2019

MASTER'S THESIS

EDI GLJUŠĆIĆ

UNIVERZA NA PRIMORSKEM
FAKULTETA ZA MATEMATIKO, NARAVOSLOVJE IN
INFORMACIJSKE TEHNOLOGIJE

MASTER'S THESIS
(MAGISTRSKO DELO)

ALIEN MARINE FLORA AND FAUNA IN HARBORS AND ADJACENT AREAS ALONG ISTRIAN PENINSULA

(TUJERODNA MORSKA FLORA IN FAVNA V PRISTANIŠČIH IN SOSEDNJIH OBMOČJIH VZDOLŽ OBALE ISTRSKEGA POLOTOKA)

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Alien marine flora and fauna in harbors and adjacent areas along Istrian peninsula

(Tujerodna morska flora in fauna v pristaniščih in sosednjih območjih vzdolž obale istrskega polotoka)

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Koper, julij 2019

Ključna dokumentacijska informacija

Ime in PRIIMEK: Edi GLJUŠĆIĆ
Naslov magistrskega dela: Tujerodna morska flora in fauna v pristaniščih in sosednjih območjih vzdolž obale istrskega polotoka
Kraj: Koper
Leto: 2019
Število listov: 79 Število slik: 46 Število tabel: 16
Število prilog: 1 Št. strani prilog: 1
Število referenc: 90
Mentor: prof. dr. Lovrenc Lipej
Delovni somentor: dr. Ljiljana Iveša
UDK: 574.2(497.57)(043.2)
Ključne besede: morske tujerodne vrste, pristanišča, Severni Jadran, istrska obala, profili vrst

Izvleček:
Zaradi velike prisotnosti pomorskega prometa je Istrska obala vse bolj pod pritiskom tujerodnih vrst. Ta študija predstavlja podatke, zbrane v poteku raziskovanja tujerodnih vrst v 10 pristaniščih in sosednjih območjih vzdolž hrvaškega dela istrske obale. Zabeleženih je bilo 14 tujerodnih vrst; od tega 4 naturalizirane, 5 invazivnih ter 5 kriptogenih v Jadranskem morju. Tekom raziskave sta bili prvič zabeleženi 2 vrsti: enakonožni rak *Paracerceis sculpta* in postranica *Caprella scaura*. Sedentarni mnogoščetinec *Hydroides elegans* je bil zabeležen drugič. Zabeležene so bile tudi interakcije vrstah in ekstrapolirani preferirani substrati. Poleg tega so bili izdelani profili za vse in situ opažene vrste ter kratek pregled zoogeografije in zgodovine raziskav tujerodnih vrst. Ta magistrska naloga lahko služi kot osnova za podobne raziskave na temo tujerodnih vrst v prihodnosti.

Key words documentation

Name and SURNAME: Edi GLJUŠĆIĆ
Title of the master thesis: Alien marine flora and fauna in harbors and adjacent areas along Istrian peninsula
Place: Koper
Year: 2019
Number of pages: 79 Number of figures: 46 Number of tables: 16
Number of appendix: 1 Number of appendix pages: 1
Number of references: 90
Mentor: Prof. Lovrenc Lipej, PhD
Working Co-Mentor: Ljiljana Iveša, PhD
UDK: 574.2(497.57)(043.2)
Keywords: marine alien species, harbors, Northern Adriatic, Istrian coastline, species profiles

Abstract:
Due to a heavy presence of marine traffic, the coastline of Istian Penninsula is increasingly burdened by non-indigenous species (NIS). This study presents data collected from surveying 10 harbors and adjacent areas along the Croatian part of the Istrian coastline. 14 alien species were recorded, 4 of which were established, 5 invasive and 5 cryptogenic in the Adriatic Sea. During the study the isopod *Paracerceis sculpta* and amphipod *Caprella scaura* were recorded for the first time on the Istrian peninsula with the tubeworm *Hydroides elegans* for the second time. Interactions and other observations were recorded and substrate preferences were extrapolated. Additionally, species profiles with recorded in situ observations were made, including a brief overview of zoogeography of the recorded species and the history of the NIS research. This thesis can serve as a base for further research into the alien species in the future.

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LIST OF ABBREVIATIONS

DAISIE- Delivering Alien Invasive Species Inventories for Europe

GISD- Global Invasive Species Database

CBD- Convention on Biological Diversity

IAS- Invasive Alien Species

IUCN- International union for the conservation of nature

MAMIAS- Marine Mediterranean Invasive Alien Species

NEMESIS- National Exotic Marine and Estuarine Species Information System

NIS- Non-indigenous species, used both as singular and plural

PBS/PBBS- Port baseline survey / Port biological baseline survey

RAS- Rapid assessment survey

WoRMS- World register of marine species

rr- Very rare

r- Rare

u- Uncommon

c- Common

cc- Very common

ACKNOWLEDGEMENTS

Firstly, I would like to thank my mentor, Prof. Dr. Lovrenc Lipej for guidance in writing this thesis. Also, I am grateful to my co-mentor Dr. Ljiljana Iveša for help with the algae identification as well as guidance and help in other aspects of this work. Additionally I would like to extend my gratitude to Domen Trkov and Ana Fortič for help in the identification of species.

I would also like to acknowledge the assistance of the employees of Center for Marine research Rovinj, for helpful advice and lab usage. Also, I am grateful to the staff of the Marine Biology Station in Piran for the help with species identification.

Also, thanks to my colleague and friend Mojca Grmek for help with my Slovenian texts.

I am also thankful to my committee members for all the constructive criticism, without which this thesis would not be the same.

Finally, I must not forget the support given by my friends, family and my colleagues.

1 INTRODUCTION

Any organism that is found “out of place”, or outside its native range is considered introduced, alien, non-native, non-indigenous (NIS) or allochthonous (as opposed to autochthonous). IUCN (International Union for Conservation of Nature) defines an alien species as: “An alien species is a species introduced by humans – either intentionally or accidentally - outside of its natural past or present distribution...“. An organism (species), once it is recorded outside its natural range can do several things:
a) Can attempt to adapt but ultimately fails and dies,
b) adapts and fills out an empty niche in the ecosystem, allowing the ecosystem to function normally; it becomes established, naturalized or acclimatized,
c) adapts and outcompetes its new (native) competitors and aggressively expands into its new range, disrupting the ecosystem’s normal functions; becomes invasive.
NIS can be placed into several categories, depending on their abundance, impact or expansion.

1.1 Terminology

1.1.1 Invasive species

When an introduced species starts to expand aggressively, disrupting the native ecosystem in the process, as well as causing measurable economic damage, it becomes an invasive species (DAISIE). Invasive alien species are plants, animals, pathogens and other organisms that are non-native to an ecosystem, and which may cause economic or environmental harm or adversely affect human health. In particular, they impact adversely upon biodiversity, including decline or elimination of native species - through competition, predation or transmission of pathogens - and the disruption of local ecosystems and ecosystem functions (CBD, 2009). Examples of such species in the Adriatic Sea are: Chlorophytes *Caulerpa cylindraea Sonder, 1845,* and *C. taxifolia,* as well as rhodophytes *Womersleyella setacea (Hollenberg) R.E.Norris, 1992* and *Asparagopsis armata* Harvey, 1855

1.1.2 Established/Naturalized species

Some introduced species fill-out an empty niche and adapt to the new environment without causing major ecological disturbances. They are considered NIS, but are not necessarily invasive (Allaby, 1998; Booth et al., 2003). These are usually dubbed as established or naturalized species, though they still hold the introduced status. MAMIAS database states the bivalve *Magallana gigas* (Thunberg, 1793) as an established species in the Adriatic Sea: it may have been invasive in the past, but has since reduced its spread and effects on the ecosystem. Sometimes, these species may even help stabilize their new environments. These should not be misidentified as invasive since only 5-20% of the introduced species become invasive (IUCN).

1.1.3 Cryptogenic species

Cryptogenic species are those whose origin is unknown or there is insufficient data to confirm their native range (Cohen & Carlton, 1998; Carlton, 1996). This can be the result of insufficient research in the past, overlooking a species, old introductions (hundreds or thousands of years ago), long-term biogeography (such as glacial isolation, Lazarus taxa, and re/colonization) or human intermediated worldwide distributions. Some cryptogenic species in the Adriatic Sea are for example the ciriped *Amphibalanus amphitrite* (Darwin, 1854) (NEMESIS) and the shipworm bivalve *Teredo navalis* Linnaeus, 1758 (Gollasch, 2006).

1.2 History of marine NIS research in Istria

Just like the geographical division of the Istrian peninsula on Croatian, Slovenian and a small Italian part, the scientific effort is also mostly spread over three countries. This has to be taken into account when browsing for previous collected data because it can easily be dispersed between several national databases and archives.
Studies of fouling communities and species have been done since the 60's (Zavodnik & Igić, 1968; Igić, 1969,) and later (Igić, 1982 a), as well as studies on fouling control (Igić, 1982 b). No studies or surveys of NIS were being done *per se* at the time, since NIS were just beginning to gain focus. This, however, does not mean that NIS were not reported. As early as the 40's, Kolosváry (1947) recorded the first specimens of the ciriped *Megabalanus tintinnabulum* (Linnaeus, 1758). Zavodnik & Igić (1968) reported on non-indigenous barnacles in fouling communities. Hrs-Brenko (1982) discovered the *M. gigas* larvae in the Lim channel for the first time. Valuable data was also collected through other studies on benthic communities or shellfish (Igić, 1986).
More specialized studies into NIS, however began with the invasion of the invasive algae of the genus *Caulerpa* in the 90's. The invasion of *Caulerpa* species was well documented with multiple publications regarding its invasion and spread (Meinesz et al., 2001; Blažina et al., 2009; Sladonja & Banovac-Kuča, 2014; Iveša et al., 2015). Orlando-Bonaca (2001) provided a summary of introduced species in the Northern Adriatic at the time, also covering the whole Istrian coastline. Schubart (2003) reported on a finding of a *Hemigrapsus sanguineus* (De Haan, 1835) crab on the Istrian coast. Just as before, the new NIS records were sometimes integrated into other studies, dealing with biodiversity, fouling, benthic communities and fish communities. Zenetos et al. (2016) reported on some non-indigenous ophistobranchs in the Adriatic Sea. Some of those, namely *Bursatella*

leachii Blainville, 1817, were found also along the Istrian coast (Lipej et al., 2018), which was covered during the research. Spagnolo et al. (2017) have surveyed harbor hard substrata for NIS and covered Trieste (IT), Koper (SLO) and Pula (CRO). Crocetta et al. (2017) (published in Lipej et al., 2017) reported on findings of *Brachidontes pharaonis* (P. Fischer, 1870) in Piran bay as well as previous records. Furthermore, Nerlović et al. (2018) presented new insights on fouling communities of mariculture areas, with a focus on *Anadara transversa* (Say, 1822), an invasive bivalve. They also briefly touch the subject of biofouling studies (in aquaculture facilities) being scarce and outdated. Malej et al. (2017) studied the invasion of the ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865 in the northern Adriatic, which affected the whole Istrian coastline. Another important research effort into NIS was through the BALMAS project (Ballast water management for Adriatic Sea protection) and was done at a larger scale. BALMAS, a port-baseline study (PBS), focuced on ports, therefore Pula, Trieste, Venice, Rijeka etc. were aslo included.. Furthermore, PBS studies seem to be gaining attention, as seen with Kraus et al. (2018) and Petrocelli et al. (2018).

1.3 Aim of the Master thesis

The aim of this thesis was to study the presence and distribution of non-indigenous marine flora and fauna found in some Croatian Istrian harbors and adjacent areas. The goal was to demonstrate whether the Istrian areas, related to maritime transport and fishery activities such harbors, small fishing ports, marinas, mariculture zones (rafts) and adjacent areas represent important recipient areas or “hotspots” for NIS.

2 STUDY AREA

2.1 Northern Adriatic Sea

The northern Adriatic is a shallow (<60 m) area of sea stretching northwards, typically divided at the Zadar-Ancona line (Bearzi et al., 2004), this data, however, can vary between publications for many different reasons (Bianchi & Morri, 2000; Bianchi, 2007; Giani et al., 2012). The Northern Adriatic sea also includes Gulf of Trieste, the northernmost biogeographic region of the Mediterranean Sea, with significant freshwater input from local rivers (mostly river Po, Italy) and North Adriatic-specific, long term oceanographical traits such as periodical hypoxic or anoxic events and low winter temperatures (Degobbis et al., 2000). This is reflected upon the species composition of the northern Adriatic, with biodiversity being a bit lower than in the rest of Adriatic. The area is also a maritime traffic corridor, a tourist destination and an important fishing area, as well. This makes it a priority for proper management and conservation; from conservationist, economic and social aspects.

The Croatian part of Istrian coastline is 570.01 km long (Paljar et al., 2017), Slovenian 46. 6 km (Thaler, 2015) and Italy controls a small portion near the town of Muggia. It contains diverse coastal types that vary from karstic coastline to muddy flats, pebbles and estuaries. The main settlements on Croatian coast are: Umag, Novigrad, Poreč, Rovinj and Pula on the western coast, Medulin at the southern edge, whereas the eastern coast is mostly devoid of major settlements, except for Rabac: a tourist town, Plomin port, Trget industrial port and various other local, small ports.

2.2 Istrian harbors

In 2016, there were 80 recognized ports in Istria: 41 public ports (1 international, 8 regional, 32 local ports) and 39 ports with special function (7 fishing, 12 nautical sports, 1 shipyard, 1 military, 3 industrial and 14 nautical tourism ports). The 14 nautical ports have a total capacity of 4434 moored ships, however, the total number with all the ports is much larger due to other local or fishing ports offering basic services and mooring (Paljar et al., 2017). Some ports are not recorded because they are either illegal (for example wild moorings), belong to a different institution (for example rehabilitation hospital in Rovinj) or may have been integrated with another port in the same town.

2.3 Sampling locations

The sampling took place at 10 different ports with various uses (Figure 1). Harbors have been picked because they represent areas where NIS are most likely to settle, since ships are the main vector of introduction and there are a lot of artificial structures present in those areas (Awad et al., 2014). Locations have been picked with practicality and ease of access in mind. This is the reason why main, large marinas were avoided. Those are typically more specialized and are quite large (they could be split into several sampling sites), while additionally, they are either closed off to public and inaccessible without a special permit or boat ownership. Furthermore, only one mariculture farm was visited, mainly because it is privately owned and small. Others were not sampled simply because of the required procedures and permissions, as well as not being near the coast.

