UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO SCIENZE DELLA VITA E DELL' AMBIENTE

Corso di Laurea Magistrale in Biologia Marina

Classe LM 6 - Biologia

ANALISI DEI SUBSTRATI IN RELAZIONE ALLE PREFERENZE ALIMENTARI DEI RECORDS DI HETEROBRANCHIA NELLA RIVIERA DEL CONERO

ANALYSIS OF SUBSTRATA IN RELATION TO FOOD PREFERENCES OF HETEROBRANCHIA'S RECORDS IN THE CONERO RIVIERA

Tesi di Laurea Magistrale di:

Alessia Colombo

Relatore Chiar.mo Prof.:

Carlo Cerrano

Correlatori:

Dott.ssa Cristina Gioia Di Camillo

Dott.ssa Agnese Riccardi

Sessione Estiva

Anno Accademico 2023/2024

RIASSUNTO

Gli Heterobranchia marini sono organismi con una dieta piuttosto specifica, nutrendosi generalmente di una (monofagia) o poche specie di prede (stenofagia). Le prede sono perlopiù organismi sessili (tra cui alghe, briozoi, poriferi e cnidari), particolarmente sensibili ai cambiamenti climatici. In questo contesto, è utile indagare se gli eterobranchi possano essere utilizzati come organismi indicatori degli effetti dei cambiamenti climatici sulle comunità bentoniche: si pensa infatti che questo fenomeno influisca sulla distribuzione di queste ultime e sulla sincronizzazione dei cicli vitali tra preda e predatore. In questo studio, abbiamo cercato di contribuire alla conoscenza, attualmente scarsa, sugli eterobranchi, raccogliendo un totale di 2177 records: 1536 per la Riviera del Conero e 641 per il resto del Mediterraneo. Molti dei dati sono stati raccolti attraverso la *citizen science*: gli eterobranchi, infatti, sono considerati specie "*flagship*", cioè specie le cui caratteristiche le rendono un'attrattiva per i subacquei di tutto il mondo. Per questo motivo, non è raro imbattersi sul web in foto di questi organismi (che possono fornire informazioni utili per il monitoraggio), e risulta abbastanza semplice coinvolgere volontari nelle attività di raccolta dati.

I dati raccolti in questo lavoro sono stati utilizzati per studiare la relazione tra eterobranchi e substrati in relazione alle loro preferenze alimentari, e per fare

un confronto tra Conero e resto del Mediterraneo. Tuttavia, la ricerca in letteratura di informazioni riguardo le prede degli eterobranchi ha rivelato una generale mancanza di conoscenza, con dati spesso privi di fonte o di metodo scientifico alla base. Questo studio, perciò, evidenzia il bisogno di svolgere studi più mirati sulla trofia di ogni specie e di raccogliere sempre più dati da tutte le fonti possibili per poter provare che lo "*small home range*" degli eterobranchi sia correlato alla loro dieta, e dunque essere in grado di definire se il loro utilizzo come organismi indicatori possa essere considerato efficace o meno.

TABLE OF CONTENTS

ABSTRACT

Marine Heterobranchia are organisms with rather specific diets, generally feeding on one (monophagy) or a few prey species (stenophagy). The prey are mostly sessile organisms (including algae, bryozoans, porifera and cnidarians), which are particularly sensitive to climate change. In this context, it is useful to investigate whether heterobranchs could be used as indicator organisms to monitor the effects of climate change on benthic communities: this phenomenon is thought to affect the distribution of the latter and the synchronisation of life cycles between prey and predators. In this study, we have tried to contribute to the currently scarce knowledge on heterobranchs by collecting a total of 2177 records: 1536 for the Conero Riviera and 641 for the rest of the Mediterranean. Many of the data were collected through *citizen science*: in fact, heterobranchs are considered flagship species, i.e. species whose characteristics make them attractive to divers all over the world. For this reason, it is not uncommon to find photos of these organisms on the web (which can provide useful information for monitoring), and it is quite easy to involve volunteers in data collection activities.

The data collected in this work have been used to study the relationship between heterobranchs and substrata in relation to their food preferences, and to make comparisons between the Conero and the rest of the Mediterranean.

Nevertheless, the literature search for information on the prey of heterobranchs revealed a general lack of knowledge, with data often unsourced or not based on a scientific method. This study therefore highlights the need to carry out more targeted studies on the trophy of each species and to collect more and more records from all possible sources in order to be able to prove that the small home range of heterobranchs is related to their diet, and thus to be able to assess whether or not their use as indicator organisms can be considered effective or not.

1. INTRODUCTION

1.1 The Heterobranchia taxon

1.1.1 Systematic Framework

The Heterobranchia is a subclass of gastropod molluscs, the most morphologically diverse of all gastropod clades (Ponder et al., 2020b).

Gastropods were originally divided into 3 groups: Prosobranchia, Pulmonata and Opisthobranchia: this classification was proposed by Alphonse Milne-Edwards in 1848 (Ponder & Lindberg, 1997).

The concept of Heterobranchia was first proposed by Gerhard Haszprunar in 1985: this includes Pulmonata, Opisthobranchia, other marine families and a freshwater group (Haszprunar, 1985). Pulmonata and Opisthobranchia were considered subclasses until recently, when molecular analyses rejected their monophyly (Grande et al., 2004), so the similarities between species of these taxa can be explained by convergent evolution.

In particular, Opisthobranchia included the majority of sea slugs and their relatives (Ponder et al., 2020b). The name of the taxon is derived from the Greek "*ópisthen*", meaning "behind", and "*bránchion*", meaning "gill", referring to the posterior position of the gills. Already in 2005, a classification defined the opisthobranchs as an informal group (Bouchet & Rocroi, 2005), and the term is now considered obsolete (Schrödl et al., 2011). Species

previously considered part of this taxon are now included in “Lower Heterobranchia” (infraclass), Acteonimorpha (subterclass), Ringipleura (subterclass), Umbraculida (order), Cephalaspidea (order), Runcinida (order), Aplysiida (order), Pteropoda (order) and Sacoglossa (superorder) *(Figure 1)*. In this work, we will refer to the “ex-opisthobranchs” with the generic term “Heterobranchia”.

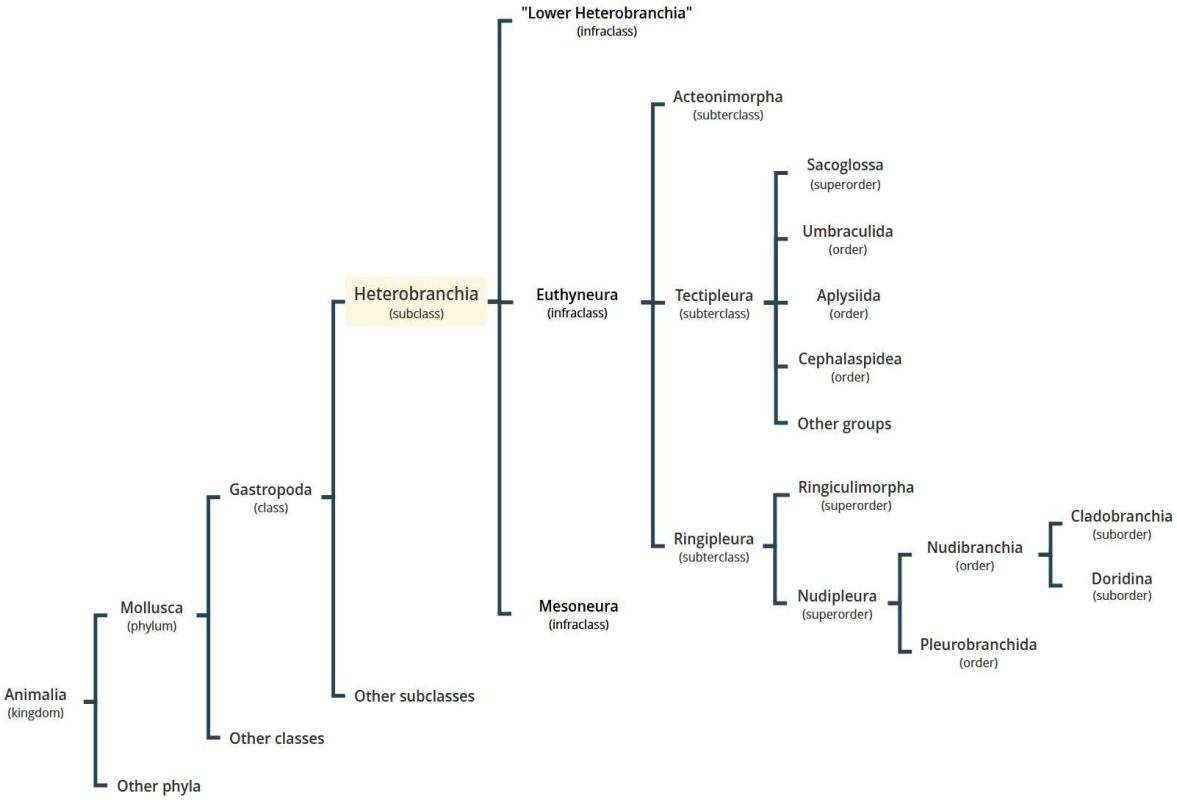


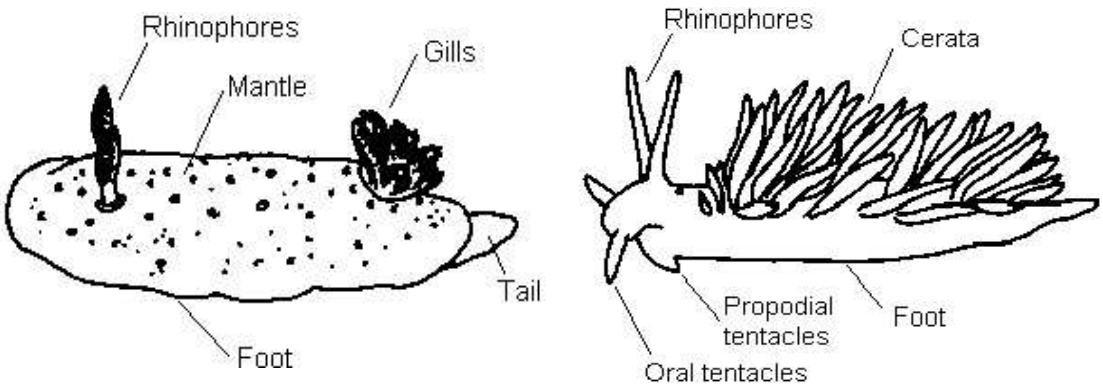
Figure 1. The scheme summarises the actual systematics of heterobranch molluscs as reported in the World Register of Marine Species (WoRMS - https://www.marinespecies.org).

1.1.2 Morphological, ecological and biological characteristics

Marine heterobranchs are colloquially known as “sea slugs” because of their morphology *(Figure 2)*: they have bilateral symmetry and an elongated

shape, a muscular foot in contact with the substrate, and at the level of the head they have two variably shaped antennae, known as rhinophores (Rinaldi, 2012), with an olfactory function (Arey, 1918) *(Figure 2; Figure 3a)*.

Some species also have two eyes, with which they are only able to distinguish light variations (Lederhendler et al., 2023) in order to facilitate the functioning of the animal's internal biological clock (Newcomb et al., 2014). The mouth is frontal and has the radula, a denticulated tongue characteristic of the Mollusca phylum, widely used for classification (Mackenstedt & Märkel, 2001). On either side of the mouth there are two more or less prominent oral tentacles, with a receptive function (Murphy & Hadfield, 1997). The anus may be postero-dorsal or lateral (Ponder et al., 2020b).

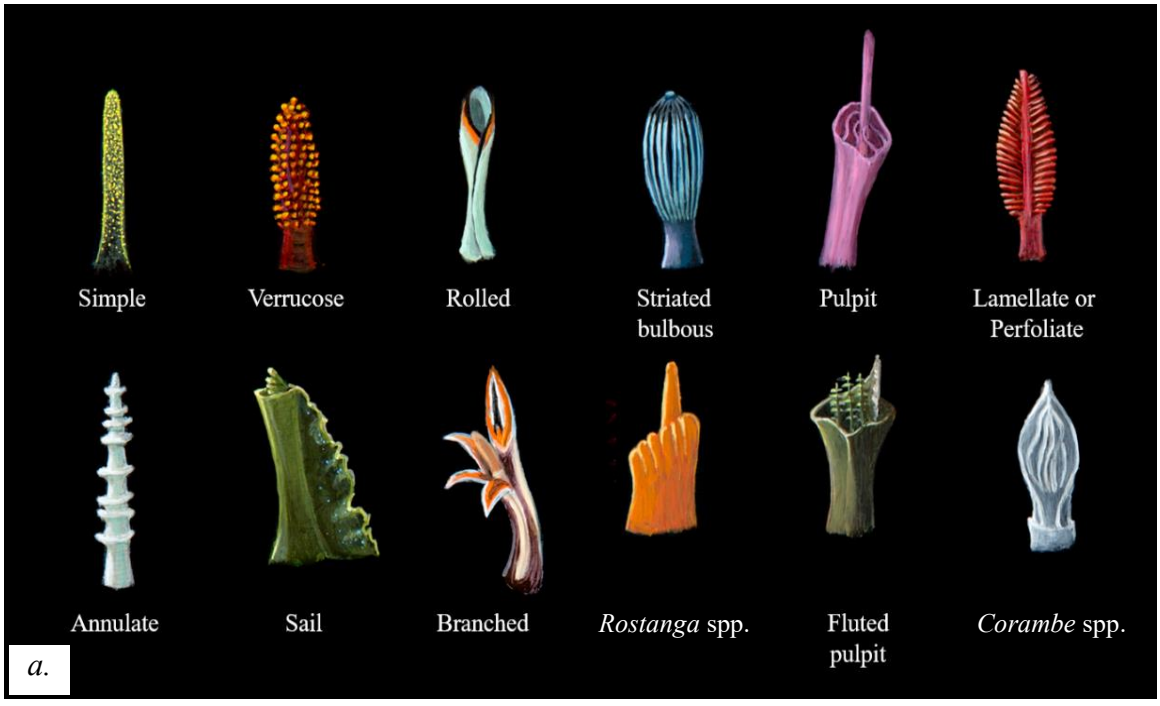


a. *b.*

Figure 2. Schematic representation of the general anatomy of two heterobranch molluscs (Source: http://www.seaslug.org.uk/nudibranchs/anatomy.html).

