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IS THE PORT OF KOPER AN INHOSPITABLE ENVIRONMENT FOR THE SETTLEMENT OF NON-INDIGENOUS MACROPHYTES?

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ABSTRACT

The area of the Port of Koper was sampled for benthic macroalgae within the BALMAS project, in order to prepare an overview of native and non-indigenous species (NIS). In the mediolittoral and upper-infralittoral belts of the port area, 28 algal taxa were found (21 Rhodophyta, 6 Chlorophyta and 1 Ochrophyta). Only one algal NIS was identified, the tetrasporophyte of the red algae Asparagopsis armata. *The species was found in low abundances only in autumn samples, collected in the lower mediolittoral and upper infralittoral belt. The aim of this paper is to discuss the limited presence and abundance of algal NIS in the area of the Port of Koper.*

Key words: macrophytes, non-indigenous species, *Asparagopsis armata,* Port of Koper, Adriatic Sea

IL PORTO DI CAPODISTRIA È UN AMBIENTE INOSPITALE PER L'INSEDIAMENTO DELLE MACROFITE NON-INDIGENE?

SINTESI

Le macroalghe bentoniche sono state campionate nell'area del porto di Capodistria al fine di preparare una panoramica delle specie autoctone e non-indigene (NIS), nell'ambito del progetto BALMAS. Nei piani mediolitorale e infralitorale superiore della zona del porto, sono stati trovati 28 taxa algali (21 Rhodophyta, 6 Chlorophyta e 1 Ochrophyta). Tra questi è stata identificata una sola NIS, il tetrasporofito dell'alga rossa Asparagopsis armata. *La specie è stata trovata in abbondanze minime solo nei campioni autunnali raccolti nei piani mediolitorale inferiore e infralitorale superiore. Lo scopo di questo lavoro è quello di discutere la presenza e l'abbondanza limitate delle alghe NIS nella zona del porto di Capodistria.*

Parole chiave: macrofiti, specie non-indigene, *Asparagopsis armata,* porto di Capodistria, mare Adriatico

INTRODUCTION

Through human-induced activities marine plants have been largely introduced to non-native locations around the world (Wonham & Carlton, 2005), and Williams & Smith (2007) recently prepared a global review on seaweeds invasions. The dominant vectors of introductions of NIS macrophytes are believed to be maritime traffic (with ballast waters, sediments in ballast tanks, hull fouling, sediments attached to anchors/chains, commercial fishing nets and gear) and aquaculture (Gollasch & Leppäkoski, 1999; Rilov & Crooks, 2009; Micael *et al*., 2014). Among marine NIS, some are recognized to be invasive, thus they have a negative impact on biodiversity (displacing native species, changing community structure and modifying habitats), ecosystem services, human health and local economies (Katsanevakis *et al*., 2014). The introduction of non-indigenous (NIS) macrophytes in the Mediterranean Sea have been quite well reviewed in the last decades (Verlaque, 2001; Boudouresque & Verlaque, 2002; Ribera-Siguan, 2002; Tsiamis *et al*., 2008; Galil, 2009; Orlando-Bonaca, 2001; Lipej *et al*., 2012; Tsiamis *et al*. 2013; Corsini-Foka *et al*., 2015).

Nowadays, the presence of 44 introduced macrophytes was reported in the northern Adriatic Sea (Orlando-Bonaca, 2001; Krmac, 2009; Curiel *et al.,* 2002; Verlaque *et al*., 2015) and four of them were found also in Slovenian waters (Orlando-Bonaca, 2010; Lipej *et al*., 2012). While *Bonnemaisonia hamifera* Hariot was found only in 1995 and *Ulva scandinavica* Bliding (Battelli & Tan, 1998) was found to be a taxonomic synonym of *Ulva rigida* C. Agardh, *Asparagopsis armata* Harvey (*Falkenbergia rufolanosa* phase) and *Codium fragile* subsp. *fragile* (Suringar) Hariot are considered to be established in Slovenian shallow coastal waters, even if both were always found in low number of specimens, with low coverage on hard substrata, and only in few localities (Battelli, 2000; Orlando-Bonaca, 2010). Within the implementation of the Marine Strategy Framework Directive (MSFD, 2008/56/EC) the Environmental status (ES) of Slovenian marine waters has been assessed also according to Descriptor 2 (Non-indigenous species). The ES was evaluated as Good since, so far, none of the NIS detected in the Slovenian Sea has shown to have a negative impact on native species and habitats (Orlando-Bonaca *et al.,* 2012).