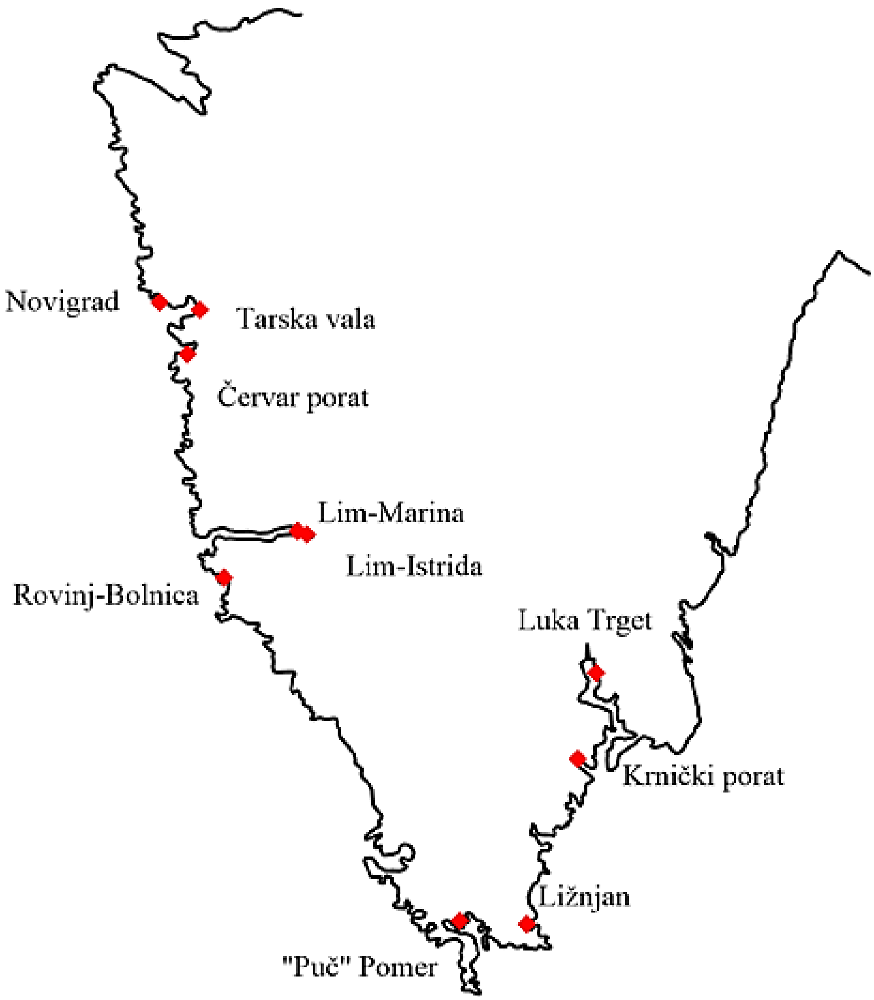

Figure 1: Map of Istria with marked sampling locations. The map of studied area was created with “Surfer“Version 8.09,2391.

3 METHODS

3.1 Sampling alien species

Sampling was performed by a hand net (Figure 2) and hand. Organisms were collected from boats, ropes, vertical blocks (stone walls) and other floating or submerged installations or structures. After collection, samples were placed in containers with seawater for 1-3 days, depending on how long it took for the organisms to emerge at the water surface[1]. This caused the submerged mobile species to float, swim or crawl out to the water surface, where they were easily picked out by hand or pincers. Sessile organisms (for example mussels, ascidians, and algae) were picked out last. Care was also taken not to invade on or damage private property (boats and other vessels). Each location was sampled once in the period between the early October and late November.

Figure 2: Sample collection using a net in Puč-Pomer

[1] Keeping organisms in seawater without oxygenation caused hypoxia, and the subsequent emergence of mobile organisms.

3.2 Laboratory analysis

3.2.1 Sorting

Material was separated either alive or dead. Soft bodied organisms were kept alive in a separate container until they were identified. Ascidians were identified from the living material since the identifying characteristics are only visible while they are still alive and erect. Other material was separated and identified using magnifiers or microscopes. Separation was done in several steps (Table 1).

Table 1: Steps taken during species separation

Step I	Morphological separation: algae, mussels, oysters, other animals
Step II	Higher taxa separation: phyla or classes
Step III	Lower taxa separation: genera or species
Step IV	Status separation: native or alien.

Species were then categorized into higher taxonomical units. Algae were all kept together in a single taxonomical unit.

3.2.2 Identification

Identification was performed with the help of several databases, online keys for determination and factsheets, as well as with the help of available expert's assistance. I used available databases on the web such as

- The Exotics Guide
- Global invasive species database (GISD),
- National Exotic Marine and Estuarine Species Information System (NEMESIS),
- Marine species identification portal,
- European Network on Invasive Alien Species (NOBANIS),
- Delivering Alien Invasive Species Inventories for Europe (DAISIE),
- World registers of marine species (WORMS) and
- The Mediterranean Science Commission, Atlas of Exotic Species in the Mediterranean (CIESM).

Taxonomical nomenclature used was in line with WORMS database.

3.2.3 Data analysis

The collected data was analyzed using Microsoft excel. Frequency was calculated as a number of samplings in which a species was detected in the total number of sampling areas (x), thus f = x / 10; with 10 being the number of sampled locations. The abundance classes were created according to studied species observed distribution, density and area coverage (Table 2).

Table 2: The assessment of abundance classes of the studied NIS.

Abundance	Class
a single specimen found	Very rare (rr)
few specimens found (2-5)	Rare (r)
some specimens found (5-10)	Uncommon (u)
species is found in moderate abundance (dozens)	Common (c)
species is found in larger numbers[2], covering large areas	Very common (cc)

Prefered substrate was extrapolated from the raw data that also includes the information on which substrate (either biotic or abiotic) the species was found, assuming the species prefers this substrate. While species can inhabit different substrates in the same location, the differences within the specific locations were not differentiated according to the abundance of the species found on them. The number of times a substrate was noted in the data, the more preferred the substrate was. This was also summed for total preference.

Table 3: Substrate categories used in graphical representation of the preferred substrate

Pontoons	Floating objects without propulsion
Hulls	Includes Boats, small ships and yachts
Ropes	Includes mooring ropes
Epibionts	Includes species growing and/or living on top of each other as well as species found together in aggregations
Vertical walls	Vertical walls forming the base of the harbor
Other	Other surfaces

[2] Several dozens or hundreds of individuals; Some organisms, such as barnacles or bryozoans were growing over large areas or in colonies and could not be effectively counted

4 RESULTS

4.1 Alien flora and fauna

Fourteen species of 7 taxa were detected in total in studied areas. They were 4 crustaceans, 3 bryozoans, 2 mollusks, 1 ascidian, 1 ctenophore, 1 polychaete, 1 rhodophyte alga and 1 chlorophyte alga. Of the crustacean species, 2 cirripeds, 1 isopod, and 1 amphipod were recorded (Table 3).

Table 4: List of recorded NIS with the identification source

	Species	Taxa	Identification source
1	*Amathia verticillata* (delle Chiaje, 1822)	Bryozoa	Jebakumar et al., 2017
2	*Amphibalanus amphitrite*	Crustacea	NEMESIS
3	*Amphibalanus improvisus* (Darwin, 1854)	Crustacea	Jensen, 2015
4	*Arcuatula senhousia* (Benson, 1842)	Mollusca	CABI
5	*Asparagopsis armata* Harvey, 1855	Algae	AlgaeBase
6	*Bugula neritina (Linnaeus, 1758)*	Bryozoa	Cohen, 2011
7	*Bugulina stolonifera (Ryland, 1960)*	Bryozoa	NEMESIS
8	*Caprella scaura Templeton, 1836*	Crustacea	Martínez & Adarraga, 2008
9	*Caulerpa cylindracea*	Algae	AlgaeBase
10	*Hydroides elegans (Haswell, 1883)*	Anellida	Zavala et al., 2017
11	*Magallana gigas*	Mollusca	NOBANIS
12	*Mnemiopsis leidyi*	Ctenophora	GISD
13	*Paracerceis sculpta (Holmes, 1904)*	Crustacea	NEMESIS
14	*Styela plicata (Lesueur, 1823)*	Chordata	GISD

The most frequently sampled species were fouling barnacles of the *A. amphitrite* species, bryozoans *Amathia verticillata*, typically associated with hull fouling, another fouling bryozoan, *Bugula neritina*, the ascidian *Styela plicata* and the bivalve *Magallana gigas*. *M. gigas* was very common and was recorded in 7 out of 10 harbors. The amphipod *Caprella scaura* is an epibiont and was found on other species with frequency of 60% (Table 5). *Arcuatula senhousia*, despite being found on half of the studied locations, was rare, only occasionally found. In total, 5 species were cryptogenic, 5 invasive and 4 established, according to referenced sources (Figure 3). Each of the species' phyla, recorded frequency, introduction status and its referenced source is presented in Table 5.

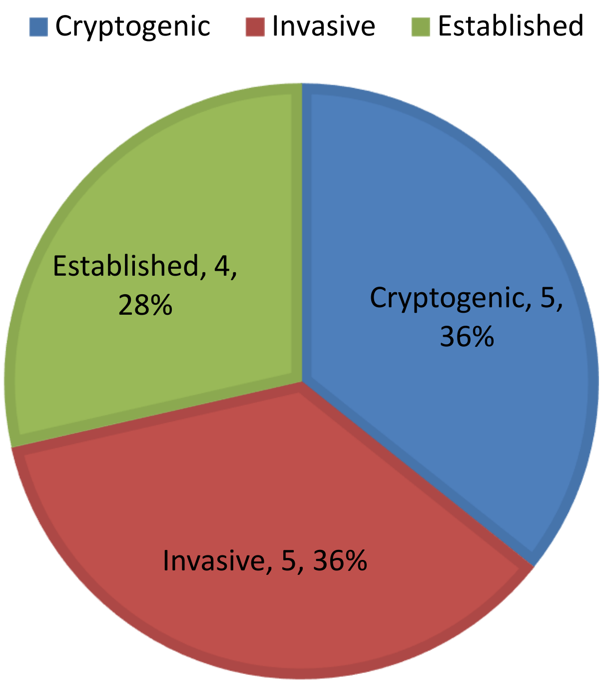

Figure 3: Ratio of the observed introduced species

Table 5: Recorded species in this study, their frequencies and introduction status in the Adriatic or Mediterranean sea[3]. Current status is referenced to the corresponding database:
a) Invasive: Species is considered invasive because it fulfills the needed criteria (paragraph "1.1.1 Invasive species").
b) Established: The species is introduced, but does not meet the criteria for invasive status (paragraph "1.1.2 Established/Naturalised species").
c) Cryptogenic: The species' native range is unknown, for any number of reasons (paragraph "1.1.3 Cryptogenic species").

	Species	Bryozoa	Frequency	Status	Reference
1	*Amathia verticillata*	Bryozoa	7	Cryptogenic	This study[4]
2	*Amphibalanus amphitrite*	Crustacea	8	Cryptogenic	NEMESIS/This study
3	*Amphibalanus Improvisus*	Crustacea	1	Cryptogenic	MAMIAS
4	*Arcuatula senhousia*	Mollusca	5	Invasive	MAMIAS
5	*Asparagopsis armata*	Algae	4	Invasive	MAMIAS

[3] When no available data was found regarding the Adriatic sea, the Mediterranean sea was used as a reference point
[4] *Amathia verticillata* has a complicated introduction status in the Adriatic. See *Amathia verticillata* species profile for more information (paragraph 5.4.1).

6	*Bugula neritina*	Bryozoa	7	Cryptogenic	NEMESIS
7	*Bugulina stolonifera*	Bryozoa	2	Cryptogenic	MAMIAS; under *Bugula stolonifera*/This study[5]
8	*Caprella scaura*	Crustacea	6	Established	MAMIAS
9	*Caulerpa cylindracea*	Algae	1	Invasive	MAMIAS
10	*Hydroides elegans*	Annelida	3	Invasive	MAMIAS
11	*Magallana gigas*	Mollusca	7	Established	MAMIAS
12	*Mnemiopsis leydii*	Ctenophora	3	Invasive	MAMIAS
13	*Paracerceis sculpta*	Crustacea	3	Established	MAMIAS
14	*Styela plicata*	Chordata	8	Established	NEMESIS/ Karachle et al. (2017)

4.2 Species preferences

Most of the recorded species exhibited a certain preference for substrate or microhabitat, except the planktonic *M. leidyi*. *A. verticillata* was mostly found fouling vessel hulls (Tables 6 – 14, Figure 4). It also supported other epibiont species, found to be crawling on it. The amphipod *C. scaura* was very abundant in most cases. Additionally, the isopod *P. sculpta* was found among the samples of *A. verticillata* where *C. scaura* was slightly less abundant. This shows that *A. verticillata* is an important species when monitoring NIS, since it is hosting other NIS (Tables 6 – 8 and 10 - 13).

S. plicata, a known ship fouling organism, was mostly found growing on ropes, hulls and pontoons (Tables 6-9 and 11-14) (Figure 4). While it is solitary, it usually formed clumps and large sized aggregations. This in turn allowed other organisms to fill-out empty space on and between the individual ascidian tunics as well as attach themselves to the aggregations. Such species were *B. neritina* and *A. armata*. Native bryozoans or *B. stolonifera* as well as *B. neritina* were filling out the voids in between; in such way cementing the aggregations. Somewhat less often, these formed together with mussels and created large growths on ropes; effectively making a small Island-habitats for many small crustaceans, flatworms, anemones, polychaetes and even fish. These clumps were diverse and housed also a lot of native species. In some cases, the ascidians also hosted *C. scaura*

[5] This species has presumably expanded its range from the atlantic, but no true native range was ever determined.

and *P. sculpta.* The two small crustaceans seem to be benefiting from the presence of other NIS, in effect “piggybacking” on the fouling species.

B. neritina, a fouling organism, represented a microhabitat as well. However, this one was much larger and wider, growing mostly on vessel hulls, but also ropes and floating objects. *B. neritina* mats housed many crustaceans, polychaetes and flatworms. It did, however, host a lesser number of organisms than *S. plycata* aggregations. On the other hand, these also present a potential threat to biodiversity due the risk of transport with the ship hulls, especially when the vessels are not cared for properly. *B. neritina* also shared the habitat with other bugulid species like the native *Bugulina flabellata* and the non-indigenous *B. stolonifera*.

Several alien species were also found growing near, or in at least a marginal association with the native bryozoan *Cradoscrupocellaria bertholletii*, such as *A. senhousia, S. plicata* and *P. sculpta*, although all were tied to their preferred host. *C. bertholletii* was in this case growing on the host of the before mentioned species and is likely to have no direct effect on the NIS presence and distribution.

A. amphitrite, a cryptogenic fouling barnacle, was found 80% of the locations (Table 5), but it was most abundant in the Lim channel, where it formed an organic belt just a few cm underwater. This was joined with large aggregations of the sedentary polychaete *Hydroides* sp. positioned just below the *A. amphitrite* belt. This was very common on pontoons (harbor platforms) and wooden barriers along the harbor (attached to pontoons). *A. amphitrite* barnacles were competing for space with conspecific organisms; growing on top of each other in large groups, but apparently successfully sequestered the vertical space with *Hydroides* sp., in effect, avoiding a strong interspecific competition. This was most evident at the Lim-Istrida sampling location and, to a lesser degree, in the Lim- marina sampling location. Occasionally, a strand or a turf of *A. verticillata*, partially encrusted by *H. elegans* and other polychates, was found attached to (but not growing on), the barnacles filled with *C. scaura* and other native amphipods and isopods, as well as vagile and sedentary polychaetes, a potential spreading method. *A. amphitrite* were found mostly on hard, artificially flat surfaced floating objects and boat hulls. It did not, however, colonize soft ropes or natural hard surfaces like stones, except when found on mussel or oyster shells.

A. senhousia was found only occasionally, in mussel aggregations and among native *C. bertholletii.* The species did not appear to do well in studied areas, but its presence should be acknowledged with caution.

M. gigas was commonly found growing on vertical walls and sometimes covering submerged objects like chains or an occasional rope. On the other hand, the reef-like aggregations provided a high surface area, diverse habitat and a habitat for other species, both native and alien.

A. armata was found at 4 locations (Table 5) mixed with other, green and red hair algae. It was found as a tetrasporophyte; a red turf covering mussels, ascidians or practically any other firm surface. This was especially noticeable in Ližnjan, where the algae literally covered all available surfaces (hulls, ropes), including other organisms, except the sediment itself, but as observed, not the solid stone vertical walls. The turf did provide a habitat for some small organisms like polychaetes or amphipods and isopods, but no NIS were found associated with it. On other locations, the alga was found on ropes and less often on hulls, occasionally overgrowing other organisms.

C. cylindracea was only observed in Červar porat, in shallow water, growing on a semi-muddy harbor bottom and covering objects such as rocks, fishermen refuse and stones.

