Diversity and behavior of sea slugs (Heterobranchia) in the rocky tide pools of Conero Riviera (western Adriatic Sea)

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Abstract
Rocky tide pools are transition environments whose communities are affected by sudden temperature, salinity and nutrient fluctuations. Furthermore, these environments are exposed to multiple stressors and can be easily altered by human trampling. In particular, specific studies on rock pool heterobranchs communities are lacking for the Mediterranean Sea. In this study, the community of Heterobranchia (Mollusca: Gastropoda) living in an anthropized rock pools system in western Adriatic (Ancona, Italy) has been investigated and a first checklist of the sea slugs in this urbanized areas is provided. During the four months survey, a total of 452 specimens, belonging to 19 species and 12 families was recorded. Notable findings were the first record of Placida dendritica for the Conero Riviera, and the first records of Doto cervicornis and Eroldania viridis for the western Adriatic Sea. Identification of trophic categories showed a diversified assemblage in terms of food sources mirroring a surprising species diversity. Moreover, we here describe the description of a peculiar behavior possibly used by sea slugs to cope with the stressful conditions within this semi-closed system.

Keywords: nudibranchs, intertidal, biodiversity, anthropization, breakwaters

Introduction
Rocky tide pools are closed or semi-closed dynamic systems widespread along the coastline worldwide. Their presence contributes to increase the spatial heterogeneity of the intertidal zone creating habitats that can host a very high variety of living organisms (Ranta 1982; Metaxas & Scheibling 1993; Griffiths et al. 2006). The exposition to sunlight, atmospheric agents and tidal currents induce a wide fluctuation of environmental variables (temperature, pH, salinity, and dissolved oxygen) leading to extreme stressful conditions for the species living in this habitat (Ganning 1970, 1971; Chan 2000; Nielsen 2001; Hulsmans et al. 2008). Nevertheless, rocky pools host a significant biodiversity providing refuge, feeding and nursery grounds for many species of intertidal invertebrates (Bussell et al. 2007; Vinagre et al. 2015) and fish (Bennett 1987; Pfister 1996; White et al. 2015).

Depending on the coastline geomorphology, rocky tide pools are marine habitats that can be easily accessible both by tourists and local people and this could represent an effective opportunity for educational activities as confirmed by the multiplicity of citizen science projects developed in this habitat, leading to an increase in public awareness on environmental issues (Fairchild et al. 2018; Lucrezi et al. 2019). Most frequently these activities result in a direct impact on marine organisms due to trampling (Keough & Quinn 1998; Pinn & Rodgers 2005), harvesting of bait and seafood (Keough & Quinn 2000), and playful interactions with people who touch and collect animals, often causing the death of the latter (Addison et al. 2008; Martens...
In addition, the growing coastal defense (e.g. seawalls, breakwaters, rip-raps) and beach nourishment pursued to protect the coastline from storms and erosion, alter the wave regime and sedimentation rate with negative implications on coastal biodiversity (Martin et al. 2005; Bulleri & Chapman 2010; Perkins et al. 2015; Afghan et al. 2020; Masucci et al. 2020; Sedano et al. 2021).

The northern and western Adriatic coast is mainly characterized by sandy and pebble beaches with the exception of few rocky promontories that interrupt this continuity (i.e. S. Bartolo and Conero Mounts) (UNEP/MAP-RAC/SPA 2015). The Conero Mount Regional Natural Park is located in the Marche region and includes 15 km of rocky coastline that represents an important substrate for hard-bottom benthos and a crucial stepping stone for larval dispersion in the western Adriatic (Di Camillo et al. 2010). Here, the presence of natural hard substrates facilitates the growth of habitat-forming species of macroalgae (e.g. Cystoseira sensu latu) (Rindi et al. 2019, 2020) and Mediterranean mussel beds (Mytilus galloprovincialis Lamarck, 1819) (Cerrano et al. 2014) that host a peculiar biodiversity of mollusks, crustaceans and fish considered rare in other areas of the Mediterranean Sea (Betti 2010, 2021; Riolo & Betti 2015). Moreover, the jagged morphology of the Conero Mount limestone coastline creates heterogeneous intertidal habitats, such as the presence of scattered rocky tide pools (Fruzzetti et al. 2011; Cerrano et al. 2014).