During 2014 the area of the Port of Koper was sampled according to the Port Baseline Protocol (PBS) prepared within the BALMAS project (IPA Adriatic Cross-Border Cooperation Programme), in order to prepare an overview of native species, NIS, harmful aquatic organisms and pathogens (HAOP) in the 12 biggest ports of the Adriatic Sea. The aim of this study was to verify how many non-indigenous macrophytes are established in marine waters of the Port of Koper and to discuss their limited presence and abundance in the Port area.

MATERIAL AND METHODS

Study area

The Port of Koper is a Slovenian multi-purpose seaport, situated in the northern part of the Adriatic Sea, connecting mainly markets of Central and Southeast Europe with the Mediterranean Sea and Far East. The marine part of the cargo port is composed of three basins (Fig. 1) and associated mooring piers and 12 specialized loading terminals. The 1st basin is designed for container cargo, with a depth at mooring piers down to 11 m. The 2nd basin is intended for the transhipment of oil, petroleum products and wood (depth down to 14 m), while in the inner part of the basin there is the mouth of the Rižana River, carrying urban and industrial waste waters. The southern part of the 3rd basin is designed for trans-shipment of iron ore and coal, with a depth down to 17 m (Geodetski Inštitut Slovenije, 2016).

In 2015, 20 million tones of goods were handled in the Port of Koper. The number of vessels was 4,611; 531 of them were passenger vessels and 4,080 were cargo vessels (Statistical Office of the Republic of Slovenia, 2016). According to David & Gollasch (2015) the discharged ballast water in the area of the Port of Koper originates almost totally from inside the Mediterranean Sea (70 % from the northern Adriatic, 15 % from the southern Adriatic, and 15% from non-Adriatic Medi-

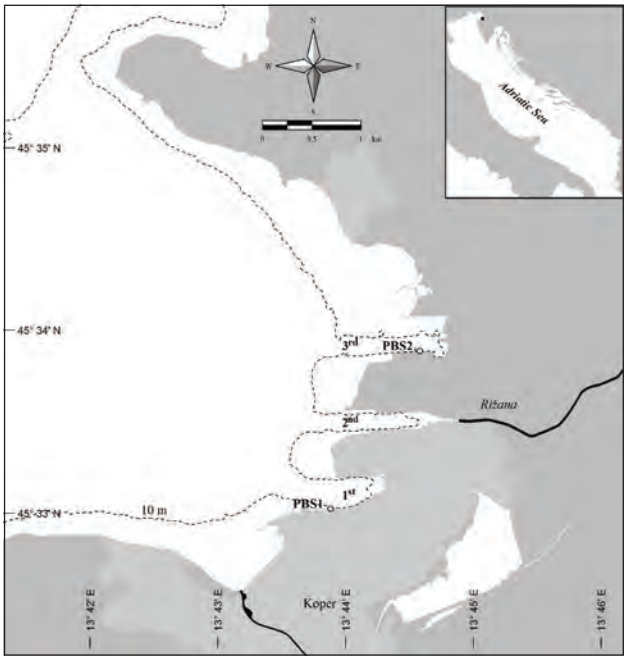

Fig. 1: *The area of the Port of Koper with basins (1st, 2nd and 3rd) and sampling stations for benthic macrophytes (PBS1 and PBS2).*
Sl. 1: Območje Luke Koper s tremi bazeni in vzorčnimi postajami za bentoške makrofite (PBS1 in PBS2).