A summary of the preferred substrates per species recorded in tables 6-15, as well as preferred substrates in general are shown in Figures 4 and 5, respectively. Several of the species were found in some kind of epibiontic asociations, but this is most evident with *C. scaura* (Figure 4), and to a lesser degree, *P. sculpta*. *A. verticillata* and *B. neritina* have both shown a strong connection with boat hulls, while S. *plicata* seems to prefer ropes. *H. elegans* has shown that it is adaptable when it comes to substrates, a trait of the invasive species. Of the recorded NIS, vertical walls seem to only be important for *M. gigas*.

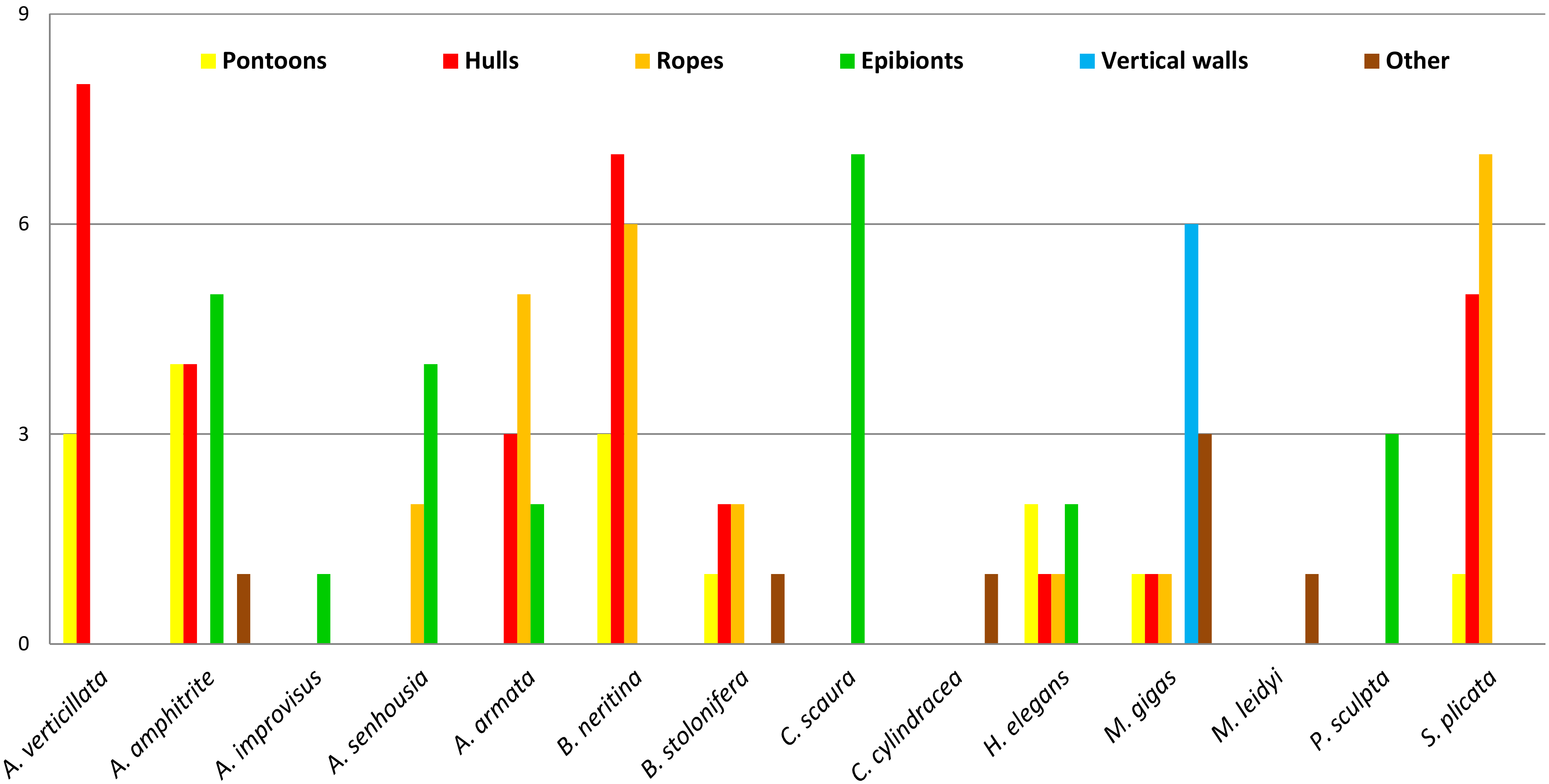

Figure 4: Collumn graph representing each of the recorded species' prefered substrate. Substrates were grouped into 6 categories, depending on how many substrates the species was found per area. Some species were found on a single substrate only while others were growing or inhabiting multiple substrates per area. The total number of noted substrate preference is 121.

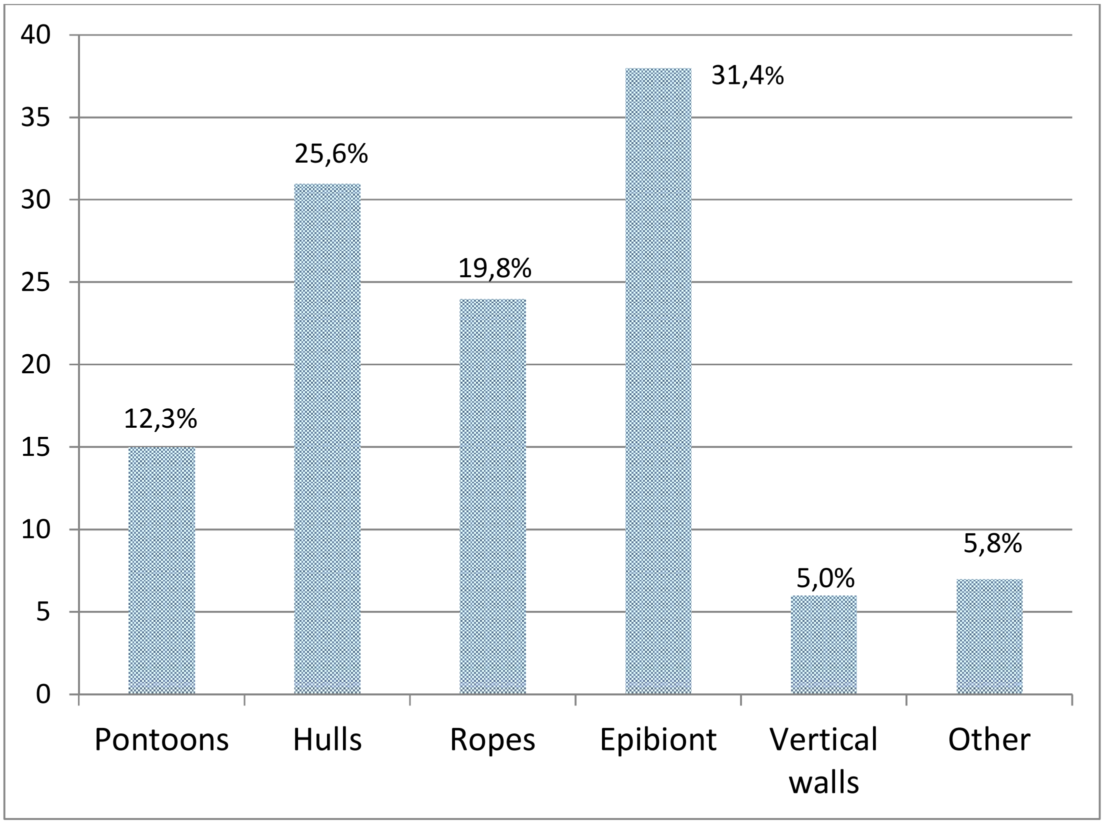

Figure 5: Prefered substrate of all the species combined. The preference is presented as the sum of the species-specific preferences. The total number of noted substrate preferences, listed in Tables 6 - 15 is 121.

When comparing preference regardless of the species (total), epibiosis seems to be the prefered lifestyle of the observed species (31,4%), but boat hulls (25,6%) remain an important substrate (Figure 5). Also, due to the lifestyle of the epibiontic species, namely *C. scaura and P. sculpta*, and the bryozoan *A. verticillata*, two categories (Hulls and Epibionts) are strongly connected with each other. This can also be extended to other fouling species, namely *B. neritina* and *S. plicata,* but also to *H. elegans.*

4.3 Studied localities and recorded species

4.3.1 Červar porat

In Červar porat (Figure 6), the most widespread fouling species were *A. amphitrite*, *B. neritina*, and *S. plicata* (Table 6). Of the epilithic species, *M. gigas* dominated. On the sea bottom, *C. cylindracea* was widely distributed, but not very densely. *A. verticillata* was common and easily found on several vessels, mostly larger, tourist yachts, but hardly widespread.

Figure 6: Červar porat harbor

Table 6: Recorded species organized into higher taxa, with the assessment of abundance and preferred substrate type. Location: Červar porat

	Species	Taxa	Abundance	Substrate
1	*Amathia verticillata*	Bryozoa	c	Pontoons, hulls
2	*Amphibalanus amphitrite*	Crustacea	cc	Pontoons, hulls
3	*Arcuatula senhousia*	Mollusca	r	Ropes, mussel aggregates
4	*Asparagopsis armata*	Algae	c	Ropes, epibiont on *S. plicata*
5	*Bugula neritina*	Bryozoa	cc	Pontoons, hulls, ropes
6	*Caprella scaura*	Crustacea	c	Epibiont on *A. verticillata*
7	*Caulerpa cylindracea*	Algae	cc	Sea bottom, sandy-mud
8	*Magallana gigas*	Mollusca	cc	Vertical walls, ropes, hulls, pontoons
9	*Mnemiopsis leidyi*	Ctenophora	c	Planktonic

10	*Styela plicata*	Chordata	cc	Hulls, ropes

4.3.2 Lim-Istrida

On the sampling location Lim-Istrida (Figure 7), the most widespread fouling species were polychaetes *H. elegans* followed by *A. amphitrite* (Table 7). Those two formed a distinctive "rim" or a "belt" on the immersed objects, just a few cm underwater. *A. verticillata* was also very common, but it too, was partially calcified by *H. elegans* and other serpulids. While only found growing in a few distinct collonies atached to floating platform, it appeared to be in healthy condition[6] and the sheer biomass of the found bryozoan suggests that it thrives in the area. *B. neritina* was also present on pontoons vessel hulls.

Figure 7: Lim-Istrida mariculture farm

Table 7: Recorded species organized into higher taxa, with approximated abundance and preferred substrate type. Location: Lim-Istrida

	Species	Taxa	Abundance	Substrate
1	*Amathia verticillata*	Bryozoa	cc	Pontoons, hulls
2	*Amphibalanus amphitrite*	Crustacea	cc	Pontoons, hulls, epibiont on mussels

[6] No dead strands or collony segments were observed in the location. When the strands of the bryozoan preserved in 75% ethanol were re-emersed in freshwater, the »turgor« of the preserved animal was completely restored.

3	*Bugula neritina*	Bryozoa	cc	Pontoons, hulls
4	*Caprella scaura*	Crustacea	c	Epibiont on *A. verticillata*
5	*Hydroides elegans*	Anellida	cc	Pontoons, epibiont on *A. verticillata*
6	*Mnemiopsis leydii*	Ctenophora	r	Planktonic
7	*Styela plicata*	Chordata	cc	Pontoons, hulls

4.3.3 Rovinj Bolnica

In the Rovinj's rehabilitation hospital marina (Figure 8), 2 NIS dominated, *M. gigas*, with dense, reef-like structures covering all vertical walls and *A. armata*, growing together with native red hair algae (from the genus *Ceramium*), encasing all the ropes in a few millimeter thick fouling layer (Table 8). Boat fouling was present, but it was not severe and it mostly consisted of *M. galloprovincialis*. Importantly, this is exactly where *A. senhousia* was found.

Figure 8: Rovinj rehabilitation hospital harbor (Rovinj Bolnica)

Table 8: Recorded species organized into higher taxa, with approximated abundance and preferred substrate type. Location: Rovinj Bolnica

	Species	Taxa	Abundance	Substrate
1	*Amathia verticillata*	Bryozoa	r	Hulls
2	*Amphibalanus amphitrite*	Crustacea	rr	Epibiont on mussels

3	*Arcuatula senhousia*	Mollusca	r	Mussel aggregates
4	*Asparagopsis armata*	Algae	cc	Ropes
5	*Bugula neritina*	Bryozoa	c	Ropes, hulls
6	*Magallana gigas*	Mollusca	cc	Vertical walls
7	*Styela plicata*	Chordata	c	Ropes

4.3.4 Krnički Porat

The area of Krnički Porat (Figure 9) was rather free of NIS except for 3 species and only *S. plicata* being very common (Table 9). It was, however, limited to submerged ropes and was not found on either hulls or on other solid objects. *M. gigas* was only found on submerged metal chains. Just like at the previous location, (Table 7), *A. senhousia* was found lodged inside mussel aggregates.

Figure 9: Krnički Porat harbor

Table 9: Recorded species organized into higher taxa, With approximated abundance and preferred substrate type. Location: Krnički porat

	Species	Taxa	Abundance	Substrate
1	*Arcuatula senhousia*	Mollusca	r	Mussel aggregates
2	*Magallana gigas*	Mollusca	u	Metal chains

3	*Styela plicata*	Chordata	cc	Ropes

4.3.5 Lim Marina

The second sampling in Lim channel was conducted in the nearby public port (Figures 10 and 11). As presented in Table 10, several species were very abundant, again with a fouling belt of *A. amphitrite* and *H. elegans* present on floating platforms, as well as some submerged wooden barriers along the harbor walls and pontoons. Interestingly, *S. plicata* was not found here, despite being very common at the nearby location Lima-Istrida.

Figure 10: Marina at the Lim Channel – lower part.

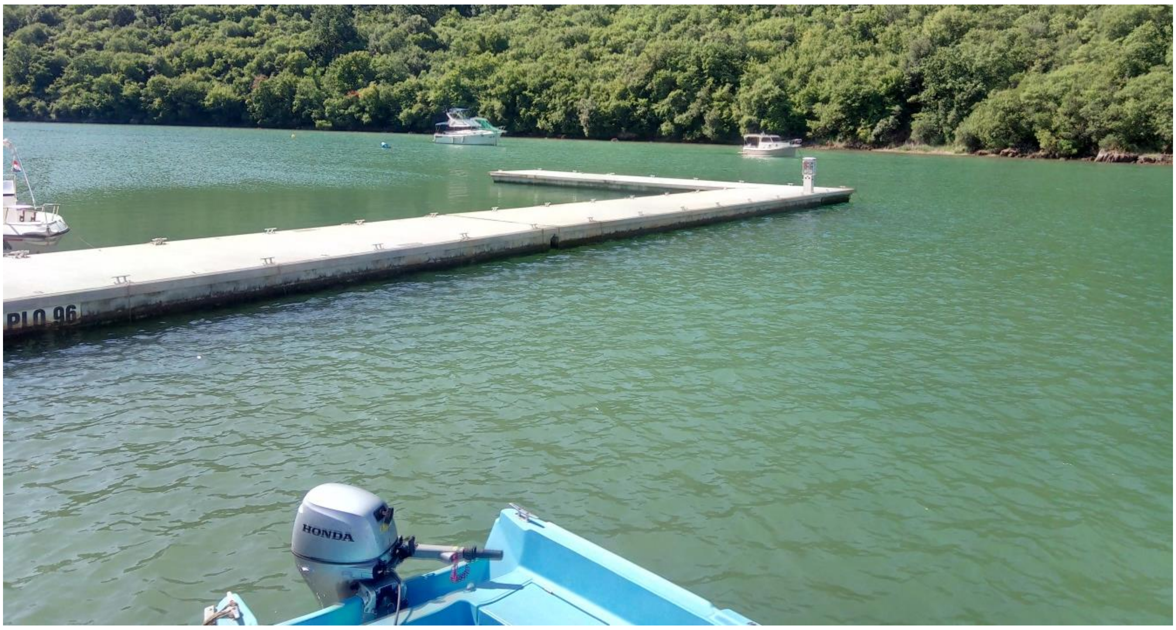

Figure 11: Marina at the Lim Channel – upper part.