Members of subclass Heterobranchia (Mollusca: Gastropoda), commonly known as sea slugs, are present in a wide range of habitats in the Mediterranean Sea (e.g. Poizat 1984; Scipione et al. 1996; Domenech et al. 2006; Poursanidis & Koutsoubas 2015; Betti et al. 2017; Chiarore et al. 2019; Canessa et al. 2021; Gerovasileiou & Bianchi 2021; Toma et al. 2022), and studies about their presence in tide pools habitat are known from the literature (Nybakken 1974, 1978; Todd et al. 1998; Lambert 2013; Nimbs et al. 2015; Cyrne et al. 2018; Armstrong et al. 2019). Nevertheless, even if some studies on these molluscs included specimens occasionally sampled from the Mediterranean intertidal zone (Lipej et al. 2008; Furfaro et al. 2020), there are no specific studies about sea slugs communities in rocky tide pools for the Mediterranean basin.

In addition, contrary to other Mediterranean localities where studies on heterobranch biodiversity have been provided (Cervera et al. 2004; Crocetta et al. 2013, 2015; Betti et al. 2017; Furfaro et al. 2020; Parera et al. 2020), including Adriatic Sea localities (Turk 2000; Lipej et al. 2008, 2012; Rinaldi 2012; Zenetos et al. 2016; Prkić et al. 2018), the heterobranch fauna of Ancona was not investigated extensively in the past. Indeed, the only sea slugs accounts from the Conero Riviera area consist of the two popular books by Betti (2011, 2021), accounting for a total of 72 species. Nevertheless, there are no quantitative assessments about heterobranch community composition of this area.

Comparison of historical data on population dynamics in the Pacific Ocean showed that sea slugs were associated to thermal anomalies and thus can be considered sentinel species for climate changes, highlighting the importance of collecting data on these communities (Schultz et al. 2011; Goddard et al. 2016).

Aim of the present study has been to assess the species and trophic diversity of Heterobranchia in the rocky tide pools of Ancona area, to provide a first baseline for the conservation of these neglected and vulnerable habitats.

Materials and methods

Study area

The study area is located in the northern part of the Conero Mount Regional Natural Park, along the waterfront of Passetto on the eastern coast of Ancona, Italy (43.618947 N, 13.531880 E; Figure 1a, b). This stretch of rocky coast represents a historical place of entertainment for the local people and it is highly frequented by tourists during the summer season. The area is renowned for the presence of more than one hundred artificial caves built between the 18th and the half of the 19th centuries, historically used by fishermen as boat shelters and now converted to houses and meeting places for the citizens (Turchetti & Tarsetti 2007). The growing popularity of the site has led over the century to an increasing anthropization. The shoreline geomorphology has been heavily altered by beach nourishments, numerous illegal concrete docks used by local fishermen as private piers and the construction in the 1956 of a public elevator that connects the city center to the shore (Figure 2; Comune di Ancona 2017). Nowadays, the natural rocky coast is restricted to the marginal sides of the bay, while the original pebble shoreline is now substituted by a promenade sidewalk interrupted by concrete docks.

