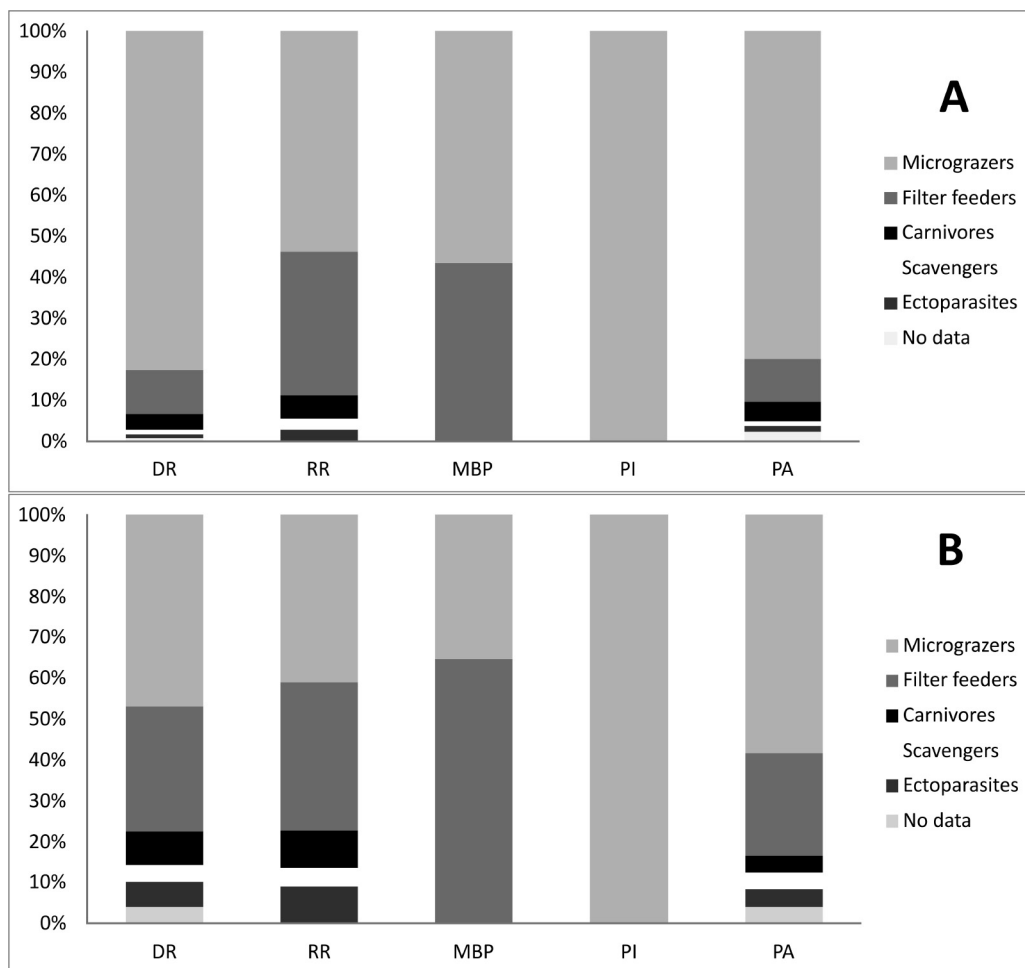
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Fig. 3: Comparison of the proportions of mollusc feeding guilds among sampling sites investigated in 2012, in terms of mean abundance (A) and richness (B) per 400 cm^2.

found between mollusc abundance and thalli density (R^2= 0.168; *df*= 23; P=0.041).

Considering both species richness and abundance, variability in mollusc assemblages between different dominant algal species (ANOSIM test, R=0.474, P=0.0003) and at different depths (ANOSIM test, R=0.335, P=0.003) was only slightly higher than variability within each algal species and each sampling depth.