Respiration is predominantly cutaneous: to increase its efficiency, some groups may have dorsal digitations of variable shape, leading to an increase in body

surface area (Ponder et al., 2020b). These digitations are called “cerata” *(Figure 2b; Figure 3b)* and may contain digestive gland diverticula or cnidosacs (Martin, 2003) for the collection of prey nematocysts (only in cnidarian-feeding species) (Greenwood & Mariscal, 1984). The evolution of cerata occurred independently in three superfamilies of the order Nudibranchia: Aeolidioidea, Proctonotoidea and Tritonioidea (Ponder et al., 2020b). In other groups, however, gill respiration can be observed instead of cutaneous respiration: secondary gills are present in some Cephalaspidea, Pleurobranchida, Aplysiida, Nudibranchia and Sacoglossa, which may be located under the mantle cavity or caudally (perianal tuft), encircling the anus (Ponder et al., 2020b) *(Figure 2a)*.



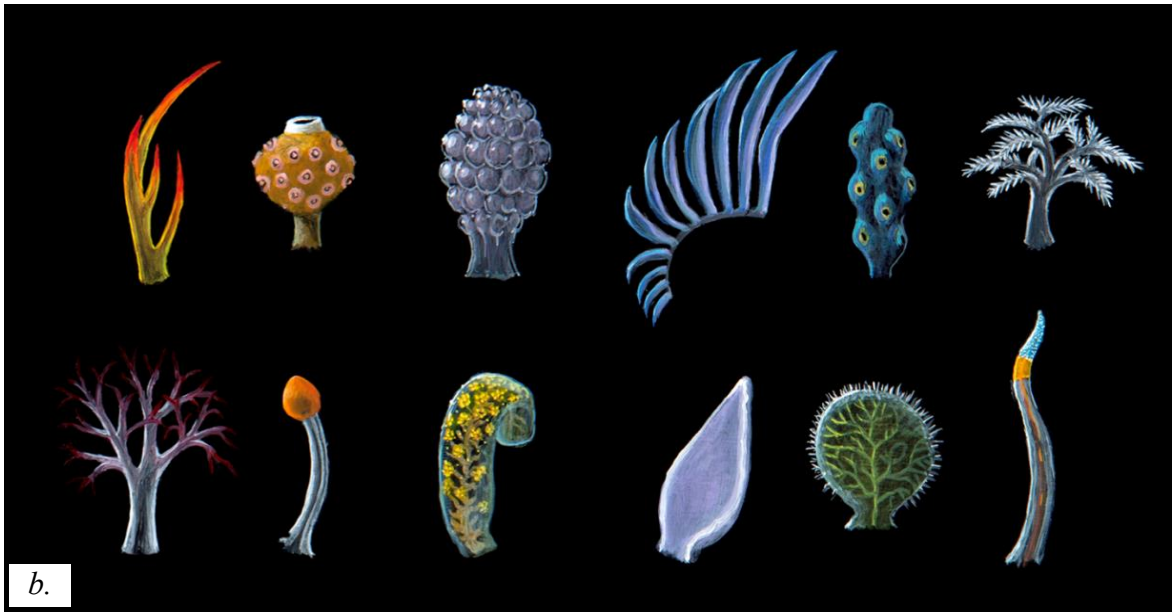


Figure 3. Diversity of rhinophores *(a)* and cerata *(b)* (Source: https://bolinasmuseum.org/exhibitions/nudibranchia-butterflies-of-the-sea/).

The mantle is the dorsal epithelium that protects the internal organs of the animal and has the function of producing the shell, which is generally reduced in Cephalaspidea, Aplysiida, Umbraculida, Pleurobranchida and Sacoglossa (Trainito & Doneddu, 2014). It is composed of organic material (<5%), which serves as a structure on which calcium carbonate crystals are deposited, making it mechanically resistant (Ponder et al., 2020a). Several proteins are also incorporated during formation, giving the shell additional strength and regulating crystal nucleation (Marie et al., 2013). In groups where it is absent, i.e. Rhodopida and Nudibranchia (Trainito & Doneddu, 2014), the shell is present only at the larval stage (Ponder et al., 2020b).

The foot can be modified to form two wing-shaped extensions on either side of the body, called parapodia (Sarà, 1990), with a swimming function, closed around the body during rest (Vorster et al., 2014) *(Figure 4)*. This has been observed in some Cephalaspidea, Aplysiida and Sacoglossa (Ponder et al., 2020b).

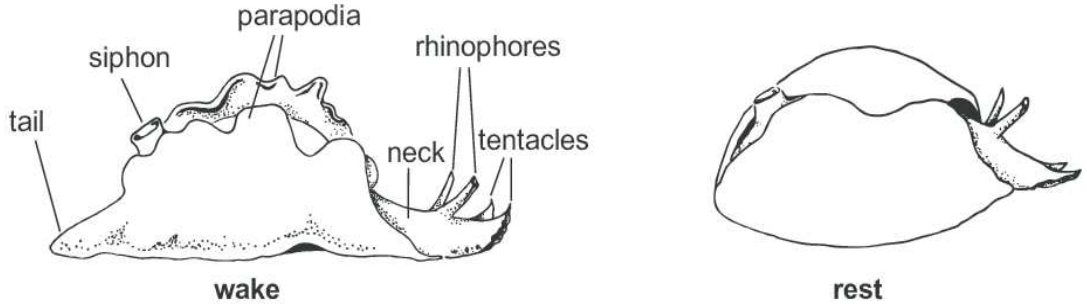


Figure 4. Schematic representation of the anatomy of *Aplysia californica* J. G. Cooper, 1863 when awake and at rest, with the foot modified into parapodia (Vorster et al., 2014).

In terms of reproduction, these are hermaphroditic animals (Sarà, 1990), i.e. they have both male and female reproductive apparatus (Heller, 1993). This is known as simultaneous hermaphroditism because the two apparatuses are active at the same time (Taraporevala et al., 2022), as opposed to sequential hermaphroditism, in which one apparatus is developed first and then sexual reversal occurs with growth (Berglund, 1990). Fertilization is internal: the genital openings are on the right side of the body, so there is cross-fertilization (Jensen, 1999) *(Figure 5)*.

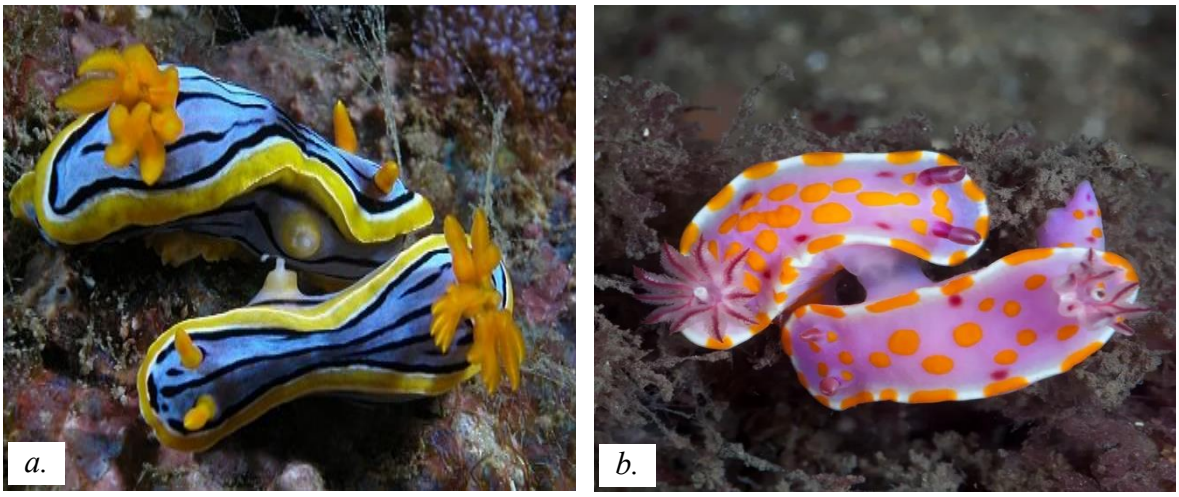


Figure 5. Two specimens of *Chromodoris elisabethina* Bergh, 1877 (*a*) and *Ceratosoma amoenum* (Cheeseman, 1886) (*b*) during a breeding event (Sources: https://nudibranchdomain.org/rudie-nudies-the-mating-game/; https://www.yesmagazine.org/issue/nature/opinion/2013/01/31/sex-in-the-wild).

Mating can be unilateral (one partner acts as male, the other as female) or reciprocal (both partners act as male and female in the same reproductive act). Ponder et al., 2020b report that most marine heterobranchs are semelparous: this means that they participate in only one reproductive event in their lifetime (Havenhand & Todd, 1989). Exceptions are some opportunistic, mostly small species, which may instead spawn several times in the same year (Ponder et al., 2020b).

Eggs are laid and held together by the secretion of a mucus that becomes gelatinous when in contact with water (Pruvot-Fol, 1954). This gelatinous mass can take various forms, depending on the anatomy of the reproductive tract and the mode of deposition. To be specific, there are four most common forms *(Figure 6)*:

- Ribbon-like *(Figure 6a)*: it is attached to the substrate on one side and is usually coiled as the animal performs a circular motion during oviposition (Alder & Hancock, 1845-1855). The eggs are surrounded by a layer of jelly and are present along the entire length of the string;
- Cylindrical string *(Figure 6b)*: it is attached to the substrate by a sheet of gelatin, it is also coiled and has eggs along its entire length;
- Gelatinous sac *(Figure 6c)*: it is attached to the substrate by a gelatinous bead;
- Small sac-like structure *(Figure 6d)*: it is attached to the substrate on one side and is typical of only a few small species. It can roll up on itself, but without making a complete turn (Hurst, 1967).

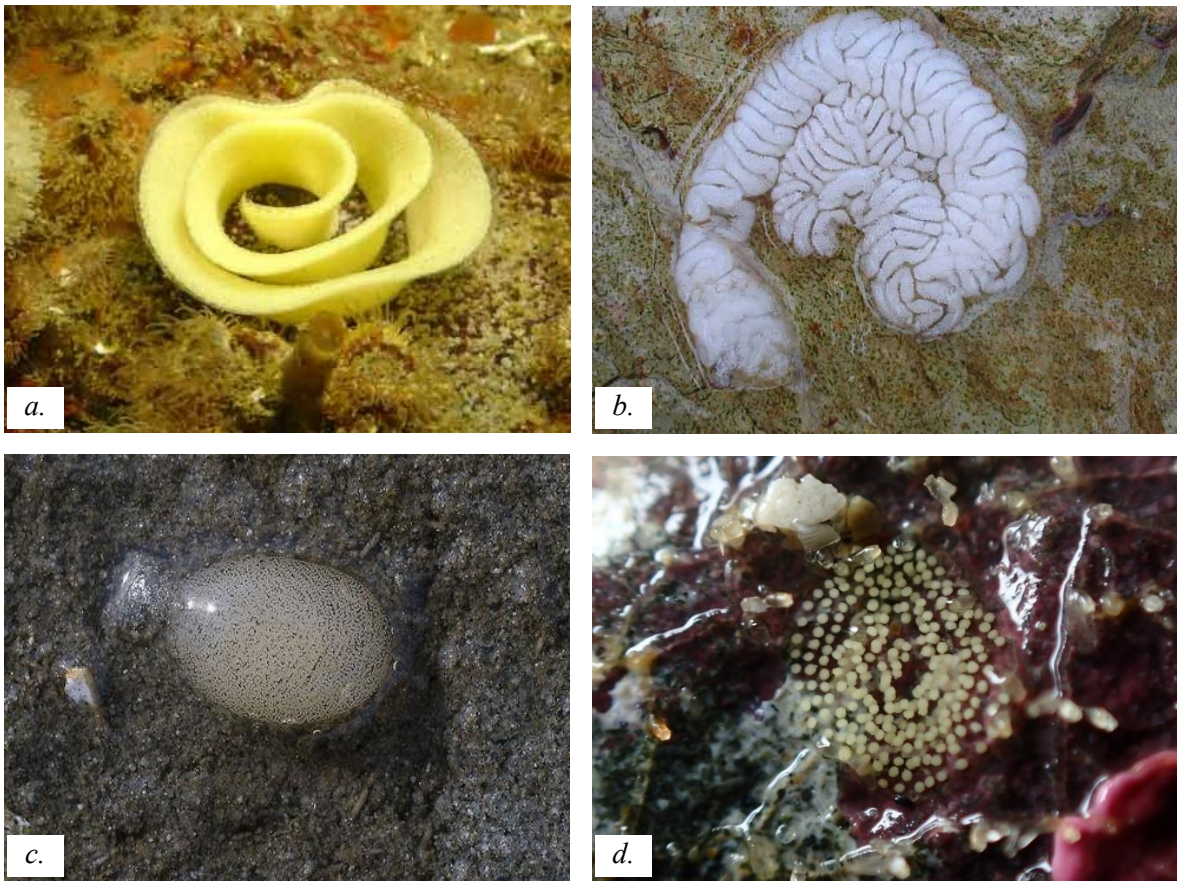


Figure 6. Egg types: *a. Doris pseudoargus* Rapp, 1827 eggs; *b. Aeolidia papillosa* (Linnaeus, 1761) eggs; *c. Melanochlamys diomedea* (Bergh, 1894) eggs; *d. Trinchesia scintillans* (M. C. Miller, 1977) eggs (Sources: https://www.marlin.ac.uk/species/detail/1856; https://www.marlin.ac.uk/species/detail/1986; https://www.jungledragon.com/specie/5490/albatross_aglaja.html; https://inaturalist.ca/observations/126576711).

Larval development in marine heterobranchs can be direct (in a minority of cases) (Hadfield, 1963), lecithotrophic (larvae feed directly in the egg at the expense of vitelline reserves), or planktotrophic (larvae swim and feed on plankton) (Todd et al., 1998; Ponder et al., 2020b).

Heterobranchs are almost exclusively marine, with a few brackish water taxa, and live mostly in shallow waters (Ponder et al., 2020b). Only two evolutionary

lineages belonging to the superfamily Acochlidioidea have independently colonized freshwater (Schrödl & Neusser, 2010): one example is *Tantulum elegans* Rankin, 1979, a Caribbean species living in mountain swamps (Rankin, 1979).

