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Faunistic, ecological, and zoogeographical survey of heterobranch fauna in the Adriatic Sea: experiences from Slovenia

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Abstract

The heterobranch fauna recorded to date in the Slovenian part of the Adriatic Sea was reviewed and analysed in order to present an updated inventory. The ecological traits and zoogeographical affiliation of the recorded heterobranchs were also analysed. The new data revealed that the heterobranch fauna in the area consists of at least 157 species with the majority being nudibranchs (61.4%). The bulk of heterobranchs were considered as Atlanto-Mediterraneans. Heterobranch diversity was found to be significantly related to the number of sampling events. The spatial pattern of heterobranch species and their composition structure in parts of the study area were linked to specific human-impacted areas (HIAs) such as coastal wetlands, ports, and mariculture facilities. Better examination of less investigated environments, new sampling techniques, and citizen science involvement will doubtlessly increase the numbers in this checklist.

Keywords: Gastropoda; Heterobranchia; checklist; ecological traits; feeding guilds; zoogeography; mapping; Adriatic Sea.

Introduction

The Gulf of Trieste is considered a cradle of marine biology and other oceanographic sciences, an area where the first organized scientific research was conducted (Casellato, 2008; Cattaneo-Vietti & Russo, 2019). Despite a centennial tradition in marine research, the list of species found and recorded in this marine area is considered far from complete. This seems to apply also to marine heterobranch sea slugs (Gastropoda: Heterobranchia). Heterobranchs, and nudibranchs in particular, are currently attracting numerous underwater photographers due to their vivid colour patterns (Smith & Davis, 2019). However, they are generally small-sized and cryptic in habits; in addition, they are generally found in low numbers (Domenech *et al.*, 2002). In the past few decades, the marine heterobranch fauna of Slovenia has drawn the attention of marine biologists and this has resulted in many publications. The number of heterobranch species was small since the data were collected mainly using sedimentary substrate sampling gear such as Van Veen and Petersen grabs. After the first checklists of marine sea slugs published by De Min & Vio (1997; 1998) and Vio & De Min (1996) for the Gulf of Trieste (GoT), and by Turk (2000) for the GoT and the adjacent Adriatic area, several studies focused on its Slovenian part in view of assessing the heterobranch fauna in the area, and this resulted in the rapid increase of the number of recorded species (Turk, 2005; Lipej *et al.,* 2008, 2012; Mavrič & Lipej, 2012, Lipej *et al.,* 2014; Zenetos *et al.,* 2016). According to Ciriaco & Poloniato (2016), at least 73 heterobranch species were recorded in the Italian part of the GoT, while 142 species were found in Slovenian part of the Gulf (Lipej *et al.,* 2018a).

The main goal of this paper is to present the results of an analysis of the heterobranch fauna (species survey) discovered in Slovenian territorial waters to date. In this study we also analysed the ecological (habitats and feeding preferences) and zoogeographical affiliations (area of distribution) of the recorded heterobranchs.

Materials and Methods

Study area

Slovenian coastal waters are located in the southern part of the GoT, which is a shallow semi-enclosed part of the northern Adriatic Sea with a maximum depth of 33 m in the waters off Piran, although it rarely exceeds

25 m. The Slovenian coastline is approximately 46 kilometres long and is made of sandstone, which is the major source of detrital material. The coastal substrate is rocky and consists of boulder fields and gravel banks that extend to a depth of approximately 10 m, while deeper it turns into a sedimentary substrate (Trkov *et al*., 2021); moreover, soft sediments of fluvial origin are also found in the shallow waters of the bays (Ogorelec *et al*., 1991). The rocky bottom is covered with a dense algae, while shallow, soft bottoms at depths between 1 and 11 m are covered with seagrass meadows (mostly *Cymodocea nodosa*) (Lipej *et al*., 2018b). The coastal plains are heavily exposed to anthropogenic influences, while the flysch cliffs are largely preserved in their natural state. Different anthropogenic activities such as urbanisation, intensive farming, and massive tourism have significantly modified the coastline with only 18% remaining in its natural state (Turk, 1999). The area is subjected to substantial salinity and temperature fluctuations, and an evident water column stratification during the summer months. Freshwater input, especially from the Isonzo River (Mozetič *et al*., 1998), influences the salinity, which ranges between 33 and 38.5‰, while the water temperature normally fluctuates between 8°C in winter and 24°C in summer (Ogorelec *et al.*, 1991).

Methodology

Heterobranchs in the Slovenian part of the Adriatic Sea were sampled using different sampling techniques such as:

a. Regular (visual census) and occasional sampling by SCUBA diving

Sampling heterobranch fauna was carried out primarily by SCUBA diving equipment to a depth of 30 m. Specimens were photographed in their natural environment before being collected by hand net. Most sea slugs collected, which we were unable to identify in their environment, were carefully deposited in plastic chambers filled with sea water and delivered to the Marine Biology Station.

b. Scratching vegetation on the surface

Not all species of heterobranchs are large-sized or brightly coloured and thus they are more difficult to detect. Some heterobranchs are small, less colourful (dull), and cryptic. Such species are difficult to detect since they inhabit complex rocky benthic communities (e.g., Zenetos *et al.*, 2016). To this end, sampling in areas covered with vegetation was performed by scratching algae patches covered with a 20 × 20 cm metal square. The vegetation samples were left untouched over-night and the presence of heterobranchs was cautiously checked on the following day.

c. Collecting fouling communities

Collections of epifauna on mariculture facilities, in harbours, ports, marinas, and coastal wetlands were performed by taking samples of epifauna on ropes, hulls, and other suitable objects. The epifauna samples were left untouched over-night and checked for heterobranchs on the following day.

d. Dredging

Collections of epifauna on secondary hard substrate was performed using a dredge. The collected volume of sampled epifauna was immediately checked for the presence of heterobranchs on board the sampling vessel. A specially designed dredge was used for collecting samples of epifauna in seagrass meadows.

e. Sampling of zooplankton

Planktonic heterobranch species such as pteropods were collected using a WP2 zooplankton net for mesozooplankton sampling. The presence of pteropods was examined under a microscope.

f. Collecting heterobranchs with hand nets

The presence of heterobranchs was checked also during low tide in rock pools, under stones, and on washed-up marine vegetation. Heterobranchs were hand-picked, photographed and preserved in ethanol.

g. Citizen science

The participation of volunteers in data collection was also important for obtaining information (Smith & Davis, 2019); some photographs were provided by underwater photographers and diving enthusiasts.

A database was created for gathering data on the collected or sighted (photographed) specimens of heterobranchs. For each heterobranch specimen, depth, broader and narrower localities, habitat description, type of substrate, and microhabitat observations were recorded. If available, we also collected data on the presence of spawn, salinity, and temperature. For every sampling station, we recorded latitude and longitude.

Based on the results of the various sampling methods, a checklist of heterobranch species was compiled following identification. Few specimens of rare or less known species or species requiring examination under the microscope for taxonomical identification, were collected for reference. After a thorough examination of the live specimens, they were released back to the environment or preserved in ethanol and added to the collection of the Marine Biology Station of the National Institute of Biology (MBS NIB). Identification was carried out based on external morphology and using different identification keys such as the Pruvot-Fol (1954), Thompson (1976), Schmekel & Portmann (1982), Thompson &

Brown (1984), Trainito (2005), Betti (2011), Trainito & Doneddu (2014), and Prkić *et al.* (2018). In some cases, such as *Haloa japonica,* specimens were anesthetized with $MgCl_2$ in order to verify the shape of Hancock's organs, which is important to assess the identity of species. Specimens collected were preserved and stored in 96% ethanol and kept in the collection of the MBS NIB. The taxonomy and nomenclature are in accordance with the World Register of Marine Species - WoRMS (www.marinespecies.org).

In order to assess the zoogeographical affiliation, distributional data for all heterobranchs were obtained from the literature, mainly from Lipej *et al.* (2018a, 2022) and references therein. Biogeographic distribution categories are based on the groupings of Realms and Provinces of coastal and shelf areas of Spalding *et al.* (2007). In addition, we tried to understand the feeding specialisation of different species. To this end, all species were grouped in different feeding guilds according to McDonald & Nybakken (1997).

The studied area, which represented the Slovenian part of the Adriatic Sea (Fig. 1), was divided into 2 × 2 km squares by applying the *Create Grid* algorithm in the QGIS environment (QGIS, 2024). The data collected for each heterobranch species were presented in terms of number of records, number of 2 × 2 km squares, number of sites, and number of specimens (abundance).

The sampling location attributes enabled spatial joining of the generated heterobranch species database with the created grid. Thus, we were able to assess possible spatial differences in species composition with respect to presence or absence in human-impacted areas (HIA). In this study we considered the HIAs that are rapidly colonised by native and alien fauna, such as ports (harbours, fishing ports, marinas), mariculture facilities (fish farms, shellfish farms) and coastal wetlands (estuaries, lagoons, brackish channels, salinas).