A slight difference was found (ANOSIM test, R=0.241, P=0.04) among mollusc assemblages collected by hand (after scraping) in 2012 and those sampled using the air-lift sampler (also after scraping) in 2008. However, considering all the samples from 2008 and 2012, there were clear differences in composition between mollusc assemblages inhabiting different algal communities at different sampling sites (ANOSIM test, R=0.694, P=0.01). These differences are shown clearly in the cluster analysis (Fig. 5). According to a 20% similarity cut, sampling sites formed 7 distinct groups. The first group of samples (from the left in Fig. 5) comprises the mollusc assemblage associated with *C. compressa* at site CM. The second group is composed of mollusc assemblages at sites PI and PA, with the presence of *C. compressa* and

C. barbata. The third group is formed of samples with molluscs inhabiting the association of *C. corniculata* at Cape Ronek. The fifth group comprises mollusc assemblages associated with *C. barbata* at sites STR and FI (exception made for sample PO3). The sixth group incorporates the mollusc assemblages related to the presence of *H. incurva* at Debeli Rtič and the seventh comprises assemblages related with *C. barbata* at sites MBP and PA (exception made for PO2). Sites DR2 and PO1 form a separate group (the fourth).

Clear differences in composition of the mollusc assemblages associated with *C. corniculata* (third group) and *H. incurva* (sixth group) were observed. At site RR, dominated by *Cystoseira corniculata*, the mollusc assemblage was characterized by a low number of juveniles (17% of total abundances) and the dominance of large adult specimens of *Jujubinus exasperatus* (26% of total abundances) and *Clanculus cruciatus* (19% of total abundances). The mollusc assemblage at site DR, dominated by *H. incurva*, was poorly structured, as shown by k-dominance analysis, with an initial dominance of more than 50% and a curve reaching the asymptote quickly (Fig. 2). This site was characterized by the dominance of small gastropods of genus *Alva-*
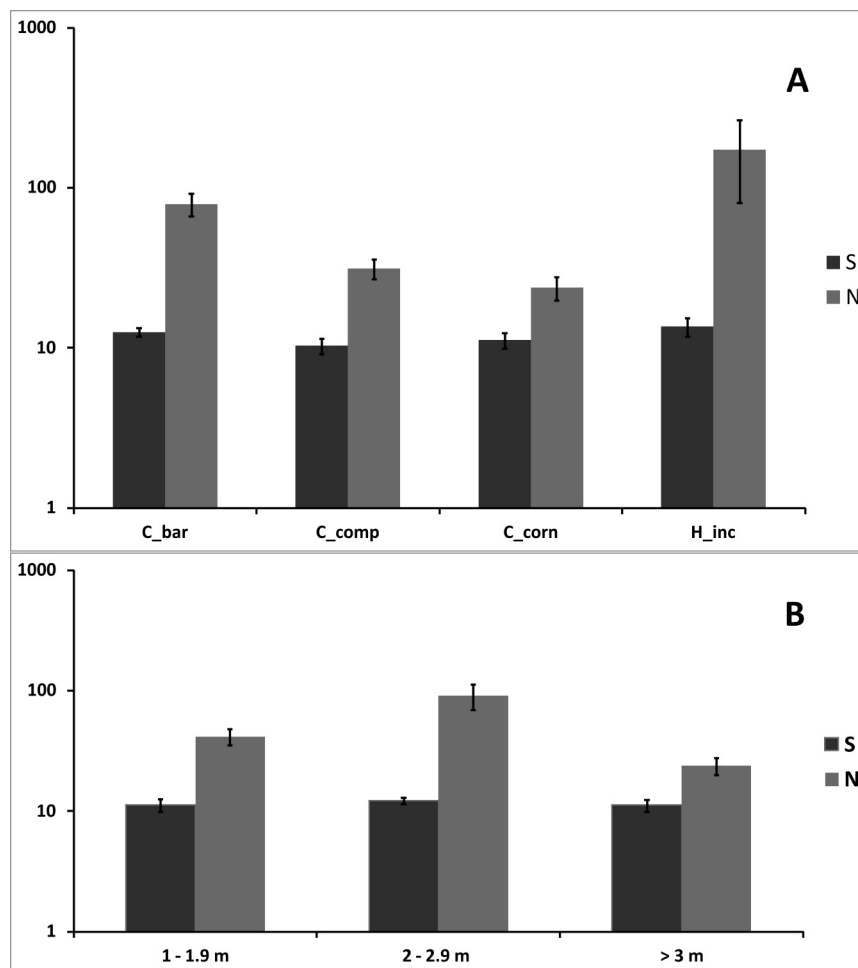
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Fig. 4: Comparison of mean mollusc richness (S) and abundance (N) per 400 cm^2 in logarithmic scale with standard error bars (±SE) in different algal species (A) and at different depths (B) at sites investigated in 2012. Samples with *C. barbata* were: MBP1-5, PI4, PA1-5, STR1-4, FI1-2; with *C. compressa* were PI1-3.5, CM1-3, PO1-3, with *C. corniculata* were RR1-5; with *H. incurva* DR 1-5. Samples collected between 1 and 1.9 m were: DR3-4, MBP-1, PI1-4, PA3-5; between 2 and 2.9 m were: DR1-2, 5, MBP2-5; PI2-3.5, PA1-2; STR 1-4, FI1-2; CM1-3, PO1-3, more than 3m were RR 1-5.