The feeding habits of this large group are very diverse: it includes both herbivorous and carnivorous (the majority) species as well as detritivorous species (Ponder et al., 2020b). In particular, the diet of these animals is very specific: each species has one (monophagy) or a few (stenophagy) prey species, mostly sessile, with which it lives in close contact (Todd et al., 2001). Among its preferred prey, porifers are of great importance, from which toxins can be absorbed and reused for defensive purposes (Proksch, 1994): in fact, about 1/3 of the extant species of sea slugs have a spongivorous diet (Belmonte et al., 2015). Another major prey type includes cnidarians belonging to several clades, including Anthozoa and Hydrozoa (Goodheart et al., 2017). Furthermore, zooxanthellae and chloroplasts (kleptoplasty) can be acquired from prey, whose metabolic products can be used by the animal (Wägele & Johnsen, 2001). It is important to note that this trophic diversity is reflected by an equal variability in the structure of the radula between the different groups (Ponder et al., 2020b): in particular, species that feed on encrusting organisms have weak jaws and broad, scraping radula *(Figure 7a)*, whereas those that feed on emergent

organisms (animals or plants) tend to have well-developed jaws and reduced radular rows *(Figure 7b)*.

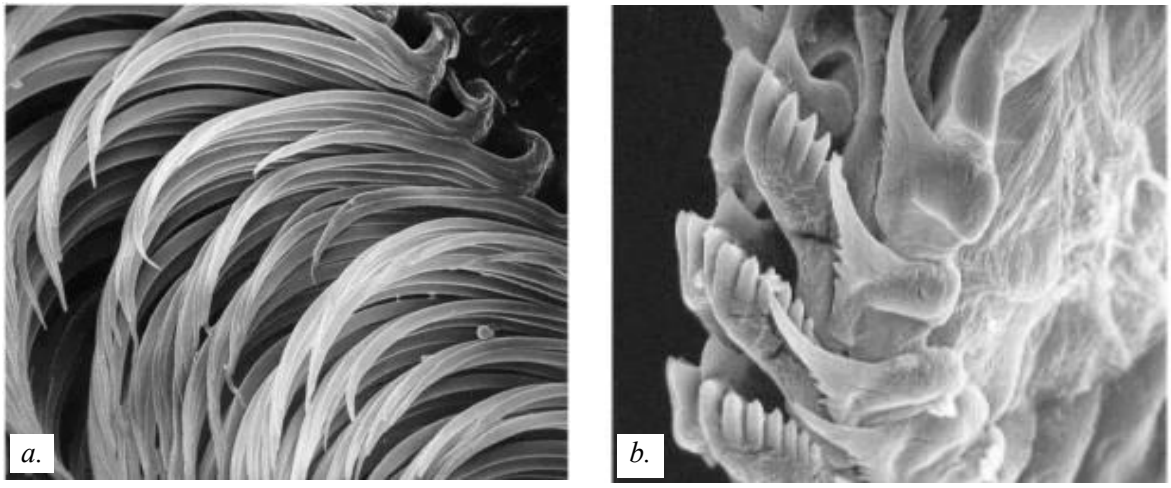


Figure 7. Examples of radulae of two organisms with different diets: *a.* Radula of *Rostanga rubra* (Risso, 1818), spongivorous; *b.* Radula of *Flabellina affinis* (Gmelin, 1791), feeding on hydroids (Source: https://opistobranquis.info/en/introduccio/alimentacio/).

These animals are often very colourful. Such conspicuous colouration is thought to be aposematic, meaning that it serves to warn predators of their lack of palatability (Ponder et al., 2020b). In almost all clades it has been observed that more recently evolved taxa tend to have brighter colours than the basal taxa of the phylogenetic tree, but whether this is important as an evolutionary strategy remains to be investigated as there is insufficient data on their toxicity (Gosliner, 2001).

Aposematism leads to another observable phenomenon, i.e. mimicry (Edmunds, 1991). In particular, predators can act as selective agents to promote similarity between multiple species: we speak of Müllerian mimicry when

several unappetizing species have similar colours and thus cooperate in educating predators to avoid them, while Batesian mimicry consists of imitating colours typically associated with unappetizing species in order to confuse potential predators (Haber et al., 2010).

Another anti-predatory strategy observed in the Heterobranchia taxon is autotomy, i.e. the voluntary detachment of a body part. An example of this phenomenon is the autotomy of cerata in response to predatory stimuli (Miller & Byrne, 2000): they can regenerate quickly and, in addition, they can continue to wriggle after detachment, thus acting as bait. Parts of the mantle such as the parapodia (Gonor, 1961) and the foot (Lewin, 1970) can also be autotomised.

Despite this diversity of defensive strategies, information on predators remains scarce. These include sea anemones (Van der Meij & Reijnen, 2012), platyhelminths, nemertines (Betti, 2021), polychaetes, pycnogonids (Rogers et al., 2000), hermit crabs, crabs, fishes (Trowbridge, 1994), starfishes, turtles and some gastropods (Valdés et al., 2013), including other heterobranchs (Paine, 1963). In some species, such as *Pleurobranchaea meckeli* (Blainville, 1825), episodes of cannibalism (intraspecific predation) have even been observed (Mazziotti et al., 2014).

Precisely because of the poor palatability of heterobranchs, most of the attacks observed by other animals on them actually ended in the rejection of the prey (Tullrot, 1994).

Since 1990, efforts have been made to catalog the heterobranch species present in the Mediterranean. The most comprehensive handbook was published in 2005 by Trainito & Doneddu. The updated version of 2014 counts and describes up to 363 species, estimated to be about 65% of the actual total in the area: of these, 48 are endemic (i.e. their presence is exclusive to the Mediterranean Sea) and 24 are alien (i.e. secondarily introduced, originating from another habitat). Heterobranchs have been studied worldwide and their presence has been documented even in extreme habitats, such as intertidal environments (Nybakken, 1978; Todd et al., 1998; Lambert, 2013; Cyrne et al., 2018): however, there are currently no specific studies on the communities present in Mediterranean rocky tide pools, except for that of Riccardi et al., 2022. There are estimated to be more than 6000 Heterobranchia species worldwide (Bouchet et al., 2017), of which Nudibranchia is the largest order (more than 3000 species) (Aikem, 2003). The current classification is mostly based on morphological characters: however, this method can easily lead to errors, as ethanol preservation can affect characteristics such as pigmentation of the specimens (Furfaro et al., 2020) and different species can be very similar to

each other (Dharmawan et al., 2021). For this reason, DNA barcoding, a technique based on genetic variation between individuals, has been increasingly used in recent years: in particular, the most widely used molecular marker in this context is the Mitochondrial Cytochrome C Oxidase Subunit I (MT-CO1), whose sequence analysis allows species to be uniquely identified (Furfaro and Trainito, 2017). This and other molecular approaches allow the continuous discovery of new species in the Mediterranean, but also worldwide: the taxonomy of the group is therefore constantly updated (Furfaro and Mariottini, 2020).

1.1.3 Heterobranchia and Climate Change

The ocean covers 71% of the Earth's surface: it has nurtured life on our planet and continues to play a dominant role in regulating climate (Hoegh-Guldberg & Bruno, 2020). Marine ecosystems are maintained by interactions between different species, such as prey-predator relationships, competition, facilitation and mutualism (Doney et al., 2020). It is the complexity of this network that allows the ocean to provide benefits that are essential for human well-being, such as fisheries and aquaculture, water purification, coastal protection or recreation: these ecosystem services can only be provided if biodiversity is maintained (Palumbi et al., 2009).

In recent years, increasing human pressures, including climate change, have had a profound impact on marine ecosystems. Specifically, increases in atmospheric Carbon Dioxide (CO_2) are leading to increases in ocean temperature and acidity, sea level rise due to increased temperature, increased ocean stratification, melting of sea ice, changes in ocean circulation patterns, precipitation and freshwater inputs, and reduced subsurface oxygen (O_2) concentrations (Doney et al., 2020).

In addition, global warming is leading to an increase in extreme weather events such as floods, cold spells and heatwaves, which as sudden events may be of greater concern than gradual temperature changes (Smale et al., 2019). Sessile species appear to be the most vulnerable in this context (Moussa et al., 2024), as they are more restricted in their movements and dispersal capabilities (Archambault et al., 2018): sponges and cnidarians, which are among the most common prey of marine heterobranchs, have therefore been the protagonists of several mass mortality events recorded along the western Mediterranean coast in recent years (Di Camillo & Cerrano, 2015).

As heterobranchs have a rather specific diet, their distribution range often coincides with that of their preys (Todd et al., 2001), i.e. benthic organisms (Canessa et al., 2021). Over the years it has been shown that the disappearance of nudibranchs from a given area, because of death or migration, is very often

due to the unavailability of prey organisms: the previous hypothesis attributing the cause to death immediately after spawning (Aboul-Ela, 1959) has thus been refuted. In molluscs, many traits are the result of co-evolution with their prey. This is easily observed in marine heterobranchs, which have specialised in feeding on organisms that have chemical defenses against predation: they have therefore acquired the ability to sequester secondary metabolites from their prey (Becerro et al., 2003). It is also thought that the development of these defenses led to the loss of the shell (Faulkner & Ghiselin, 1983).

Recent studies have shown how, precisely because of this species-specific diet, sea slugs can be used as bioindicators of climate change (Adiwijaya et al., 2021): indeed, shifts in the distribution of some species of sea slugs have been recorded as a result of water warming, which, as mentioned above, primarily affects the development of the sessile benthic communities on which these organisms feed. Some tropical species are expanding their range, overlapping with that of typically temperate species (Nimbs et al., 2015). This behaviour has been observed in many other marine taxa, that are increasingly moving toward the poles (Goddard et al., 2011). Numerous studies have been conducted on heterobranchs and their range shifts due to climate change in the Pacific, particularly in Australia (Nimbs & Smith, 2018; Nimbs et al., 2016; Nimbs & Smith, 2016), while in the Mediterranean the effects of climate change on

different types of organisms have been studied except for marine heterobranchs (Albouy et al., 2012; Chevaldonné & Lejeusne, 2003; Bianchi et al., 2019). However, it is reasonable to assume that where their populations are declining, it is due to the same factors as in the Pacific communities, and therefore global warming is the cause.

Global warming not only affects water temperature but also the intensity of currents: this can facilitate the establishment of new species, which can alter existing ecosystem interactions (Nimbs et al., 2016) and lead to the collapse of native species, especially in the Eastern Mediterranean, that is the warmest basin (the effects of global warming are more evident) (Albano et al., 2021). The phenomenon is also favored by the warming of the waters, which leads to a reduction in winter mortality (Trainito et al., 2022): this facilitates the process of "tropicalization" of the Mediterranean Sea, i.e. the establishment of allochthonous species of tropical origin (Coll et al., 2010).

Climate change not only affects the individual distributions of organisms, but also interferes with their interactions: different trophic levels respond at different times to rapid changes in abiotic parameters. The result is a disruption of the synchronisation of life cycles between prey and predators (Damien & Tougeron, 2019).

In this context, knowledge of species diversity, ecology, distribution and food preferences of Heterobranchia can be very important for determining changes in species composition and thus for monitoring the effects of climate change (Adiwijaya et al., 2021).

Heterobranchs can be considered “flagship species” due to their morphological diversity and colourful patterns (Chan et al., 2022): this means that their monitoring consequently leads to the conservation of a significant number of other species belonging to different taxonomic groups, but which are considered “less glamorous” (Dietz et al., 1994), such as their sessile preys (sponges and cnidarians). In recent years, several organizations and agencies have begun to use certain taxa as “flagships” to help engage and inform the public about the importance of conservation efforts (Bowen-Jones & Entwistle, 2002). The highly colourful nature of heterobranchs makes them very photogenic and attractive to divers worldwide: this makes it easier to involve non-scientists in data collection (Smith & Nimbs, 2022). In fact, within the World Wide Web (WWW), among the various websites and social media, there is a huge database unintentionally created by the publication of underwater photos/videos, from which a large amount of information can be extracted, constituting a source of passive citizen science (Di Camillo et al., 2018).

Concerning the heterobranch molluscs of the Mediterranean and Black Sea, an important data collection project has been set up by Reef Check Italia ETS, a non-profit scientific association founded in 2008 (https://www.reefcheckmed.org/italiano/reef-check-med/nudibranchi/): it uses iNaturalist, one of the most important global citizen science platforms for collecting biodiversity data, where anyone can register and upload photos of their observations (Seltzer, 2019), which are later validated by experts.

1.2 The Conero Riviera and its Heterobranchs

The Conero Riviera is a stretch of coastline of 20 km (Masini et al., 2007) in the north-western Adriatic Sea, stretching from the port of Ancona to that of Numana. It takes its name from Monte Conero (lat. 43.560017; long. 13.613717) (Irving et al., 2009), a promontory overhanging the sea that is part of the Umbria-Marche Apennine and reaches a height of 572 m (Aringoli et al., 2014). The formation dates to 5 million years ago (Pliocene), after a long process of marine sedimentation that began in the Jurassic period.

The entire area was declared Conero Natural Regional Park in 1987 (Law L.R. 23/04/1987, n. 21), covering 6011 hectares and distributed among the municipalities of Ancona (53%), Sirolo (21%), Numana (16%) and Camerano (10%) (Osmani, 2010).

In the area, there are different levels of protection. The Natura 2000 sites (Directive 92/43/EEC "Habitat") cover 51,86% with SPA (Special Protection Area) (29,50%), and SCIs (Sites of Community Interest) (22,36%), concentrated in the north-eastern area, including the sea cliff and the upper part of the mountain (Osmani, 2010). In concrete terms, there are 3 SCIs: "Costa tra Ancona e Portonovo" (IT5320005), "Portonovo e falesia calcarea a mare" (IT5320006) and "Monte Conero" (IT5320007), which are included in the SPA "Monte Conero" (IT5320015) *(Figure 8)*. The Area floristica protetta "Monte Conero" (L.R. 30/12/1974, n. 52), the Vincolo Idrogeologico (R.D. 2367/26) and the PPAR Marche (D.A.C.R. 3/11/1989, n. 197) have also been established. Despite this, some of the Priority Habitats (i.e. "Banchi di sabbia a debole copertura permanente di acqua marina", Habitats Directive code 1110, and "Scogliere", Habitats Directive code 1170) and species (*Pinna nobilis* Linnaeus, 1758 and *Lithophaga lithophaga* (Linnaeus, 1758)) recognised by the European Directive do not fall within the area of the Park, which is why the management plan should be reviewed to guarantee their monitoring.