The HIA information layer (shape files) was generated using the most recent national ortho-photo imagery (GURS, 2024) owned by the national Surveying and Mapping Authority, which operates under the Ministry of Natural Resources and Spatial Planning. In the next step, we intersected this layer with our 2 km grid, in the QGIS environment (QGIS, 2024) and thus determined squares with HIAs and those without. To bypass the spatial sampling bias, the study area was divided into three parts (red, green, blue) by applying the *k-means* clustering algorithm (distance squared Euclidean), in the R statistical environment (R Core Team, 2024), with the following two (log-transformed) variables per each square: (1) number of heterobranch samplings and (2) total number of heterobranch species. Here, the species abundance variable was ignored since different sampling techniques generated bias in these data per each square. However, to visualize the effect of sampling size on heterobranch species composition in the study area and simultaneously prove the validity of only within-study area part comparison, a NMDS analysis was performed with heterobranch species presence/absence data regarding the factor study area part. However, to test the assumption of heterobranch composition inequality between areas with and without HIAs within the red study area parts (= highest sampling effort), a multivariate permutation analysis of variance (PERMANOVA; 999 permutations; distance matrix = Jaccard) was performed via the vegan package (Oksanen *et al.,* 2024) in the R statistical environment (R Core Team, 2024). Control tests regarding homogeneity of group/factor and permutation dispersion (the *betadisper* and *permutest* algorithms) were performed in the following steps. In the case of both dispersion tests yielding insignificance ($p > \alpha$; $\alpha = 0.05$), the *simper* function was implemented to identify key contributors of heterobranch differentiation along the HIA contrast within study area part.

To further explore and evaluate the functional relationship between HIA distribution in the red study (= highest sampling effort) and heterobranch species number (and diversity), distance variables from specific HIAs such as coastal wetlands, mariculture facilities and ports, were calculated in the QGIS environment with the raster proximity function (QGIS, 2024). Then, the mean distance value per each square was calculated by applying

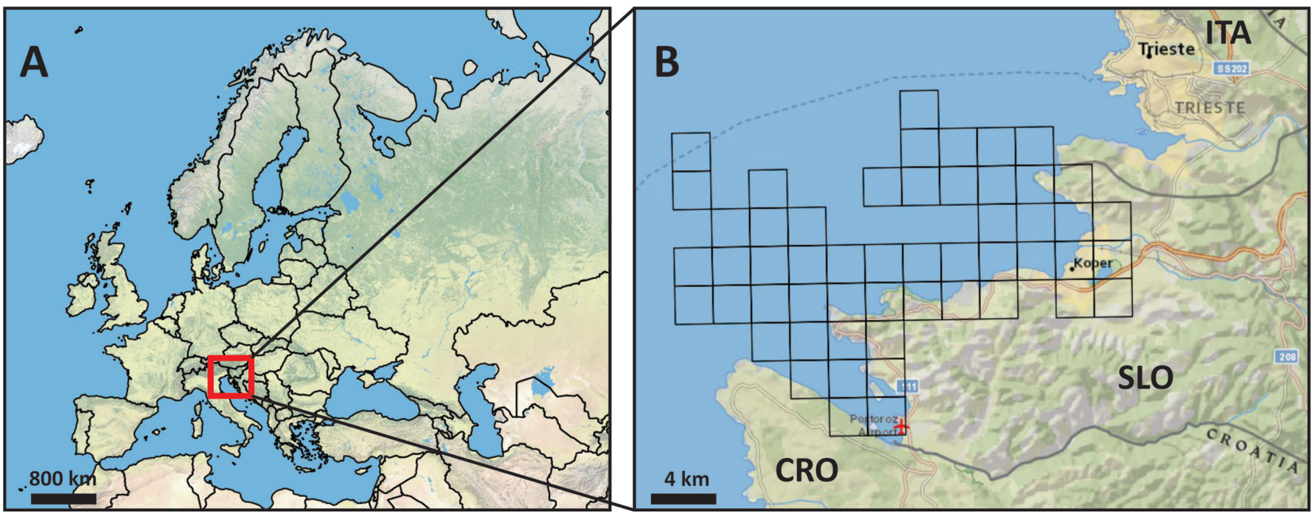

Fig. 1: The location of the study area within the Adriatic and Mediterranean Sea (A) and the Slovenian part of the Gulf of Trieste (B), divided into 2 × 2 km squares. Only those squares where at least one heterobranch species was found are shown.

the *Zonal Statistics* algorithm within Raster analysis toolbox in the QGIS environment (QGIS, 2024).

Accordingly, a second response variable for each square (along species number), the Shannon-Weaver index (*H'*) (Shannon & Weaver, 1949) was calculated using the equation $H' = (\Sigma\ pi*\ln\ pi)$. However, it should be pointed out that in our case *H'* values should be interpreted with caution since multiple sampling techniques generated unwanted bias in heterobranch abundance data. Finally, the effect of HIA distribution and availability in the red part of the study area (= highest sampling effort), represented as distance-based variables per each square, on the number and diversity of heterobranch species was evaluated using generalized linear (GLM;) and additive (GAM) models (family = Poisson [for species number as dependent variable] or gaussian [for *H'* as dependent variable]) in the R statistical environment (R Core Team 2024). For that purpose, the *rcmdr* (Fox & Bouchet-Valat, 2024) and *mgcv* (Wood, 2011) packages were applied. The performance of both models, in the final methodological step, was measured with Akaike Information Criterium (AIC) values and they were compared by means of ANOVA analysis.

Results

General heterobranch survey

In the period from 1970 to 2023, a total of 2229 heterobranchs were recorded in the territorial waters of Slovenia, with an evident upward trend of new species found in the area (Fig. 2). The obtained data revealed that the heterobranch fauna of the Slovenian part of the Adriatic Sea consists of (at least) 157 species (Table 1), which belong to 50 families. The most represented families are Discodorididae with ten species, followed by Facelinidae with nine species, Chromodorididae and Limapontiidae with eight species each, and Dotidae, Goniodorididae, Haminoeidae, and Trinchesiidae with seven species each. In terms of records (samples) the highest number of findings were recorded for *Thuridilla hopei* (206 records), followed by *Cratena peregrina* (93) and *Elysia timida* (81) (Table 1). In terms of the number of sites, the species found in the highest number of localities was *T. hopei*, (32 sites), followed by *Philine quadripartita* and *Dendrodoris limbata* (26 sites each), and *C. peregrina* and *Elysia viridis* (24 sites each) (Table 1).

If we consider the presence in 2 × 2 km squares, the most distributed species was *P. quadripartita* (present in 19 squares), *Tethys fimbria* (16), *T. hopei* and *D. limbata* (both with 14 squares). Twenty-nine species occurred on only one occasion (1.3% of all records and 18.35% of all recorded species).

Taxonomic aspect

From the taxonomical point of view, the majority of heterobranchs are nudibranchs (61.15%), followed by cephalaspids (14.01%), sacoglossans (10.19%), sea hares (3.82%), pleurobranchids (3.82%), pteropods (3.18%), runcinids (1.91%), umbraculids (less than 1%), ringiculomorphs (< 1%), and acteonimorphs (< 1%) (Fig. 3a). Among the nudibranchs, 52 species belonged to the suborder Cladobranchia and 44 to the suborder Doridina.

Sampling techniques

SCUBA diving proved to be the most adequate method for detecting, collecting, and photographing heterobranchs (Fig. 3b), since 35 species were recorded in such a way. Scratching vegetation, especially algae such as *Corallina* and *Cystoseira* also proved to be a very important collecting method, since many very small and cryptic species (24 species) were detected. The collection of epifauna samples, especially in ports, harbours, marinas, and

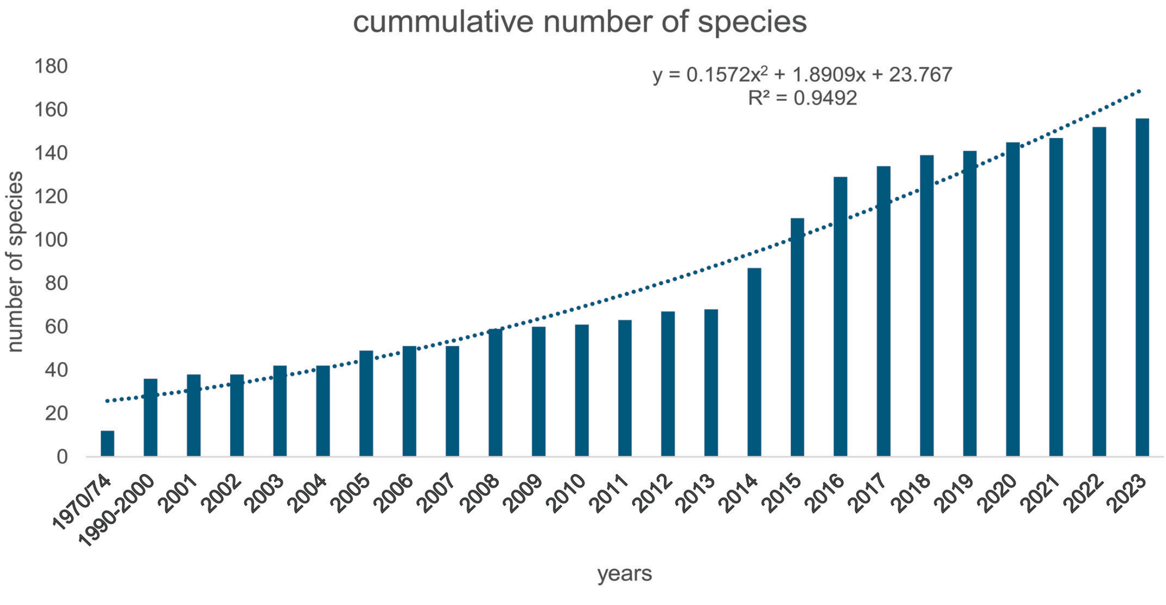

Fig. 2: Number of heterobranch species in the period from 1970 to 2023 in the territorial waters of Slovenia.