nia (56% of total abundance at the site) and *Rissoa* (17%) and a high number of juveniles (65% of total abundances). Conversely, there was no clear difference between mollusc assemblages associated with *C. barbata* and *C. compressa* (they were grouped together in the first, second, fifth and seventh group).

Discussion

Composition of the mollusc assemblage

Macroalgal associations in Slovenian coastal waters provide suitable microhabitats for a large number of mollusc species. The most frequent and top dominant mollusc species found inhabiting *Cystoseira* associations, the gastropods *Jujubinus exasperatus*, *Rissoa guerinii* and *Bittium reticulatum*, have previously been reported in similar macroalgal associations in different parts of Mediterranean Sea (Poulicek, 1985; Vio & De Min, 1996; Sánchez-Moyano *et al.*, 2000; Chemello & Milazzo, 2002; Antoniadou & Chintiroglou, 2005; Antoniadou *et al.*, 2005). These are micrograzer-detritivore

species, feeding preferentially on diatoms and epiphyte microalgae, as well as food trapped in sediments retained by algal thalli (Paine, 1984; Ávila, 2003). They have been observed migrating from the base to the apex of the shoots at night, where epiphytes are more abundant (Gambi & Morri, 2008; Rueda *et al.*, 2008).

The dominant and most frequent bivalves, such as *Modiolus barbatus* and *Musculus costulatus,* were also reported by other authors in similar algal stands (Poulicek, 1985; Vio & De Min, 1996, Sánchez-Moyano *et al.*, 2000; Chemello & Milazzo, 2002; Antoniadou & Chintiroglou, 2005). They are mostly sessile species, living on hard bottoms among holdfasts of macroalgae or attached to the algae themselves (Hrs-Brenko & Legac, 2006). Exceptions were *Galeomma turtoni*, whose mode of movement resembles that of gastropods, and bivalves *Lima hians* and *Mimachlamys varia,* which are known to live anchored to the substrate with their byssum and also to move freely (Hrs-Brenko & Legac, 2006). It is known that macroalgae play an important role in trapping sediment. The layer of trapped sand covering hard bottom
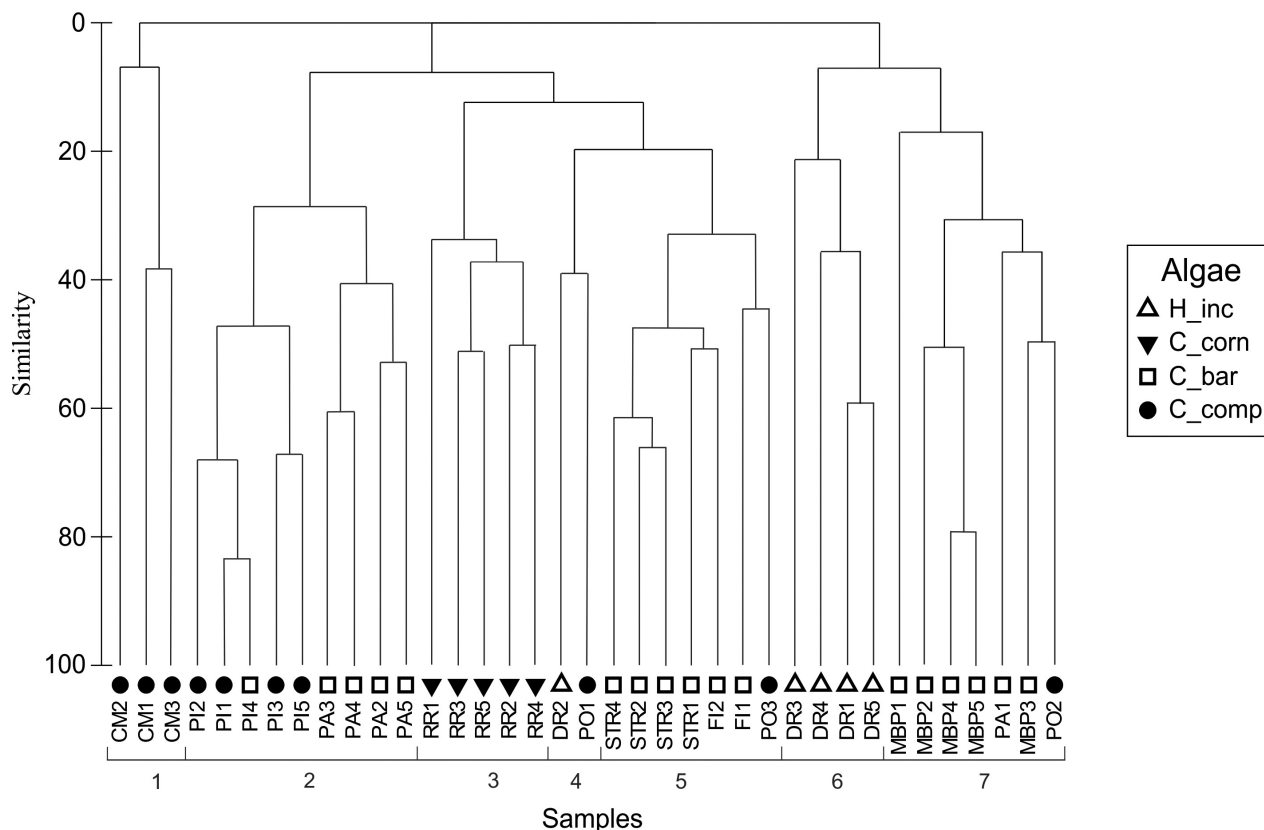
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Fig. 5: Cluster of samples associated with four canopy-forming algal species (H_inc=*H. incurva*; C_corn=*C. corniculata*; C_bar=*C. barbata*; C_comp=*C. compressa*), based on mollusc abundance data. Groups were formed with a cut at 20% of similarity.