Tab. 1: Average coverage of macroalgal taxa recorded in the Port of Koper in 2014. UM = Upper Mediolittoral; LM = Lower Mediolittoral; UI = Upper Infralittoral.
Tab. 1: Povprečna pokrovnost makroalg v Luki Koper v 2014. UM = zgornji mediolitoral; LM = spodnji mediolitoral; UI = zgornji infralitoral.

Sampling station		**Passenger terminal in the Port of Koper**					
Sampling belt and depth range (m)		UM (0-0.5)	LM (0.5-1)	UI (1-2)	UM (0-0.5)	LM (0.5-1)	UI (1-2)
Samping date		2.6.2014	2.6.2014	2.6.2014	7.10.2014	7.10.2014	7.10.2014
Phylum	**Taxa**	**Average coverage within three sampling frames (20 cm x 20 cm)**					
Chlorophyta	*Blidingia minima* (Nägeli ex Kützing) Kylin	1.33	0.00	0.00	0.00	0.00	0.00
Chlorophyta	*Cladophora coelothrix* Kützing	0.33	3.33	3.33	0.00	0.17	0.00
Chlorophyta	*Cladophora prolifera* (Roth) Kützing	0.00	0.00	0.00	0.00	0.00	0.67
Chlorophyta	*Cladophora* sp.	0.00	0.00	2.67	0.00	0.00	0.00
Chlorophyta	*Rhizoclonium riparium* (Roth) Harvey - Adriatic Sea (Sfriso. 2011)	0.07	0.00	0.00	2.00	0.10	0.00
Chlorophyta	*Valonia macrophysa* Kützing	0.00	0.00	0.00	0.00	0.00	0.33
Ochrophyta	*Sphacelaria fusca* (Hudson) S.F.Gray	0.33	0.00	0.00	0.00	0.17	0.00
Rhodophyta	*Aglaothamnion caudatum* (J.Agardh) Feldmann-Mazoyer	0.00	0.00	0.00	0.00	0.00	0.17
Rhodophyta	*Antithamnion cruciatum* (C.Agardh) Nägeli	0.00	0.00	0.17	0.00	0.00	0.00
Rhodophyta	*Ceramium codii* (H.Richards) Mazoyer	0.00	0.00	0.00	0.00	0.00	0.17
Rhodophyta	*Compsothamnion thuyoides* (Smith) Nägeli	0.00	0.83	0.17	0.00	0.17	0.33
Rhodophyta	*Dasya hutchinsiae* Harvey	0.00	0.33	0.00	0.00	0.33	0.50
Rhodophyta	*Dasya ocellata* (Grateloup) Harvey	0.33	0.00	0.33	0.00	0.17	0.17
Rhodophyta	*Erythrotrichia carnea* (Dillwyn) J.Agardh	0.00	0.00	0.00	0.00	0.07	0.00
Rhodophyta	***Asparagopsis armata* Harvey**	0.00	0.00	0.00	0.00	**2.00**	**2.00**
Rhodophyta	*Gelidium* sp.	0.00	0.00	0.00	0.00	0.00	0.17
Rhodophyta	*Gelidium spathulatum* (Kützing) Bornet	0.00	3.00	0.00	0.67	4.00	0.00
Rhodophyta	*Herposiphonia tenella* (C.Agardh) Ambronn	0.00	0.00	0.00	0.00	0.00	0.17
Rhodophyta	*Lithophyllum incrustans* Philippi	0.00	0.00	0.67	0.00	0.00	0.00
Rhodophyta	*Lomentaria verticillata* Funk	0.00	0.17	0.00	0.00	0.00	0.00
Rhodophyta	*Peyssonnelia rubra* (Greville) J.Agardh	0.00	1.33	3.67	0.00	0.00	4.00
Rhodophyta	*Peyssonnelia* sp.	0.00	0.00	0.00	0.00	0.00	1.00
Rhodophyta	*Polysiphonia* sp.	0.00	0.00	0.00	0.00	0.17	0.17
Rhodophyta	*Pterocladiella melanoidea* var. *filamentosa* (Schousboe ex Bornet) M.J.Wynne	1.00	0.33	0.33	0.00	0.67	0.00
Rhodophyta	*Pterosiphonia pennata* (C.Agardh) Sauvageau	0.00	0.00	0.00	0.00	0.50	0.17
Rhodophyta	*Pterothamnion crispum* (Ducluzeau) Nägeli	0.00	0.00	0.00	0.00	0.00	0.33
Rhodophyta	*Rhodymenia ardissonei* (Kuntze) Feldmann	0.00	5.67	10.67	0.00	4.00	5.00
Rhodophyta	*Titanoderma pustulatum* (J.V.Lamouroux) Nägeli	0.00	0.00	0.00	0.00	0.00	0.67
Total average coverage		**3.40**	**15.00**	**22.00**	**2.67**	**12.50**	**16.00**