Table 10: Recorded species organized into higher taxa, With approximated abundance and preferred substrate type. Location: Lim-marina

	Species	Taxa	Abundance	Substrate
1	*Amathia verticillata*	Bryozoa	c	Hulls
2	*Amphibalanus amphitrite*	Crustacea	cc	Hulls, pontoons, wooden planks, epibiont on *M. gigas*
3	*Bugula neritina*	Bryozoa	cc	Ropes, hulls, pontoons
4	*Bugulina stolonifera*	Bryozoa	cc	Ropes, hulls, pontoons
5	*Caprella scaura*	Crustacea	c	Epibiont on *A. verticillata* and *B. neritina*
6	*Hydroides elegans*	Annelida	cc	Hulls, pontoons, wooden planks
7	*Magallana gigas*	Mollusca	u	Vertical walls

4.3.6 Tarska vala

The location Tarska vala (Figures 12 and 13) is a very small fishing harbor, having relatively few mooring spots, but it hosted several non-native species (Table 11), possibly due to river Mirna runoff. While the only widespread NIS was fouling bryozoan *B. neritina*, 11 other alien species were present, with *S. plicata*, *M. gigas* and *A. armata* being widely distributed, but without dense populations. As previously seen, *C. scaura* was again found on *A. verticillata*, but in this case sharing the host with *P. sculpta* isopods.

Figure 12: Moorings at Tarska vala

Figure 13: Moorings at Tarska vala

Table 11: Recorded species organized into higher taxa, With approximated abundance and preferred substrate type. Location: Tarska vala

	Species	Taxa	Abundance	Substrate
1	*Amathia verticillata*	Bryozoa	c	Hulls, pontoons
2	*Amphibalanus amphitrite*	Crustacea	r	Pontoons, hulls
3	*Arcuatula senhousia*	Mollusca	r	Mussel aggregates
4	*Asparagopsis armata*	Algae	c	Ropes, hulls, epibiont on mussels
5	*Bugula neritina*	Bryozoa	cc	Ropes, hulls
6	*Bugulina stolonifera*	Bryozoa	r	Ropes, hulls
7	*Caprella scaura*	Crustacea	c	Epibiont on *A. verticillata*
8	*Hydroides elegans*	Annelida	u	Mussel Aggregates
9	*Magallana gigas*	Mollusca	c	Vertical walls, boulders
10	*Paracerceis sculpta*	Crustacea	c	On *A. verticillata* and other Bryozoa
11	*Styela plicata*	Chordata	c	Ropes, Hulls

4.3.7 Novigrad

On the location in the Novigrad fishing port (Figure 14) the most abundant NIS were *M. gigas* and *B. neritina* (Table 12). *A. verticillata* was also found in lesser densities than in nearby Tarska vala, but was host to *C. scaura* and *P. sculpta*, similar to the previous location (Table 11). *S. plicata* was also found, fouling mostly ropes, but also boat hulls. Furthermore, it also hosted *P. sculpta* isopods.

Figure 14: Main harbor in the town of Novigrad

Table 12: Recorded species organized into higher taxa, With approximated abundance and preferred substrate type. Location: Novigrad

	Species	Taxa	Abundance	Substrate
1	*Amathia verticillata*	Bryozoa	c	Hulls
2	*Amphibalanus amphitrite*	Crustacea	r	Epibiont on mussels and oysters
3	*Bugula neritina*	Bryozoa	cc	Ropes, hulls
4	*Caprella scaura*	Crustacea	c	Epibiont on *A. verticillata*
5	*Magallana gigas*	Mollusca	cc	Vertical walls
6	*Paracerceis sculpta*	Crustacea	c	On *S. plicata* and *A. verticillata*
7	*Styela plicata*	Chordata	c	Ropes, hulls

4.3.8 Puč[7]-Pomer

In the location in Pomer, a small, sport-fishing harbor (Figure 15), the most abundant NIS was *S. plicata*, which formed large clumps on ropes and sometimes grew on hulls. They were also covered with *C. scaura* amphipods, sometimes in large aggregations. *A. verticillata* was also present, but, in contrast to most previous encounters, did not host any *C. scaura*. *B. neritina* fouled the ropes and ship hulls and *A. armata* covered ropes and hulls, forming a thin carpet-like surface. Amongst the many mussel aggregates, a few *A. senhousia* specimens were also found (Table 13).

Figure 15: Puč – Pomer harbor

Table 13: Recorded species organized into higher taxa, With approximated abundance and preferred substrate type. Location: Puč-Pomer

	Species	Taxa	Abundance	Substrate
1	*Amathia verticillata*	Bryozoa	c	Hulls
2	*Amphibalanus amphitrite*	Crustacea	r	Epibiont on mussels, ropes, substrate for *Shizoporella* spp.
3	*Amphibalanus improvisus*	Crustacea	rr	Epibiont on mussels
4	*Arcuatula senhousia*	Mollusca	r	Mussel aggregates
5	*Asparagopsis armata*	Algae	cc	Ropes, hulls

[7] "Puč"is the name of the small port in the town of Pomer.

6	*Bugula neritina*	Bryozoa	cc	Ropes, hulls
7	*Caprella scaura*	Crustacea	cc	Epibiont on *S. plicata*
8	*Magallana gigas*	Mollusca	u	Vertical walls, wooden Poles
9	*Mnemiopsis leydii*	Ctenophora	r	Planktonic
10	*Paracerceis sculpta*	Crustacea	rr	On *S. plicata*, but outnumbered by *Caprella*
11	*Styela plicata*	Chordata	cc	Ropes, hulls

4.3.9 Ližnjan

At the Ližnjan harbor (Figure 16), only 3 NIS were found (Table 14). The concrete walls, however, did have an intertidal belt, where substantial biomass could be found. The only widely distributed NIS was *A. armata*, which covered every available surface, including other organisms such as algae and mussels. Of other species, *A. verticillata* was found on many moored boats, but, not so dense and this time it hosted no animal species.

Figure 16: The harbor near the town of Ližnjan

Table 14: Recorded species organized in higher taxa, With approximated abundance and preferred substrate type. Location: Ližnjan

	Species	Taxa	Abundance	Substrate
1	*Amathia verticillata*	Bryozoa	c	Hulls
2	*Styela plicata*	Chordata	r	Ropes

3	*Asparagopsis armata*	Algae	cc	Everywhere

4.3.10 Luka Trget

The location in Trget, a small port town near Raša river mouth (Figure 17), was completly dominated by native mussels, but among them a single *A. amphitrite* was found on a mussel shell (Table 15). More NIS could possibly be lurking in the nearby floating mussel farms, a likely source of the found species.

Figure 17: The harbor at the small town of Trget

Table 15: Recorded species organized in higher taxa, with approximated abundance and preferred substrate type. Location: Luka Trget

	Species	Taxa	Abundance	Substrate
1	*Amphibalanus amphitrite*	Crustacea	rr	On mussel shells

5 DISCUSSION

5.1 Suitability of the methods used

The methods used to sample NIS in harbors and adjacent areas are the most common available, yet effective enough to make the studies on harbor colonization of NIS feasible and easily achieved. The results, however, are very dependent on the person doing the research. In this case, some taxa or groups of organisms were neglected or received less attention than others due to the difficulty in identifying the correct species or lower taxonomical unit, such as the polychaetes. Their identification (specifically the errant, vagile taxa) presents a real difficulty to anyone who is not an expert. The barnacles were another problematic group. While several species have been identified, most of the recorded barnacles belong to the *A. amphitrite* complex, where species are remarkably similar to each other. While the literature presents examples where the differences are visible, the sampled individuals were very morphologically diverse (as seen in Figures 22 and 23). This caused serious obstacles in proper identification, thus they were identified as *A. amphitrite*.

The analisys of the preferred substrate is understandably biased towards artificial surfaces, since most of the recorded species were fouling species. Also, the epibionts included cases where species were found just gouped inside aggregations of other species, namely *A. senhousia* in mussel aggregates and *A. armata* simply expanding over the available area. This was done in an attempt to reduce the number of possible categories, so when interpreting the graph, it is important to observe the tables which show preferred substrates more specifically.

Important distribution data might be found through a more sophisticated MDS analysis of the recorded distributions, determining possible differences between specific locations. However, the current data is too descriptive for such analysis and it should be preformed independently.

5.1.1 Sampling and localities

The selection of sampling spots on the location was not random, meaning that boats, ropes or other harbor installations were chosen depending on the amount of growth on them (Figure 18). As such, the study focused more on fouling species than others. This is likely to skew the results towards fouling species, however, other species were found. Another problem in study design is the asymmetric distribution of ports and moorings on the Istrian coast; the western coast having disproportionally more ports than the eastern Istrian coast. This is also the case with harbor quality, the western coast having better maintained harbors. The number of species, however, was larger in the western coast harbors, despite them being better maintained. This also reflects the disproportional maritime traffic and tourism sector in Istria County. Furthermore, studied locations did not include the main harbors due to practical reasons as well as accessibility. Some harbor areas of the studied locations were closed-off, possibly due to safety concerns. This presented a limitation to the study effort. In addition, some observed organisms were not accessible by hand or net.

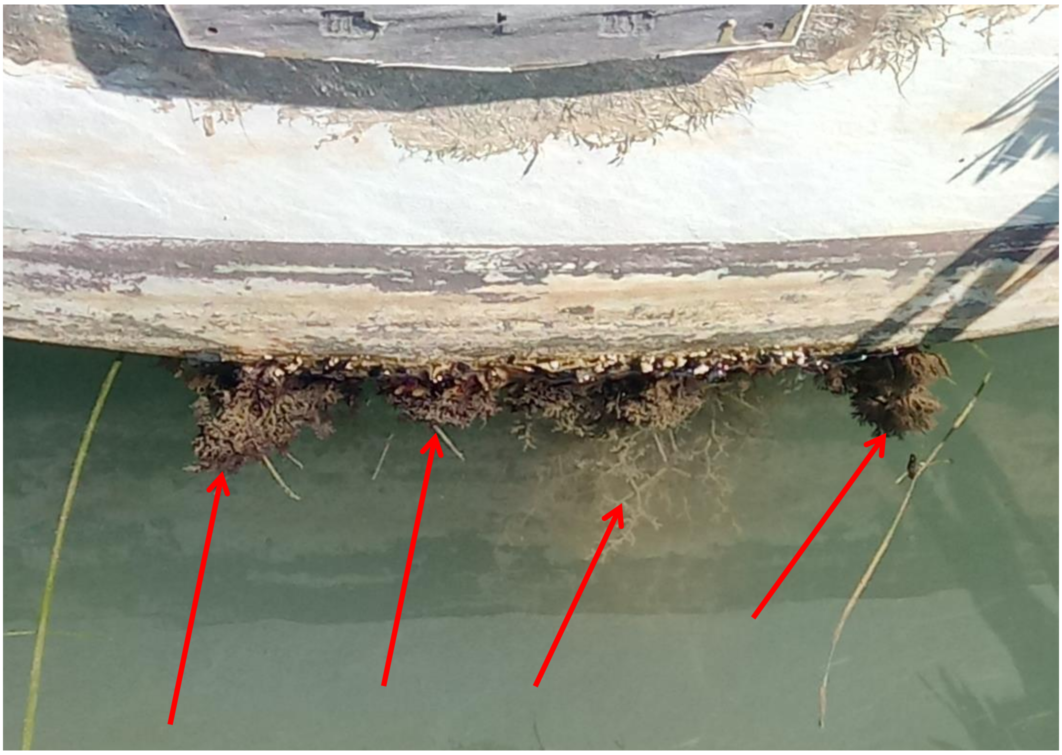

Figure 18: Specific boat hull picked for its extensive fouling

5.1.2 Observations of species

The disproportion between algae and animal NIS in the results is likely just apparent. While sampling, several algae species were found, but almost all were native. Pećarević et al (2013) present 16 different algae NIS found allong the croatian coastline, only 2 of which were recorded during this study, with at least *Codium fragile ssp. fragile* being found in some areas in Istria, despite not being found in this study. The reason for missing algal NIS might be some abiotic or biotic factor, or some seasonal variations. It is, however, most likely that they are present in some larger marina or commercial port or deeper in the infralitoral. It is interesting though; that the number of zoobenthic NIS on croatian coastline stated by Pećarević et al. (2013) is 44; more than double when compared to NIS algae.

5.1.3 Seasonal factors

This study was done in a period from the beginning of October to late November and is, thus limited to late summer[8] and autumn species. To extend this study to other seasons would greatly improve and expand the results. This seasonal limitation could also explain the missing species of algae, but also the lack of NIS in Trget port, which was sampled last, well into November, when the temperatures dropped sharply.

5.2 Zoogeographical review and vectors of introduction

The Lessepsian migration or the arrival of NIS through the Suez channel is the largest source of introductions in the Mediterranean Sea (Por, 1971; Petrocelli et al., 2018). This is the main pathway for Indo-Pacific species, but not necessarily the only one. The organisms can arrive via human intermediation (boats, ballast waters) or by themselves, if they can "manage the trip". Of the species sampled during this study, *A. senhousia* is thought to have arrived via Suez channel (Orlando-Bonaca, 2001) (Table 16). It is also one of the possible pathways for the introduction of *A. armata*. Others may have come multiple times over the centuries.

Table 16: Observed alien species and their native distribution

	Species	Origin	Reference
1	*Amathia verticillata*	Unknown; either native to Mediterranean or Caribbean; the latter most likely	Marchini et al, 2015
2	*Amphibalanus amphitrite*	S Atlantic, Pacific and Indian Oceans, uncertain	NEMESIS
3	*Amphibalanus improvisus*	Western Atlantic	NEMESIS
4	*Arcuatula senhousia*	W Pacific, from Siberia, the Kurile Islands, Japan, and Korea along the China coast to Singapore	Galil, 2006; under syn. *Musculista senhousia*
5	*Asparagopsis armata*	Pacific SW	MAMIAS
6	*Bugula neritina*	Unknown	CABI, NEMESIS
7	*Bugulina stolonifera*	NW Atlantic	NEMESIS
8	*Caprella scaura*	Indian Ocean to the SW Pacific, uncertain	Cohen, 2011

[8] T9he early autumn of 2018 still had summer temperatures.

9	*Caulerpa cylindracea*	SW Australia	Verlaque et al, 2003; Algabase
10	*Hydroides elegans*	Unknown, presumed to be somewhere in the Indo-Pacific	NEMESIS
11	*Magallana gigas*	NW Pacific	Minchin & Gollasch, 2008
12	*Mnemiopsis leidyi*	Western Atlantic	Didžiulis, 2013
13	*Paracerceis sculpta*	Pacific, California, Mexico	Ariyama & Otani, 2004
14	*Styela plicata*	NW Pacific (based on genetics)	de Barros et al, 2009

Most of the observed NIS, whose origins can be traced, originate from the Pacific Ocean, with Atlantic Ocean being the second. It is unfortunate that over a third of the observed NIS origins are as of yet not fully resolved or uncertain (Figure 19).