among algae increases habitat complexity and represents a suitable niche for a number of soft bottom species (Antoniadou & Chintiroglou, 2005; Antoniadou *et al.*, 2005). At the sites studied for this work, the above layer offers a suitable environment for the bivalves *Kurtiella bidentata* and *Gouldia minima* and for the gastropod *Mangelia attenuata*.

The large number of juvenile molluscs collected confirms the importance of *Cystoseira* spp. associations for mollusc recruitment. The majority of the species collected were characterized by a short life cycle (1-2 years) and with larval stages developing inside eggs (Gambi & Morri, 2008). As was first hypothesised by Pérès (1967) and confirmed by following studies (Poulicek, 1985; Antit *et al.*, 2013), photophilic algae represent a preferential habitat for egg deposition and larval settlements. The high number of juveniles collected resulted in a bias in species determination, because juveniles could resemble adults of different species, which could lead to a potential underestimation of species number. For these reasons, the number of juveniles plays a relevant role in the determination of community structure and ecological status, and deserves wider attention.

Comparison of sampling sites investigated in 2012

Although sampling sites were located at the same depth range and hosted similar algal assemblages, some differences in mollusc composition were observed. These differences could be related to different factors acting locally. Specifically, low diversity at site MBP was due to a clear dominance of a few species, especially of genus *Bittium*. These species are generally dominant and frequent in photophilic assemblages elsewhere in the Mediterranean Sea (Poulicek, 1985; Vio & De Min, 1996; Sánchez-Moyano *et al.*, 2000; Chemello & Milazzo, 2002), but such a high abundance, as found at site MBP, is unusual. According to Antoniadou *et al.* (2005), thalli with complex and branching shape offer suitable conditions for the trophic demands of these grazer/detritivore species, trapping a large amount of organic material. In our case, the macroalgal species at site MBP (*C. barbata*) was the same as the other site with lower abundance of *Bittium* spp., but at MBP algal thalli were significantly more developed in height (Table 4) and consequently displayed a more complex structure. This could have led to trapping of a larger amount of organic material.

