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# Unusual bloom of tetrasporophytes of the non-indigenous red alga *Asparagopsis armata* in the northern Adriatic Sea

Martina ORLANDO-BONACA*, Borut MAVRIČ, Domen TRKOV and Lovrenc LIPEJ

***Marine Biology Station, National Institute of Biology, Fornače 41, 6330 Piran,Slovenia***

* ***Corresponding author, e-mail: Martina.Orlando@nib.si***

*The tetrasporophyte of the non-indigenous red alga Asparagopsis armata (the Falkenbergia stage) is considered to be established in Slovenian coastal waters. However, until 2016, it was found only in low coverage and in few localities with hard substrata. The paper reports a recent bloom of these tetrasporophytes in the mediolittoral belt of the Bay of Piran, where thalli of this red alga overgrew the articularted coralline alga* Corallina officinalis*. The sites affected by this large expansion of tetrasporophytes of A. armata should be regularly monitored in the future, in order to point out which environmental factors are responsible for such phenomenon, and to formulate proper conclusions on the status of this non-indigenous alga in the coastal area concerned.*

**Key words:** *non-indigenous species; Asparagopsis armata; tetrasporophyte; outburst; northern Adriatic Sea*

## INTRODUCTION

The introduction and spreading of non-indigenous (NIS) macrophytes in the Mediterranean Sea were quite well reviewed in the last decades (VERLAQUE, 2001; BOUDOURESQUE & VERLAQUE, 2002; RIBERA-SIGUAN, 2002; TSIAMIS *et al*., 2008; GALIL, 2009; TSIAMIS *et al*. 2013; CORSINI-FOKA *et al*., 2015; VERLAQUE *et al.*, 2015). Nowadays, the Mediterranean and the NE Atlantic are known to support the highest number of macrophytes introductions in the world (PACIOS *et al*., 2011). The main vectors of this process are confirmed to be maritime traffic (hull fouling, ballast waters and sediments in ballast tanks, sediments attached to anchors/chains, commercial fishing gear), aquaculture (GOLLASCH & LEPPÄKOSKI, 1999; RILOV & CROOKS, 2009; MICAEL *et al*., 2014), aquarium trade and exchange (STAM *et al*., 2006), and through the Suez Canal in the Mediterranean Sea since 1869 (GALIL *et al.*, 2014). Some NIS macrophytes are defined as invasive. According to the definition of the EUROPEAN COMMISSION (2002), invasive are those NIS "*whose introduction and/or spread outside their natural past or present distribution threaten biological diversity*". They may have a negative impact not only on local biodiversity (displacing native species, changing community structure and modifying habitats), but also on human health, ecosystem services, and local economies (KATSANEVAKIS *et al*., 2014).

Two species of the tropical/temperate red algal genus *Asparagopsis* Montagne (order Bonnemaisoniales), *A. armata* Harvey and *A. taxiformis* (Delile) Trevisan de Saint-Léon, include populations that have been introduced in the Mediterranean Sea (BOUDOURESQUE & VERLAQUE, 2002). However, for *A. taxiformis*, only the lineage 2 is confirmed to have an Indo-

Pacific origin and it is considered invasive (ANDREAKIS *et al*, 2009). On the contrary, the lineage 3 of *A. taxiformis,* with the type specimen collected in Alexandria (Egypt) well before the opening of the Suez Canal, is supposed to have an Atlantic–Mediterranean origin (ANDREAKIS *et al*, 2009).

The diploid epiphytic tetrasporophyte of the genus *Asparagopsis* is known as the '*Falkenbergia*' stage, small "pompon" naturally occurring as epiphyte, sometimes as a free floating thallus deriving from detachment (CHIHARA, 1961). Both mentioned *Asparagopsis* species are present in the southern Adriatic Sea (MAČIĆ *et al*., 2014), while in the northern and central Adriatic only *A. armata* was found up to date (ANTOLIĆ *et al*., 2013).