The survey was conducted in a semi-closed rocky pool system, formed by the coastal limestones located at the westernmost end of the Passetto promenade. The area investigated is shown in Figure 1b (yellow and red squares). This system of pools has
an overall surface extension (wet and rocky area) of about 750 m² (Figure 1b). The rock formation follows the coastal slope and is excavated by two main crevices perpendicularly to the coastline. The southernmost crevice is narrower and deeper (maximum ~0.4 m deep at lowest tide; red area in Figure 1b) while the northernmost is larger and shallower (maximum ~0.2 m deep at lowest tide; yellow area in Figure 1b, c), and here majority of the pools are formed during low tide. Additional pools form also along the southernmost crevice proximally to the coastline, while its outermost part is well connected to the open sea. During the surveys, the minimum tide was ~0.68 m (30/01/2018, 17:10, +1 GMT), while the maximum 0.64 m (11/03/2018, 17:10, +1 GMT), with a mean low water (MLW) of ~0.14 ± 0.1 m (data retrieved from RMN – Rete Mareografica Nazionale, ISPRA, available at https://

Figure 1. Map of the study area. (a) The exact location in Ancona and Italy of the study area. (b) Aerial view of the rocky tide pools system: areas investigated where pools form are highlighted in yellow (northern crevice) and in red (southern crevice). (c) View of the rocky pools formed on the northern crevice. Image b copyrights to Apple, California.

Figure 2. (a) View of the pristine coastline of the Passetto (1928), before the construction of the docks and fishermen caves. Picture modified from Turchetti and Tarsetti (2007). (b) Nowadays view of the Passetto waterfront from the same angle, showing break walls, beach nourishments, caves and in the back the uppermost floor of the elevator.
www.mareografico.it). This tide pool system, during the surveyed period, was characterized by a diversified macroalgal assemblage, rich in Dictyotales Bory, Corallinales P. C. Silva & H. W. Johansen, Ceramiales Nägeli and Ulvales Blackman & Tansley. Local intertidal fauna is mainly composed by cnidarians such as Exaipatia diaphana (Rapp, 1829) and Obelia sp. Péron & Lesueur 1810, the bryozoan Bugula neritina (Linnaeus, 1758), and crustaceans, like Macropodia sp. Leach, 1814 [in Leach, 1813–1815], Pachygrapsus marmoratus (J. C. Fabricius, 1787), Perforatus perforatus (Bruguière, 1789), and Palaemon elegans Rathke, 1836. Among vertebrates, Blenniidae Rafinesque, 1820 as Aidablennius sphynx (Valenciennes, 1836), Lipophrys trigloides (Valenciennes, 1836), Parablennius incognitus (Bath, 1968), Salaria pavo (Risso, 1810), and Gobiidae Cuvier, 1816 like Gobius cotitis Pallas, 1814, are common sightings.

Field activities and data analysis

Monitoring of heterobranchs was carried out twice per month, with a minimum interval of 10 days, from January to April 2018 (8 surveys in total; see Table I for replicates abbreviation used) with visual census and photographic methods. Considering that the rocky pool system was highly dynamic and characterized by sudden variations in the amount and volume of the pools, we decided to visually assess the whole area available during the daily minimum low tide. Each survey was carried out for a total of 60 minutes with the lowest tide after 30 minutes from the beginning in order to better cover the lowest tide period, to avoid the rising tide, and thus to facilitate specimen spotting. Specimens were photographed with a Canon G16 equipped with Inon S200 strobe for identification purposes at the lowest possible taxonomic level using the most up to date literature (i.e. Pruvot-Fol 1954; Schmekel & Portmann 1982; Lipej et al. 2008, 2012; Betti 2011; Trainito & Doneddu 2014; Zenetos et al. 2016; Prkić et al. 2018; Korshunova et al. 2019, 2020; Carmona 2020; Ghanimi et al. 2020). Diversity and abundance of species and trophic groups are reported. The assignment of trophic groups categories used here refers to Betti et al. (2017).