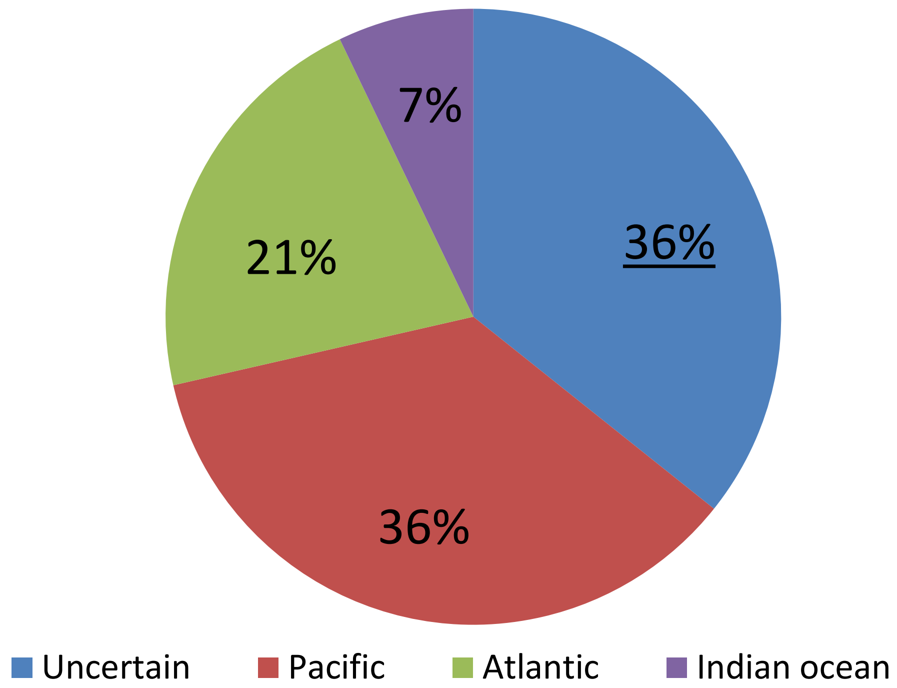

Figure 19: Pie-chart representing the origins of the found species. Any species without fully known origins are placed into unknown category.

While many species arrived by boats as fouling organisms or hitchhikers on the ship fouling (DAISIE), the size of these ships (inability to dock), makes the spread of NIS in coastal waters and small harbors impossible. This role is then taken by the smaller recreational and fishing boats (Zabin et al., 2014) which can also be overgrown. This was confirmed during the study, especially in mixed-function ports. Hull fouling (including ropes and floating objects) is the most prominent vector of introduction for the observed species. *A. verticillata* and bugulid bryozoans are most likely spread this way. Of the cryptogenic species, *B. neritina* is already well-established in this region, as is *A. amphitrite.*

Ballast waters are another important vector of NIS, albeit a lesser one in the Mediterranean, with cca 1% of the macrophyte species arriving via ballast waters (Boudouresque & Verlaque, 2002) and animals having a few more examples such as *M. leidyi*, which is believed to have come with the ballast waters. Despite most ports in the Adriatic Sea have an import function, which means that the ballast water is being pumped in, and not discharged, several species are thought to have arrived this way (Petrocelli et al., 2018), such as *Callinectes sapidus* (Rathbun, 1896) and *M. leidyi*. Mariculture is a prominent vector of NIS in the Mediterranean (DAISIE). Some of these were observed, like *M. gigas* while others have been missed either due to depth or inaccessibility (*Mya arenaria*, *Ulva rigida*, *Venerupis philipinarum*…), or have not yet established their presence on the Istrian coast (*Unidaria pinnatifida, Sargassum muticum*…).

Of the species recorded during this study *A. senhousia*, *A. armata*, *A. senhousia*, *C. scaura*, *P. sculpta, S. plicata* and *A. improvisus* likely arrived via shipping vessels as fouling, while *M. leidyi*, *H. elegans* and possibly *C. cylindracea* have arrived via ballast tanks (MAMIAS). Some species were cryptogenic, so the real vector of introduction is impossible to deduce, but it can be linked to the preferred substrate type and specific habitats where they thrive. For example, while *A. verticillata*, a cryptogenic species, is typically found on vessel hulls or some floating objects (Marchini et al., 2015), which can explain its spread and presence in most harbors. Similarly, *B. neritina* is distributed on fixed or floating objects in harbors, as is *A. amphitrite*. Also, while it is possible for *B. stolonifera* to have naturally expanded its range into the whole Mediterranean, it could have easily been spread as fouling on ropes and ship hulls.

5.3 A note on old introductions

Some species in the Mediterranean and the Adriatic may have been introduced centuries or even millennia ago. The introduction status of these species is, in fact, more of a historical debate than species introduction research, since they have been introduced and spread even by galleys and trade ships from old civilizations surrounding the Mediterranean Sea. Such species include mostly cryptogenic species *A. amphitrite* (Carlton et al., 2011), *A. verticillata*, (Marchini et al., 2015) *B. neritina*, *T. navalis* (Gollasch, 2006), *S. plicata* (de Barros et al., 2009) and likely others. The question forms around the time it takes for the species to "become native". While 100-300 years ago (Gollasch, 2006, Didžiulis, 2011, Jebakumar et al., 2017), at least some species were being reported as non-native, are species that have possibly been introduced over 2000 years ago (Carlton et al., 2011) as ship fouling considered native or introduced?

5.4 Recorded species profiles

5.4.1 *Amathia verticillata* (delle Chiaje, 1822)

This soft bodied, semi-transparent, spaghetti-looking bryozoan can be confused with a hydrozoan or algae when submerged (Figure 20). Interestingly, in the past, it was described as both a cnidarian and algae (Jebakumar et al., 2017). When taken from the water, it looks like long semi-transparent strands of spaghetti or noodles (Figure 21), sometimes exceeding 50 cm in length, but typically branching (Gordon et al., 1992). This species was first described in the gulf of Naples in 1882. The early description from the gulf of Naples would make it a native species, however, the status of *A. verticillata* has changed from native to "semi-native" because it was found that it was probably introduced centuries ago from the Caribbean (Marchini et al., 2015) where its predator, a nudibranch *Okenia zoobotryon* natively lives. Karachle et al. (2017) did, however present it as established in Croatia. It is almost exclusively limited to ports, and mostly ship hulls (Figure 18). It is a filter feeder, thriving in highly productive areas (Marchini et al., 2015). Despite its limited distribution and thus, low risk of actual invasion, it can still contain other species some of which can be alien (Marchini et al., 2015). I will state this species' status as cryptogenic, for easier classification since its status is still debated.

Figure 20: *A. verticillata* growing on a boat hull.

Figure 21: *A. verticillata*, preserved in alcohol and re-immersed in freshwater

5.4.2 *Amphibalanus amphitrite* (Darwin, 1854)

A small to medium sized, pink-striped acorn barnacle with a troubling taxonomy. It is recognized by the pink, vertical stripes along its shell (Figures 22 and 23), hence the name, Pink-striped acorn barnacle. When described by Darwin in 1854, this species was already globally distributed. Its apparent native range is somewhere in the Indian Ocean or SW pacific, where the Pleistocene fossil records place it (Foster, 1978). Fossil records however, also place it in Argentina, where it is not considered native (Orensanz et al., 2002). In addition, there were also remains of *A. amphitrite* found in archeological site in Tunisia, dating to 200 BC (Carlton et al., 2011). Furthermore, this appears to be a complex of multiple, similar and related species (Henry & McLaughlin, 1975). WORMS lists 15 subspecies of *A. amphitrite* to date, with several others being recognized as separate species. It was abundant in most study locations, and was found covering hard substrate, usually artificial (hulls, pontoons,). It was exceptionally abundant in Lim bay, likely due to high water productivity. As a typical acorn barnacle, it is a filter feeder and thrives in productive, enclosed areas (NEMESIS). While it is considered cryptogenic at the Mediterranean scale (NEMESIS) where it likely arrived centuries ago, there is no classification data for it in the Adriatic Sea. Thus, it is probably accurate to consider it cryptogenic in the Adriatic as well.

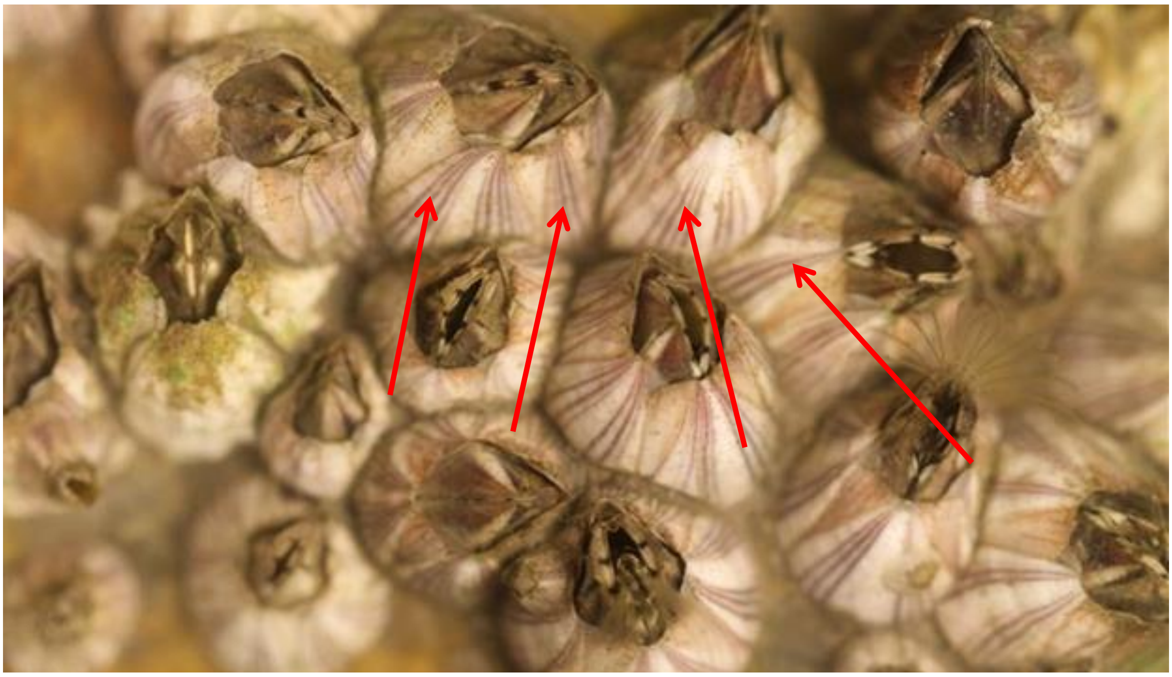

Figure 22: *A. amphitrite* barnacles (by Frey, M.). The red arrows mark the characteristic pink stripes.

Figure 23: *A. amphitrite*, living sample. Notice the shell deformities when compared to previous figure. The red arrows mark the characteristic pink stripes.

5.4.3 *Amphibalanus improvisus* (Darwin, 1854)

This species can resemble several other barnacles, especially when they are of different ecophenotypes (NEMESIS). When compared to regularly grown barnacles of other similar species, it can be told apart by its "tooth-shaped" orifice and 6 smooth plates with no lines (Figure 24) (Jensen et al., 2015). The more accurate way to identify this species is by the shape of its tergum and scutum. It is a filter-feeding barnacle species with a preference for brackish water and it grows on solid objects in harbors as well as other shelled animals, like mussels (Jensen et al., 2015) It can be quite widespread in certain areas, but in this case, it was very rare, only encountered once. While it is known to inhabit the Adriatic Sea, its introduction status is still questionable (MAMIAS) and varies among databases. It is, thus stated as cryptogenic in this thesis.

Figure 24: *A. improvisus*, with its characteristic 6 smooth plates (by Jensen, K. R)

5.4.4 *Arcuatula senhousia* (Benson, 1842)

A small mytilid NIS previously listed as *Musculista senhousia*. Though it can marginally resemble small mussel species, it is easily told apart by its greenish coloration and pattern (Figure 25). It originates from Western pacific coast (Galil, 2006) First found in Savudrija/Salvore bay in 2003 (Crocetta, 2011), it spread all over the northern Adriatic. In my surveys, it was found always among mussels, sometimes associated with native bryozoans. It was rare likely because it prefers freshwater influx. Just like other mytilids, it is a filter-feeder on phytoplankton and detritus. It has been reported as a serious invader in some relatively nearby brackish localities (Lipej et al., 2012) in Slovenia. This is supported by Karachle et al. (2017), who, on the other hand present it as established in Croatia. It is, despite its observed scarcity during this study, considered invasive in both Adriatic and Mediterranean seas. Thus, its mere presence should be acknowledged with caution.

Figure 25: *A. senhousia* shells, encircled in red.

5.4.5 *Asparagopsis armata* Harvey, 1855 syn. *Falkenbergia rufulanosa*

The presence of two species in *Asparagopsis* genus, namely *A. taxiformis* and A. *armata*, has been known in the Mediterranean for at least a hundred years (Algabase). However, both of them are NIS (Andreakis et al., 2007). The distribution data for these algae, on the other hand, is a bit ambiguous and may be unreliable due to easy misidentification of the tetrasporophyte phase (Andreakis et al., 2007), which is common in the Northern Adriatic, as opposed to gametophyte. Orlando-Bonaca et al. (2017) reported the unusual blooming of the *A. armata* in the Slovenian Istrian coast (Piran), which is connected by marine traffic to the rest of the Istrian coast. The algae is known to be present in large areas of the Istrian coastline (Ljiljana Iveša, *pers. communication*), both in anthropogenic and natural settings. When found during my surveys, it was covering any available surface (biotic or abiotic) including ropes and attached organisms, as well as boat hulls.

Figure 26: *A. armata* in *Falkenbergia* phase (tetrasporophyte) growing on and around other algae. The tetrasporophyte resembles a pinkish-red hairy ball or a cotton turf (inside encircled areas). (by Bárbara, I.)

The algae appears as a fine red turf (sometimes brighter or darker) (Figures 26 and 27) growing on ropes, mussels, ascidians, bryozoans, rocks, other algae and any surface in general. It is also a well-known aquarium pest, where it arrives as a hitchhiker on rocks and other organisms. This can also potentially be a serious vector of transmission. It grows together with some *Ceramium* species which, if careless, can be mixed up as the same hair-like algae and the identification requires the use of a microscope. While Karachle et al. (2017) state it as established in Croatia, MAMIAS, except on country level, presents it as and invasive species with invasive catacteristics.

Figure 27: *A. armata* tetrasporophyte growing on the tube of *Sabella spalanzanni*. Samples collected in Puč-Pomer and photographed in aquarium.

5.4.6 *Bugula neritina* (Linnaeus, 1758)

B. neritina is a bryozoan that grows in upright, bushy, branching tufts, up to 15 cm in height that are often mistaken for seaweed. They are usually a dark red-purple or purple-brown, though occasionally they are a dull, dark red (Cohen, 2011) (Figure 28). When immersed in freshwater, it will release a purple colored liquid (Kennedy, 1979), which is one way to identify it. It is a fouling organism typically growing on ship hulls, floating objects and ropes (Figure 29). It can easily be mistaken for red algae, but its true form becomes apparent even under smaller magnifications. It can form dense colonies and it typically houses other, smaller organisms inside (flatworms, caprellids, isopods, gastropods, crabs, polychaetes). Furthermore, as a fouling organism it can also potentially clog propellers, intake and exhaust pipes and increase drag on the ship's hull. It is a suspension feeding bryozoan, thriving in highly productive, enclosed areas, where it is a common fouler (Cohen, 2011). Its current introduction status in the Adriatic and Mediteranean is cryptogenic (NEMESIS) because its native range has not been discovered yet (Cohen, 2011) and it was first recorded over 200 years ago. It is, however, starting to cause problems as a fouling species in our areas (Istrida employes, *pers. communication*).

Figure 28: *B. neritina*, preserved in alcohol

Figure 29: *B. neritina* growimg an a submerged rope

5.4.7 *Bugulina stolonifera* (Ryland, 1960)

A bugulid bryozoan that forms tufts 3-4 cm in height, often grey-buff in color (NEMESIS). Up-close, without a microscope, it resembles *B. flabellata* in color, but with a much more irregular growth, more akin to *B. neritina* (Figure 30). Just like its relatives, it is a filter feeder thriving in enclosed, productive areas. In the studied areas, it was found in 2 ports, both having some freshwater input, where it grew mostly as rope and hull fouling. It seems to be introduced (NEMESIS) to the Adriatic Sea; MAMIAS database states that it expanded its range from the Atlantic, and then possibly spread as hull-fouling into the Adriatic, but it could have sprad naturally. Without a confirmed native range however, I can only state it as cryptogenic, until the status is fully resolved..