*A. armata* is a temperate species, native to New Zealand and southern Australia (HORRIDGE, 1951; BONIN & HAWKES, 1987). In the North Atlantic, it can be found in coastal waters ranging from the British Isles to the Canary Islands, as well as from Salvage Islands to the Senegal coasts (DIXON & IRVINE, 1977; PRICE *et al.*, 1986). The known temperature tolerance data indicate that it naturally occupies cold to warm temperate zones (NÍ CHUALÁIN *et al*., 2004). In the Mediterranean Sea, *A. armata* was firstly reported from the Algerian coast in 1923 (FELDMANN & FELDMANN, 1942), and it is currently considered as invasive (BOUDOURESQUE & VERLAQUE, 2002). Its gametophytes are regularly found mostly in the western Mediterranean basin (SOUTH & TITTLEY, 1986; GUIRY & DAWES, 1992; SALA & BOUDOURESQUE, 1997; VERLAQUE *et al.*, 2015). Due to morphological difference, the tetrasporophyte of *A. armata* was originally believed to be a separate species from the gametophyte and was incorrectly classified as *F. rufolanosa* (Harvey) Schmitz (FELDMANN & FELDMANN, 1942). It is reported to occur in subtidal communities all year around, primarily as an epiphyte, mostly from late winter to spring (VERLAQUE *et al.*, 2015).

Nowadays, the tetrasporophyte of *A. armata* is considered to be established in Slovenian coastal waters, while the gametophyte has never been found (LIPEJ *et al*., 2012). However, until now, it was found only at few sites with hard substrata and in low coverage (BATTELLI, 2000; ORLANDO-BONACA, 2001, 2010; ORLANDO-BONACA *et al*., 2016), and it didn't show any negative impacts on native species and habitats (ORLANDO-BONACA *et al*., 2012).

The aim of this paper is to report a recent bloom of tetrasporophytes of *A. armata* on hard bottom segments along the Slovenian coastline, and to discuss the possible reasons for this extensive coverage in shallow waters, previously never recorded.

## MATERIAL AND METHODS

### Study area

The Bay of Piran is the SW part of the Slovenian Sea, which is a part of the Gulf of Trieste, a shallow semi-enclosed embayment situated in the northernmost part of the Adriatic Sea, extending from Cape Savudrija (Croatia) to Grado (Italy). Although the sea-bed of the Slovenian sea is predominantly soft sedimentary of fluvial origin, the bottom along the coastline (approximately 46.7 km) is mostly rocky, consisting mainly of Eocene Flysch layers, with alternating solid sandstone and soft marl (OGORELEC *et al*., 1997).

### Fieldwork and laboratory work

In October 2016, visual surveys of benthic habitat types were performed by SCUBA diving on 50 m long transects parallel to the coast, in the mediolittoral and upper-infralittoral belts in front of the town of Piran (Fig. 1, sampling site A). In addition, a sampling frame (20 cm x 20 cm) was placed on the surface at different depths (1 m, 2m, 3m). Quantitative sampling was performed by carefully scraping all algae within the sampling frame and placing the material into collecting bags.

The biological material was then transported to laboratory of the Marine Biology Station of the National Institute of Biology for analysis. Each sample was fixed in 4% formaldehyde solution. Identification of macroalgal species

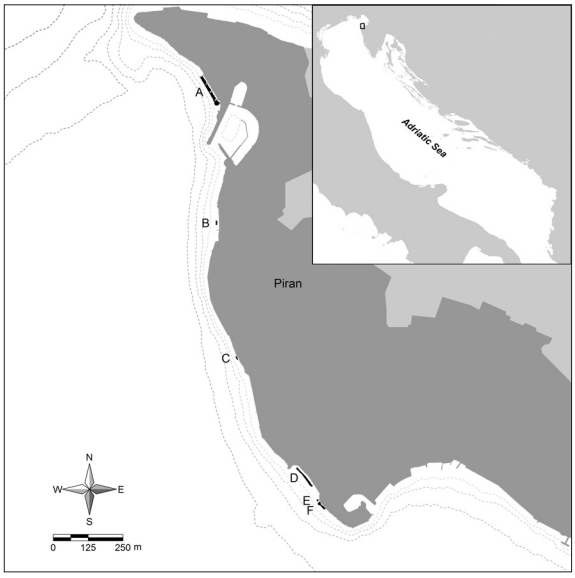


*Fig. 1. Map with coastal segments densely covered by tetrasporophytes of A. armata in the Bay of Piran*