terranean areas), while less than 1% from outside the Mediterranean basin.

Fieldwork and laboratory work

On the 2nd and 12th of June 2014, and on the 7th of October 2014, samplings of macroflora were performed in the first and third basin of the Port of Koper. The samplings were done on the wall of the passenger terminal (1st basin) and on three piles (3rd basin), by SCUBA diving. Three vertical transects at each station were performed during the period of high tide. Along every transect, one sampling frame (20 cm x 20 cm) was fixed to the pile or wall surface at different depths (upper mediolittoral down to 0.5 m of depth, lower mediolittoral down to 1.0 m, and upper infralittoral down to 2.0 m of depth). Such a surface (400 cm^2) is considered to be the minimal sampling area in the case of the Mediterranean communities (Boudouresque & Belsher, 1979). Quantitative sampling was performed by carefully scraping all organisms inside these nine sampling frames (three per each depth) per sampling station into collecting bags. On the research vessel, each collected sample was immediately sieved through a 0.5 mm mesh and fixed in formalin (4-5 %).

All material was transported to the Marine Biology Station of the National Institute of Biology laboratory for analysis. Species identification of macroalgae was carried out in the laboratory by using a binocular microscope, in accordance with Ribera *et al*. (1992), Gallardo *et al*. (1993), Gómez Garreta *et al*. (2001), and Bressan & Babbini (2003). Each sample was sorted carefully and the surface covered by each species (the vertical projection) was quantified in cm^2 (4 cm^2 = 1 % of the sampling surface; Orlando-Bonaca *et al*., 2008). Species names were checked in AlgaeBase (Guiry & Guiry, 2016).

RESULTS

On three sampled piles under the pier in the third basin of the port of Koper (on the 12th of June 2014), no macrophyte specimens were found, while on the wall of the passenger terminal (in the first basin) 28 algal taxa were found (Tab. 1). Among them, 21 were Rhodophyta, 6 were Chlorophyta and only one was Ochrophyta. In June 2014 only 15 taxa were collected, while in October of the same year 23 taxa were determined. Totally, seven species were present in the upper mediolittoral belt, 15 in the lower mediolittoral belt and 22 in the upper infralittoral belt.

The coverage of macroalgae was quite low in all sampled belts and in both seasons (Tab. 1). The lowest coverage value was calculated as the average coverage of three samples collected in the upper mediolittoral belt in October, while the highest value was the average coverage of three samples collected in the upper infralittoral belt in June. Among all taxa, *Rhodymenia ardissonei* (Kuntze) Feldmann had the highest average coverage (10.67% in the upper infralittoral belt in June). The most frequently found species were *Cladophora coelothrix* Kützing, *Compsothamnion thuyoides* (Smith) Nägeli, *Dasya ocellata* (Grateloup) Harvey, *Pterocladiella melanoidea* var. *filamentosa* (Schousboe ex Bornet) M.J.Wynne, and *R. ardissonei*.

Only one NIS was identified, the tetrasporophyte (*Falkenbergia rufolanosa* phase) of *Asparagopsis armata*. The species was found only in October's samples, collected in the lower mediolittoral and upper infralittoral belt. The abundance of the species was low in all samples – with an average coverage of 2% of the sampling surface (Tab. 1).