Due to its naturalistic importance, the Conero Riviera has been identified as Marine Recovery Area (MRA) (Laws n. 979/1982 and n. 394/1991): in fact, only the establishment of the Costa del Monte Conero MPA (Marine Protected Area) could guarantee the conservation of the organisms present and the

balance between the submerged and emerged parts of the coast (Comitato Promotore per il Referendum sull'istituzione dell'Area Marina Protetta Costa del Conero, 2021). Despite this, for several years critical issues, limited to specific stakeholders, have slowed down its implementation.

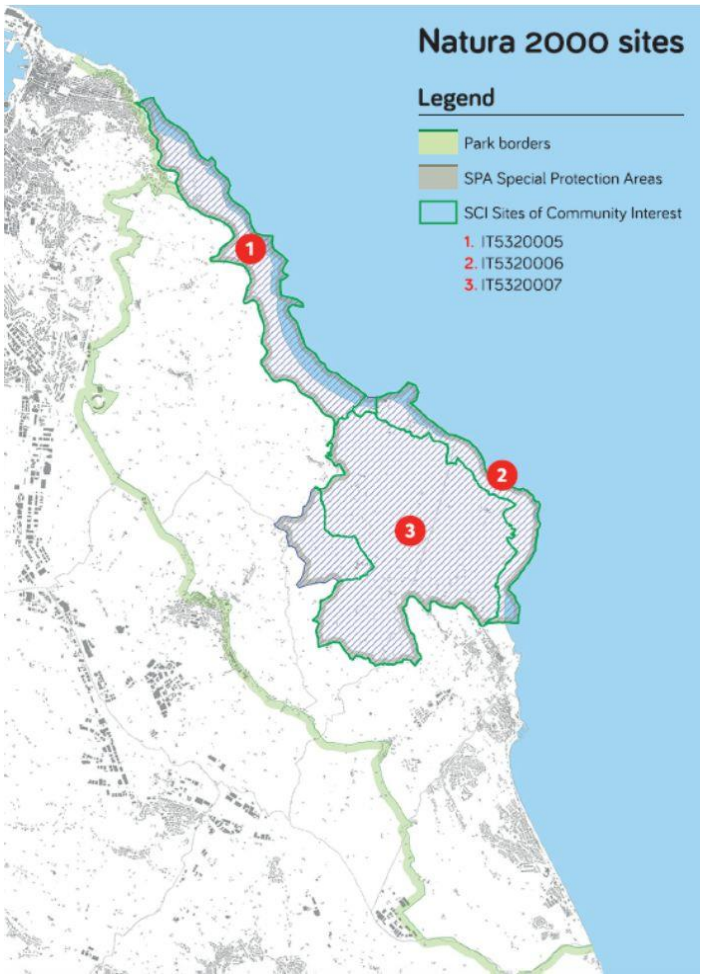


Figure 8. Map of the Conero Park highlighting Special Protection Area (SPA) and Sites of Community Interest (SCIs) (Source: https://ambiente.regione.marche.it).

From a geographical and morphological point of view, the area can be divided into three main zones: the first zone is represented by the coastal strip, with different lithological and structural types; the second one is identified by the

hills of the inland sector, where pelitic-arenaceous and marly deposits of the Mio-Pliocene age outcrop; the third and last zone is represented by the Monte Conero relief, characterised by calcareous rocks with numerous faults and fractures that form a complex network (Biondi et al., 2000). As far as the coast is concerned, between Ancona and Portonovo the cliff is marly-arenaceous, while between Portonovo and Sassi Neri it is calcareous. The sea in front of the marly-arenaceous cliff is dotted with rocky outcrops, the result of the different erosive action on the different strata: among these is the Trave, which juts out into the sea for about 450 m. The calcareous cliff, on the other hand, is subject to a different type of erosive process: the waves cause the formation of furrows and cavities at the foot of the cliff, which cause instability and, consequently, landslides that cause the promontory to extend further and further into the sea (Biondi et al., 2000). In fact, Monte Conero is a rocky promontory that interrupts the continuity of sandy beaches stretching from Rimini to the Gargano (UNEP/MAP-RAC/SPA, 2015; Di Camillo et al., 2010). This heterogeneity of the seabed favours a high diversity of animal and algal species, especially sessile ones: this is why the Conero coast is a destination for recreational divers, often dedicated to the observation of biodiversity and underwater photography (Riolo & Betti, 2015).

The seabed is shallow: most of the Riviera has a depth of about 5-6 m, with a maximum of 14 m (Rindi et al., 2020). This factor, together with the contribution of cold river waters (from the Po and other smaller rivers), means that the water temperature is very variable throughout the year: the average surface temperature in winter is 7 °C, which can reach up to 27 °C in summer (Di Camillo et al., 2012). The fluvial inputs, combined with the semi-enclosed nature of the Adriatic basin, result in a high availability of nutrients: this makes it an ideal habitat for phytoplankton, which, in addition to being involved in photosynthesis and being the base of the trophic web, is the cause of the greenish colour that characterizes these waters, especially in summer (Betti, 2010).

As anticipated, the coastline along the Conero promontory is characterised by rocky reefs alternating with sandbanks. The reefs are rich in plants and animals that structure themselves along the depth depending on light penetration and hydrodynamics (Cerrano et al., 2014). Facies with bioeroders are the main component of benthic communities, dominated by the sponge *Cliona adriatica* Calcinai, Bavestrello, Cuttone & Cerrano, 2011 and the bivalve *Rocellaria dubia* (Pennant, 1777). Moreover, there are extended algal-dominated facies, mainly represented by *Cystoseira* species and closely related genera of brown seaweeds such as *Ericaria* and *Gongolaria*: these are important ecosystem

engineers, forming canopies that play a key role in the functioning of the ecosystem (Rindi et al., 2020).

However, the most important biocenotic components of the area are the mussel beds formed by *Mytilus galloprovincialis* Lamarck, 1819: this species is also considered an ecosystem engineer, as it increases the three-dimensional complexity of the substrate and hosts a large number of associated organisms (Cerrano et al., 2014). *M. galloprovincialis* is also important from a socio-economic point of view, as it is a food resource that is widely exploited in the region (Slow Food Presidium since 2004).

Two species of bivalve molluscs of community interest present in the area are *Lithophaga lithophaga* (Linnaeus, 1758) and *Pinna nobilis* Linnaeus, 1758: these are included in Annex IV of the Directive 92/43/EEC "Habitat", as they require strict protection measures due to their decline as a result of human overexploitation. In addition, there is *Pholas dactylus* Linnaeus, 1758, which is not listed as a species of community interest but is nevertheless included in both the Bern Convention (19-09-1979) and the ASPIM Protocol (Barcelona Convention for the Mediterranean, 1995), as it is considered a "vulnerable" species by the IUCN (International Union for Conservation of Nature) (Cerrano et al., 2014). Other species characteristic of rocky habitats are also present: examples include *Cladocora caespitosa* (Linnaeus, 1767) (an endemic

scleractinian coral of the Mediterranean included in the IUCN Red List (Zunino et al., 2018)), *Sabellaria alcocki* Gravier, 1906 (a gregarious tubicolous polychaete) and *Cereus pedunculatus* (Pennant, 1777) (an anemone with tentacles arranged in the shape of a crown (Badreddine et al., 2022)) (Cerrano et al., 2014).

Sandbanks, on the other hand, are very representative habitats of the Italian North Adriatic coast and are of great importance for several reasons: first, they host a great biodiversity in the first few centimetres of the substrate, which plays a key role in stabilizing and oxygenating it; then, these habitats represent a nursery for vagile species, among which the gastropods *Neverita josephinia* Risso, 1826 and *Hexaplex trunculus* (Linnaeus, 1758), polychaetes of the family Arenicolidae or crustaceans such as *Squilla mantis* (Linnaeus, 1758) stand out.

With regard to heterobranchs, along the Conero Riviera they have always been an important underwater tourist attraction for both local and foreign divers because of their high diversity. In fact, data on the heterobranch molluscs of the Conero Riviera are very scarce: the most complete work on the subject is certainly that of Betti 2011, later updated in 2021, which counts 72 species. Among these, the most abundant species are *Flabellina affinis* (Gmelin, 1791)

and *Cratena peregrina* (Gmelin, 1791), which are particularly abundant near the hydroids of the genus *Eudendrium* (Betti, 2021) *(Figure 9)*.

Figure 9. Flabellina affinis (Gmelin, 1791) (on the left) and *Cratena peregrina* (Gmelin, 1791) (on the right) on *Eudendrium* sp., on which they usually both feed, photographed at Scoglio del Trave (pictures by Marco Boncompagni).

Betti, 2021 provides us also with data on the food preferences of most of the species present along the Conero Riviera: however, it is not clear whether these are exclusively based on direct observations or whether they have been supplemented by other information from the literature (bibliography is not cited). Nevertheless, an extensive photographic archive is included, which provides valuable information about the substrata on which almost all the species were found.

Rocky tide pools habitat of the Passetto beach

Passetto is one of the main landmarks of the Ancona city coast, in the northernmost part of the Riviera. It is an area that has become increasingly anthropized over the years due to the touristic value it assumes, especially in

summer (Turchetti & Tarsetti, 2007; Riccardi et al., 2022). The seabed consists of large rocks (Betti, 2010) and there is a semi-enclosed system of rocky tide pools at the foot of the Seggiola del Papa (a characteristic rock in the middle of the sea, so called because of its shape, reminiscent of the Pope's chair), with a total area of 750 m²: it consists of a rock formation with two main cracks parallel to the coastline, along which the pools form at low tide (Riccardi et al., 2022) *(Figure 10)*.

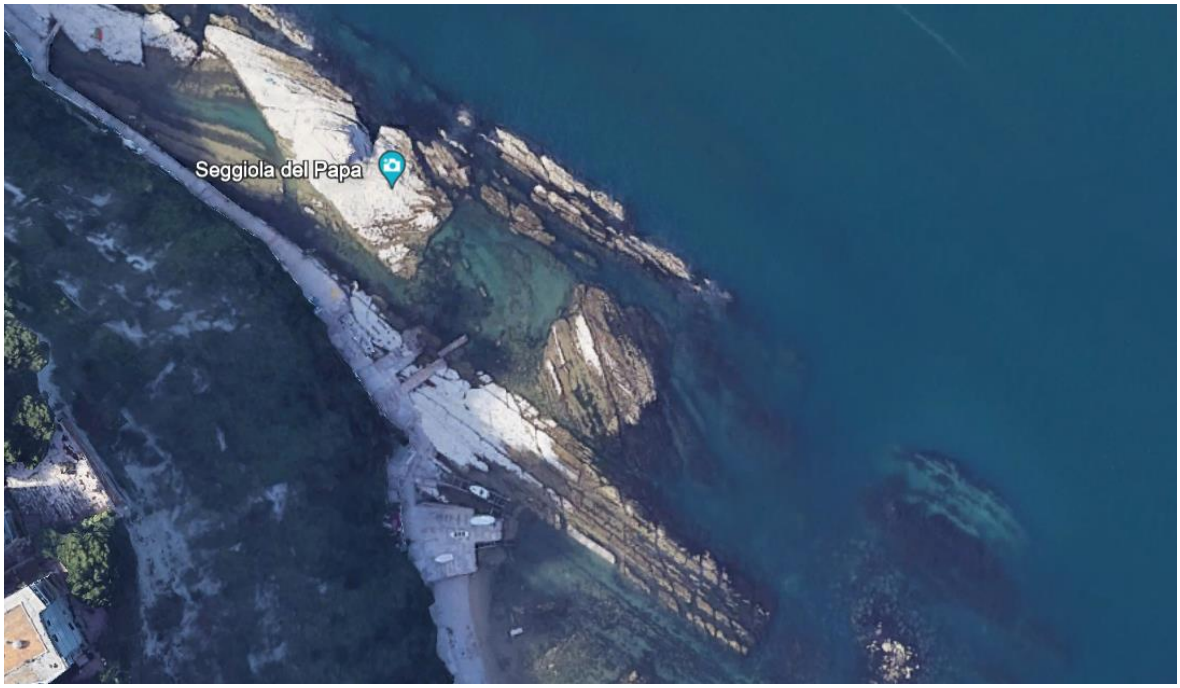


Figure 10. Top view of the rocky tide pools system at Passetto, with the Pope's Chair in evidence (Source: Google Earth).

Rocky intertidal pools are ubiquitous features of rocky coastlines in many parts of the world and can support rich biodiversity (Firth et al., 2014): indeed, they provide refuge, feeding habitat or breeding grounds for many marine species (Mendonça et al., 2018). This environment is subject to diurnal variations in

physical and chemical parameters such as temperature, light, pH, salinity, O_2 and CO_2 concentrations: this exposes the individuals living there to extreme conditions (Huggett & Griffiths, 1986).

The Passetto tide pools host a macroalgal assemblage of at least 48 species (Toffanin, 2021): these support a rich community of benthic invertebrates such as sponges, cnidarians, bryozoans and ascidians, which can act as a food source for heterobranchs of different trophic groups.

A study carried out in this intertidal habitat recorded 19 species of heterobranchs belonging to 12 different families in the rocky tide pools system. Three of them were first records: *Placida dendritica* (Alder & Hancock, 1843), recorded for the first time along the Conero Riviera, *Doto cervicenigra* Ortea & Bouchet, 1989 and *Ercolania viridis* (A. Costa, 1866), both of which had never been recorded in the western Adriatic Sea (Riccardi et al., 2022) (the most recent checklist of Adriatic heterobranchs previously counted 154 species for the western area (Zenetos et al., 2016)).

Data on food preferences are also included in the study, obtained from the literature, but it is unclear whether they are based on simple substrate preference or food sources identified through more specific analyses. Despite this, it was found that the heterobranchs present belonged to 6 different trophic

groups, the majority of which were cnidarian-eaters, immediately followed by herbivores.

At Passetto, many individuals (belonging to 11 out of 19 species) were observed lying upside-down on the surface of the water, taking advantage of the surface tension *(Figure 11)*. The reasons for this behaviour are not yet clear, although it is known from the literature and has also been observed in captivity: the hypothesis is that this is a locomotion technique used by heterobranchs as a response to the stressful conditions of the tide pools (Riccardi et al., 2022). Undoubtedly, this study highlights the need for further focused studies on this type of habitat.