Table 1. Heterobranch fauna, collected in the Slovenian part of the Adriatic Sea. Legend: m – massive occurence, ind. – individuals.
Zoogeographic affiliation is according to that of Spalding *et al.* (2007): M – Mediterranean, EAM – East-Atlanto-Mediterranean, IAM – Indian Ocean and Atlanto-Mediterranean, NTAM – North & tropical East-Atlanto-Mediterranean, NEAM – North-East Atlanto-Mediterranean, WEAM – warm temperate East-Atlantic-Mediterranean, WIAM –- West Indian Ocean and Atlanto-Mediterranean, A – (non-Mediterranean) amphiatlantic, AM – Atlanto-Mediterranean, NAM – North-Atlantic Mediterranean, PIA – West Pacific, Indian Ocean & Atlanto-Mediterranean, CG – circumglobal, CTT – Circumtropical & warm temperate including Mediterranean, WP – western Pacific, IP – Indo-Pacific, NIP – northern Indopacific.

n	Species	higher taxa	n records	n squares	n sites	n ind.	Zoogeogr. zone affiliation
1	*Acteon tornatilis* (Linnaeus, 1758)	Acteonimorpha	21	11	17	60	NEAM
2	*Aegires leuckartii* Vérany, 1853	Nudibranchia	8	2	3	8	M
3	*Aegires palensis* Ortea, Luque & Templado, 1990	Nudibranchia	6	2	3	8	M
4	*Aeolidiella alderi* (Cocks, 1852)	Nudibranchia	3	2	2	3	NEAM
5	*Akera bullata* O. F. Müller, 1776	Aplysiida	19	10	15	21	NEAM
6	*Antiopella cristata* (Delle Chiaje, 1841)	Nudibranchia	20	7	14	29	NEAM
7	*Aplysia depilans* Gmelin, 1791	Aplysiida	4	3	3	4	NEAM
8	*Aplysia fasciata* Poiret, 1789	Aplysiida	1	1	1	1	NAM
9	*Aplysia punctata* (Cuvier, 1803)	Aplysiida	43	4	11	564	NEAM
10	*Aplysiopsis elegans* Deshayes, 1853	Sacoglossa	19	3	5	47	EAM
11	*Archidoris pseudoargus* (Rapp, 1827)	Nudibranchia	22	9	14	29	NEAM
12	*Armina rubida* (A. Gould, 1852)	Nudibranchia	1	1	1	1	IP
13	*Armina tigrina Rafinesque, 1814*	Nudibranchia	1	1	1	1	M
14	*Ascobulla fragilis* (Jeffreys, 1856)	Sacoglossa	1	1	1	1	M
15	*Atagema rugosa* Pruvot-Fol, 1951	Nudibranchia	1	1	1	1	AM
16	*Atalodoris camassae* Furfaro & Trainito, 2022	Nudibranchia	1	1	1	3	M
17	*Atalodoris pictoni* (Furfaro & Trainito, 2017)	Nudibranchia	2	2	2	2	NEAM
18	*Baptodoris cinnabarina* Bergh, 1884	Nudibranchia	6	5	5	6	NEAM
19	*Berghia coerulescens* (Laurillard, 1832)	Nudibranchia	22	6	10	23	AM
20	*Berghia verrucicornis* (A. Costa, 1867)	Nudibranchia	10	5	6	16	AM
21	*Bermudella polycerelloides* Ortea & Bouchet, 1983	Nudibranchia	4	4	4	76	A
22	*Berthella ocellata* (Delle Chiaje, 1830)	Pleurobranchida	6	3	4	6	NEAM
23	*Berthella plumula* (Montagu, 1803)	Pleurobranchida	7	4	5	8	NEAM
24	*Berthellina edwardsii* (Vayssière, 1897)	Pleurobranchida	6	3	8	8	EAM
25	*Bosellia mimetica* Trinchese, 1891	Sacoglossa	13	3	9	22	AM
26	*Bulla striata* Bruguière, 1792	Cephalaspidea	1	1	1	1	AM
27	*Bursatella leachii* Blainville, 1817	Aplysiida	60	12	20	240	CG
28	*Calliopaea bellula* A. d’Orbigny, 1837	Sacoglossa	4	2	4	24	NEAM
29	*Calmella cavolini* (Vérany, 1846)	Nudibranchia	2	2	2	2	M
30	*Candiella manicata* (Deshayes, 1853)	Nudibranchia	11	4	6	13	WEAM
31	*Capelinia doriae* Trinchese, 1874	Nudibranchia	2	2	2	6	NEAM
32	*Caloria elegans* (Alder & Hancock, 1845)	Nudibranchia	5	2	3	5	EAM
33	*Camachoaglaja africana* (Pruvot-Fol, 1953)	Cephalaspidea	11	1	4	22	WEAM
34	*Cratena peregrina* (Gmelin, 1791)	Nudibranchia	93	11	24	221	WEAM
35	*Creseis acicula* (Rang, 1828)	Pteropoda	32	11	11	m	CG
36	*Creseis conica* A. Costa, 1869	Pteropoda	1	1	?	1	CG
37	*Cumanotus beaumonti* (Eliot, 1906)	Nudibranchia	2	2	2	3	EAM
38	*Cuthona perca* (Er. Marcus, 1958)	Nudibranchia	5	1	5	5	WP

Continued

Table 1 continued

n	Species	higher taxa	n records	n squares	n sites	n ind.	Zoogeogr. zone affiliation
39	*Cylichna cylindracea* (Pennant, 1777)	Cephalaspidea	18	12	14	20	WEAM
40	*Dendrodoris grandiflora* (Rapp, 1827)	Nudibranchia	33	11	18	38	NEAM
41	*Dendrodoris limbata* (Cuvier, 1804)	Nudibranchia	68	14	26	109	NEAM
42	*Diaphorodoris alba* Portmann & Sandmeier, 1960	Nudibranchia	1	1	1	1	NEAM
43	*Diaphorodoris papillata* Portmann & Sandmeier, 1960	Nudibranchia	3	3	3	3	EAM
44	*Dicata odhneri* Schmekel, 1967	Nudibranchia	4	3	4	5	NEAM
45	*Doriopsilla areolata* Bergh, 1880	Nudibranchia	13	5	8	13	WEAM
46	*Doris bertheloti* A. d'Orbigny, 1839	Nudibranchia	9	3	3	17	EAM
47	*Doris ocelligera* (Bergh, 1881)	Nudibranchia	36	8	17	64	NEAM
48	*Doto acuta* Schmekel & Kress, 1977	Nudibranchia	2	2	2	2	M
49	*Doto cervicenigra* Ortea & Bouchet, 1989	Nudibranchia	40	10	14	159	M
50	*Doto coronata* Gmelin, 1791	Nudibranchia	16	7	9	28	NEAM
51	*Doto floridicola* Simroth, 1888	Nudibranchia	1	1	1	1	WEAM
52	*Doto koenneckeri* Lemche, 1976	Nudibranchia	2	2	2	2	NEAM
53	*Doto paulinae* Trinchese, 1881	Nudibranchia	4	2	3	5	M
54	*Doto rosea* Trinchese, 1881	Nudibranchia	3	3	3	4	WEAM
55	*Edmundsella pedata* (Montagu, 1816)	Nudibranchia	14	8	12	36	WEAM
56	*Elysia gordanae* T. E. Thompson & Jaklin, 1988	Sacoglossa	16	5	9	20	WEAM
57	*Elysia timida* (Risso, 1818)	Sacoglossa	81	7	14	118	AM
58	*Elysia viridis* (Montagu, 1804)	Sacoglossa	68	10	24	182	EAM
59	*Ercolania coerulea* Trinchese, 1892	Sacoglossa	12	3	5	17	M
60	*Ercolania viridis* (A. Costa, 1866)	Sacoglossa	38	6	12	79	NAM
61	*Eubranchus exiguus* (Alder & Hancock, 1848)	Nudibranchia	29	9	13	191	NEAM
62	*Eubranchus viriola* (Korshunova, Malmberg, Prkić, Petani, Fletcher, Lundin & Martynov, 2020)	Nudibranchia	4	2	2	9	NEAM
63	*Eubranchus farrani* (Alder & Hancock, 1844)	Nudibranchia	10	6	8	15	NEAM
64	*Eubranchus linensis* García-Gómez, Cervera & F. J. Garcia, 1990	Nudibranchia	14	6	7	16	NEAM
65	*Facelina annulicornis* (Chamisso & Eysenhardt, 1821)	Nudibranchia	4	2	4	5	NEAM
66	*Facelina auriculata* (O. F. Müller, 1776)	Nudibranchia	2	2	2	7	NEAM
67	*Facelina dubia* Pruvot-Fol, 1948	Nudibranchia	7	3	5	52	NAM
68	*Facelina fusca* Schmekel, 1966	Nudibranchia	18	6	8	69	M
69	*Facelina rubrovittata* (A. Costa, 1866)	Nudibranchia	2	2	2	2	WEAM
70	*Facelina vicina* (A. Costa, 1866)	Nudibranchia	10	3	4	18	NEAM
71	*Favorinus branchialis* (Rathke, 1806)	Nudibranchia	43	9	16	184	NEAM
72	*Felimare gasconi* (Ortea, 1996)	Nudibranchia	3	1	1	3	AM
73	*Felimare orsinii* (Vérany, 1846)	Nudibranchia	7	4	5	7	M
74	*Felimare picta* (R. A. Philippi, 1836)	Nudibranchia	26	10	13	45	NEAM
75	*Felimare tricolor* (Cantraine, 1835)	Nudibranchia	17	6	9	18	NEAM
76	*Felimare villafranca* (Risso, 1818)	Nudibranchia	56	7	20	78	NEAM
77	*Felimida krohni* (Vérany, 1846)	Nudibranchia	19	5	14	21	NEAM
78	*Felimida luteorosea* (Rapp, 1827)	Nudibranchia	25	6	8	27	NEAM
79	*Felimida purpurea* (Risso, 1831)	Nudibranchia	3	3	3	3	NEAM