DISCUSSION

The algal community found in the Port of Koper in 2014 resulted to have lower species richness and coverage than other ten sampling sites along the hard bottom Slovenian coastline, sampled with the same methodology and in the same depth range in June 2008 (Quaggiotto, 2010; Pitacco *et al*., 2013). During those surveys performed in non-port areas, 14 algal taxa were found in the upper and 27 in the lower mediolittoral subbelt (Pitacco *et al*., 2013), while 39 taxa were collected in the upper infralittoral belt (Quaggiotto, 2010), and no algal NIS was found. In June 2009, the sampling station in the first basin of the Port of Koper was surveyed with the same methodology, and two algal taxa in the upper mediolittoral, five taxa in the lower mediolittoral, and nine taxa in the upper infralittoral belt were found, but no algal NIS was collected (Orlando-Bonaca *et al*., 2010). From our results therefore appears that from 2009 to 2014 the number of algal species in the Port of Koper area slightly raised, and that in this period one NIS algal species settled down on the vertical wall of the first basin. The tetrasporophyte of *A. armata* was recorded for the first time in Slovenian coastal waters and in the northern Adriatic Sea in 1991 (M. Richter, *pers. comm*. in Orlando-Bonaca, 2001). The alga, which originates from Australia and New Zealand, was introduced to the Mediterranean Sea unintentionally with oysters (Ribera & Boudouresque, 1995). In 1997 gametophyte thalli of *A. armata* were recorded in Croatian waters near Senj (M. Richter, *pers. comm*.), but were never found in Slovenian waters.

The evidence that *A. armata* was recently collected within the Port of Koper during target surveys, leads to the assumption that in the Port's area other NIS algal taxa could settle down in the nearby future. Potentially, around 30 NIS could arrive from Italian Adriatic waters (Verlaque *et al*., 2015). Among them, at least 11 species were confirmed to be established in the Venetian Lagoon. The most invasive are *Undaria pinnatifida* (Harvey) Suringar, *Sargassum muticum* (Yendo) Fensholt, and *Antithamnion pectinatum* (Montagne) J.Brauner (Curiel *et al*., 1994, 1995, 1996, 1998), that were introduced

in the late 1960s into European waters with *Crassostrea gigas* (Critchley *et al*., 1983; Rueness, 1989). Other 8 NIS macroalgae are also well known for the Venetian Lagoon: three brown algae (*Sorocarpus* sp., *Ectocarpus siliculosus* var. *hiemalis* (P.L.Crouan & H.M.Crouan) Gallardo, and *Punctaria tenuissima* (C.Agardh) Greville), and five red algae (*Polysiphonia morrowii* Harvey, *Polysiphonia mottei* Lauret, *Desmarestia willii* Reinch, *Lomentaria hakodatensis* Yendo, and *Aglaothamnion feldmanniae* Halos) (Curiel *et al*., 1999, 2002, 2003, 2006). Moreover, the presence of the NIS red alga *Acrothamnion preissii* (Sonder) E.M. Wollaston was reported from the Marano and Grado Lagoon (Falace *et al*., 2009). Eventually, two NIS are confirmed to be established in the Italian part of the Gulf of Trieste: *C. fragile* subsp. *fragile*, and the tetrasporophyte of *A. armata* (Falace, 2000; Ceschia *et al*., 2007).

Among the 16 NIS macroalgae confirmed in Croatian marine waters (Pećarević *et al*., 2013), two are considered to be established and invasive in northern Adriatic waters. The most invasive is the green alga *Caulerpa cylindracea* Sonder (Žuljević *et al*., 2003; Despalatović *et al*., 2008) that was recently found very near to the Croatian-Slovenian border (Sladonja & Banovac-Kuča, 2014; Iveša *et al*., 2015). The species is however affecting less than 1% of the entire west Istrian coastline, probably because low winter seawater temperatures (8°C) cause an abrupt decrease of *C. cylindracea* biomass (Iveša *et al*., 2015). The probability that the species will be found in the nearby future in the Slovenian sea is therefore high, but given the low winter temperatures of the area, it may not find the environment suitable to settle down and become established. Secondly, the invasive red alga *Womersleyella setacea* (Hollenberg) R. E. Norris is frequently found all along the Croatian coastline, where its dense monospecific turfs are covering native algal assemblages (Battelli & Arko Pijevac, 2005; Despalatović *et al*., 2008, Nikolić *et al*., 2010). Lastly, *Caulerpa taxifolia* (M. Vahl) C. Agardh was considered highly invasive during the first period of its introduction, but later it proved to have a lower invasive potential, and was partially eradicated from Adriatic areas (Žuljević & Antolić, 1998). Nowadays, it seems that the species has disappeared from the northern Adriatic Sea (*A. Žuljević, pers. obs*.). However, green algae like several *Caulerpa* species, *Codium fragile* subsp. *fragile* and subsp. *atlanticum,* are known to be highly successful invaders that compete directly with native species also in other marine areas (Williams & Smith, 2007).