was carried out by using a stereomicroscope Olympus SZH-ILLK, and a microscope Olympus BX51. A set of useful diagnostic characters reported in ZANOLLA *et al.* (2014) was checked in order to discriminate between tetrasporophytes of *A. armata* and *A. taxiformis*. Their findings contradicted previous observations reporting that the tetrasporophyte stages of these species were morphologically identical (ANDREAKIS & SCHAFFELKE, 2012), since they can be clearly distinguished by the dimensions of the axial cells and the width of the apical cell (ZANOLLA *et al.,* 2014).

Once the presence of tetrasporophytes of *A. armata* in the samples was confirmed, the bottom area affected by this alga was photographed and filmed. Its extension was detected by SCUBA diving and then measured by the use of satellite imagery (Google Earth) and the GIS program Manifold®.

Additionally, during low tide periods, the mediolittoral belt along the whole coastline of the Bay of Piran was visually surveyed from the land, and additional thalli of *A. armata* were collected from October 2016 to January 2017 (Fig. 1, sampling sites B-F). Bottom areas covered by *A. armata* were photographed and their extension was again measured by the use of satellite imagery (Google Earth) and the GIS program Manifold®.

## RESULTS

In front of the town of Piran (Fig. 1, sampling site A), at 1 m of depth the hard substrata composed by limestone boulders were mostly colonized by the red alga *Corallina officinalis* Linnaeus (Table 1). Its calcified thalli were densely overgrown by the tetrasporophyte of *A. armata* (Fig. 2). Both species were covering

*Table 1. Macroalgal cover (%) within a sampling frame of 20 cm x 20 cm collected at 1 m, 2 m and 3 m of depth, at the sampling site A in front of the town of Piran*

| | | **Depth (m)** | **1** | **2** | **3** |
|---|---|---|---|---|---|
| **Class** | **Taxa** | **Functional Group** | | | |
| Phaeophyceae | *Cystoseira compressa* | IA | 0 | 0 | **4** |
| Phaeophyceae | *Padina pavonica* | IB | 0 | 0 | **15** |
| Phaeophyceae | *Dictyota dichotoma* | IIA | 0 | **10** | **5** |
| Phaeophyceae | *Halopteris scoparia* | IIA | **1** | **4** | 0 |
| Florideophyceae | *Jania virgata* | IC | **3** | **8** | **12** |
| Florideophyceae | *Corallina officinalis* | IC | **60** | **43** | **48** |
| Florideophyceae | *Peyssonnelia squamaria* | IC | 0 | **6** | **8** |
| Florideophyceae | ***Asparagopsis armata*** | **IIA** | **60** | **1** | **1** |
| Florideophyceae | *Ceramium* spp. | IIB | 0 | 0 | **2** |
| Ulvophyceae | *Cladophora* spp. | IIB | 0 | 0 | **2** |

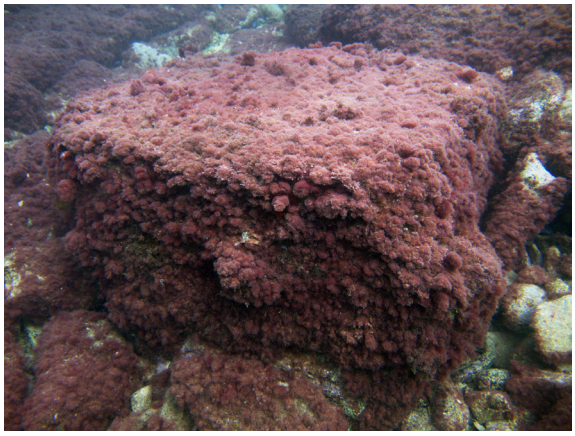

*Fig. 2. Dense coverage of tetrasporophytes of* A. armata *in the Bay of Piran. They developed as ephyphytes on the red alga Corallina officinalis (photo: L. Lipej)*

60 % of the surface of the sampling frame. At 2 m depth, *C. officinalis* covered 43% of the frame surface, while *A. armata* only 1%. At 3 m depth *C. officinalis* covered 48% of the sampling frame surface, while *A. armata* again only 1% of it. At the site A, the extension of the area covered by the tetrasporophyte of *A. armata* belt was estimated to be about 994 $m^2$ (Table 2).