Continued

Table 1 continued

n	Species	higher taxa	n records	n squares	n sites	n ind.	Zoogeogr. zone affiliation
80	*Flabellina affinis* (Gmelin, 1791)	Nudibranchia	14	4	6	14	WEAM
81	*Gargamella rosi* (Ortea, 1979)	Nudibranchia	9	5	5	9	NEAM
82	*Geitodoris planata* (Alder & Hancock, 1846)	Nudibranchia	20	8	15	33	NAM
83	*Haloa japonica* (Pilsbry, 1895)	Cephalaspidea	51	5	9	944	IP
84	*Haminoea hydatis* Linnaeus, 1758	Cephalaspidea	7	4	4	11	NEAM
85	*Haminoea navicula* (da Costa, 1778)	Cephalaspidea	24	10	19	45	NEAM
86	*Haminoea orteai* Talavera, Murillo & Templado, 1987	Cephalaspidea	4	3	3	18	NEAM
87	*Hancockia uncinata* (Hesse, 1872)	Nudibranchia	1	1	1	1	NEAM
88	*Heliconoides inflatus* (A. d'Orbigny, 1835)	Pteropoda	16	6	8	m	CTT
89	*Hermaea bifida* (Montagu, 1816)	Sacoglossa	1	1	1	1	NEAM
90	*Hermania scabra* (O. F. Müller, 1784)	Cephalaspidea	1	1	1	1	NTAM
91	*Idaliadoris depressa* (Alder & Hancock, 1842)	Nudibranchia	2	2	2	2	IAM
92	*Idaliadoris neapolitana* (Delle Chiaje, 1841)	Nudibranchia	21	5	9	35	M
93	*Janolus hyalinus* (Alder & Hancock, 1854)	Nudibranchia	1	1	1	1	NEAM
94	*Jorunna tomentosa* (Cuvier, 1804)	Nudibranchia	29	10	13	36	NEAM
95	*Limacia clavigera* (O. F. Müller, 1776)	Nudibranchia	1	1	1	1	NEAM
96	*Limacina trochiformis* (A. d'Orbigny, 1835)	Pteropoda	7	4	4	m	CTT
97	*Limapontia capitata* (O. F. Müller, 1774)	Sacoglossa	9	4	6	67	NEAM
98	*Limenandra nodosa* Haefelfinger & Stamm, 1958	Nudibranchia	1	1	1	1	IAM
99	*Melibe viridis* (Kelaart, 1858)	Nudibranchia	1	1	1	1	IP
100	*Nemesignis banyulensis* (Portmann & Sandmeier, 1960)	Nudibranchia	3	3	2	3	WEAM
101	*Okenia elegans* (Leuckart, 1828)	Nudibranchia	1	1	1	1	NEAM
102	*Paradoris indecora (Bergh, 1881)*	Nudibranchia	14	6	11	15	NEAM
103	*Paraflabellina gabinieri* (Vicente, 1975)	Nudibranchia	1	1	1	1	M
104	*Paraflabellina ischitana* (Hirano & Thompson, 1990)	Nudibranchia	55	7	13	106	WEAM
105	*Pelagella castanea* (A. E. Verrill, 1880)	Nudibranchia	14	6	11	23	WEAM
106	*Petalifera petalifera* (Rang, 1828)	Aplysiida	1	1	1	1	EAM
107	*Philine catena* (Montagu, 1803)	Cephalaspidea	8	4	5	12	NEAM
108	*Philine punctata* (J. Adams, 1800)	Cephalaspidea	1	1	1	1	NEAM
109	*Philine quadripartita* (Ascanius, 1772)	Cephalaspidea	42	19	26	62	EAM
110	*Philine vestita* (Philippi, 1844)	Cephalaspidea	1	1	1	2	NEAM
111	*Philinopsis depicta* (A. E. Verrill, 1901)	Cephalaspidea	8	5	6	8	AM
112	*Phyllidia flava* (Aradas, 1847)	Nudibranchia	1	1	1	1	WEAM
113	*Piseinotecus sphaeriferus* (Schmekel, 1965)	Nudibranchia	3	2	2	6	EAM
114	*Placida cremoniana* (Trinchese, 1892)	Sacoglossa	3	3	3	3	IAM
115	*Placida dendritica* (Alder & Hancock, 1843)	Sacoglossa	6	3	4	9	CG
116	*Placida viridis* (Trinchese, 1874)	Sacoglossa	1	1	1	1	M
117	*Platydoris argo* (Linnaeus, 1767)	Nudibranchia	10	5	6	10	WEAM
118	*Pleurehdera stellata* (Risso, 1826)	Pleurobranchida	15	7	13	21	CTT
119	*Pleurobranchaea meckeli* (Blainville, 1825)	Pleurobranchida	4	2	3	5	EAM
120	*Pleurobranchus membranaceus* (Montagu, 1815)	Pleurobranchida	1	1	1	1	EAM
121	*Pneumoderma mediterraneum* (Oken, 1815)	Pteropoda	1	?	?	1	CG
122	*Polycera hedgpethi* (Er. Marcus, 1964)	Nudibranchia	16	8	10	32	IP
123	*Polycera quadrilineata* (O. F. Müller, 1776)	Nudibranchia	22	5	11	57	NEAM

Continued

Table 1 continued

n	Species	higher taxa	n records	n squares	n sites	n ind.	Zoogeogr. zone affiliation
124	*Polycerella emertoni* (A. E. Verrill, 1880)	Nudibranchia	20	9	15	103	A
125	*Retusa laevisculpta* (Granata-Grillo, 1877)	Cephalaspidea	6	4	5	6	M
126	*Retusa mammillata* (Philippi, 1836)	Cephalaspidea	4	2	3	40	NEAM
127	*Retusa truncatula* (O. F. Müller, 1776)	Cephalaspidea	2	2	2	2	EAM
128	*Retusa umbilicata* (Montagu, 1803)	Cephalaspidea	2	1	2	2	EAM
129	*Ringicula auriculata* (Ménard de la Groye, 1811)	Ringiculimorpha	1	1	1	1	AM
130	*Rostanga rubra* (Risso, 1818)	Nudibranchia	3	2	2	3	EAM
131	*Roxaniella jeffreysi* (Monterosato, 1874)	Cephalaspidea	9	7	8	13	WEAM
132	*Rubramoena amoena* (Alder & Hancock, 1845)	Nudibranchia	1	1	1	1	NEAM
133	*Runcina adriatica* (Kress, 1970)	Runcinida	22	3	6	32	WEAM
134	*Runcina brenkoae* (Schmekel, 1972)	Runcinida	3	1	2	3	M
135	*Runcina ferruginea* (Kress, 1977)	Runcinida	26	3	6	74	NEAM
136	*Scaphander lignarius* (Linnaeus, 1758)	Cephalaspidea	1	1	1	1	EAM
137	*Spinoaglaja wildpretii* (Ortea, Moro & Espinosa, 1999)	Cephalaspidea	2	2	2	2	WEAM
138	*Spurilla neapolitana* (Delle Chiaje, 1823)	Nudibranchia	34	9	18	45	WIAM
139	*Stiliger fuscovittatus* (Labbé, 1923)	Sacoglossa	2	2	2	35	IP
140	*Tayuva maculosa* (Bergh, 1884)	Nudibranchia	9	5	8	15	CG
141	*Tenellia adspersa* (Nordmann, 1845)	Nudibranchia	5	3	5	38	WIAM
142	*Tenellia maua* (Er. Marcus & Ev. Marcus, 1960)	Nudibranchia	3	2	2	8	AM
143	*Tergipes tergipes* (Forsskål in Niebuhr, 1776)	Nudibranchia	9	4	5	18	AM
144	*Tethys fimbria* (Linnaeus, 1767)	Nudibranchia	25	16	23	60	EAM
145	*Thecacera pennigera* (Montagu, 1815)	Nudibranchia	5	1	1	15	PIA
146	*Thordisa filix* (Pruvot-Fol, 1951)	Nudibranchia	2	2	2	2	WEAM
147	*Thuridilla hopei* (Vérany, 1853)	Sacoglossa	206	14	32	288	WEAM
148	*Trinchesia genovae* (O'Donoghue, 1929)	Nudibranchia	1	1	1	1	M
149	*Trapania lineata* (Haefelfinger, 1960)	Nudibranchia	5	4	4	9	M
150	*Trapania maculata* (Haefelfinger, 1960)	Nudibranchia	22	5	10	29	NEAM
151	*Trinchesia caerulea* (Montagu, 1804)	Nudibranchia	3	2	2	9	NEAM
152	*Trinchesia foliata* (Forbes & Goodsir, 1839)	Nudibranchia	2	2	2	6	AM
153	*Trinchesia genovae* (O'Donoghue, 1929)	Nudibranchia	60	10	18	144	WEAM
154	*Trinchesia miniostriata* (Schmekel, 1968)	Nudibranchia	11	3	6	16	M
155	*Tylodina perversa* (Gmelin, 1791)	Umbraculida	3	3	3	3	NEAM
156	*Volvulella acuminata* (Brugnone, 1873)	Cephalaspidea	2	1	2	2	NEAM
157	*Weinkauffia turgidula* (Forbes, 1844)	Cephalaspidea	9	4	6	30	NEAM