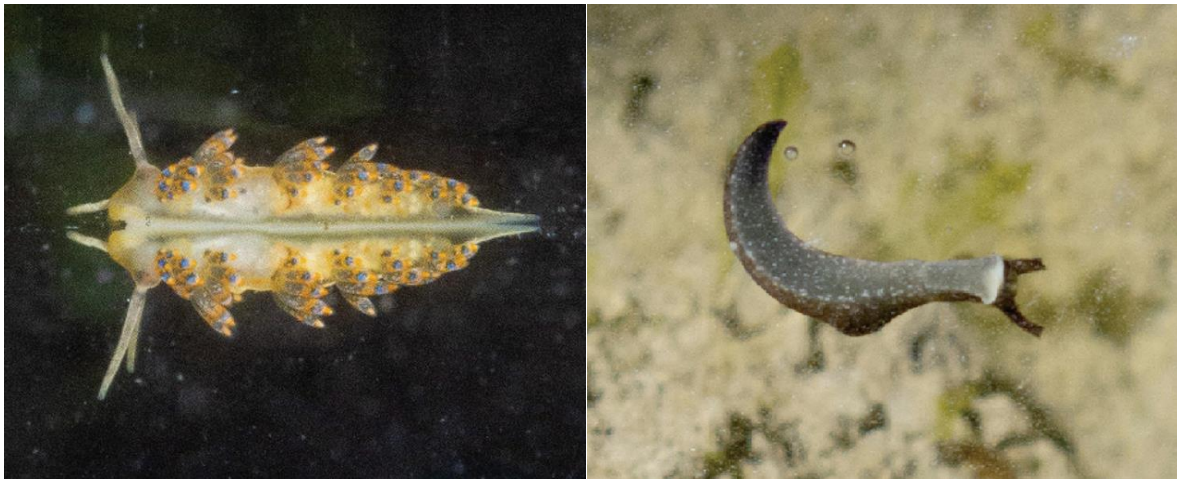


Figure 11. Trinchesia morrowae Korshunova, Picton, Furfaro, Mariottini, Pontes, Prkić, Fletcher, Malmberg, Lundin & Martynov, 2019 (on the left) and *Elysia viridis* (Montagu, 1804) (on the right) showing an upside-down floating locomotion behavior (Riccardi et al., 2022).

Therefore, as local biodiversity hotspots, tide pools, which are part of the reef habitat, are included in the Directive 92/43 EEC “Habitat” as an environment to be particularly protected at European level.

Considering their accessibility and ease of manipulation, tide pools can also be a resource from which to derive models for understanding the complex processes that regulate food webs, which are otherwise more difficult to study in open ecosystems (Mendonça et al., 2018). Moreover, again because of their accessibility, it is easy to organize volunteer projects for non-scientists interested in contributing to scientific knowledge through data collection (Jacob, 2008).

1.3 Objectives of the study

Marine heterobranchs are animals with a small home range: they live closely associated to the substrate, using it for anchoring, shelter, feeding and reproduction (Adiwijaya et al., 2021). Despite the fact that heterobranchs are among the most photographed marine invertebrates (Andrimida & Hermawan, 2020), the information about their diet is not exhaustive, which hinders a full understanding of their trophic behaviour and ecological role. The underwater observation of heterobranchs through *visual census* and photography can be an effective non-destructive method to contribute to the understanding of their food preferences and to find the link between their life cycles and the life histories of other benthic organisms. For several terrestrial species, climate change has been observed to disrupt the synchronization of prey and predator

life cycles (Damien & Tougeron, 2019) or flowering time (Geissler et al., 2023). The monitoring of heterobranchs, if carried out over the long term, can therefore be useful in determining a possible shift in trophic behavior and phenological synchrony of benthic organisms due to climate change (Adiwijaya et al., 2021).

Unfortunately, general knowledge about this group of organisms is currently scarce (Camacho-García et al., 2014; Kaligis et al., 2018; Jovanović et al., 2019; Carvajal-Florian & Gracia, 2022; Furfaro et al., 2022); even the available information about heterobranchs' diet is not exhaustive, since the food sources may vary in relation to the locality.

Thus, the main objective of this work is to increase knowledge of the ecology of marine heterobranchs in the Mediterranean, with particular regards to trophic behaviour.

The study area, the Conero Riviera, was chosen due to the great diversity of heterobranchs that can be observed here, even at low depths (Betti, 2021; Riccardi et al., 2022). Subsequently, data were collected on sightings of heterobranchs, both along the Riviera itself and in the rest of the Mediterranean, to reconstruct their ecology and identify any patterns of species-substrate-food sources correlation. The sources used for this collection were direct experience through *visual census* (dives at main Conero dive sites and dry *visual census* at

the Passetto tide pools), Local Ecological Knowledge (LEK), Citizen Science (CS), the World Wide Web (WWW) and literature. A detailed literature search was carried out to make comparisons between the food preferences reported in the Mediterranean literature and the substrata detected.

A secondary objective in this context was also to compare the species present on natural and artificial substrata: indeed, the type of substrate is thought to influence the sessile benthic communities present, and consequently the upper trophic levels (Sedano Vera et al., 2019).

Given the paucity of data on the Conero Riviera, further analysis has been carried out, in particular to characterize the tide pools habitat: the aim is to promote local and easily accessible habitats, such as that of the Passetto, in order to raise citizens' awareness and facilitate their involvement in citizen science projects.

2. MATERIALS AND METHODS

2.1 Data collection on substrate preferences of Heterobranchia in the Conero Riviera

First of all, in order to obtain more information on the heterobranchs present in the Conero Riviera, a specific checklist was drawn up, highlighting for each species the taxonomic classification according to the WoRMS (World Register of Marine Species). The sources used to obtain this checklist were mainly the literature (Betti, 2021 and Riccardi et al., 2022), supplemented by citizen science data (from iNaturalist and observations reported by volunteers), LEK and information from direct observation, both in the tide pools of Passetto and in the main dive sites of the Conero Riviera (Cava Davanzali, Due Sorelle, Relitto M/N Nicole, Passetto (no tide pools), Sassi Neri, Secca dell'Ospedale, Scoglio della Vela) during the period from September 9th, 2021 to February 6th, 2024.

A careful search was then made for records of heterobranchs along the Conero Riviera. Records were taken from direct experience through *visual census*, Local Ecological Knowledge (LEK), Citizen Science (both iNaturalist and direct reports from citizens), Web Ecological Knowledge (WEK) (websites/social media) and literature. For each record, information collected were:

- Date and location of the find (with coordinates when available);
- Sampling protocol (ARA scuba diving, visual census out of water or snorkeling);
- Source (Citizen Science, Local Ecological Knowledge, literature, present work or Web Ecological Knowledge);
- Species (with the taxonomic classification as reported on WoRMS);
- Depth (in metres);
- Temperature (expressed in Celsius degrees);
- Presence or absence of mucous filaments;
- Substrate (indicated by acronyms);
- Nature of substrate (natural or artificial);
- Type of exposure (if the individuals were exposed or hidden);
- Abundance.

It is important to emphasize that when we talk about natural or artificial substrate, we mean secondary substrate: in fact, individuals were always found on natural substrate, which in turn was immersed in a natural (e.g. reefs, sandy bottoms, ...) or artificial (artificial reefs, wrecks, ...) habitat type.

In the end, any other relevant note was added (for example presence/absence of eggs, reproductive behaviour, size of the individuals, surrounding habitat, time of the day, …).

In addition, a unique ID was assigned to each record and, where possible, a picture was included. All data were collected in an Excel spreadsheet, with each row corresponding to one record, for a total of 58 categories/records. The registration began on December 13th, 2023 and ended on April 30th, 2024. Next, graphs were created for each significant parameter.

2.1.1 Visual Census (scuba diving and tide pools) as a data source

Five main dive sites along the Conero Riviera were initially identified for data collection, which are (from north to south) *(Figure 12)*:

- Secca dell'Ospedale, in Sirolo (lat. 43.560017; long. 13.613717): this is located about 300 metres south of Scoglio della Vela and is characterised by the presence of huge piled-up boulders, rich in cracks. The seabed is detrital and the depth ranges from 6 to 12 metres;
- Cava Davanzali, in Sirolo (lat. 43.554043; long. 13.620391): this is a small cove where one of the first quarries for the white Conero stone was located, named after its owner, Cesare Davanzali (1895-1958). The area is characterised by the presence of rocks from landslides. The seabed is sandy and muddy (Betti, 2010), with an average depth of about 10 metres;

- Due Sorelle, in Sirolo (lat. 43.548825; long. 13.628522): there are two imposing stacks very close to the shore, delimiting a small bay to the south. In terms of habitat, it is very similar to Cava Davanzali (Betti, 2010) and is 6-13 metres deep: one of the main differences is the presence of the remains of the Potho wreck, which sank in 1962;
- Sassi Neri, in Sirolo (lat. 43.534303; long. 13.623298): it owes its name to the presence of large dark rocks along the coast. The seabed in front of it is one of the most sheltered in the area and is made up of very large rocky areas (Betti, 2010). The maximum depth is 10 metres;
- Relitto M/N Nicole, in Numana (lat. 43.50744; long. 13.63287): located 2 miles off the coast of Numana, it is the wreck of a large cargo ship, 138 metres long, dating from 2003. The ship is split into two parts and lies on a muddy seabed (Betti, 2010) at a depth of about 14 metres. The minimum depth is 7 metres.

As data collected by other operators were added to the present work, some other sites were considered, bringing the total number of sites to 9: these new sites are La Spiaggiola, Passetto (no tide pools), Scoglio della Vela and Spiaggia del Frate *(Figure 12)*. Data in these sites were collected by underwater *visual census*.

Figure 12. Dive sites of the Conero Riviera where the *visual census* was carried out (red markers) (Source: Google Earth).

In addition, a separate standardized *visual census* out of water was carried out at the Passetto tide pools *(Figure 13)*, which is considered a separate site from Passetto (no tide pools) because of the unique conditions of the habitat described above. Pictures were sometimes taken with an iPhone 13 Promax (12MP camera). Specifically, data collection took place according to the following scheme *(Table 1)*:

Period	Number of sessions	Sampling protocol	Method	Sites	Operators
Sep 9th, 2021 - Jun 15th, 2022	19	ARA scuba diving	Standardized dives of 60 minutes each	Cava Davanzali, Due Sorelle, Sassi Neri, Scoglio della Vela, Secca dell'Ospedale, Passetto (no tide pools), Relitto M/N Nicole	Ricardo Mendes
Jul 19th, 2022 - Apr 23rd, 2023	12	ARA scuba diving	Non-standardized dives	Due Sorelle, Spiaggia del Frate, Relitto M/N Nicole, La Spiaggiola and Secca dell'Ospedale	Alessia Colombo, Gloria Vono
Feb 2nd, 2023 - May 31st, 2023	8	*Visual census* out of water	Standardized sessions of 60 minutes each, twice a month, with the peak of low tide in the middle of the session (to facilitate monitoring activities)	Passetto (tide pools)	Alessia Colombo, Elisa Fasano, Maria Riccardi
Jun 17th, 2023 - Jul 17th, 2023	10	ARA scuba diving	2 standardized dives per site of 60 minutes each	Cava Davanzali, Due Sorelle, Sassi Neri, Secca dell'Ospedale, Relitto M/N Nicole	Alessia Colombo, Maria Riccardi

Table 1. Overview of the *visual census* sessions held along the Conero Riviera.

For all individuals found during each *visual census* session, whether underwater or not, as much information as possible was recorded, including, where available, the size and the observation of any reproductive events.

Figure 13. Visual census activity carried out at the Passetto rocky tide pools (pictures respectively by Agnese Riccardi and Maria Riccardi).

2.1.2 Local Ecological Knowledge as a data source

For biodiversity monitoring, Local Ecological Knowledge (LEK) can also play a very important role (Crocetta et al., 2017), which consists of involving local people in the collection of relevant biological information on target organisms, based on personal observations (Gadgil et al., 2003) or transmitted through social and cultural practices (Benham, 2017): indeed, this method is extremely faster than using scientific protocols for data collection (Anadón et al., 2009).

Regarding the Conero heterobranchs, LEK interviews were conducted with two regular divers in the area who have a historical perception, Marco Giuliano, owner of the Centro Sub Monte Conero diving centre, and Marco Boncompagni, an expert local diver and photo-naturalist. Experts provided their perception of eterobranchs' distribution, seasonality, range of abundances and common substrate. For inclusion in the dataset, it was decided to use the highest number in the range reported by the two divers for each species, in order to have an integer number of individuals. No information was provided on the possible presence of eggs/reproductive behaviour.

2.1.3 Citizen Science as a data source

Another very important data collection tool is Citizen Science, a term coined in the 1990s to describe the active involvement of the public in scientific research alongside scientists (Vohland et al., 2021). Recently many organisations have preferred to call it *Community Science*, as the term "citizen" is seen as a barrier to inclusion. Despite this, it is still being considered whether the two terms can be considered interchangeable, as it has been observed that participants in citizen science are predominantly white, with above average incomes and university degrees (Patenam et al., 2021; Allf et al., 2022), so it would not currently represent a truly egalitarian variant of science (Cooper et

al., 2021). In any case, it is a powerful resource, both for collecting data over large spatial and temporal scales, and for helping to increase public knowledge and awareness of the marine environment (Krželj et al., 2020). As noted above, volunteer participation in marine heterobranch census programmes is facilitated compared to other taxa by the fact that these animals tend to be very colourful and therefore highly photogenic (Smith & Nimbs, 2022), attracting the attention of underwater macro-photographers and others. In this context, new technologies are of great importance for communication between scientists and citizens (Krželj et al., 2020), who can contribute to a better knowledge of biodiversity by publishing photos/videos on scientific platforms (such as iNaturalist), from which information on the distribution and seasonality of certain organisms can be obtained (Smith & Davis, 2019).

It is precisely from the iNaturalist platform that data on the heterobranchs of the Conero Riviera have been obtained: in particular, the observations made between June 9th, 2015 and June 23rd, 2023 were taken into account. The species identifications on iNaturalist were double-checked, which was made possible by the fact that all observations are accompanied by one or more photographs. The presence of photographs also made possible to identify the substrate of the record. Furthermore, notes on the time of day/surrounding habitat were included in many of these data.