Figure 30: *B. stolonifera* (by De Blauwe, H)

5.4.8 *Caprella scaura* Templeton, 1836

Caprellids are very elongated amphipods also known as "Skeleton shrimp". While there are native caprellid populations present on the Istrian coastline, the individuals found were in fact *C. scaura*, easily recognized by the well-developed occipital spine on the head (Figures 31 and 32) in both sexes (Martínez & Adarraga, 2008). These can reach large populations and possibly displace native amphipods or caprellids. Usually, they are found on algae or any other branched organisms where they have taken on a suspension-feeder strategy. In this case, the majority were found on *A. verticillata* and some on a *S. plicata* clump. The species most likely originates from somewhere in the Indian ocean, around Mauritius, where it was recorded for the first time, but this is still uncertain (Krapp et al., 2006; Cohen, 2011). MAMIAS treats this species as established in the Adriatic Sea.

Figure 31: Magnified observation of *C. scaura*. Notice the occipital spine.

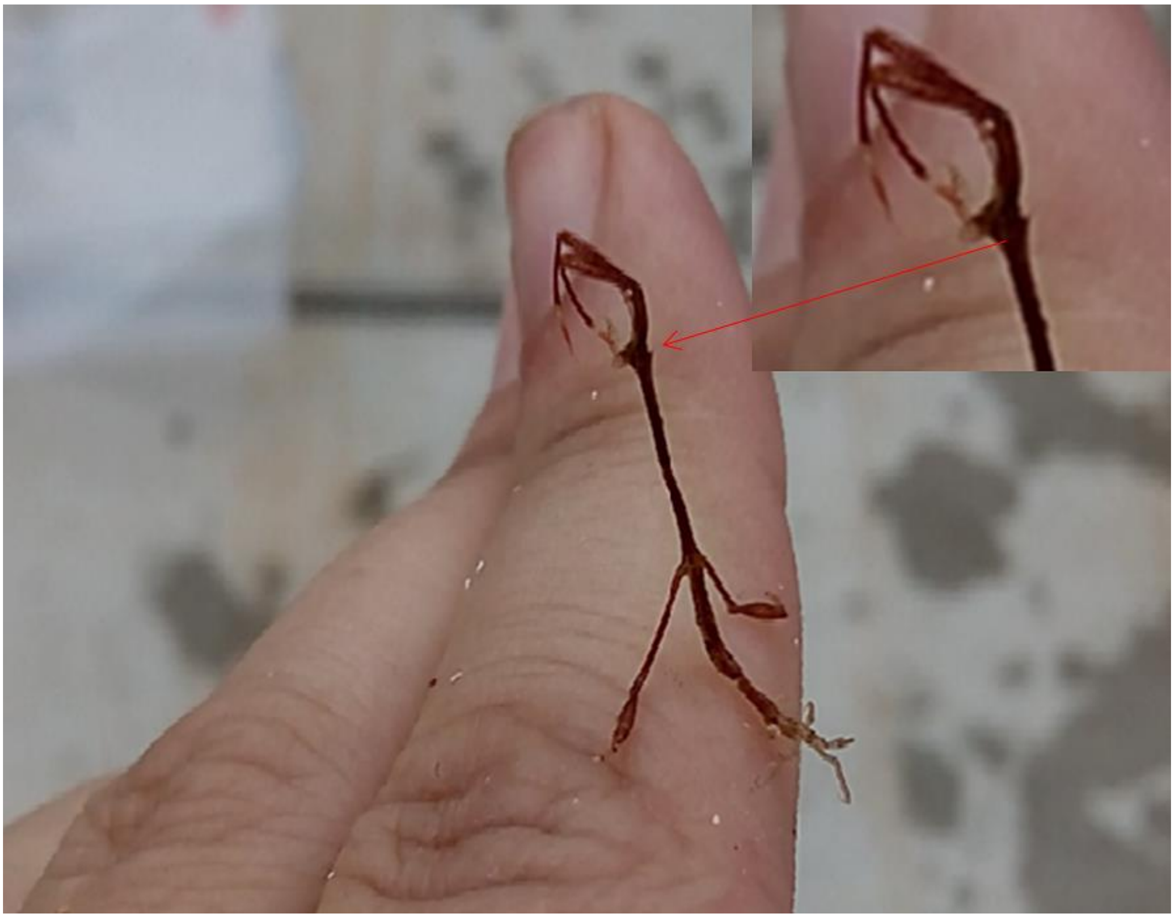

Figure 32: A particularly large *C. scaura* specimen found in Lim channel. Notice the visible occipital spine.

5.4.9 *Caulerpa cylindraea* Sonder, 1845

A highly invasive green algae, commonly known as "Grape Caulerpa" is easily recognized by its shape: a creeping thallus with berry-like growths on top (Figure 33). It has invaded Mediterranean by unidentified means (ballast, shipping, aquarium trades...) (Algaebase) and is now widespread. It was recorded in one location in a harbor during this study (Location 1: Červar porat), covering the muddy-sandy bottom of a well-protected harbor, but its presence is known from all over the western Istrian coastline (Sladonja & Banovac-Kuča, 2014), especially near ports. This is further supported by Iveša et al. (2015), where the role of ports, anthropogenic activity and low wave exposure in the spread of this species is explained.

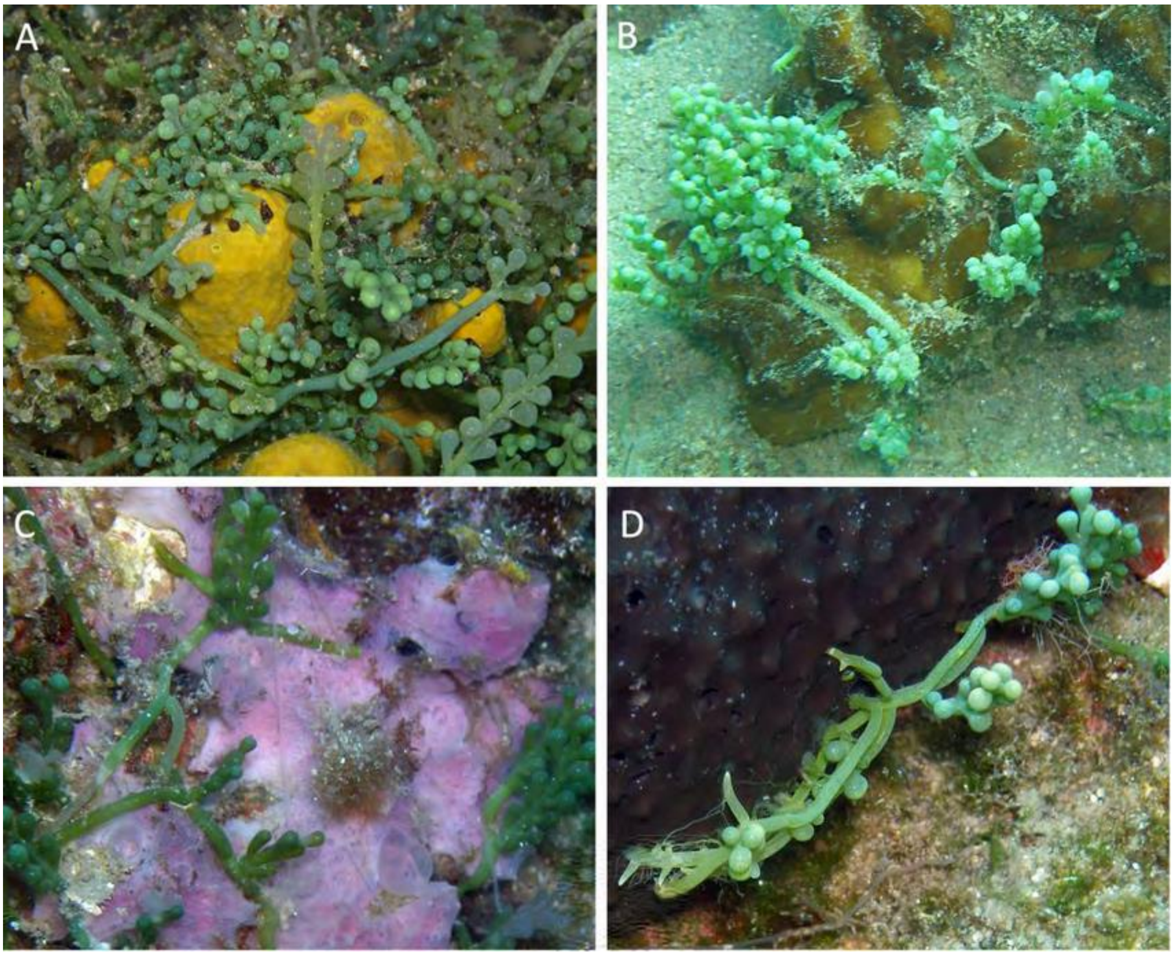

Figure 33: *C. cylindracea*, colonizing different substrates (Çinar, Bilecenoglu, 2015):

5.4.10 *Hydroides elegans* (Haswell, 1883)

Hydroides is a well-known genus of tube-dwelling serpulid polychaetes. Many of them are fouling species; the reason for their broad distribution with some even having invasive potential. Several such species, including *H. elegans* and *H. dianthus* have been introduced in the Mediterranean within the last 150 years. Both are also present in the Adriatic (MAMIAS). Additionally, *H. dianthus* was proven to be a species complex of at least 2 species with invasive potential (Sun et al., 2017). *H. elegans* was identified by the specifically serrated shape of its operculum (Figures 34 and 35) (Bastida-Zavala et al, 2017). This was further confirmed by observing the shape of the worm's spines below the operculum. The abundance of these polychaetes on some locations was staggering and other species were observed, but the true species identity was too difficult to determine. They likely aggregated in the Lim channel because of high productivity and low wave exposure. This species presumably comes from somewhere in the Info-Pacific (NEMESIS), but is globally distributed now, likely due to ship fouling, thus its place of origin is still uncertain.

Figure 34: Operculum of *H. elegans*, the main distinguishing feature (by Keppel, E).

Figure 35: *H. elegans* preserved in ethanol. A specimen encircled in red was removed from its tube. The red arrow shows the operculum.

5.4.11 *Magallana gigas* (Thunberg, 1793)

This bivalve is known commonly as a Pacific oyster, after its native area. It is a rather large bivalve growing up to 30 cm in length and more. It feeds on phytoplankton and detritus, typically in the coastal area, where it can be widespread (GISD). It is very variable in shape and size, but generally, the right valve tends to be more concave than the left (CABI), hence the name “Cup oyster” (Figure 36). As observed in studied locations, it can form reef-like structures on vertical surfaces and span from intertidal to infralitoral area. The reefs can support more oysters or more organisms in general. These reefs present threats to people, due to their sharpness, as well as boats, while, on the other hand, establishing a new biogenic formation. While its invasive potential cannot be ignored, (Nehring, 2011) it is considered established in the Mediterranean (MAMIAS). The cause of its spread is aquaculture escape due to release of spat (larvae) to enhance the stocks.

Figure 36: *M.* gigas, living sample fouled with epibionts. The red line shows the curvature of the right (lower) valve.

5.4.12 *Mnemiopsis leidyi* A. Agassiz, 1865

This planktonic ctenophore, sometimes confused as a jellyfish, is native to the Atlantic, along the American coast of the Atlantic, from Narragansett Bay, USA to the Valdez Peninsula, Argentina (Didžiulis, 2013). It is recognised by the seemingly, although not truly fluorescent ciliated combs that actually reflect light, creating rainbow-colored effects (Figure 37). It has eight (four long and four short) rows of small, but numerous, ciliated iridescent bioluminescent combs (Didžiulis, 2013). It was recorded for the first time in the Northern Adriatic in 2005, but has only recently started blooming during the summer (Malej et al., 2017) only to die out in later months. This planktonic species is stated here as a well-known invader with a strong invasive potential. Furthermore, the jellies tend to aggregate in coastal areas such as harbors, in large numbers. During this study, it was sighted at 3 locations, but only as a remnant of this summer's bloom. It is a carnivorous opportunist feeding on a large variety of larval animals and eggs, as well as each other. It is considered an invasive species in the Adriatic Sea (MAMIAS).

Figure 37: *M. leidyi* (by Vidar A), with reflective ciliated combs.

5.4.13 *Paracerceis sculpta* (Holmes, 1904)

This isopod is easily recognized by the large uropods in males and a deep indentation in the apex of the pleotelson (NEMESIS). In females, the telson is simple and the uropods are small and less ornamented (NEMESIS). Their carapace is also serrated (Figure 38). They were typically found on seaweed, seagrass and other branched organisms such as *A. verticillata* in the studied area. While the adults are herbivores, juveniles are carnivorous and even cannibalistic. It is native to Pacific ocean, California and Mexico (Ariyama & Otani, 2004), but has been introduced across the planet, including the Mediterranean Sea (CABI) and the Adriatic Sea (Forniz & Maggiore, 1985).

Figure 38: *P. sculpta*, male with large uropods (encircled) and female. The red arrows also show the serrated carapace.

5.4.14 *Styela plicata* (Lesueur, 1823)

This species is a relatively large, solitary ascidian with no peduncle, and a lumpy, leathery surface. The oral siphon is terminal, and the atrial siphon is a little behind it – both siphons are short, with square apertures with rounded humps on each side. The color of the tunic is whitish with 8 (4 double) brown or black stripes radiating from the siphons (Figures 39 and 40) (GISD). Thought it typically comes in some variety of white or tan (GISD), even almost brown specimens were observed in this study. Most of the time, they were overgrown with other organisms. It is a filter-feeding ascidian growing well in productive, enclosed areas. In my surveys, it was mostly found fouling submerged ropes, sometimes in large aggregations, some on floating objects, rarely on ship hulls and never on vertical, stone or concrete walls. NEMESIS database states that *S. plicata* is considered introduced in Mediterranean although centuries ago. It could be classified as cryptogenic due to supposed age of introduction, but since it's most probable native range (NW Pacific) has been found (de Barros et al., 2009), its presence has been known since the 19th century and no invasive behaviour in the region has yet been reported, it can be stated as established, which is supported by Karachle et al. (2017).

Figure 39: *S. plicata* aggregation (by Cilsick, R.)

Figure 40: *S. plicata* clump, live specimens. Collected from Pomer bay and photographed in aquarium.. Notice the 4 brown double lines around the siphon

5.5 Possibly misidentified/suspected species

A few of the records, although found species, were excluded from the results due to likely misidentification, degraded sample state or other reasons. This data may be useful in future, more detailed surveys. These are listed below:

5.5.1 Unconfirmed barnacle species

Occasionally, some species samples were too damaged or deformed to perform a proper identification. Some barnacles are among those set apart. In the first case, the dilemma was between *Balanus trigonus* and *A. amphitrite subsp. amphitrite*. *B. trigonus* is a robust barnacle, similar to some species of *A. amphitrite* complex, so they can be mixed-up. *B. trigonus*, however has significantly deeper ridges, in contrast to the smoother surface of *A. amphitrite* and one to six longitudinal lines of pits on the scutum (which was unfortunately missing) (Darwin, 1854). It is typically recognized by a roughly triangular shape of the orifice. Native to Pacific Ocean (Darwin, 1854), it has been introduced by boats to most parts of the world. Its presence in the Adriatic is the example of the anthropogenous cosmopolitanism (Igić, 2007). This sample was present in 1 studied location only (Červar porat), in the form of dead (no scutum or tergum) and sufficiently eroded shells to make the identification impossible. Also, the triangular orifice might have been damaged or deformed (As seen with *A. amphitrite*. See species profile). It is, however, possible that this is in fact a variation from *A. amphitrite* complex named *A. amphitrite amphitrite,* as seen by the noticeable, but eroded deep vertical ridges on the outside of the shell (Figure 41). In the end however, this specimen appears to be unusable due to excessive erosion.