mariculture facilities was useful for obtaining data on 13 heterobranch species. Many of them are alien and cryptogenic species, such as *Polycerella emertoni, Bermudella polycerelloides*, and *Thecacera pennigera*. Other techniques accounted for less than 10% of species recordings.

Ecological traits

The great majority were benthic species (96.82%) while five were planktonic heterobranchs (3.18%). In terms of feeding guilds, heterobranchs preying on hydrozoans were the most represented (37 species), followed by spongivores (29), herbivores (28), bryozoan feeders (14), and anthozoan feeders (10). Other feeding guilds such as heterobranchs feeding on molluscs, tunicates, foraminiferans, kamptozoans, crustaceans, and others accounted for fewer than ten heterobranch species (Fig. 3c).

Zoogeographic affiliation

Most species were east Atlantic-Mediterranean heterobranchs; 76% of the total number of species. An

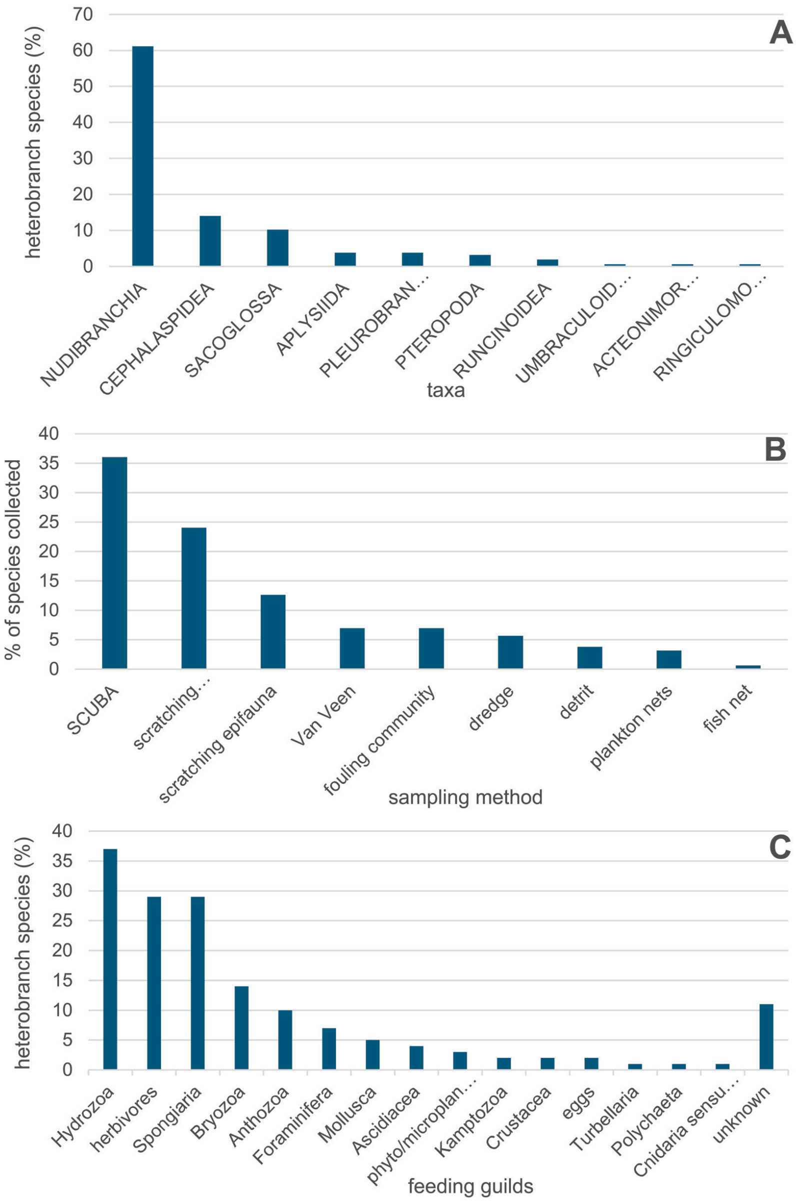

Fig. 3: Heterobranch fauna in the studied area: a – Number of heterobranch species per higher taxonomical category, b – percentage of the total number of heterobranch in relation to the main methodological approach used, and c – feeding guilds of heterobranch species (expressed in percentage).

additional 10.1% were amphi-Atlantic-Mediterranean species (Fig. 4). Twenty-one species (13.3%) were Mediterranean endemics. Ten species were recorded as alien species, of which *Bursatella leachii* has a circumglobal distribution while the other nine species are considered as non-Mediterranean species. Among them, nine accounted for 5.73%, with 4.46% of them being of Indo-Pacific origin and 1.27% of Atlantic origin.

Mapping heterobranchs in squares

Heterobranchs were detected in 54 squares (75.0%) out of a total of 72 2 × 2 km squares. All squares in which no heterobranchs were detected were located off the coast. In the majority of squares (61.0%), 1-5 heterobranch species were detected, while in 22.2% of squares 6-20 species were detected (Fig. 5). In 35.2% of the squares only a single species was detected. The highest number of heterobranch species per square was 95, followed by 78 and 64 species detected.

As the sampling at the different sites varied in extent, caution is required when comparing species composition and richness at the different collection sites. Considering the number of samplings performed (with different sampling methods) per square and comparing it with the mean Shannon diversity index, we found that heterobranch diversity is significantly related to the number of samplings performed (Fig. 6A).

From this perspective, Figure 6 conveys the message that due to the spatial bias in sampling, comparisons of heterobranch species composition and abundance, in relation to factors such as distribution and availability of HIAs, are only meaningful within parts of the study area

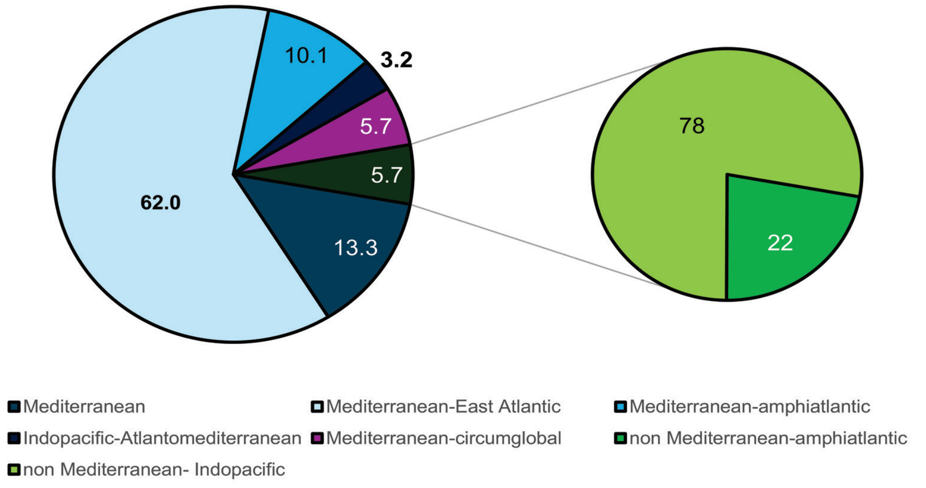

Fig. 4: Zoogeographic affiliation of heterobranch species (in percentages). The smaller pie diagram represents the portion of non-Mediterranean species (5.73 %).