In addition to iNaturalist, other citizen science records reported by volunteers aware of this collection work were included in the dataset. These reports date from the period between March 5th, 2021 and March 18th, 2024. Again, notes on the conditions of the find were often included, as well as photographs in some cases.

2.1.4 World Wide Web as a data source

The publication of photos and videos that can be used for scientific purposes can also often happen unconsciously, for example through websites or social media. This unconscious data source present on the World Wide Web (WWW) is called Web Ecological Knowledge (WEK) (Di Camillo et al., 2018). As far as Conero heterobranchs are concerned, a very relevant site is Marco Boncompagni's blog webpage (the same naturalist photographer expert interviewed with LEK) publishes a very detailed report, year by year, of all his diving seasons along the Conero Riviera, all accompanied by very high-quality photographs. This site has therefore been considered for the compilation of the Excel spreadsheet, in particular the heterobranch photographs from the diving years 2015 to 2022. The photos were analysed to understand the substrate on which each individual was found, while the information on the species and the location was provided by Marco Boncompagni himself. As far as the period of

the sightings is concerned, the website only gives the months and not the exact dates, so by convention the 15th of each month was chosen as the approximate date. As the photographer also added many comments about the sea/climate conditions and the abundance of the different species found, this information has been included in the notes.

2.1.5 Literature as a data source

At this point, a comparison between the species in the collected records and the Conero checklist showed that some species had not been reported. These gaps were filled with the data of Betti, 2021: for these species, the main sighting locations and seasonality (if present), the substrate and its nature were therefore included in the dataset.

Furthermore, it was decided to include data extrapolated from Betti, 2021 also for other species on the checklist for which there is little information in the literature from the rest of the Mediterranean.

2.2 Data collection on substrate preferences of Conero heterobranchs in the Mediterranean basin

In addition to the search for heterobranch records along the Conero Riviera, a further search was carried out for the rest of the Mediterranean.

Starting from the Conero checklist, iNaturalist (CS), Social Media (WEK) and data from the Mediterranean literature (excluding the Conero Riviera) were used to enrich the Excel spreadsheet.

Specifically, data from iNaturalist were entered into the dataset in the same way as for the Riviera del Conero, and photos were analysed to confirm species recognition on the platform. The period of recorded sightings is from May 26th, 2013 to February 5th, 2024. Not all observations available on iNaturalist for the Mediterranean were considered, as they were too numerous: mostly recent ones (2023/24) were included, and only a few dated from previous years.

Social media were also “scoured” for information about sightings, looking for photos or videos unwittingly posted by users of these platforms. In particular, the research was carried out on the Facebook platform, with only a few data coming from Instagram. The photos in question were analyzed for recognition of the immortalized species, and as much information as possible was extracted from them to be included in the dataset.

The final source used to obtain data on the behaviour of Conero heterobranchs in the rest of the Mediterranean was the literature. Specifically, for each species on the checklist, information documented for the Mediterranean Sea was searched in the literature and inserted into the dataset. For these records,

information on date/year of report and location was included, while for the other parameters no information was provided.

These data were collected to make a comparison with those obtained for the same species along the Conero Riviera, by constructing specific graphs.

2.3 Data collection on food preferences of Conero heterobranchs from the literature of the Mediterranean basin

At the same time as collecting the records, a more detailed search was carried out in the Mediterranean literature. In fact, starting from the checklist of the heterobranchs of the Conero Riviera, information on the feeding behaviour of each species was searched in the Mediterranean (excluding the Conero, therefore neither Betti, 2021, nor Riccardi, 2022) literature. Since it was observed that many articles reported on the feeding habits of the species without indicating the source of the information, it was thought that it might be important to indicate how the information was obtained (where indicated) in order to distinguish the species for which there was a valid scientific method behind it.

This study was carried out to be able to compare, at a later stage, the data on the substrata of the Conero heterobranchs obtained from the records with the food preferences of the individual species, in this way identifying a possible

correlation. For this purpose, the data on all the substrata on which the different species were found were taken from the Excel spreadsheet and added to the substrata observed in the photographic repertoire of Betti, 2021: in this way, a more or less varied list of substrata was obtained for each species, from which the substrate-food preferences correspondence could be calculated.

When making the comparison, it is important to bear in mind that not all substrates are also food sources (e.g. rocks, mussel shells, ...).

3. RESULTS

A checklist of the heterobranch molluscs of the Conero Riviera was compiled: 92 species belonging to 18 superfamilies were recorded *(Table 2)*. The three superfamilies with the highest number of species are Aeolidioidea (18 species), Fionoidea (15 species) and Doridoidea (12 species), while there are superfamilies with only one species found in the waters of the Conero, such as Akeroidea, Arminoidea, Phyllidioidea, Proctonoidea and Umbraculoidea.

CONERO CHECKLIST	
Aeolidioidea	**18**
Aeolidiella alderi	
Aeolidiella sanguinea	
Berghia coerulescens	
Berghia verrucicornis	
Calmella cavolini	
Cratena peregrina	
Edmundsella pedata	
Facelina annulicornis	
Facelina auriculata	
Facelina bostoniensis	
Facelina dubia	
Facelina rubrovittata	
Facelina vicina	
Favorinus branchialis	
Flabellina affinis	
Nemesignis banyulensis	
Paraflabellina ischitana	
Spurilla neapolitana	
Akeroidea	**1**
Akera bullata	
Aplysioidea	**3**
Aplysia fasciata	
Aplysia punctata	
Bursatella leachii	
Arminoidea	**1**
Armina tigrina	
Chromodoridoidea	**7**
Felimare gasconi	
Felimare picta	
Felimare tricolor	
Felimare villafranca	
Felimida krohni	
Felimida luteorosea	
Felimida purpurea	
Dendronotoidea	**3**
Doto cervicenigra	
Doto coronata	
Tethys fimbria	
Doridoidea	**12**
Baptodoris cinnabarina	
Discodoris rosi	
Discodoris stellifera	
Amphorina linensis	
Calma glaucoides	
Catriona aurantia	
Coryphella lineata	
Cuthona spp.	
Eubranchus tricolor	
Eubranchus vittatus	
Tergipes tergipes	
Trinchesia caerulea	
Trinchesia cuanensis	
Trinchesia diljuvia	
Trinchesia genovae	
Trinchesia morrowae	
Haminoeoidea	**2**
Haloa japonica	
Haminoea navicula	
Onchidoridoidea	**7**
Idaliadoris depressa	
Idaliadoris neapolitana	
Okenia elegans	
Okenia mediterranea	
Pelagella castanea	
Trapania lineata	
Trapania maculata	
Philinoidea	**2**
Philine quadripartita	
Philinopsis depicta	
Phyllidioidea	**1**
Dendrodoris limbata	
Plakobranchoidea	**7**
Calliopaea bellula	
Elysia viridis	
Ercolania viridis	
Limapontia capitata	
Placida dendritica	
Placida viridis	
Thuridilla hopei	
Pleurobranchoidea	**5**
Berthella perforata	
Berthellina edwardsii	
Pleurehdera stellata	
Pleurobranchaea meckeli	
Pleurobranchus membranaceus	

Doris bertheloti	
Doris ocelligera	
Doris pseudoargus	
Jorunna tomentosa	
Paradoris indecora	
Platydoris argo	
Rostanga rubra	
Thordisa azmanii	
Thordisa filix	
Fionoidea	**15**
Amphorina andra	
Amphorina farrani	
Polyceroidea	**3**
Limacia clavigera	
Palio nothus	
Polycera quadrilineata	
Proctonoidea	**1**
Antiopella cristata	
Tritonioidea	**3**
Candiella cincta	
Candiella manicata	
Marionia blainvillea	
Umbraculoidea	**1**
Tylodina perversa	

Table 2. Heterobranchia checklist of the Conero Riviera: superfamilies are shown in bold, with the respective species below. The total number of species is 92.

A total of 2177 records were collected, of which 1536 for the heterobranchs of the Conero Riviera and 641 for the rest of the Mediterranean Sea. Of these, 819 were from the *visual census* (present work), 260 from the LEK, 430 from citizen science, 386 from the WEK and 282 from the literature, as summarised in the diagram below *(Table 3)*:

	Conero Riviera	**Mediterranean Sea (Conero Riviera excluded)**	**TOTAL**
PRESENT WORK	819	nn	819
LEK	260	nn	260
CITIZEN SCIENCE	227	203	430
WEK	183	203	386
LITERATURE	47	235	282
TOTAL	*1536*	*641*	***2177***

Table 3. Total number of records by source and area (Riviera del Conero and the rest of the Mediterranean)

As for the *visual census*, this was carried out both during the dives and out of the water, for a total of 41 and 8 sessions respectively. Of the 819 records, 747

came from the dives at the Cava Davanzali, Due Sorelle, Spiaggia del Frate, Relitto M/N Nicole, Passetto (no tide pools), Sassi Neri, Secca dell'Ospedale, La Spiaggiola and Scoglio della Vela sites, while the remaining 72 records came from observations at the Passetto tide pools.

Citizen science (430 records) consists mainly of data from iNaturalist (293 records), both for the Conero Riviera and the rest of the Mediterranean, while direct reports from citizens exist only for the Conero (137 records).

The WEK records consist only of data from Marco Boncompagni's website for the Conero, and from social media for the rest of the Mediterranean (201 records from Facebook, 2 from Instagram).

In total, therefore, taking all sources into account, 14 different sites have been identified for the Conero (15 if we consider the tide pools of Passetto as a separate site), listed in alphabetical order: 4 Grotte, Cava Davanzali, Due Sorelle, La Spiaggiola, Passetto (no tide pools and tide pools), Ramona, Relitto M/N Nicole, Sassi Neri, Scalaccia, Scoglio del Trave, Scoglio della Vela, Secca dell'Ospedale, Spiaggia del Frate and Spiaggia della Vedova.

The protocol used to collect the data was mainly ARA scuba diving (236 records), with only a minority of data coming from snorkeling (97 records) and *visual census* out of the water (88 records). There are few data where the method of collection is not specified (9 records).

It is important to emphasize that the number of records does not reflect the abundance of individuals, as each record may report the presence of several individuals sighted at the same site.

All these data were used to construct the graphs shown in the following paragraphs. A small histogram has also been added to each graph, highlighting the reference sources of the data considered. Records for which no benchmark data were available (indicated by "nn" in the spreadsheet) were excluded from any representation.

3.1 Analyses of data on substrate and food preferences of heterobranchs in the Conero Riviera

Analysis of substrate preferences

A total of 1536 records were collected for the Conero Riviera, in which the present work played a significant role, accounting for about half of the records. In particular, the records are distributed among the different sites as shown in the graph in *Figure 14*. For the four sites with fewer records (i.e. Ramona, Spiaggia della Vedova, 4 Grotte and Scalaccia) there are no data related to the present work, but only data from CS and WEK. Data for Scoglio del Trave were not collected in the present work either, but were obtained from LEK, literature and WEK.

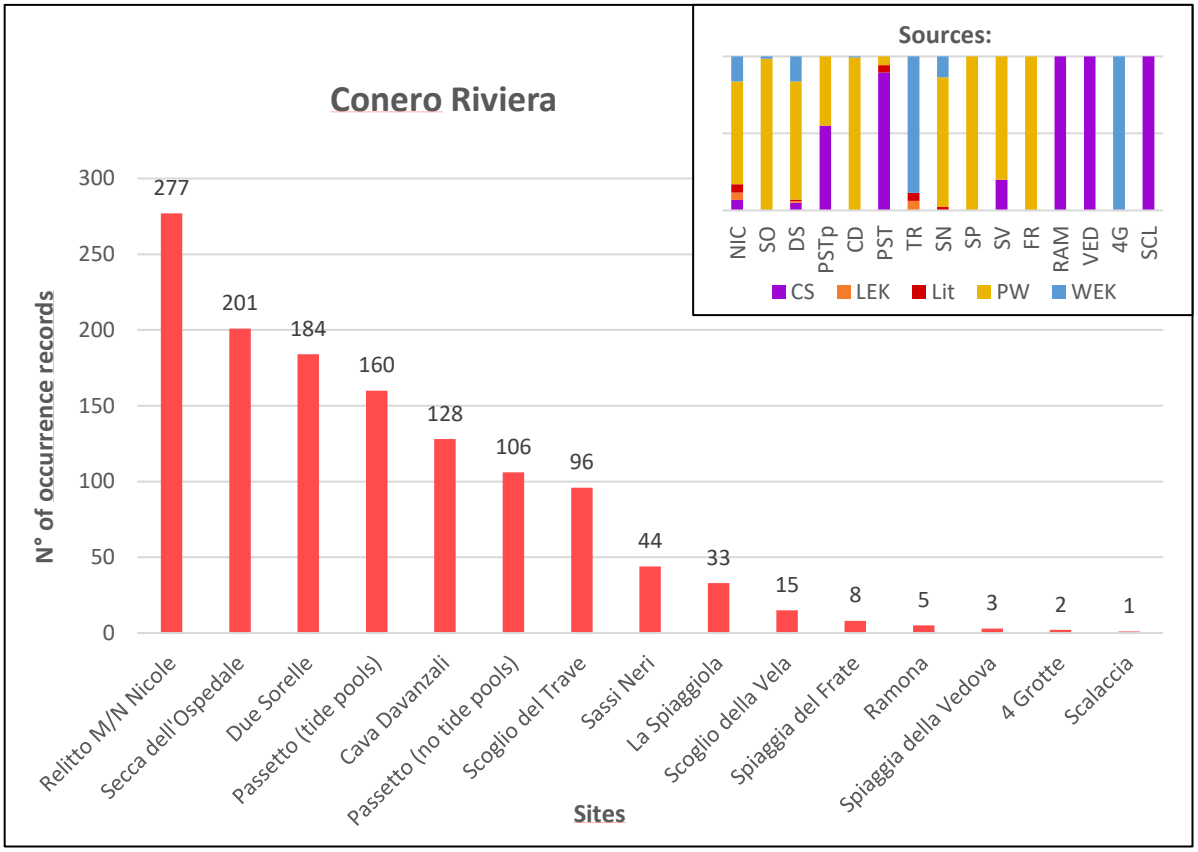


Figure 14. Number of records for each site of the Conero Riviera. The hystogram above shows the contribution of the different sources for each site.