Results

During the 4 months of survey a total of 452 specimens of Heterobranchia have been found, belonging to three orders, 12 families and 19 species (Table I, Figure 3). The most abundant and diversified family was Facelinidae Bergh, 1889 (Heterobranchia: Nudibranchia) with 244 specimens and 5 species:Facelina rubrosvittata (A. Costa 1866), n = 236; Cratena pergerina (Gmelin, 1791), n = 2; Facelina dubia Pruvot-Fol, 1948, n = 2; Facelina vicina (Bergh, 1882), n = 2; Favorinus branchialis (Rathke, 1806), n = 2. The Family Plakobranchidae Gray, 1840 and Limapontiidae Gray, 1847 (Heterobranchia: Sacoglossa) were respectively the second and third most abundant families. Family Plakobranchidae was only represented by the species Elysia viridis (Montagu, 1804), n = 94, while Limapontiidae by Placida dendrítica (Alder & Hancock 1843), n = 29, and Ercolania viridis (A. Costa 1866), n = 3.

Comparison between replicates revealed a distribution pattern within the temporal interval surveyed (January to April) for some species while others were constantly or occasionally present. Abundances for the most representative species are reported (Figure 4). Facelina rubrosvittata was the most abundant species in all months and was present in every survey together with Elysia viridis (Table I; Figure 4). The first showed high fluctuations between replicates in March and April, while Elysia viridis had an abundance peak in the second replicate of February (F2, n = 30). Polycera quadrilineata (O.F. Müller, 1776) was absent only in the first replicate of April and its highest abundance was recorded in January and February (F2 and F1, n = 3) (Table I; Figure 4). The species P. dendriticà, Amphorina andra Korshunova et al. 2020 and Edmundella pedata (Montagu, 1816) were more abundant in F2 (n = 17), M2 (n = 15), and A2 (n = 4) respectively (Table I; Figure 4). Favorinus branchialis, Felimare villagranca (Risso, 1818) and forunma tomentosa (Cuvier, 1804) were found only in one replicate (Table I). Regarding species diversity per month, February was the most diversified month with 15 species in total, found respectively 12 in F1 and 10 in F2, followed by January and April with 11 species and March with eight species (Table I).

A total of six trophic groups were assigned: sponge-eaters (SP), omnivorous and opportunistic (OM), ophagous (OO), bryozoan-eaters (BR), herbivores (HE), and cnidarian eaters (CN) (Table I; Figure 5). CN, HE and BR have been found in all the replicates, while SP, OO and OM appeared sporadically (Table I; Figure 5). CN and HE showed the highest average percentage in every month, but HE presence decreased considerably in March and April while CN increased during these months (Table I; Figure 5). BR were found constantly but in low abundance, ranging from 1.2%
Table I. List of Heterobranchs found in the rocky pool system at Passetto (Ancona, Italy). Number of specimens and species per each replicate and occurrence of upside-down behavior for each species are reported. Trophic groups abbreviations: SP = sponge-eaters; OM = omnivorous and opportunistic; OO = oophagous; BR = bryozoan-eaters; HE = herbivores; CN = cnidarian-eaters. Upside-down – observations of heterobranchs that shown the upside-down walking behavior during the surveys. The two replicates per month have been abbreviated as follows: J = January; F = February; M = March; A = April.