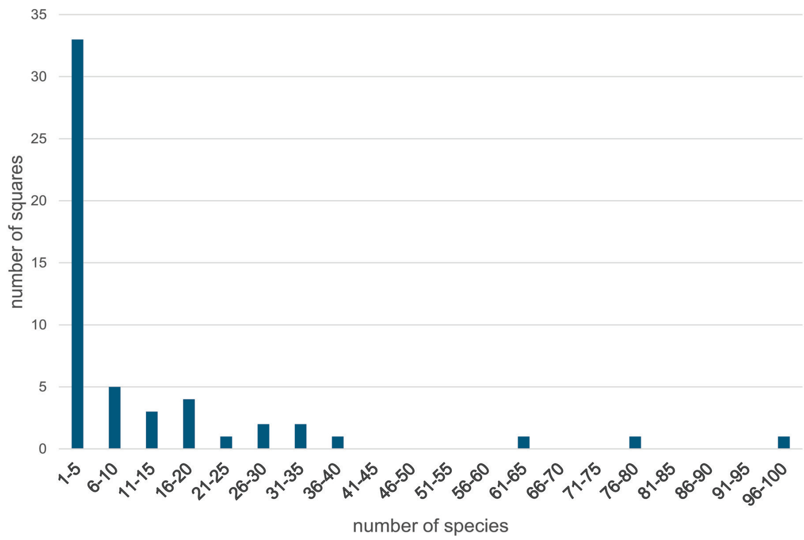

Fig. 5: Heterobranch species per number of 2 × 2 km squares.

(red, green, blue) that were objectively determined by simultaneously considering the number of heterobranch samplings and the total number of heterobranch species. The effect of sampling frequency on heterobranch species composition [and indirectly on diversity (Figure 6)] is clearly visible when plotting the presence/absence species matrix in NMDS space (distance = Euclidean) and considering the above-mentioned parts of the study area determined by the described clustering methods (Fig. 7A, B).

In addition, significant ($p < \alpha$; $\alpha = 0.05$) differences in heterobranch species composition were detected within the red part of the study area (Fig. 8A) by considering the absence (N) or presence of HIA (coastal wetland & mariculture [CW_M], coastal wetland & port [CW_P], port [P]) factor. The statistically insignificant ($p = 0.41$; $\alpha = 0.05$) *betadisper* and *permutest* tests indicated that the PERMANOVA results can be trusted. Heterobranch species such as *Berghia verrucicornis (> CW_M)*, *Bursatella leachii (> CW_M)*, *Spurilla neapolitana (> CW_M)* and *Eubranchus linensis (> CW_M)* contributed to the differences in species composition between areas with no HIAs (N) and those with coastal wetland and mariculture *(CW_M)* (in the red part of the study area). The neutral area-ports contrast *(N-P)* showed that these heterobranch species contributed significant differences in species composition: *Elysia gordanae* (only N), *Polycerella emmertoni* (only P), *Cylichna cylindracea* (> N), *Weinkauffia turgidula* (only N) and *Roxaniella jeffreysi* (> N). The differences in the composition of heterobranch species were most pronounced between the *N-CW_P* contrast. Species such as *B. leachii* (< *CW_P*), *Dendrodoris limbata* (> N), *Ercolania viridis* (> *CW_P*), *H. japonica* (only in *CW_P*), *Limapontia capitata* (only in *CW_P*) and *Tenellia adspersa* (only in *CW_P*) contributed significantly to the observed difference in composition. Greater similarities in the composition of heterobranch species in the red part of the study area were evident when comparing the *CW_M* and *P* contrast, where the differences in species composition could be associated with (only) three heterobranch species: *Berghia verrucicornis* (> *CW_M*), *Stiliger fuscovittatus* (only in *CW_P*) and *Calliopaea bellula* (only in *CW_P*). The *CW_M* and *CW_P*

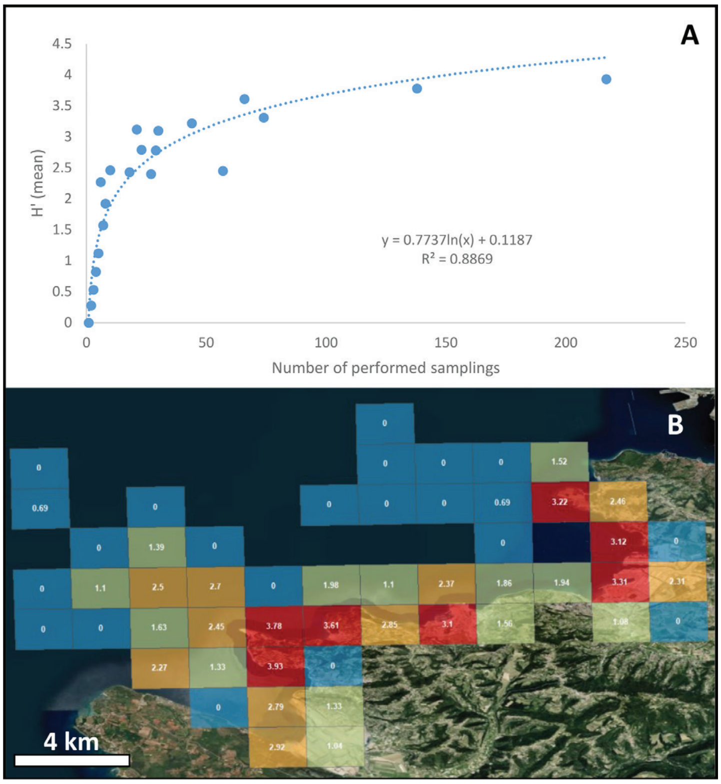

Fig. 6: Heterobranch diversity (calculated as mean H' per square) in relation to the number of samplings (A) and its spatial distribution (in colour ramp [red=high H' values, blue=low H' values]) in the study area (B).

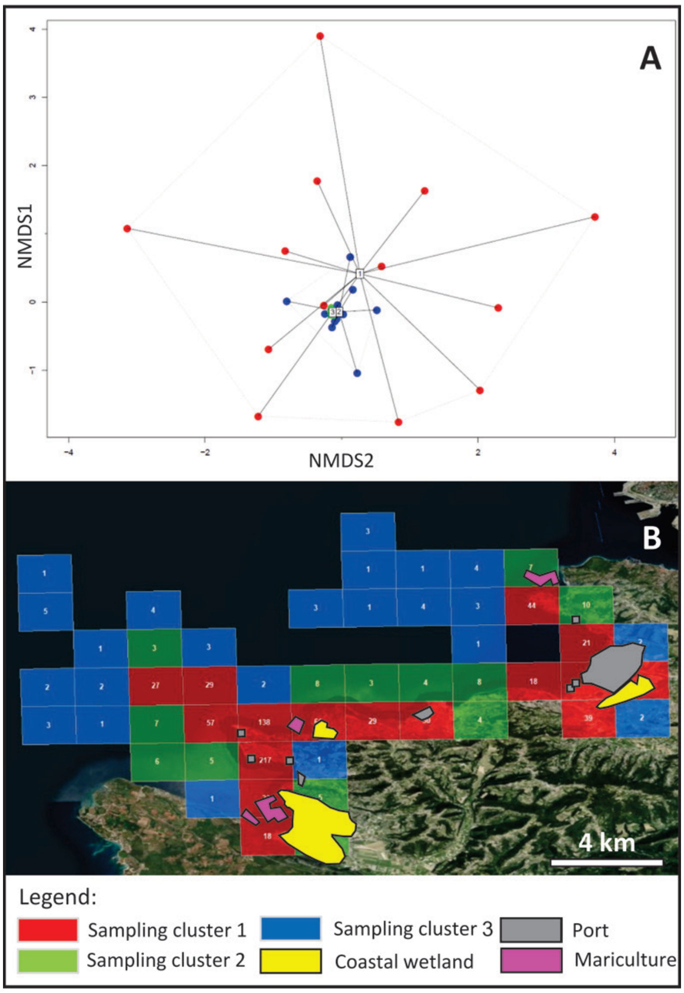

Fig. 7: Differentiation of the study areas with respect to spatial sampling bias (A) and the corresponding variability of heterobranch species composition in NMDS space by considering the defined areas (red, green, blue) (B). White numbers represent the numbers of performed samplings per square.