The very poor mollusc assemblage at site PI, domi-

nated by *C. compressa*, with an extremely simplified trophic structure, could be explained by the fact that at this site the canopy layer formed by *Cystoseira* spp. was poorly developed, with short thalli (see Table 4), thus offering limited surfaces for molluscs to hide. The lower height of *Cystoseira* thalli at this site could be a consequence of intense grazing by sea urchins. During our sampling surveys it was observed that this site is abundantly inhabited by the sea urchin *Paracentrotus lividus*. Sea urchins still have a negative impact on macroalgal associations, although to a much lesser extent than in the past, when sea urchins caused a severe decline of infralittoral macroalgal associations off the Slovenian coast (Turk & Vukovič, 1994).

Relationship between mollusc assemblage and algal associations

Many authors have reported that the presence of different canopy forming algal species supports different mollusc assemblages in photophilic stands (Chemello & Milazzo, 2002; Ávila, 2003; Antoniadou & Chintiroglou, 2005). Relationships between macroalgal associations and mollusc assemblages have generally been explained in the past more in terms of habitat modifications than trophic relationships. In fact, grazers found in the present work do not directly consume macroalgal thalli, but rather sporelings and attached epiphytes, as already observed by other authors (Paine, 1984; Ávila, 2003).

Complex macroalgae should encourage a more abundant and well-diversified associated fauna (Chemello & Milazzo, 2002). Consequently, during our study, differences in mollusc assemblages between associations composed of different macroalgal species were expected. Our results show a diverse composition of mollusc assemblages at different sites, in some cases probably due to the different morphology or developmental stage of canopy-forming algae. Specifically, *Cystoseira barbata* and *C. compressa* displayed a similar architecture during the early summer, with a high degree of branching and similar height and width, and this could explain why no clear distinction was observed between mollusc assemblages in relation with these two species. *Halopithys incurva* and *C. corniculata* instead, have a more flattened thallus and different architecture. *C. corniculata*, present only at site RR, has primary branches completely covered with thorns, and the available surface for hiding, feeding and reproduction of mollusc species is thus much reduced. This could explain the low number of juveniles found associated with *C. corniculata* and the dominance of adults of species such as *J. exasperatus* and *C. cruciatus*, reaching a larger size compared with molluscs dominant at other sites. Conversely, fine branches with short and pointed ramification on the upper side, typical of *H. incurva*, offer suitable shelter for small animals. This could explain the high dominance of small-sized gastropods (*Alvania* and *Rissoa* spp.) and juveniles at site DR, where *H. incurva* was dominant.

Nevertheless, macroalgal species morphology alone is not sufficient to explain all the variability of mollusc assemblages. Samples with dominant *C. barbata* and *C. compressa* were grouped (Fig. 5) according to sampling site rather than algal association, which suggests that some other site-specific factors influence the diversification of mollusc assemblages in synergy with the presence of canopy-forming algal species. As already observed by other authors (Sánchez-Moyano *et al.*, 2000; Gozler *et al.,* 2010), the same sub-association exposed to different hydrographical conditions hosts different invertebrate assemblages. These factors may act indirectly, influencing macroalgal growth and development, or may have a direct effect on mollusc assemblages. According to recent investigations (Sánchez-Moyano *et al.*, 2000; Antoniadou & Chintiroglou, 2005; Gozler *et al.,* 2010), the most important abiotic factors directly influencing mollusc communities associated with macroalgae are water movement, food availability, sedimentation rate and substrate characteristics.

Also, the use of different sampling methods could have influenced our results. Most samples collected using the air-lift pump clustered together in groups 1 and 5 (Fig. 5) but only a slight difference was found between mollusc assemblages collected with the two methods (ANOSIM test). Given the variability of mollusc assemblages between sampling sites and the close relationship between benthic mollusc species and the surfaces on which they live (both abiotic substrates and biotic structures, like algal thalli), we could conclude that both methods entail no loss of relevant information about the mollusc assemblage.