For each site, the most representative substrata were identified *(Figure 15)*. For ease of illustration, acronyms have been used for both sites and substrata. In 304 out of 1536 records, the substrate was not specified: these records were excluded from the graph.

For sites with ≥ 100 records (Relitto M/N Nicole, Secca dell'Ospedale, Due Sorelle, Passetto (tide pools), Cava Davanzali and Passetto (no tide pools)), the frequency of sightings of the different heterobranch superfamilies was also plotted *(Figure 15 a÷f)*.

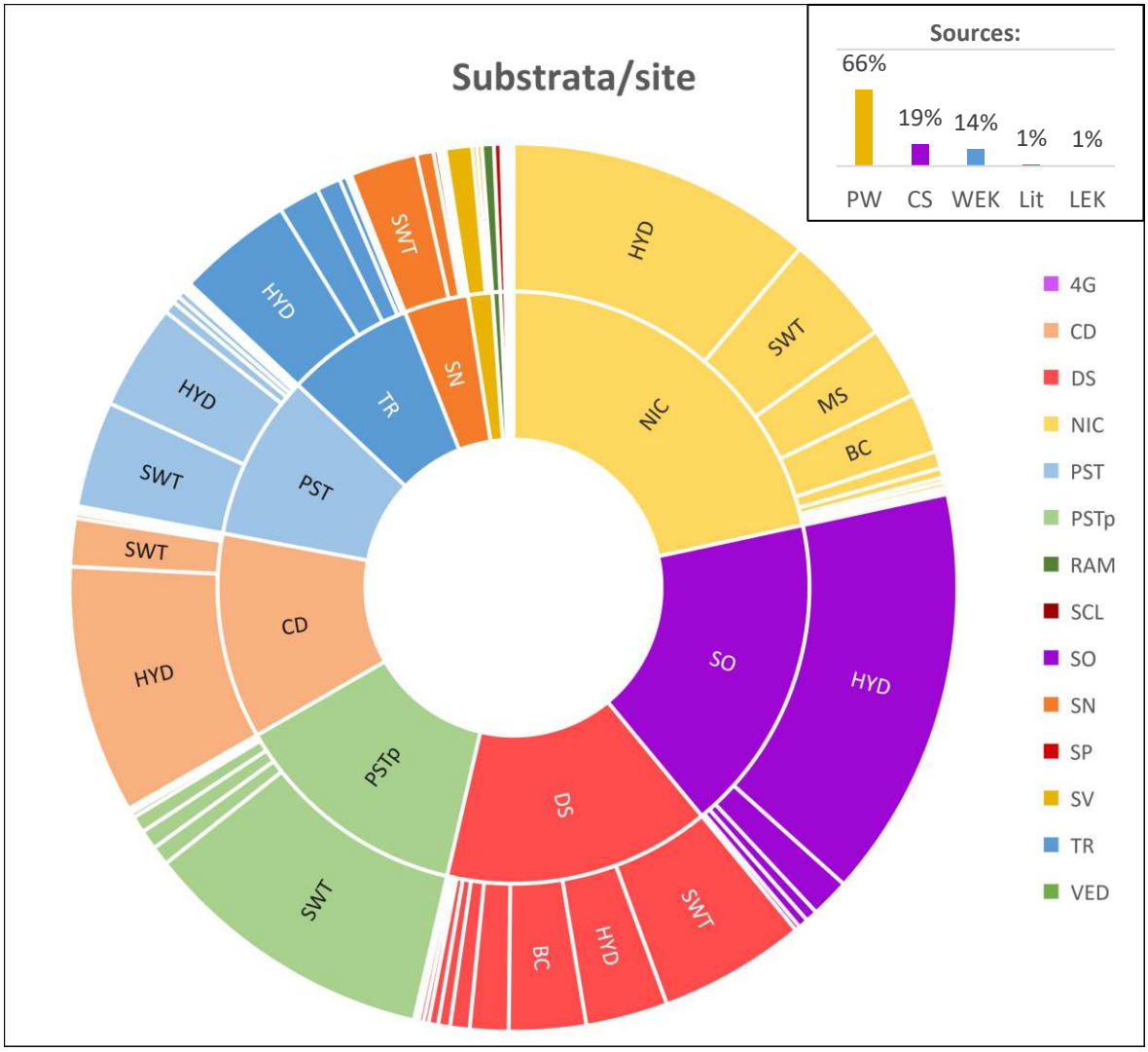
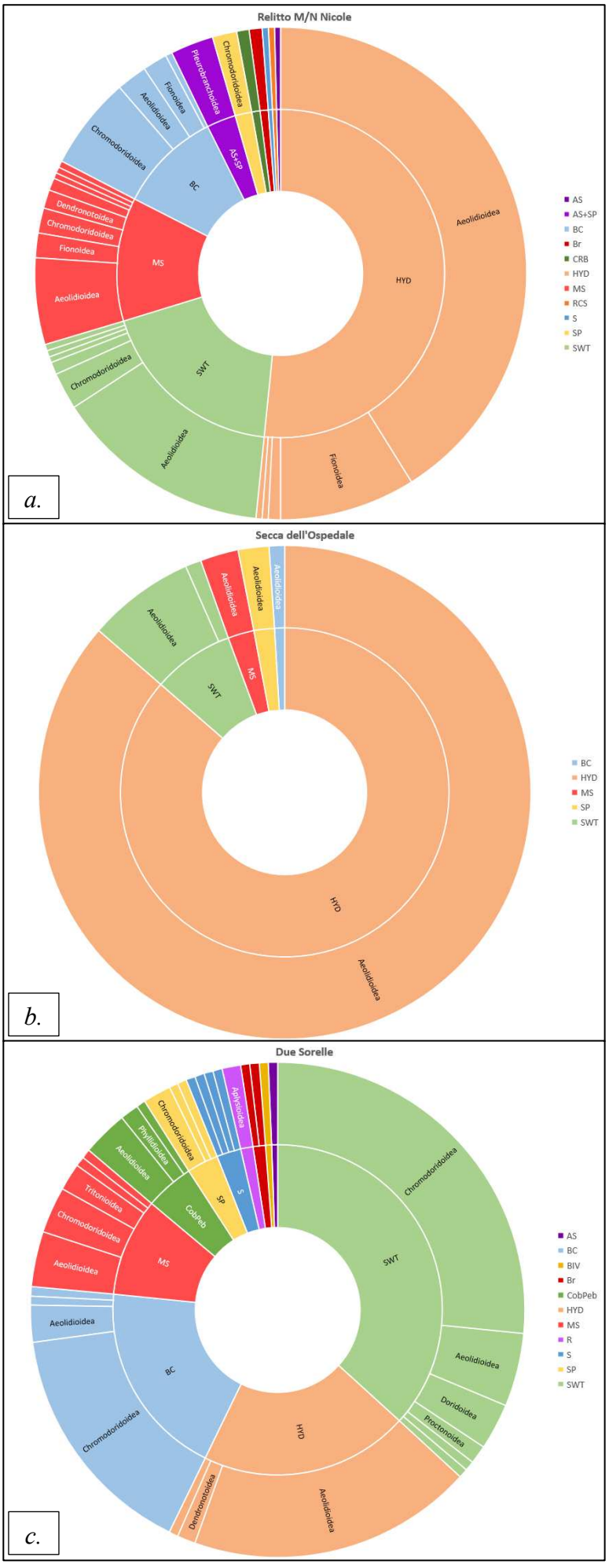
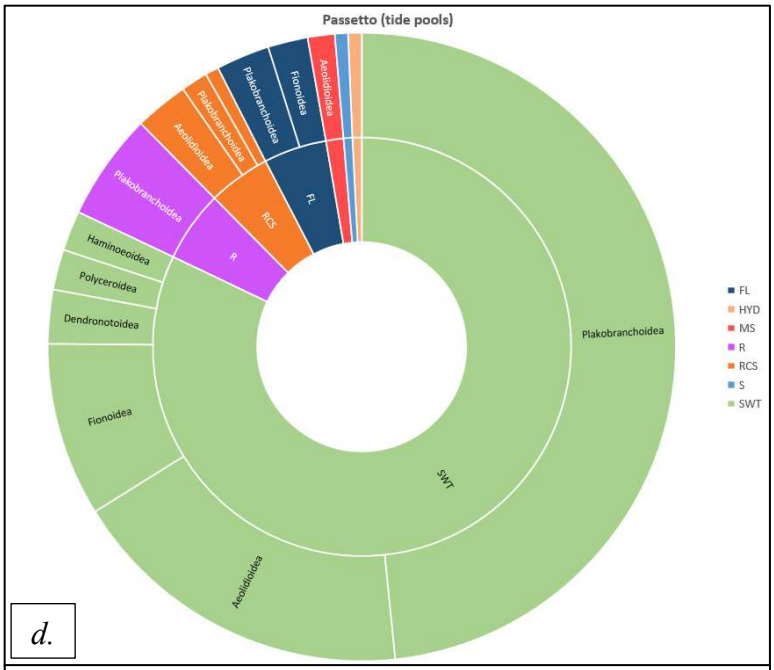


Figure 15. Relative frequency of substrata (outer pie) in each sampling site of the Conero Riviera (inner pie). The small hystogram above represents the contribution of each source. The charts below *(a÷f)* underline the relative frequency of the superfamilies for each substrate in the respective site.

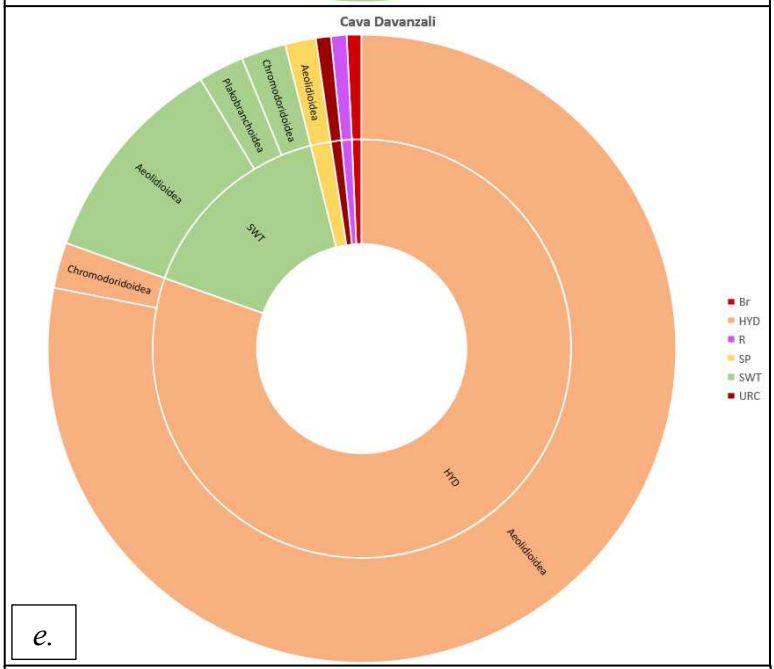
The following codes are used:

- **Sites:** 4G = 4 Grotte; CD = Cava Davanzali; DS = Due Sorelle; NIC = Relitto M/N Nicole; PST = Passetto (no tide pools); PSTp = Passetto (tide pools); RAM = Ramona; SCL = Scalaccia; SO = Secca dell'Ospedale; SN = Sassi Neri; SP = La Spiaggiola; SV = Scoglio della Vela; TR = Scoglio del Trave; VED = Spiaggia della Vedova.
- **Substrata:** ANT = Anthozoans; AS = Ascydians; AS+SP = Ascydians+sponges; BC = Rock covered by encrusting organisms/anellids; BIV = Bivalves; Br = Bryozoans; CobPeb = Cobbles or pebbles; CRB = Crab; FL = Floating; HYD = Hydroids; MS = Mussel shells; R = Rock; RCS = Rock covered by seaweeds; S = Sediment; SP = Sponges; SWT = Seaweeds/turf; URC = Sea urchin.
- **Sources:** CS = Citizen Science; LEK = Local Ecological Knowledge; Lit = Literature; PW = Present work; WEK = Web Ecological Knowledge.

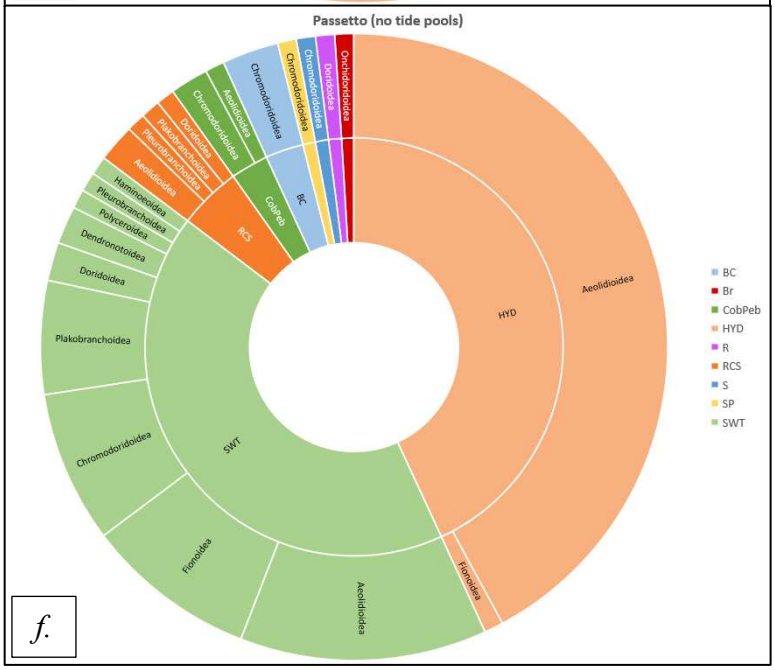

Relitto M/N Nicole
Aeolidioidea
Fionoidea
HYD
Aeolidioidea
Chromodoridoidea
SWT
Aeolidioidea
Fionoidea
MS
Dendronotoidea
Chromodoridoidea
Fionoidea
Aeolidioidea
BC
Chromodoridoidea
Aeolidioidea
Fionoidea
AS+SP
Pleurobranchoidea
Chromodoridoidea
AS
AS+SP
BC
Br
CRB
HYD
MS
RCS
S
SP
SWT
a.
Secca dell'Ospedale
Aeolidioidea
HYD
Aeolidioidea
SWT
Aeolidioidea
MS
Aeolidioidea
Aeolidioidea
BC
HYD
MS
SP
SWT
b.
Due Sorelle
Chromodoridoidea
SWT
Aeolidioidea
Doridoidea
Proctonoidea
HYD
Aeolidioidea
Dendronotoidea
BC
Chromodoridoidea
Aeolidioidea
MS
Aeolidioidea
Chromodoridoidea
Tritonioidea
Aeolidioidea
Phyllidioidea
Chromodoridoidea
Aeolidioidea
CobPeb
SP
S
Polycerioidea
AS
BC
BIV
Br
CobPeb
HYD
MS
R
S
SP
SWT
c.