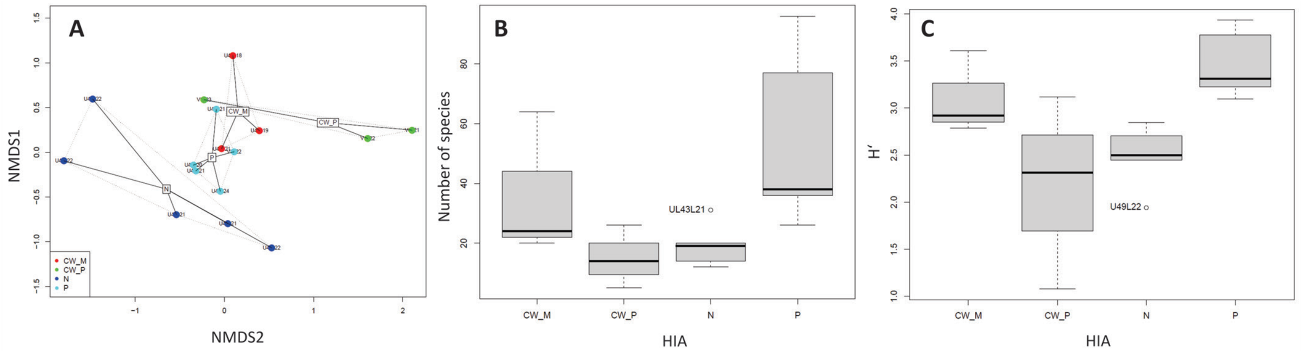

Fig. 8: Differences/similarities in the composition of heterobranch species in relation to the absence (N) or presence of HIAs (CW_M=coastal wetland & mariculture, CW_P=coastal wetland & port, P=port) in the red part of the study area (A) and the corresponding boxplots showing differences/similarities in the number of heterobranch species (B) and H' values per HIA factor (C).

environments differed in the heterobranch species *B. verrucicornis* (only in *CW_M*), *Amphorina linensis* (only in *CW_M*), *T. adspersa* (only in *CW_P*), *S. fuscovittatus* (only in *CW_M*), and *C. bellula* (only in *CW_M*). The last contrast *CW_M-P*, in the red part of the study area, differed significantly as regards the composition of heterobranch species by considering *Dendrodoris grandiflora* (only in *P*), *C. cylindracea* (only in *P*), *Felimare villafranca* (only in *P*), *Elysia gordanae* (only in *P*).

An analysis of the frequency distributions of the variable number of species and *H'* in relation to factors HIA *N, CW_M, CW_P*, and *P* (Fig. 8B, C) suggests (based on significant [$p < \alpha$; $\alpha = 0.05$] Kruskal-Wallis and Dunn post-hoc tests) that areas with HIAs are characterised by significantly higher heterobranch species richness and (potentially; taking into account that *H'* values are influenced by abundance data that are biased due to the different sampling methods used in this study) greater diversity.

However, Figure 9 shows how the number of heterobranch species (A-C) and diversity (D-E) behave as a function of the distance to the HIAs considered in the red part of the study area. The results of the GAM model were significantly ($p < \alpha$; $\alpha = 0.05$) better than the GLM model (AICGAM = 112.5141; AICGLM = 213.4163), indicating that only the variable "distance to coastal wetland" (disCW) had a linear effect on the number and diversity of heterobranch species. In contrast, a significant non-linear relationship was found for the other two distance-based predictors (distance from mariculture facilities [disM], distance from ports [disP]). From the disM perspective, it was evident that the number and diversity of heterobranch species increased up to a certain threshold (0.02 decimal degrees [DD]) as we moved away from these

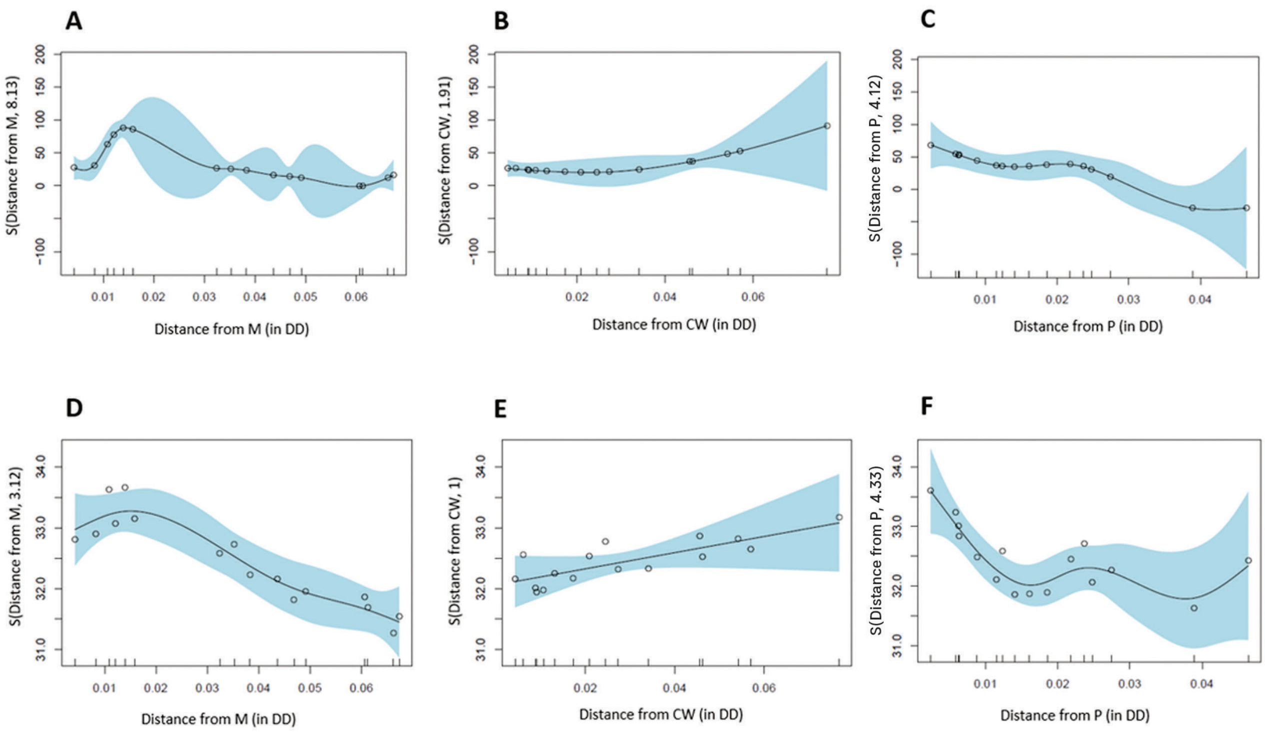

Fig. 9: Partial GAM effect plots showing the interaction between the number of heterobranch species (A to C) and diversity (H') (D to F) in relation to the predictor variables mean distance from coastal wetland (disCW), mariculture (disM) and ports (disP) in the red part of the study area.

receptor sites. After the 0.02 decimal degrees threshold, the relationship reversed in both cases, and the number and diversity of heterobranch species began to decrease. In the case of disP, the influence on both response variables is predominantly negative. Here, the number of heterobranch species decreased constantly (with some fluctuations) with increasing distance from the harbours. However, the relationship between the variables *H'* and disP was more complex. At certain distance thresholds (from 0.02 to 0.028 DD and from 0.04 DD), heterobranch species diversity can nevertheless increase.

Discussion

Number of species

The number of confirmed heterobranch species in the territorial waters of Slovenia is similar in number to the heterobranch fauna from Malta (Sammut & Perrone, 1998), where 168 species were recorded. The data obtained on heterobranchs highlighted the fact that along the Slovenian coastline the number of species per square varied considerably, with the main determining factor being the number of samplings performed. Research on heterobranch fauna in the study area has steadily increased during the past 20 years, which is evident in the growing number of species. The increase in species is mainly related to factors such as heterobranch detectability, the availability of suitable habitat type, in terms of feeding and grazing (Lipej *et al.*, 2008), the improvement of sampling techniques (Lipej *et al.*, 2012), and also citizen scientists, due to the popularity of heterobranchs for recreational divers and naturalists (Smith & Davis, 2018).

This account increases the number of heterobranch species that were confirmed as being present in the territorial waters of Slovenia to 157. To date, more than 600 heterobranch species have been recorded in the Mediterranean Sea (Trainito & Doneddu, 2014); thus, the heterobranch species reported from the waters of Slovenia represent nearly a quarter of all Mediterranean species, which is still an impressive number. In our opinion, the actual heterobranch diversity in the studied area is likely to be considerably higher. Sampling success is related to various environmental factors; however, detectability seems to be one of the most important issues (Lipej *et al.,* 2008). At the same time, many heterobranchs feed on a single or few prey items and, therefore, their availability is highly dependent on the presence of their preferred prey item. Future more intensive sampling in less explored environments such as the circalittoral biocoenoses and other habitat types, which received less sampling effort, is likely to increase the number of heterobranch species present in the area.

Another important issue to be taken into consideration is the lack of night sampling as already pointed by Türkmen & Demirsoy (2009). Recently, an alien nudibranch *Armina rubida* (Gould, 1852), with nocturnal habits was discovered in the area of Fiesa (Knapič *et al.*, 2024).