Comparison between the Gulf of Trieste and other areas

Comparing results from different areas is a challenging task, because of the natural variation among geographic zones and because of the different sampling methodologies used. Despite these difficulties, some similarities can be found regarding mollusc assemblages associated with upper infralittoral algal associations (Table 6). Assemblages are generally dominated by gastropods, with a few species of bivalves and chitons (Poulicek, 1985; Vio & De Min, 1996; Sánchez-Moyano *et al.*, 2000; Chemello & Milazzo, 2002; Antoniadou & Chintiroglou, 2005). Species belonging to the family Rissoidae are usually abundant and very frequently found, together with species of genera *Bittium, Tricolia, Jujubinus, Gibbula* and *Calliostoma* (Poulicek, 1985; Sánchez-Moyano *et al.*, 2000). Similar results were also obtained for the Black Sea (Çulha *et al.*, 2010; Gozler *et al.*, 2010), where physico-chemical conditions limit the number of species, and in the Azores in the Atlantic Ocean (Costa

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Table 6. Comparison of results and sampling methods between the present study and research work in other areas. AS=Adriatic Sea, WM=Western Mediterranean, BS=Black Sea, AO=Atlantic Ocean. *Cyst_barb=C. barbata*, *Cyst_comp=C. compressa*; *Cyst_corn=C. corniculata; Hal_inc=H. incurva; Cyst_barbat=C. barbatula, Cyst_spin=C. spinosa, Sar_vulg=Sargassum vulgare; Hal_scop=Halopteris scoparia, Dict_fasc=Dictyota fasciola, Dic_dic=D. dichotoma, Asp_arm=Asparagopsis armata, Hal_fi =Halopteris filicina, Zon_tou=Zonaria tournefortii, Acr_ver=Acrosorium verulosum.* Taxa=total taxa of molluscs, Biv=Bivalvia, Ga=Gastropoda, Pol=Polyplacophora, CoSp= Species also found in the present work.

Area	Depth (m)	Dominant algal species	Mollusc Taxa	Biv	Ga	Pol	CoSp	Sampling method	N. of samples	Authors
AS	1 - 4	*Cyst_barb, Cyst_comp, Cyst_corn, Hal_inc*	51	17	33	1	/	20 cm × 20 cm frame, scraper	25	Present work (2012)
AS	2	*Cyst_barb, Cyst_comp*	46	19	25	2	23	20 cm × 20 cm frame, air lift sampler, scraper	12	Present work (2008)
WM	1 - 5	*Cyst_barbat, Cyst_spin, Sar_vulg, Hal_scop, Dic_fasc, Dic_dic*	57	2	55	/	12	algal specimen collection, chisel and hammer	18	Chemello & Milazzo, 2002
WM	3 - 10	*Hal_scop*	89	13	76	/	10	algal specimen, bag	260	Sánchez-Moyano *et al.*, 2000
WM	3 - 12	*Cystoseira* spp.	74	7	64	3	15	air lift pump, extraction by freshwater and by asphyxiation on algae in labo-ratory	66	Poulicek, 1985
BS	0 - 3	*Cyst_barb*	7	1	5	1	1	20 cm x 20 cm frame, spatula	20	Gozler *et al.*, 2010
BS	0 - 5	*Cyst_barb*	14	2	11	1	4	20 cm x 20 cm frame, spatula/ shovel	16	Çulha *et al.*, 2010
AO	5 - 15	*Halopteris* spp.	36	7	29	/	2	algal specimens	70	Costa & Ávila, 2001
AO	4 - 30	*Dyct_dic, Asp_arm, Hal_fil, Zon_tou, Acr_ver*	71	13	56	2	/	50 cm x 50 cm frame, scraper	51	Ávila, 2003

& Ávila, 2001; Ávila, 2003), where certain endemic species are present. Overall, mollusc assemblages associated with algal associations show high species richness and diversity.

In conclusion, this study confirms that *Cystoseira* species associations are very important at different levels for the mollusc assemblage, especially for juveniles. In the Gulf of Trieste, these associations are distributed in a restricted shallow area and, therefore, affected by many anthropogenic factors (Orlando-Bonaca *et al.,* 2008a, b). The results of this study have to be improved by long-term investigations in the near future. Moreover, researches into other invertebrate taxa, such as polychaetes and crustaceans, would further elucidate the importance of *Cystoseira* species associations for benthic communities. Such habitat types deserve more research efforts and conservation actions for the maintenance of their good ecological status (Orlando-Bonaca *et al.*, 2008a) in the studied area.

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