| TAXON                                      | Trophic groups | J1 | J2 | F1 | F2 | M1 | M2 | A1 | A2 | Upside-down |
|--------------------------------------------|----------------|----|----|----|----|----|----|----|----|-------------|
| Order PLEUROBRANCHIDA                      |                |    |    |    |    |    |    |    |    |             |
| Family Pleurobranchidae Gray, 1827         |                |    |    |    |    |    |    |    |    |             |
| Berthella perforata (Philippi, 1844)       | SP             | 1  |    | 1  | X  |    |    |    |    |             |
| Order NUDIBRANCHIA                         |                |    |    |    |    |    |    |    |    |             |
| Suborder DORIDINA                          |                |    |    |    |    |    |    |    |    |             |
| Family Discodorididae Bergh, 1891          |                |    |    |    |    |    |    |    |    |             |
| Jorunna tomentosa (Cuvier, 1804)           | SP             | 1  |    |    |    |    |    |    |    |             |
| Family Polyceridae Alder & Hancock, 1845   |                |    |    |    |    |    |    |    |    |             |
| Polycera quadrilineata (O.F. Müller, 1776) | BR             | 2  | 3  | 3  | 1  | 1  | 1  | 1  | X  |             |
| Family Chromodorididae Bergh, 1891         |                |    |    |    |    |    |    |    |    |             |
| Felimare villafranca (Risso, 1818)         | SP             |    |    |    |    |    |    |    | 1  |             |
| Suborder CLADOBRANCHIA                     |                |    |    |    |    |    |    |    |    |             |
| Family Dotidae Gray, 1853                  |                |    |    |    |    |    |    |    |    |             |
| Doto coronata (Gmelin, 1791)               | CN             | 3  | 2  | 1  | 1  | 1  | X  |    |    |             |
| Family Janolidae Pruvo-Fol, 1933           |                |    |    |    |    |    |    |    |    |             |
| Antiopella cristata (Delle Chiaje, 1841)   | BR             | 1  |    | 2  |    |    |    |    |    | X           |
| Family Eubranchidae Odhner, 1934           |                |    |    |    |    |    |    |    |    |             |
| Anphorina andra                            | CN             | 1  | 2  | 1  | 5  | 15 | 1  | X  |    |             |
| Korshunova et al. 2020                     |                |    |    |    |    |    |    |    |    |             |
| Anphorina linensis                         | CN             | 1  | 1  | 1  | 1  |    |    |    |    |             |
| (Garcia-Gomez, Cervera & Garcia, 1990)     |                |    |    |    |    |    |    |    |    |             |
| Family Flabellinidae Bergh, 1889           |                |    |    |    |    |    |    |    |    |             |
| Edmundsellia pedata (Montagu, 1816)        | CN             | 3  | 2  | 2  | 4  |    |    |    |    |             |
| Family Trinchesiidae F. Nordsieck, 1972   |                |    |    |    |    |    |    |    |    |             |
| Trinchesia morosaee Korshunova et al. 2019 | CN             | 3  | 3  |    |    |    |    |    |    | X           |
| Trinchesia sp.                             | -              | 9  |    | 2  |    |    |    |    |    | X           |
| Family Facelinidae Bergh, 1889             |                |    |    |    |    |    |    |    |    |             |
| Cratena peregrina (Gmelin, 1791)           | CN             | 1  | 1  |    |    |    |    |    |    | X           |
| Facelina dubia Pruvo-Fol, 1948             | OM             |    | 2  |    |    |    |    |    |    | X           |
| Facelina nubmmeissata (A. Costa 1866)      | CN             | 19 | 17 | 42 | 28 | 9  | 58 | 3  | 60 | X           |

(Continued)
Table 1. (Continued).

| TAXON | Family | Species                     | n. species | n. specimens |
|-------|--------|-----------------------------|------------|--------------|
| Superorder SACOGLOSSA | Family Limapontidae Gray, 1847 | Facelina vicina (Bergh, 1882) | HE | 1 | 2 |
| | | Favorinus branchialis (Rathke, 1847) | HE | 6 | 30 |
| | | Eryodina viridis (A. Costa 1866) | HE | 6 | 6 |
| | | Placida dendritica (Alder & Hancock 1843) | HE | 4 | 24 |
| | | Elysia viridis 1804) | HE | 4 | 5 |

Discussion

Owing to their interesting adaptive and aesthetic characters, heterobranchs have always been an attractive topic among scientists and naturalists, engaging also numerous passionate people, especially among SCUBA divers. Nevertheless, poor knowledge about their local diversity, ecology and life history at large is still wide for many sectors of the Mediterranean Sea. In the studied area, within a complex system of rocky pools, we found around the 26% of the heterobranch fauna reported for this trait of coast, considering previous assessment from the Conero area (Betti 2011, 2021).