When the results of all sampling surveys performed during the BALMAS project will become available, it will be possible to evaluate how many of these NIS are present also in Adriatic port's areas, since according to Williams & Smith (2007) hull fouling is a very important vector of introduction of seaweeds, especially of filamentous and weedy corticated foliose genera, while ballast water introductions are less common for seaweeds than documented for other marine species. Despite the high diversity of algal non-native species in adjacent Adriatic areas, some limiting factors appear to prevent the colonization of algal NIS on hard substrata in the Port of Koper. The reduced light availability due to the high resuspension rate of sediments (Orlando-Bonaca *et al*., 2010) could be assumed to be the most important limiting factor for the settlement of native and NIS macroalgae in Port waters. This hypothesis is supported by the low coverage of macroalgae recorded in the upper infralittoral belt at the Port's sampling stations. In shallow areas outside the Port of Koper, the macroalgal coverage in this depth range could be also higher than 100% (Quaggiotto, 2010). Moreover, the evidence that no macroalge were found growing on the three sampled piles in the third basin of the port of Koper, suggests that the construction of the piers on piles would be helpful in the prevention of the settlement of algal NIS in such areas. Since the piles are located under the pier, the light that reaches them seems to be insufficient for the growth of any macroalgae.

Regardless the low presence and abundance of algal NIS in Slovenian waters, at least the Port's area should be regularly sampled in order to quickly report any new settlement of non-native algal species, since the number of taxonomic units involved in bio-invasion is currently underestimated (Provan *et al*., 2008). It should be considered also that the Mediterranean Sea and the NE Atlantic support the highest number of successful algal introductions (Williams & Smith, 2007). Therefore, a regular national monitoring program for NIS should be established in accordance with MSFD requirements, accompanied by a scientific evaluation of key environmental factors, in order to develop effective management solutions in the case of introduction and establishment of invasive species. As the future of introduced NIS is unpredictable, the enforcement of the international collaboration in the Adriatic Sea, such as within the BALMAS project, is crucial to constantly update the global data bank on NIS and HAOP, in order to prevent or at least decrease their potentially negative impacts on native species and receptive habitats along Adriatic coasts.

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JE KOPRSKO PRISTANIŠČE NEGOSTOLJUBNO OKOLJE ZA NASELJEVANJE TUJERODNIH MAKROFITOV?

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POVZETEK

Na območju Luke Koper so avtorji vzorčili bentoške makroalge z namenom, da v okviru projekta BALMAS pripravijo pregled avtohtonih in tujerodnih vrst (NIS). V mediolitoralu in zgornjem infralitoralu so na območju pristanišča ugotovili 28 taksonov alg (21 Rhodophyta, 6 Chlorophyta in 1 Ochrophyta). Med temi je bila le ena NIS in sicer tetrasporofit rdeče alge Asparagopsis armata. *Le-ta se je pojavljala v nizkih abundancah in v jesenskih vzorcih, nabranih v spodnjem mediolitoralu in zgornjem infralitoralu. V članku avtorji razpravljajo o omejeni prisotnosti in nizki številčnosti NIS alg na območju Luke Koper.*

Ključne besede: makrofiti, tujerodne vrste, *Asparagopsis armata,* Luka Koper, Jadransko morje

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