As pointed by Zenetos *et al.* (2016), the number of species per country depends on the country's coastline and the level of scientific recording effort. Therefore, the checklist of recorded species should be considered as far from complete. In fact, Graeffe (1903) found many opisthobranchs in the broader area of the GoT, which have not yet been confirmed in the waters of Slovenia and the same applies to other recent surveys of heterobranch fauna (e.g., Zenetos *et al.*, 2016; Ciriaco & Poloniato, 2016; Ciriaco *et al.*, 2023). In addition, shelled heterobranchs are in general rarely reported in Mollusca inventories (Türkmen & Demirsoy, 2009) and the same applies to a number of small enigmatic heterobranch groups, which are morphologically and biologically highly aberrant such as the Acochlidia (*sensu* Jörger *et al.*, 2010). Since we did not sample meiofaunal habitats, no acochlidid heterobranchs were recorded in the studied area. However, they have been reported in adjacent areas (Eder *et al.*, 2011), which means that their presence would further enlarge the checklist of species in Slovenia. The five species of pteropods in the study area, which is character-

Table 2. Some rare, less well-known, and alien species reported in the study area. Legend: *–first record in the Adriatic Sea. **–second record in Mediterranean Sea, ***–second record for the Adriatic Sea.

Species	Year of discovery in Slovenian waters	Status	Source
Atalodoris camassae	2020*	Newly described species	Furfaro *et al.,* 2023
Atalodoris pictoni	2021***	Overlooked species	Fortič *et al*., 2021
Armina rubida	2023***	Alien species	Knapič *et al.,* 2024
Bursatella leachii	2001	Alien species	Lipej *et al*., 2008
Cuthona perca	2015**	Alien species	Yokes *et al*., 2018
Haloa japonica	2015	Alien species	Lipej *et al*., 2018
Melibe viridis	2017	Alien species	Lipej & Mavrič, 2017
Stiliger fuscovittatus	2016	Alien species	Lipej *et al*., 2018
Thecacera pennigera	2020***	Cryptogenic species	Bariche *et al.,* 2020
Polycera hedgpethi	2016	Alien species	Lipej *et al*., 2018
Polycerella emertoni	2016	Alien species	Lipej *et al*., 2018
Cumanotus beaumonti	2005*	Rare and less known species	Turk, 2005

ized as a semi-enclosed shallow gulf, are more or less expected. Three of them, *Heliconoides inflatus, Limacina trochiiformis* and *Creseis acicula* are among the most abundant pteropods in the Mediterranean Sea (Johnson *et al.*, 2023). In addition, heterobranch taxonomy is subject to frequent changes in recent times. New species were described for the first time, in the studied area as well, as is the case of *Atalodoris camassae* (Furfaro *et al.*, 2023), while others have been recorded only once or in a restricted area (see Table 2).

In our opinion, the participation of recreational underwater photographers and divers in marine research, facilitated by rapid spreading of information using electronic means (*sensu* Poursanidis *et al*., 2009), is already enriching the number of heterobranch records and will also significantly enhance our knowledge of heterobranch fauna in the studied area in the future. The organized mapping of heterobranchs plays a significant role; it encourages underwater photographers to share their data and contribute to filling gaps in the knowledge of these marine molluscs.

Feeding guilds

Many of the heterobranch species recorded in the studied area are stenophagous and feed exclusively on specific marine invertebrates. The majority of them feed on hydrozoans and sponges, followed by herbivores. The wide availability of potential food items such as hydrozoans (*Pennaria disticha, Obelia* sp*., Eudendrium* sp.) and bryozoans (*Bugula neritina, Schizoporella errata, Amathia verticillata*), which were found in fouling communities on artificial structures in harbours and mariculture facilities (personal observations), is probably the main reason for the large number of specimens and species of heterobranchs in such areas. Arborescent bryozoans in particular, with their three-dimensional body structure, provide space or substrate for many other species (e.g., Marchini *et al*., 2015) and thus an additional habitat and potential food source for heterobranchs. Many species of macroalgae, e.g., the genera *Corallina* and *Cystoseira*, also play a similar role as a substrate on which heterobranchs find food and hiding places. In addition, *Cystoseira* algae have already been recognised as a very important element in the environment where molluscs find food and shelter but rarely constitute their food (Orlando-Bonaca *et al.*, 2022). However, some species are known to be related to the presence of invertebrate spawn or fish eggs. We confirmed the presence of the egg-preying nudibranchs *C. bellula* and *Favorinus branchialis*. The latter species has also been observed to feed on eggs of the alien species *H. japonica* in aquaria (Lipej *et al*., 2018a) and thus has the potential to limit the impact and spread of alien species. We expect that in the future some other sea slug species will be recorded in the studied area, especially since some missing species on our checklist have already been reported in the broader area of the GoT (Ciriaco & Poloniato, 2016; Ciriaco *et al*., 2023).

Zoogeographic affiliation

The zoogeographic affiliation of heterobranchs shows that the great majority of species are of Atlanto-Mediterranean origin, as previously noted by Zenetos *et al.* (2016). In their work, the authors considered *Cumanotus beaumonti* as an alien species, which was assessed differently by us (Table 2). Poursanidis *et al.* (2009) estimated that almost 40% of the total heterobranch fauna is represented by Atlanto-Mediterranean species. However, according to Sammut & Perrone (1998), only 26.4% of the species found in the archipelago of the Maltese islands are of Atlanto-Mediterranean origin, which is probably due to the different criteria applied to define zoogeographic affiliation. In fact, their ratio of Mediterranean species is greater than in this work (21.5% versus 12.7%), while the percentage of alien species of Indo-Pacific origin is lower (2.4% vs. 4.5%). Since the study area is facing severe global warming phenomena (as witnessed in other Mediterranean areas), it will certainly be colonised in the future by other alien heterobranch species.

The effect of human impacted areas

When analysing the composition and abundance of heterobranch species in the HIAs, special attention should be paid to the areas with a higher number of heterobranch sampling events, total abundance of heterobranch species and total number of heterobranch species (red squares). The high species richness and *H'* values in many of the squares studied reflect the diversity of macro- and microhabitats and environmental conditions. Indeed, the rocky bottom environment is characterised by high diversity of habitat types and species. According to Hutchinson (1961), the spatial heterogeneity and interspecific niche differentiation are essential ingredients in the mediation of long-term species coexistence. Since then, it has been demonstrated by many studies that the abundance and distribution patterns of benthic organisms reflect the influence of spatial heterogeneity (Zuschin *et al.,* 2001; Bouchet *et al.,* 2002). Therefore, the greatest mollusc richness is frequently associated with hard substrates such as rocky or reef corals (coral-rock). In our case, squares with the highest number of heterobranch species recorded (from 64 to 95) are characterised by rich algal vegetation (dominated by brown algae) and high spatial heterogeneity. The Natural Reserve Strunjan, for example, is very important in this regard since it hosts 64 species that were detected by 66 samplings, compared to the surrounding, more frequently sampled squares with 217 and 138 samplings, where 95 or 76 species were recorded, respectively. Since many heterobranchs have a species-specific diet, the presence of the prey reflects the presence of the predator (heterobranch). Spatial heterogeneity is also reflected in the higher number of species representing abundant prey, which are preyed upon by many heterobranch species grouped in different feeding guilds.

However, our results suggest that the areas with the highest abundance and diversity of heterobranch species are also associated with HIAs such as ports, harbours and marinas, mariculture facilities, and coastal wetlands. This is especially true for squares that were sampled most frequently (Fig. 6). Currently, marinas are known to be a favourable HIA environment for settling of many fouling organisms, such as cryptogenic and alien species of bryozoans (Ferrario *et al.*, 2017; Fortič *et al*., 2019). Many species of hydrozoans and bryozoans in fouling communities provide food or important hiding places and feeding grounds for many different heterobranch species. As a result, the species diversity of heterobranchs differs and is even higher than in the adjacent natural habitats (Parera *et al.,* 2020) The most important species transfers occur by biofouling, i.e., organisms attached to and associated with underwater surfaces (*sensu* Davidson *et al.*, 2010) and fishing vessels. This also results in unnoticed transfer of species to the new environment and thus plays a key role in alien species introductions into the new environment (Afonso *et al.*, 2020). With increasing distance from mariculture facilities and ports, the number of heterobranch species and their diversity significantly decreased. Artificial structures such as fish farms are considered as high spatially structured habitats in the marine pelagic system where high population densities of invertebrates can be found associated with fish farm fouling communities (Fernandez-Gonzalez *et al*., 2021). Ports are sheltered and confined environments that are strongly influenced by several anthropic stressors (Cognetti & Maltagliati, 2005), characterised by a fouling community rich in arborescent bryozoans, but also suitable areas for early colonisation of some alien invertebrate species (see for example Ferrario *et al.*, 2017). On the other hand, the number of species increased with increasing distance from coastal wetlands. This trend seems plausible, as coastal wetlands are unpredictable euryhaline environments that are exposed to strong salinity and temperature fluctuations, which means that species richness is often lower than in adjacent coastal areas (de Witt, 2011). In contrast, some areas affected by various anthropogenic activities harbour a high diversity of heterobranchs, such as the port of Koper, where 38 species (in 74 samplings) were detected.

Although pollution is known to have a negative impact on heterobranch fauna (Poizat, 1984), some authors share the opinion that this impact does not affect heterobranchs, as they have found higher species richness in polluted areas (Ah Shee Tee *et al.*, 2022). In addition, ports and harbours are known to host many alien species (Spagnolo *et al.*, 2017; Travizi *et al.*, 2019) and can be considered a starting point for colonisation of adjacent areas.

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