Along the coastline of the Piran Bay, the tetrasporophyte of *A. armata* was found to overgrow thalli of *C. officinalis* in the mediolittoral belt (depth down to 1.0 m) at five other locations, where the hard substrata consist of limestone boulders, as well. The second largest patch of *A. armata* was recorded as site D, covering an area of approximately 403 $m^2$, while the smallest was the patch E, covering around 20 $m^2$ (Table 2).

At all sites the cover of the tetrasporophyte of *A. armata* drastically decreased in the second half of December 2016, and only thalli of *C. officinalis* remained. Eventually, in January 2017 *A. armata* disappeared completely from the mediolittoral belt of the Bay of Piran.

## DISCUSSION

Due to its strong invasive behavior, *A. armata* is included in the list of the "Worst invasive alien species threatening biodiversity in Europe" and also in the list of the "100 Worst Invasives in the Mediterranean Sea", without specifying whether the gametophyte or the tetrasporophyte is considered invasive (STREFTARIS & ZENETOS, 2006). OTERO *et al*. (2013) reported that the genus *Asparagoposis* as a whole appears to have a high invasive potential, and that once *A. armata* becomes invasive, eradication and even control are almost impossible. Tetrasporophytes of *A. armata* are known to have a negative impact on the survival of native algal taxa, since they densely overgrow their host macrophytes (such as thalli of *C. officinalis* in the Bay of Piran), consequently decreasing the light irradiance available for these species (KATSANEVAKIS *et al*., 2014). Moreover, these tetrasporophytes are avoided by Mediterranean herbivores, since they are highly toxic both in summer and winter (MARTÍ *et al*., 2004). The results of ecotoxicological experiments with a gastropod mollusc, *Gibbula umbilicalis* (da Costa, 1778), as test organism, showed that *A. armata* exudates sig-

*Table 2. Coordinates, length of the coastal segments and areas densely covered by tetrasporophytes of A. armata at sampling sites in the Bay of Piran*

| Sampling site | Longitude (E) | Latitude (N) | Length of the coastal segment (m) | Area covered ($m^2$) |
|---|---|---|---|---|
| A | 13°33.983 | 45°31.675 | 121.44 | 993.76 |
| B | 13°34.002 | 45°31.417 | 14.51 | 47.36 |
| C | 13°34.058 | 45°31.155 | 12.67 | 29.41 |
| D | 13°34.245 | 45°30.925 | 87.11 | 402.78 |
| E | 13°34.282 | 45°30.879 | 6.20 | 20.26 |
| F | 13°34.292 | 45°30.868 | 31.07 | 164.69 |

nificantly affect the survival of this mollusc, without specifying whether they used the gametophyte or the tetrasporophyte of the alga (DA CRUZ JACINTO, 2015).

According to OZA (1989), on the European Mediterranean coasts water temperature and day length are in the favourable range for the growth of tetrasporophytes of *A. armata*, mainly in November and December. The photoperiod results too long for their growth before October, and again from January onwards, when also water temperatures are reported to be too low for the survival of thalli (OZA, 1989). However, the presence of the species in the northernmost part of the Mediterranean Sea has been very limited and sporadic in the last two decades, even in November and December. Our data collected in the Bay of Piran suggest that some environmental factors have changed in order to lead to the first recorded bloom of this red alga in the northern Adriatic Sea. Nevertheless, sea-bottom physical characteristics didn't change in the Bay of Piran in the last few years. According to the EEI-c methodology (ORFANIDIS *et al.*, 2011), *A. armata* belongs to the functional group of opportunistic species (ESG II). Its high coverage at 1 m of depth in the bay of Piran, with the coexistence of the late-successional species (ESG I), indicates intermediate environmental conditions. Some researchers concluded that disturbance (especially trophic) is an important triggering factor that leads to the invasiveness of non-indigenous species (SCHAFFELKE & HEWITT, 2007; TSIAMIS *et al.*, 2013). However, the Slovenian national monitoring program of macrophytes in the upper-infralittoral belt has never revealed local pollution sources in the coastal area of the Piran town (ORLANDO-BONACA *et al.*, 2008; unpublished monitoring data). Moreover, recent studies concerning the reduction of chlorophyll *a* concentrations in the northern Adriatic, consistent with the decrease in phosphate and ammonia concentrations, highlighted the oligotrophication of the basin over the last decade (MOZETIČ *et al.*, 2010, 2012). At the moment it is therefore impossible to formulate any valid hypothesis about the factors responsible for the considerable development of these tetrasporophytes in the area.