Therefore, the Passetto rocky tide pools host a considerable heterobranch abundance and biodiversity during the surveyed period, suggesting that this littoral restricted habitat can sustain massive presence of heterobranchs in syntopy, with complex food-webs dynamics (Mendonça et al. 2018).

The macroalgal assemblage of the tide pools provides substrate and resources to a rich community (e.g. sponges, hydroids, bryozoans, ascidians) that result in a diversified food availability for heterobranchs. In this regard, the heterobranch community of the Passetto rocky pools was diversified also in terms of trophic groups. Hydrozoan-eaters were the most abundant and diversified (eight species) in all the replicates: *F. rubrovittata*, the most abundant species in our study, feeds on hydrozoans of the genus *Eudendrium* Ehrenberg, 1834, together with the other cladobranchian *C. peregrina, Doto coronata* (Gmelin, 1791) (Schmekel & Portmann 1982; Traini & Doneddu 2014), and *Trinchesia morrowae* Konshunova et al. 2019 (possibly also on *Sertularella* spp. Gray, 1848; Cattaneo-Vietti et al. 1990). Other hydrozoan-eaters found were the Eubranchidae Odhner, 1934 *A. andra* and *A. linensis* (Garcia-Gomez, Cervera & Garcia, 1990), which possibly feed on *Obelia* spp. and *Tubularia* spp. Linnaeus, 1758 (McDonald & Nybakken 1997, 1999). Only recently the systematics of the genera *Eubranchus* Forbes, 1838 and *Amphorina* Quatrefages, 1844 have been updated by Korshunova et al. (2020). Both *A. andra* and *Eubranchus farrani* (Alder & Hancock, 1844) show highly polymorphic color patterns but with specific diagnostic characters in the pigmentation and spot positioning. Specimens encountered showed exclusively patterns of *A. andra* (Figure 3h), coherently with previous findings in this area (see *E. farrani sensu* Betti 2011 and *A. 
Figure 3. Heterobranchs found during the tide pools surveys at Passetto. Order Pleurobranchida. Family Pleurobranchidae: (a) *Berthella perforata*. Order Nudibranchia. Suborder Doridina. Family Discodorididae: (b) *Jorunna tomentosa*. Family Polyceridae: (c) *Polycera quadrilineata*. Family Chromodorididae: (d) *Felimare villafranca*. Suborder Cladobranchia. Family Dotidae: (e) *Doto cervicenigra*; (f) *Doto coronata*. Family Janolidae: (g) *Antiopella cristata*. Family Eubranchidae: (h) *Amphorina andra*; (i) *Amphorina linensis*. Family Flabellinidae: (j) *Edmundella pedata*. Family Trinchesiidae: (k) *Trinchesia morrowae*; (l) *Trinchesia* sp. Family Facelinidae: (m) *Facelina dubia*; (n) *Facelina rubroventata*; (o) *Facelina vicina*; (p) *Favorinus branchialis*. Superorder Sacoglossa. Family Limapontiidae: (q)–(r) *Ercolania viridis*; (s) *Placida dendritica*. Family Plakobranchiidae: (t) *Elysia viridis*. All scale bars around 1 cm.
Moreover, both species are now known to be present in the Adriatic (Korshunova et al. 2020). In view of this, we identified the two species as *Amphorina andra* and *Amphorina linensis*. Among cladobranchian, we also add to the account of the species for the western Adriatic the Dotidae Gray, 1853 *Doto cervicenigra* Ortea & Bouchet, 1989, although only two specimens were found in the rocky pools out of the survey in the same period, and therefore not included in the results (Figure 3e).