Figure 41: Partial (eroded) sample of barnacles from Červar porat

Another dubious barnacle finding is *Amphibalanus eburneus.* A small to medium size barnacle, known as an “Ivory barnacle”, this species can be easily confused with *A. improvisus* which prefers brackish habitats (NEMESIS; Furman & Yule, 1991). Without taking into account the various ecophenotypes (NEMESIS), *A. eburneus* is generally more flat and stocky; while *A. improvisus* is a bit more tooth-shaped, but with smoother plates. This, however, is not the most valid identification feature and tergum/scutum shapes need to be observed (which were unfortunately missing). *A. eburneus* has a shell which varies from conical to cylindrical, depending on the amount of crowding. The orifice is round or slightly toothed, and its width is usually more than ½ its height. The plates have wide longitudinal spaces (radii), narrowing towards the top of the shell plates, while the tops (summits) of the shell plates are thick and rough (Henry & McLaughlin, 1975). *A. eburneus* is native to the Western Atlantic, from the southern Gulf of Maine to Panama, Colombia and Venezuela. It ranges further south, to Uruguay and Argentina (Henry & McLaughlin, 1975; Young, 1994), where it may be cryptogenic (NEMESIS). During the study, it was noted only occasionally, with a few individuals on two locations: Lim-Istrida and Pomer bay, but with the missing internal structure, this barnacle cannot be properly identified as *A. eburneus* (Figure 42).

Figure 42: *Amphibalanus eburneus* (likely), found on *M. galloprovincialis* shell

5.5.2 *Botrylloides violaceus* (*Botryloides sp*)

This Ascidian species vary in color and shape a lot, and it has a similar and native relative in the Mediterranean (and Adriatic) Sea: *Botrylloides leachii*. Generally, it is an encrusting colonial ascidian, with a soft tunic that can easily be torn (NEMESIS; Cohen, 2011). It also dissolves easily making sample preservation difficult, which is exactly what happened in this case. While alive and erect, the zooid pattern resembles a zipper opening, positioned in lines (Figure 43), in opposition to the *Botryllus* genus, which are typically star-shaped (Figure 44). The main problem in identifying this species is the profound variations between colonies. This species is considered established in the Adriatic Sea and Mediterranean (MAMIAS). It originates from the NW Pacific (MAMIAS) or southern Siberian coast, Japan and southern China (Cohen, 2011) and its probable vectors stated by MAMIAS are aquaculture and shipping. The mistake made while doing this study shows that some organism types should be picked-out fresh or early-on in the waiting period. It was only found in Tarska vala.

Figure 43: *Botrylloides sp.* colony growing on mussel shell (by Solórzano, Luis A.)

5.5.3 *Botryllus schlosserii* (Pallas, 1766)

Another encrusting colonial ascidian, a sample of which was found in Pomer bay, also fits on the exclusive list due to early sample degradation and dubious introduction status, as well as troubling identification. The differences between *Botryllus* and *Botrylloides* are mainly in the zooid growth pattern, the former being star-shaped (Figure 44), and the latter ladder or zipper-shaped (Figure 43). They can both come in varying colors, although *B. schlosserii* is usually brown-black-yellow and *Botrylloides sp.* orange to red (NEMESIS; Cohen, 2011). The sample did survive in aquarium for a brief period of time (where it was kept temporarily until its status could be confirmed), but was not successfully preserved. Also, it was very small, shedding doubt to its proper identification. Some databases state this species as native in the whole Mediterranean, while others state it as cryptogenic (NEMESIS) or have, in fact excluded it from the NIS lists (MAMIAS). Due to these reasons it was excluded from the main list.

Figure 44: *B. schlosserii* (possibly) colony growing on mussel - *Halimeda tuna* aggregation. Notice the star-shaped patterns shown by the red arrows.

5.5.4 *Hydroides dianthus* (Verrill, 1873)

While comparing this serpulid tubeworm to another on the outside, it is impossible to properly define the species, but the opercula show profound differences. While this is how the *H. elegans* was recognized (serrated pattern on the operculum) (Bastida-Zavala et al., 2017) and later confirmed by observing setae, other opercula have shown similarities to *H. dianthus* (See Figure 45). For this reason, its complex taxonomical status and due to its dubious status as an introduced species (MAMIAS states it as introduced, but Mikac (2015) in her manuscript, does not), it was removed from the main list. *H. dianthus* serpulids, just like *H. elegans* have been reported before in the Adriatic and their presence is not surprising. Both species, including some native, similar tubeworms, were growing all together. Since some of the *Hydroides* species are considered invasive (several in the Mediterranean), it would be wise to monitor the situation on floating fish and shellfish farms, as well as other objects where *Hydroides* species tend to aggregate

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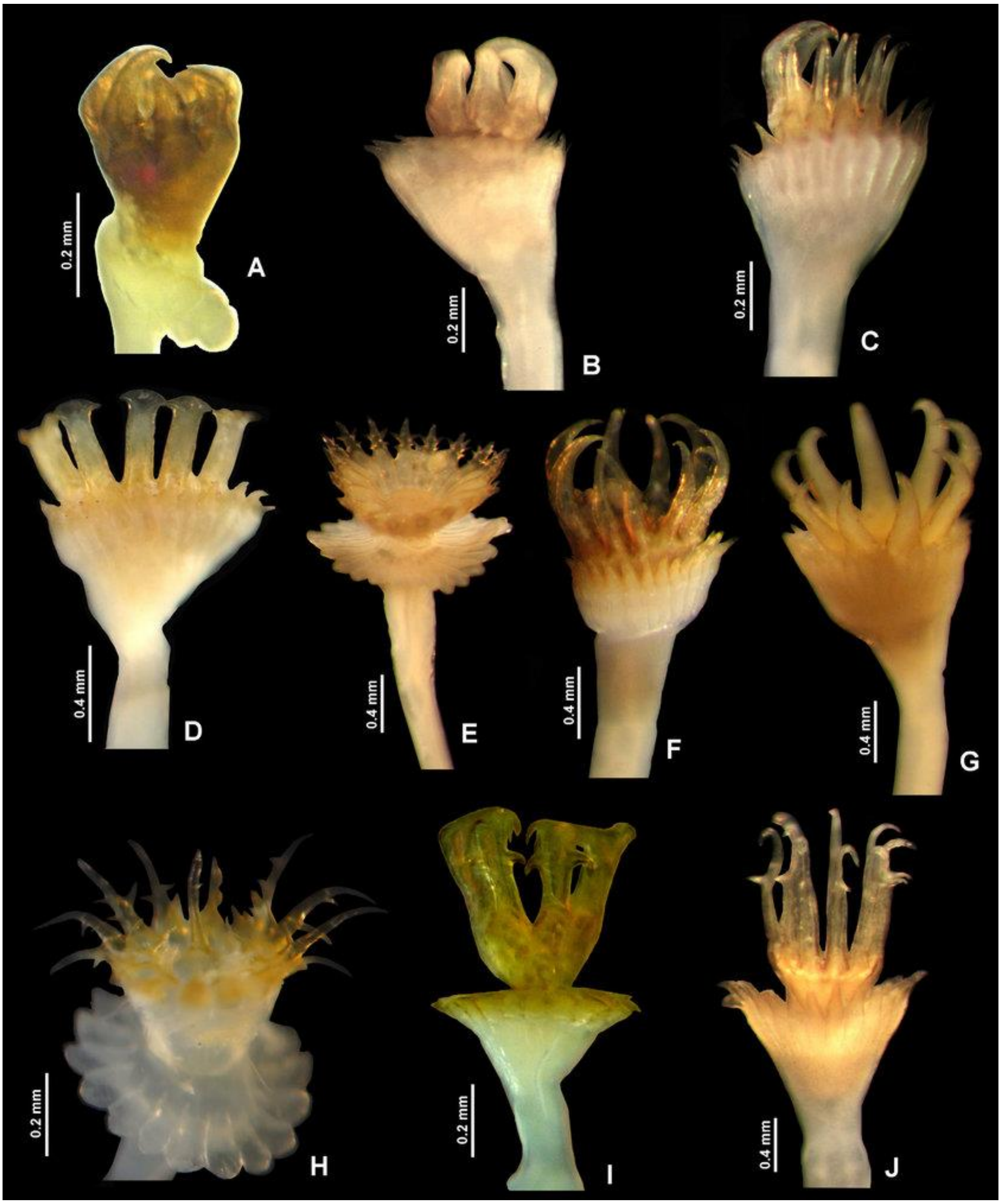

Figure 45: *Hydroides* species opercula: *H. bispinosa* - A, *H. cf. brachyacantha* - B, *H. dianthus* - C, *H. dirampha* - D, – *H. elegans* - E, *H. floridana* - F, *H. gracilis* - G, *H. longispinosa* - H, *H.s parva* - I, *H. sanctaecrucis* – J (Bastida-Zavala et al., 2017)

5.5.5 *Callinectes sapidus* (Rathbun, 1896)

Lastly, while not directly observed in the wild, *Callinectes sapidus* presence was reported as a personal communication by a fishermen's grandson (who recognized the crab shown on Figure 46 from the picture after telling me of strange crabs being caught) from Medulin elementary school in Šćuza lagoon, near Pomer bay, as well as other locals who caught them by accident. The species obviously stands out as "an alien" among the native crabs and is easily recognized as such, although, other portunid crabs are common in nearby areas. Unexpectedly, but not surprisingly, the species is already being widely collected for its tasty meat, which is one possible way to contain the invasion. *C. sapidus* is native to the Western Atlantic, from Nova Scotia, Maine and northern Massachusetts to Argentina, including Bermuda and the Antilles (CABI). While the CABI database states that *C. sapidus* doesn't have invasive characteristics in the Mediterranean, MAMIAS states contrary; that it has. Since the species was not directly observed during the surveys and is only in proximity of a studied area (Puč-Pomer, See Figure 1), coupled with the unreliability of the record, the species was not included in the main list.

Figure 46: *C. sapidus*, known as the Blue Crab (by Kaveney, W.)

5.6 Comparison with other studies in the nearby areas

Many of the studies included Istrian coastline as a part of the covered area, namely Zenetos et al. (2016), as well as Lipej et al. (2018) reported on the anaspidean seaslug *B. leachii* being present (in the whole Adriatic). However, it was not observed during this study; in fact not a single alien or native ophistobranch was observed. While this is unexpected, it is likely just a sampling error or the result of avoiding sediments due to practicality issues (primarily depth). Another missed species of sea slug is *Okenia zoobotryon*, a predator and epibiont of *A. verticillata*, as well as many alien bugulid bryozoans like *B. neritina*. In spite of the sheer biomass (several kilograms) of collected *A. verticillata* and *B. neritina*, not a single specimen was found. Other studies also mentioned several algae species, such as *Codium fragile* (Orlando-Bonaca, 2001). While *C. fragile* is common, it was not observed in studied harbors. This is a sampling anomaly because *C. fragile* is well distributed on the Istrian coast (Ljiljana Iveša, *pers. communication*) with several locations being in the vicinity of ports (Poreč, Rovinj, Val Saline-Rovinj…) While studies have been done, much of this data remains unpublished or they are too scarce to be published. Nerlović et al. (2018) studied the fouling communities in Lim channel and Pomer bay mariculture areas, focusing especially on the invasive *A. transversa*, which was also not observed. This is likely because this bivalve lives in the infralitoral area, only sporadically attaching to rocks or other larger objects. Also, the studied mariculture areas were distanced from the harbors and the coast. The observations of *A. senhousia* in Škocjanski zatok, Slovenia (Lipej et al., 2012) differ from my findings, since they report its invasion in very shallow areas of the Škocjan inlet, close to the city of Koper. As mentioned before, this species was not growing well in the studied areas, but other localities should be well studied, especially brackish water areas, where this bivalve is known to invade. Most other studies also focused either on a single or few groups, or made detailed benthic (or fouling) community surveys, typically surveying all the species: native with the inclusion of NIS, while this one focused on several taxa (some in the fouling community, others epilithic…) in a limited area and depth (1 meter at most). This can explain the discrepancy in species records. Also, various known data was not yet published (Ljiljana Iveša, *pers. communication*), which could explain the apparently "unreported", but relatively common species. This is but a fraction of the missing and/or easily accessible (and locatable) data.

The comparison with the study by Lipej et al. (2012) showed that only 2 species, namely *M. gigas* and *A. armata*, were common in both studies. *M. leidyi* was reported as casual at the time, but has started blooming massively in the summer periods since 2016 (Malej et al., 2017). In Lipej et al. (2012) many species were not reported such as *C. scaura*, *P. sculpta*, *and A. verticillata*, or cryptogenic bugulid bryozoans. This, however, does not have to mean they were not present on the Istrian coastline since study by Lipej et al. (2012) was limited to the Slovenian coastline. This discrepancy is likely tied to temporal factors due to studies being conducted 6 years apart from each other, as well as spatial division.

5.7 Comparison with similar studies

NIS monitoring programs have become very attractive both in environmentalist, marine scientist and popular-scientific circles. PBBS (Port Biological Baseline Surveys) are a form of coastal monitoring for NIS, typically spread by ballast waters, but also ship hulls (Awad et al., 2014). While designed for large, commercial ports, these methods can be applied to smaller harbors and marinas. PBBS have been done in the Adriatic Sea by Petrocelli et al. (2018) with abundant results. Twelve large Adriatic ports were surveyed during BALMAS' PBS, with all available substrates included, according to BALMAS Port Baseline Survey Protocol (Ninčević-Gladan et al., 2014). Similar approaches named "Rapid Assessment Survey" (RAS) have been conducted for at least 2 decades. While RAS studies have similar methodology to this thesis (they do include native species in their surveys), they consist of a larger team of people such as scientists, students and volunteers. Coupled with much larger covered areas, their findings are thus, more expanded. Examples of such surveys are Cohen et al. (2005) in California, Wells et al. (2014) in New England, Vercaemer & Sephton (2014) in Nova Scotia, Mathieson (2016), focusing on seaweeds from southern Maine to Rhode Island and others. Arenas et al. (2006) made a RAS of marinas in the southern England in 2004 with a similar methodology to this thesis. Their results understandably vary from this survey, but some species have been found sharing common preferences, mainly bugulid bryozoans, *A. verticillata* and ascidians.

5.8 Potential impact on the native biota

Alien species may bring about a range of ecological, economic, and human health impacts. (CIESM, 2002) Except for the highly invasive species such as *C. cylindracea* and *A. armata*, which can cover large areas and outcompete other species for space, as well as *M. leydi* the voracious plankton-feeding ctenophore, the sampled NIS appeared to be mostly harmless. The oyster farm workers did, however mention the problems fouling causes in their jobs. This risk is also present with other species not observed during this study. Other recorded species were either naturalized, cryptogenic or had their ranges limited to harbors, docks, pontoons or vessels (*A. verticillata*, *B. neritina*…). Some of the recorded species such as *A. senhousia* are, however known to invade certain locations in neighboring countries especially in estuarine areas (Lipej et al., 2012). *M. gigas*, while considered established, may not have stabilized its population yet. It is widespread in most harbors and the attachment of larvae to other oyster shells (due to higher surface area) only accelerates the population growth. Ultimately however, most of these species are limited to harbors and do not appear to survive "out in the open". This is facilitated, in addition, by the cold winters in the north Adriatic.

5.9 Implication for the future work

The collected data from this study can be very useful when designing further studies into the matter. The observations of species and their associations show that, even if some species are not invasive, they can be useful in monitoring other species spread and introductions, as shown with *A. verticillata, B. neritina, S. plicata* or even native mussel aggregations. This also presents potential risk due to facilitated spread of NIS, or NIS "Piggybacking". Despite the fact that only a portion of the known alien species in the Northern Adriatic were observed and that others lurk along the coasts, hopefully other studies will pick up and the problems presented by tropicalisation and NIS spread will be faced properly.