Since gametophytes *A. armata* are confirmed to be abundant in several Mediterranean marine areas (VERLAQUE *et al.*, 2015), many researchers have tried to assess not only the impact of the species (both gametophytes and tetrasporophyetes) on the marine environment, but also any possible utility in terms of ecosystem services. In NW France they started to extract bioactive substances (for cosmetic and medical products) from cultivated, vegetatively propagated gametophytes in the mid 1990s (KRAAN & BARRINGTON, 2005). Moreover, farming of gametophytes *A. armata* started also in Ireland by the end of the 1990s, since it was confirmed that the species produces biologically active secondary metabolites that are valuable natural medical ingredients, having antibacterial and antifungal activity (STEINBERG *et al.*, 2001; KRAAN & BARRINGTON 2005; PAUL *et al.*, 2006; SALVADOR *et al.*, 2007). Additionally, SCHUENHOFF *et al.* (2006) reported that tetrasporophytes of *A. armata* were successfully tank-cultivated as a continuous biofilter for the effluent of a commercial fish farm in southern Portugal. Tanks with these tetrasporophytes proved to be more efficient biofilters for effluents from a fish farm than the conventionally used *Ulva rigida* (MATA *et al.,* 2010).

The sites affected by the massive spread of tetrasporophytes of *A. armata* in the Bay of Piran have to be regularly monitored in the future, in order to produce long-term datasets that can be used to formulate proper conclusions on the status of this NIS species in the coastal area concerned. Additionally, any changes in environmental factors have to be studied to point out which are responsible for the recent spread of the species. At the same time, new visual surveys should be conducted in other northern Adriatic areas. If tetrasporophytes of *A. armata* will become abundant in the northern Adriatic basin, the already verified possibility of its exploitation should be taken into consideration, and the ratio between the impact and the benefit for the local environment should be assessed.

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# Neuobičajeno cvjetanje tetrasporofita nezavičajnih crvenih algi *Asparagopsis armata* na sjevernom Jadranu

Martina ORLANDO-BONACA*, Borut MAVRIČ, Domen TRKOV i Lovrenc LIPEJ

*Kontakt e-adresa: Martina.Orlando@nib.si

## SAŽETAK

Tetrasporofite ne-autohtone crvene alge *Asparagopsis armata* (*Falkenbergia* faza) smatra se prisutnom u slovenskim obalnim vodama. Međutim, do 2016. godine, ustanovljena je isključivo mala pokrivenost i to na nekoliko lokaliteta s tvrdom podlogom (sediment). U ovom radu se navodi nedavni procvat tetrasoprofita u mezolitoralnom pojasu Piranskog zaljeva gdje je crvena alga *A. armata* nadjačala koraljnu algu *Corallina officinalis.*

U budućnosti će se redovito nadzirati mjesta na koja utječe ova velika ekspanzija tetrasporofita, kako bi se istaknulo koji su ekološki čimbenici odgovorni za takav fenomen, te da bi se mogli formulirati odgovarajući zaključci o statusu ove ne-autohtone alge u tim obalnim područjima.

**Ključne riječi:** nezavičajne vrste, *Asparagopsis armata,* ekspanzija algi, tetrasporofite, sjeverni Jadran