Herbivores showed a high abundance of individuals but a lower number of species (n = 3). *Elysia viridis* is a generalist herbivore, feeding on different green and red algae (Schmekel & Portmann 1982), while *P. dendritica* and *Ercolania viridis* are known to feed on green macroalgae, respectively on *Bryopsis* sp. J. V. Lamouroux and *Chaetomorpha* sp. Kützing (Trinchese 1874; Schmekel & Portmann 1982; Micaroni et al. 2018). It is noteworthy that the two Limapontiidae *P. dendritica* and *Ercolania viridis* found during the surveys represent new records for Ancona and the Conero coasts, with *Ercolania viridis* being the first record from the western Adriatic (Figure 3q–s). The distribution of this species was recently updated with new records from the Ionian Sea (Salento Peninsula in Micaroni et al. 2018; Furfaro et al. 2020) and eastern Adriatic (Rovinji and Vranjic, Croatia in Prkić et al. 2018) but it was never recorded before at this latitude in western Adriatic.
During our surveys, sacoglossan specimens were attributed to *P. dendritica* in view of the white tipped auriculate rhinophores and cerata, the latter being not particularly pointed apically with the digestive gland diverticula branching through the cerata and showing lack of violet longitudinal bands along the foot (Alder & Hancock 1843; Costa 1866; Trinchese 1874, 1892; Pruvot-Fol 1954; Schmekel & Portmann 1982). However, *P. dendritica* is known to be highly polymorphic through its wide distribution range, from temperate to subtropical areas, showing a variety of color morphs according to its diet (Schmekel & Portmann 1982; Trowbridge 1992, 1997) and locality (Bleakney 1989; Rudman 2002). Following recent updates on the phylogeny of its congeneric *Placida cremoniana* (Trinchese 1892), which revealed cryptic diversity within this apparently widespread species (McCarthy et al. 2019; Durucan & Crocetta in Tsagarakis et al. 2021), it is likely that *P. dendritica* may also belong to a complex of species. Although in Betti (2021) *Placida cf. viridis* is instead reported as an occasional sighting in the Passetto area, from our findings, specimens encountered (Figure 3s) were closer to the typical *P. dendritica* described from Torquay (UK) by Alder and Hancock (1843). *Placida dendritica* is not a common finding in this sector of the Mediterranean Sea, being more commonly found in the western Mediterranean (Rinaldi 2012; Trainito & Doneddu 2014). Although further molecular studies would be necessary to clarify the identification of this population, we record this species for the first time in Ancona to our knowledge.

It is also noteworthy that, during our surveys, several specimens of heterobranchs were found crawling upside-down the water surface of the tide pools (Table I; Figure 6). We recorded this behavior for 11 species belonging to eight families and all the three orders found (Pleurobranchida, Nudibranchia, and Sacoglossa) for a total of 29 specimens.

![Figure 6. Upside-down floating locomotion behavior assessed during the surveys. (a) Trinchesia morrowae; (b) Facelina rubroviata; (c) Polycera quadrilineata; (d) Cratena peregrina; (e) Berthella perforata; (f) Facelina dubia drifting in the tidal current; (g) Elysia viridis. All scale bars around 1 cm.](image-url)
Contrary to pleustonic heterobranchs (i.e. Glauicidae Gray, 1827) which are specialized in such lifestyle (Farmer 1970), benthic heterobranchs occasionally may use the surface tension of the water using mucous secretions to slide upside-down the water surface (Ponder et al. 2020). Reasons for this behavior are poorly understood, being seldomly described both in captivity (Colgan 1914; Alqudah et al. 2016) and in nature (Colgan 1914; Bertsch et al. 1972; Behrens 2018; Cyrne et al. 2018). In captivity, sea slugs have been seen crawling upside-down the surface soon after being moved to aquaria (Colgan 1914; Bertsch et al. 1972). Moreover, ventral-gilled nudibranchs as Phyllidiidae Rafinesque, 1814 have been seen displaying gills once on the surface, as in anoxic condition (Alqudah et al. 2016). Hence, it is possible that the heterobranchs of the Passetto tide pools system take advantage of this locomotion technique as a response to stressful conditions occurring in tide pools. Along with flowing, also active and passive upside-down drifting through tidal currents have been noticed (Figure 6). Locomotion tasks as food hunting in heterobranchs are driven by chemosensory feedbacks (Wyeth et al. 2006; Ponder et al. 2020), but is still not known if the upside-down floating locomotion may be driven by specific tasks and further studies on this peculiar behavior are needed.