A special look must be reserved for the Lim channel in this study. The abundance of organisms in the area, as well as many of them being NIS (all categories), makes the area especially interesting. The area, other than being an important spawning location and aquaculture area, seems to be the hub for NIS associated with harbors. As mentioned previously, the organisms on floating objects formed layers, visibly separated. The polychete abundance was especially intriguing, as was the identification of *H. elegans* among them, which was first reported in the port of Raša just a few months prior to this study, although the samples were collected back in 2011 (Travizi et al., 2018). The population of those tubeworms was mixed as was observed by comparing their opercula. Another species likely present is *H. dianthus*, a species marked as introduced and invasive by MAMIAS database, as mentioned before. Others may easily lurk among the aggregated foulers as well, especially among bugulid bryozoans and *A. verticillata.*

For a few species, namely *C. scaura* and *P. sculpta*, this is also the first written record on the Croatian part of Istrian coastline. The species may have been overlooked before, or the recorded findings were never published by other authors. *H. elegans* was recorded for the second time on the Istrian Coastline, first being in Raša on the eastern coast (Travizi et al., 2018), and this being the first on the opposite, west coast, showing the spread of the species.

Monitoring NIS can also be a great citizen science area, especially where interest blooms. One such example is presented by Mannino & Balistreri (2018) and shows how citizen science can be used to monitor the distribution and presence of specific invasive species, or even other categories of NIS. Zenetos et al. (2013), while reporting on the Greek example, stated that: "*Engaging citizen scientists to survey local biota and detect non-native marine species incursions is expected to result in the collection of significant data sets, which could potentially be used for an early warning*..." The increasingly marine tourism-dependent coastal areas of the Istrian coastline (and beyond) are a prime target for these activities. Other than citizen science, students should be encouraged to do work on this subject, as it presents a comparatively simple, yet easily available and rich educational resource.

6 CONCLUSION

This study attempted to look into the presence and distribution of non-indigenous marine flora and fauna inhabiting harbors and adjacent areas. Ten ports on the Croatian part of Istrian peninsula were sampled for alien marine flora and fauna. Fourteen NIS were found, twelve animal species and two algae. Their frequencies and introduction status have been stated. Species profiles were written and observations were noted for each of the recorded species. Observed distributions, preferred habitat in the each sampled locations, as well as the origins of the NIS were also noted. As such, the goal of the study was achieved. Coupled with a brief overview of the local research and discussion on the subject, this thesis will, hopefully, be useful in facing the threat of NIS the Northern Adriatic and the larger Mediterranean.

7 POVZETEK MAGISTRSKEGA DELA V SLOVENSKEM JEZIKU

Vsak organizem, ki se najde na območju, kjer naravno ne pripada, se šteje kot vneseni, alohtoni ali tujerodni organizem, ali vrsta. IUCN definira tujerodno vrsto kot: „Tujerodna vrsta je vrsta, ki so jo prinesli ljudje – zmerno ali slučajno – izven svoje pretekle ali sedanje distribucije.“ Razlikujemo več kategorij tujerodnih vrst.

INVAZIVNE VRSTE

Če se tujerodna vrsta začne širiti nekontrolirano in agresivno ter škoduje avtohtonemu ekosistemu, lahko med samim procesom povzroči tudi ekonomsko škodo, taka vrsta potem postane invazivna. Takšne vrste so lahko živali, rastline, patogeni ali pa drugi organizmi, ki so tujerodni in lahko motijo avtohtone, naravne procese v ekosistemu na način, da delajo ekološko, okoljsko, ekonomsko in antropološko škodo. Škodo začnejo tipično delati tako, da zmanjšujejo stopnjo biodiverzitete, ker prihajajo v kompeticijo z avtohtonimi organizmi, kar destabilizira celotni ekosistem, vključno s človekom. Nekateri primeri takšnih organizmov v morju so: *Caulerpa taxifolia, Caulerpa cylindracea, Womersleyela setacea* in *Asparagopsis armata.*

NATURALIZIRANE VRSTE

Nekatere vrste, ko pridejo v alohtono okolje, preprosto izpolnijo prosto nišo v ekosistemu. Pri tem ne delajo (značilne) škode ali pa lahko še bolj stabilizirajo ekosisteme. Takšne tujerodne vrste imenujemo naturalizirane, čeprav še vedno držijo tujerodni status. Pomembno je, da se ta skupina vrst ne zamenja oz. napačno identificira kot invazivna, ker je to širša skupina vrst, od katere vsaj 5-20 % vrst postane invazivnih. V primeru da se to zgodi, lahko pride do večje škode pri upravljanju s takšno vrsto, kot bi bilo brez intervencije. Eden izmed primerov morskih naturaliziranih vrst je Japonska ostriga *Magallana gigas*, ki je bila mogoče na začetku invazivna, ampak je takšen status izgubila zaradi zmanjšane reprodukcije in vpliva na ekosistem.

KRIPTOGENE VRSTE

Če se za vrsto ne ve, ali je avtohtona, alohtona in od kod prihaja, se ta vrsta imenuje kriptogena. Do tega lahko pride iz več razlogov: omejeno raziskovanje v preteklosti, vrsta ni bila opažena, vrsta je bila vnesena pred več sto in ali tisoč leti, dolgoročna biogeografija (glacialna izolacija, Lazarjevi taksoni, rekolonizacija) ali pa antropogena svetovna distribucija. Nekatere kriptogenične vrste v Jadranu so: *Amphibalanus amphitrite, Teredo navalis* in *Bugula neritina*.

DOSEDANJE RAZISKAVE TUJERODNIH VRST NA OBMOČJU ISTRSKEGA POLOTOKA

Severni Jadran je zelo zanimivo področje za večje število raziskovalcev in držav. Raziskave, narejene pred letom 1990 v okolici istrskega polotoka, ki dominira severnemu Jadranu, generalno nimajo neposrednih podatkov o tujerodnih vrstah, ampak to ne pomeni, da te vrste niso bile opažene. Veliko raziskav je bilo fokusirano na obraščanje v morju (Zavodnik & Igić, 1968; Igić, 1969) (eng: fouling), kar je bil en način, kako so se odkrile nekatere tujerodne vrste. Del podatkov ni bil niti objavljen ali pa so bili podatki vključeni v druge raziskave.

Večji interes za raziskovanje tujerodnih vrst je prišel z invazijo roda morskih alg *Caulerpa*. Invazija v Istri je dobro opisana skozi več objavljenih člankov (Meinesz in sod., 2001; Blažina in sod., 2009; Sladonja & Banovac-Kuča, 2014; Iveša in sod., 2015). Po letu 2000 je interes akademskih krogov značilno porasel. Začele so se, danes že številne raziskave na temo tujerodnih vrst v celem Jadranu, vključno z Istrsko obalo, kjer so raziskave opravljali inštituti iz Hrvaške, Slovenije in Italije, enako kot sodelavci izven te regije. Kljub temu, raziskovani podatki na Istrski obali še vedno niso popolnoma zbrani.

RAZISKOVANO OBMOČJE

Hrvaški del istrske obale je dolg 570,01 km, kar je več kot desetkrat daljše od slovenske obale, ki meri 46,6 km. Obstaja tudi mali del obale, ki pripada Italiji. Istrska obala je zelo raznolika, z obalnimi tipi, kot so kraška obala, majhni blatni poloji, prodniki, lagunarne obale ter estuariji. Raziskovano je bilo vse skupaj 10 lokacij, razporejenih po zahodni, vzhodni in južni obali hrvaške Istre. Cilj so bila pristanišča majhne in srednje velikosti. Velika pristanišča se niso raziskovala zaradi možnih pravnih in praktičnih težav. Raziskovane lokacije so prikazane na karti (Slika 1) in so:

- Červar-Porat
- Lim-Istrida
- Rovinj-Bolnica
- Krnički porat
- Lim-marina
- Tarska vala
- Novigrad
- Puč-Pomer
- Ližnjan
- Luka Trget

METODE

VZORČEVANJE IN ANALIZA

Organizmi so se vzorčili ročno in s pomočjo vodne mreže. Nabirali so se organizmi, ki so rastli po čolnih, vrveh, vertikalnih blokih ali potopljenih instalacijah in antropogenih strukturah. Po končanem vzorčenju so se organizmi hranili 1-3 dni v morski vodi, odvisno koliko je bilo potrebno, da potopljeni organizmi izplavajo na površino vode. Inducirana hipoksija je prisilila vagilne organizme, da so zapustili svoja zavetišča in mikrohabitate ter začeli bežati, tako se jih je lahko pobralo s pinceto ali ročno. Sesilni organizmi so bili pobrani zadnji.

Material je bil separiran živ in mrtev, odvisno od organizma. Organizmi mehke zgradbe, kot so plaščarji, so se izolirali živi za potrebe identifikacije. Ostanek materiala se je separiral in determiniral s pomočjo lupe in mikroskopa. Separacija se je izvedla skozi več korakov (Tabela 1).

Frekvenca se je izračunala kot delež števila lokacij, na katerih so bile najdene posamezne vrste. Karta lokacij je bila izdelana s pomočjo računalniškega programa Surfer »Version 8.09,2391«. Klase abundance so bile izdelane na podlagi opazovanj številčnosti, distribucije in površine substrata. (Tabela 2).

Preferirani substrat je ekstrapoliran iz zabeleženih podatkov, ki govorijo o tem, kjer, oz. na katerem substratu (podlagi) so se sposamezne vrste najdele. Lahko je vrsta imela več preferiranih podlag (kot so vrvi, čolni ipd…). Substrati so bili postavljeni v eno izmed 6 kategoriji, ki so prikazane v Tabeli 3.

REZULTATI IN DISKUSIJA

TUJERODNA FLORA IN FAVNA

V raziskavi je bilo najdenih vse skupaj 14 vrst v 7 taksonov. Od tega so bili 4 raki, 3 mahovnjaki, 2 mehkužca ter po ena vrsta plaščarjev, rebrač, mnogoščetincev, rdečih alg in zelenih alg. 2 vrsti rakov sta bili iz skupine vitičnjakov (Cirripedia). Dve drugi vrsti rakov sta bili iz skupin postranic (Amphipoda) in enakonožnih rakov (Isopoda) (Tabela 4). Zabeležene vrste so bile: *Amathia verticillata, Amphibalanus amphitrite, Amphibalanus improvisus, Arcuatula senhousia, Bugula neritina, Magallana gigas, Styela plicata, Caprella scaura, Bugulina stolonifera, Paracerceis sculpta, Caulerpa cylindracea, Hydroides elegans, Asparagopsis apmata* in *Mnemiopsis leidyi.* Vrste z največjo frekvenco so bile *A. amphitrite, A. verticillata, B. neritina* in *M. gigas.* Nasprotno, najmanj pogoste vrste so bile *C. cylindracea, A. Improvisus* in *B. stolonifera.* Nekaj vrst je bilo tudi v medsebojni interakciji. *C. scaura* je bila večinoma najdena na mahovnjaku *A. verticillata*, enako kot *P. sculpta*, s katero je bila v kompeticiji za živeči prostor. To nakazuje na to, da

obe vrsti preferirata sekundarne, razvejane habitate. Vrste *S. plicata, B. neritina, B. stolonifera* so skupaj z avtohtonimi organizmi (*Mytilus galloprovincialis, Bugulina flabellata, Cradoscrupocellaria bertholletii...*) gradili obrast po vrveh, čolnih in ladjah ter *M. gigas* po vertikalnih blokih. Preferirani substrati za vsako najdeno tujerodno vrsto so zabeleženi v tabelah 5 – 14.
Vektorji zabeleženih tujerodnih vrst so lahko balastne vode, v primeru grozdaste kaulerpe in *M. leidyi*, pobeg iz akvakulture, v primeru *M. gigas*, ali pa transport v obrastu na ladjah ter disperzija s pomočjo majhnih ladij ali pa čolnih po pristaniščih, v primeru skoraj vseh drugih vrst. Vektorji kriptogenih vrst niso poznani, ampak vse se lahko širijo po obali s pomočjo obrasta na čolnih in ladjah.
Frekvenca vzorčenih vrst je bila seveda zelo povezana z dostopnostjo, tako da je npr. v primeru grozdaste kaulerpe, frekvenca zelo nizka, čeprav je ta vrsta precej razširjena. Frekvenca in prisotnost vrst sta tudi zelo odvisni od vremena in klime. Raziskovanje se je izvajalo le od septembra pa do konca novembra, kar pomeni da so bile zabeležene tiste vrste, ki so bile prisotne na koncu poletnega in v jesenskem obdobju. Nekatere vrste so lahko ostale tudi nezabeležene. In sicer, brezcevni mnogoščetinci (npr. Nereidae) se niso raziskovali zaradi zelo kompleksne določevalne metodologije. Zanimivo je tudi, da v raziskavi ni bil najden niti eden polž zaškrgar, kar je nenavadno. V rezultatih se lahko opazi tudi velika razlika med številom živali in alg, vendar je ta razlika popolnoma naključna. Druge vrste tujerodnih alg so lahko prisotne, ampak niso bile dostopne z uporabljeno metodologijo (npr. *Codium fragile*) ali niso rastle na raziskovanih lokacijah.

PRIMERJAVA Z DRUGIMI RAZISKAVAMI

Druge raziskave so ponavadi vključevale večja pristanišča, kot so Pula, Trst, Benetke, Reka ipd... kar značilno vpliva na rezultate. Poleg tega, večje raziskave vključujejo večje število raziskovalcev, specializiranih v določevanju posameznih skupin in vrst, kar lahko pomaga pri izdelavi raziskave. Podobno, bolj specializirane raziskave, kot so za eno skupino organizmov (npr. tujerodne školjke), dajejo bolj specifične rezultate.

PRIMERJAVA S PODOBNIMI RAZISKAVAMI

Opravljena raziskava se lahko opiše kot RAS študija (Rapid assesment survey), pogosto izvedena v severnih državah (Združeno Kraljestvo, ZDA, Kanada...), ampak RAS upošteva tudi avtohtone vrste, kako bi se potencialno pravočasno opazilo spremembe v sestavi vrst. PBBS je bolj natančna in podrobna študija, fokusirana na tujerodne vrste, ki se izvaja v pristaniščih. Obe vrsti sta vsebinski podobni izdelani magistrski nalogi, z določenimi variacijami. RAS študije se pogosto izvajajo v manjših pristaniščih in, čeprav so izdelane na severu Evrope, ali pa v Ameriki, pogosto vsebujejo nekatere skupne vrste in podobne rezultate, seveda z razlikami zaradi razdalj.

ZAKLJUČEK

V študiji tujerodne morske flore in faune v pristaniščih hrvaške Istre se je poskusilo raziskovati prisotnost in razširjenost alohtonih organizmov na tem območju. Na 10 lokacijah je bilo zabeleženih vse skupaj štirinajst tujerodnih vrst, dvanajst živali in dve algi. Izračunana je bila njihova frekvenca in naveden njihov tujerodni status. Poleg tega so bili izdelani profili in obzervacije za vse opažene vrste. Dodatno, navedeni so bili tudi distribucija, preferirani habitati ter poreklo najdenih vrst. S pomočjo pridobljenih podatkov in obzervacij lahko zaključim, da je navedena hipoteza študije potrjena ter cilj študije izpolnjen. Z dodatkom kratkega pregleda dosedanjih raziskav ter diskusije na to temo, upam da bo ta magistrska naloga koristna pri soočanju z grožnjo tujerodnih vrst v Severnem Jadranu in Sredozemnem morju.

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APPENDIX A

Figure authors and sources

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Figure 24: Jensen, K. R. (2015): NOBANIS - Marine invasive species in Nordic waters - Fact Sheet: *Amphibalanus improvisus*. Identification Key to Marine Invasive Species in Nordic Waters, 1–3. at: https://www.nobanis.org/globalassets/speciesinfo/a/amphibalanus-improvisus/amphibalanus-improvisus2.pdf on 3.1.2019.

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