It is acknowledged that majority of heterobranchs taxa are stenophagous (McDonald & Nybakken 1997, 1999), commonly undergoing seasonal fluctuations according to prey life cycles (Miller 1962; Todd 1981; Cattaneo Vietti & Balduzzi 1991; Sisson 2005; Canessa et al. 2021) with population blooms occurring locally on a year-basis for many species (Trowbridge 1997; Davis et al. 2017; Lombardo et al. 2020). For example, the occurrence of several specimens of Trinchia sp. only in one replicate, as well as the massive presence of P. dendritica in February, are likely due to the sudden populations exploit which these species are subjected to. Nevertheless, reasons for the appearance and disappearance of massive amounts of heterobranchs specimens in such a short time frame in tide pools environments are not completely understood and several hypotheses have been proposed to explain such fluctuations (Cyrne et al. 2018). Among these, horizontal migrations from nearby areas to mate/spawn or fortuitous larval settlement followed by subsequent death by lack of food are supposed (Miller 1962; Nybakken 1974, 1978). As a matter of fact, heterobranchs are often seen reproducing in stressful conditions (Cyrne et al. 2018) and dying post spawning (Nybakken 1978; Domènech et al. 2002), although in our survey we did not observe mating behavior nor egg masses (together with a scarce presence of oophagous species), suggesting that the aggregations found were likely driven by food availability more than reproduction purposes. Nevertheless, the disappearance dynamics observed are not clear, but are potentially related to post-bloom food decrease or to environmental factors (e.g. temperature shifts, storms) (Cyrne et al. 2018). A more extended survey, covering also summer and fall months, could better clarify populations dynamics, prey–predator relations and possible anthropogenic stressors of heterobranch species here reported.

Rocky reefs, and thus also the tide pools, are listed among protected habitats in the EU Habitat Directive (92/43/EEC, code 1170), deserving further protection (UNEP/MAP-RAC/SPA 2015). These environments are still exposed to anthropogenic pressures that may affect their biota, such as human pressure through trampling (Pinn & Rodgers 2005) and construction of coastal defenses (Airoldi et al. 2005).

The western Adriatic Sea is affected by intense coastal erosion due to winter storms that led to the extensive construction of coastal defenses (Matteucci et al. 2010; Rosskopf et al. 2018). In particular, in the Marche region, more than 70% of the coast is protected by breakwaters (Lorenzoni et al. 2016), including a breakwater placed close to the study area in front of an artificial beach. Although these structures represent a suitable hard substrate for many species, the complex three-dimensionality of adjacent rocky pools and natural hard substrates support higher biodiversity than artificial ones (Bulleri & Chapman 2004; Airoldi et al. 2005; Moschella et al. 2005; Firth et al. 2013, 2014; Lai et al. 2018; Sedano et al. 2019, 2021). Moreover, placement of breakwaters modify the coastal current flows and sedimentation rates, thus altering benthic assemblages at intertidal and upper infralittoral level with cascade effects that can contribute to the general loss of biodiversity, not only at local scales (Airoldi et al. 2005; Afghan et al. 2020; Masucci et al. 2020).

We herein highlight the key role this semi-closed environment can play as a hotspot for local biodiversity, underlining that anthropic interventions such breakwaters placement and beach nourishment should consider the conservation of this important, albeit neglected, habitat in the Mediterranean Sea.

Authors contribution

A.C., A.R. and R.V. conceived the study, performed sampling activities and managed collected data. A.
C. and R.V. wrote the original draft. A.C., A.R., R.V. and C.C reviewed and edited the manuscript. All the authors agreed to publish this version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s)

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