Passetto (tide pools)
Aeolidioidea
Fionoidea
Plakobranchoidea
Plakobranchoidea
Aeolidioidea
Plakobranchoidea
Haminoeoidea
Polyceroidea
Dendronotoidea
Fionoidea
Aeolidioidea
FL
RCS
R
SWT
Plakobranchoidea
FL
HYD
MS
R
RCS
S
SWT

d.


Cava Davanzali
Aeolidioidea
Plakobranchoidea
Chromodoridoidea
Aeolidioidea
Chromodoridoidea
SWT
HYD
Aeolidioidea
Br
HYD
R
SP
SWT
URC

e.


Passetto (no tide pools)
Chromodoridoidea
Chromodoridoidea
Dorisoidea
Onchidoridoidea
Chromodoridoidea
Aeolidioidea
Chromodoridoidea
Dorisoidea
Plakobranchoidea
Pleurobranchoidea
Aeolidioidea
Haminoeoidea
Pleurobranchoidea
Polyceroidea
Dendronotoidea
Doridoidea
Plakobranchoidea
Chromodoridoidea
Fionoidea
Aeolidioidea
RCS
CobPeb
BC
SWT
HYD
Aeolidioidea
Fionoidea
BC
Br
CobPeb
HYD
R
RCS
S
SP
SWT

f.

The choice to represent superfamilies rather than species was made for ease of representation: within the same superfamily we can assume more or less the same ecological characteristics. We can observe that the superfamily most frequently found on the different substrata is the Aeolidioidea, immediately followed by the Chromodoridoidea. In terms of substrata, hydroids and seaweeds/turf are the most common for the majority of sites.

Comparison between tide and no tide pools substrata

A comparison of the sighting substrata in the Passetto tide pools with those in the rest of the Conero *(Figure 16)* shows that there is less diversification of substrata in the pools. Seaweeds/turf is the most common substrate in the pools, but only second to hydroids in the rest of the Conero. In the tide pools there is also a high proportion of records not related to a specific substrate, but to heterobranchs found floating upside-down at the water surface, taking advantage of the surface tension, as already reported by Riccardi et al. 2022. The sources for obtaining data for the two types of environment are different, as there are no LEK, literature and WEK data for the pools environment, which are instead available for the dive sites along with citizen science and the present work.

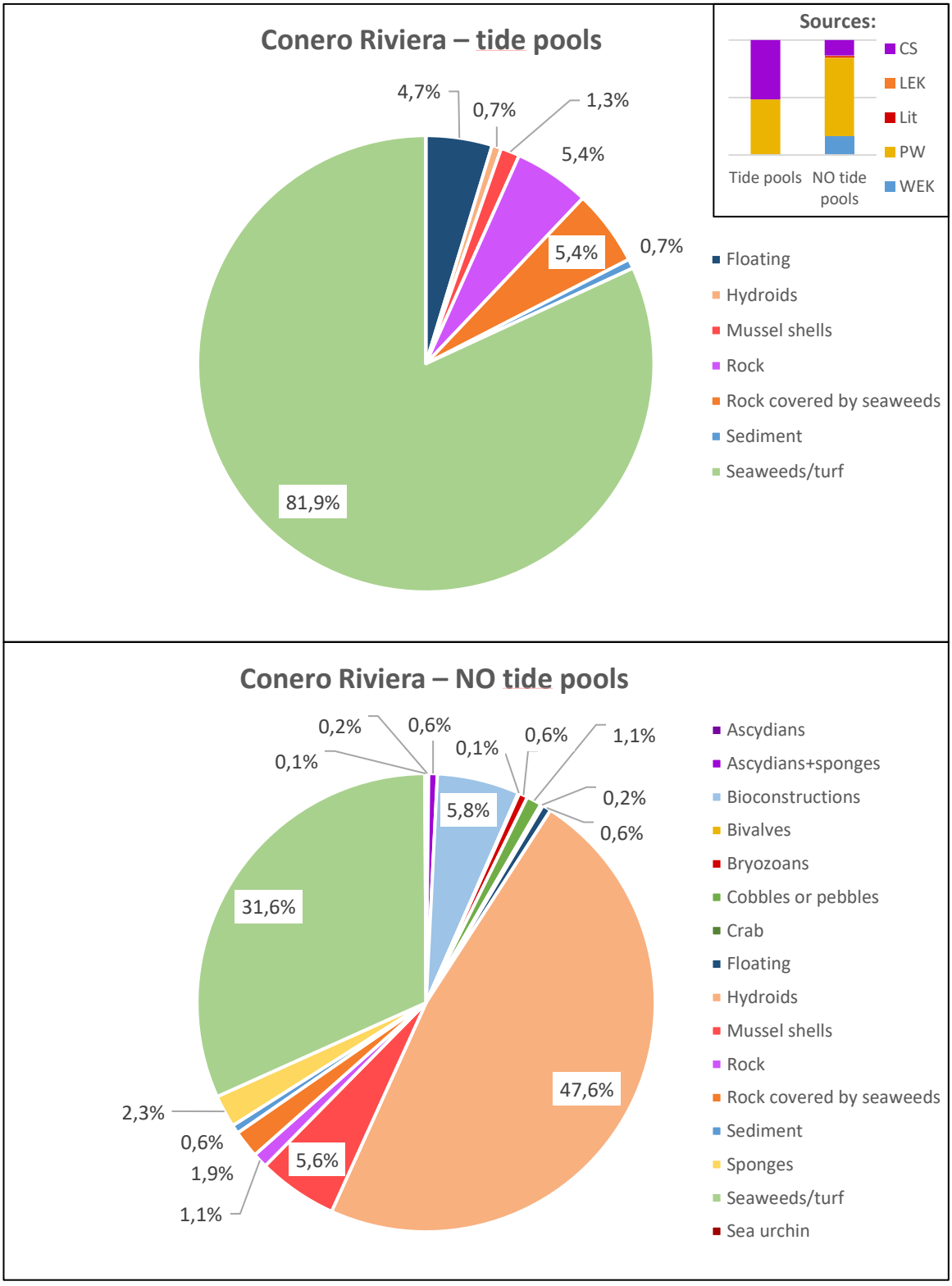


Figure 16. Comparison of substrata between tide pools habitat (at Passetto) and sites with no tide pools (4 Grotte, Cava Davanzali, Due Sorelle, Relitto M/N Nicole, Passetto, Ramona, Scalaccia, Secca dell'Ospedale, Sassi Neri, La Spiaggiola, Scoglio della Vela, Scoglio del Trave and Spiaggia della Vedova) in the Conero Riviera. The hystogram above shows the comparison between the contribution of the different sources.

Comparison between natural and artificial substrata species

For the Conero checklist, a comparison was made between species found on natural and artificial substrata. The comparison was made by means of a table *(Table 4)*, which showed that of the Conero species sighted (87 species), 49 species were found exclusively on natural substrate (57%), while only 11 were found exclusively on artificial substrate (13%): the latter belong to 5 of the 17 superfamilies present, while species exclusive to natural substrata are found in all superfamilies except Proctonoidea.

The percentage of species common to both substrata was also calculated. These species are more or less equally distributed in half of the superfamilies.

Superfamily	Conero species	Nature of substrate
Aeolidioidea	*Aeolidiella alderi*	Both
Aeolidioidea	*Aeolidiella sanguinea* *	Natural
Aeolidioidea	*Berghia coerulescens* *	Natural
Aeolidioidea	*Berghia verrucicornis*	Both
Aeolidioidea	*Calmella cavolini*	Both
Aeolidioidea	*Cratena peregrina*	Both
Aeolidioidea	*Edmundsella pedata*	Both
Aeolidioidea	*Facelina annulicornis* *	Natural
Aeolidioidea	*Facelina auriculata* *	Natural
Aeolidioidea	*Facelina bostoniensis* **	Artificial
Aeolidioidea	*Flabellina affinis*	Both
Aeolidioidea	*Nemesignis banyulensis* *	Natural
Aeolidioidea	*Paraflabellina ischitana*	Both
Aeolidioidea	*Spurilla neapolitana*	Both
Aplysioidea	*Aplysia* sp.*	Natural
Aplysioidea	*Aplysia fasciata* *	Natural
Aplysioidea	*Aplysia punctata* *	Natural
Aplysioidea	*Bursatella leachii* **	Artificial
Arminoidea	*Armina tigrina* *	Natural
Chromodoridoidea	*Felimare gasconi* *	Natural
Chromodoridoidea	*Felimare picta*	Both
Chromodoridoidea	*Felimare tricolor*	Both
Chromodoridoidea	*Felimare villafranca*	Both
Chromodoridoidea	*Felimida krohni*	Both
Chromodoridoidea	*Felimida luteorosea*	Both
Chromodoridoidea	*Felimida purpurea*	Both
Dendronotoidea	*Doto* sp. *	Natural
Dendronotoidea	*Doto cervicenigra* *	Natural
Dendronotoidea	*Doto coronata*	Both
Dendronotoidea	*Tethys fimbria* *	Natural
Doridoidea	*Discodoris rosi*	Both
Doridoidea	*Doris bertheloti*	Both
Doridoidea	*Doris ocelligera* *	Natural
Doridoidea	*Jorunna tomentosa*	Both
Doridoidea	*Paradoris indecora*	Both
Doridoidea	*Platydoris argo* *	Natural
Doridoidea	*Rostanga rubra*	Both
Doridoidea	*Thordisa azmanii* *	Natural
Doridoidea	*Thordisa filix* *	Natural
Fionoidea	*Amphorina* sp. *	Natural
Fionoidea	*Amphorina andra* *	Natural
Fionoidea	*Amphorina farrani* *	Natural
Fionoidea	*Amphorina linensis* *	Natural
Fionoidea	*Calma glaucoides* *	Natural

Superfamily	Conero species	Nature of substrate
Fionoidea	*Coryphella lineata* **	Artificial
Fionoidea	*Cuthona* sp. *	Natural
Fionoidea	*Eubranchus tricolor* **	Artificial
Fionoidea	*Eubranchus vittatus* *	Natural
Fionoidea	*Tergipes tergipes* **	Artificial
Fionoidea	*Trinchesia* sp.	Both
Fionoidea	*Trinchesia caerulea*	Both
Fionoidea	*Trinchesia cuanensis* *	Natural
Fionoidea	*Trinchesia diljuvia* *	Natural
Fionoidea	*Trinchesia genovae* *	Natural
Fionoidea	*Trinchesia morrowae* *	Natural
Haminoeoidea	*Haloa japonica* *	Natural
Haminoeoidea	*Haminoea* sp. *	Natural
Haminoeoidea	*Haminoea navicula* *	Natural
Onchidoridoidea	*Idaliadoris depressa* **	Artificial
Onchidoridoidea	*Idaliadoris neapolitana*	Both
Onchidoridoidea	*Okenia elegans* *	Natural
Onchidoridoidea	*Okenia mediterranea* **	Artificial
Onchidoridoidea	*Pelagella castanea* **	Artificial
Onchidoridoidea	*Trapania lineata* *	Natural
Onchidoridoidea	*Trapania maculata* *	Natural
Philinoidea	*Philine quadripartita* *	Natural
Philinoidea	*Philinopsis depicta* *	Natural
Phlyllidioidea	*Dendrodoris limbata* *	Natural
Plakobranchoidea	*Calliopaea bellula* *	Natural
Plakobranchoidea	*Elysia viridis* *	Natural
Plakobranchoidea	*Ercolania viridis* *	Natural
Plakobranchoidea	*Facelina rubrovittata*	Both
Plakobranchoidea	*Favorinus branchialis* *	Natural
Plakobranchoidea	*Limapontia capitata* *	Natural
Plakobranchoidea	*Placida dendritica* *	Natural
Plakobranchoidea	*Placida viridis* *	Natural
Plakobranchoidea	*Thuridilla hopei* *	Natural
Pleurobranchoidea	*Berthella perforata* *	Natural
Pleurobranchoidea	*Berthellina edwardsii* **	Artificial
Pleurobranchoidea	*Pleurehdera stellata* **	Artificial
Pleurobranchoidea	*Pleurobranchaea meckeli* **	Artificial
Polyceroidea	*Limacia clavigera**	Natural
Polyceroidea	*Polycera quadrilineata*	Both
Proctonoidea	*Antiopella cristata*	Both
Tritonioidea	*Candiella cincta*	Both
Tritonioidea	*Marionia blainvillea* *	Natural
Umbraculoidea	*Tylodina perversa* *	Natural

	Total	Exclusive	%
Natural substrata	75	49	**57%**
Artificial substrata	38	11	**13%**
Both	27	-	**31%**

Table 4. Conero species for which is recorded their presence on natural, artificial substrate or both in the Conero Riviera. The darker horizontal lines delineate different superfamilies. The percentages of exclusive (* = Natural; ** = Artificial) and in common species are calculated.

3.2 Comparative analyses with data collected for species found in the rest of the Mediterranean

For the Mediterranean Sea (excluding the Conero Riviera), as already mentioned, 641 records were collected, equally distributed among the 3 reference sources (CS, WEK and literature).

In particular, these records span the period from 2004 to 2024 (with 183 records in 2024, 181 in 2023 and 208 records with no year specified) and are mainly concentrated in Italy (268 records), Spain (184 records) and France (146 records).

Comparison between food preferences in the Mediterranean Sea of Conero heterobranchs and substrata in the Conero Riviera

The literature search in the Mediterranean (excluding the Conero Riviera) on the trophy of the heterobranchs of the Conero checklist revealed that there are 17 out of 92 species for which the trophy in the Mediterranean has not been recorded. Of the remaining 75, there are 52 species for which we have information on the method of obtaining the data, while for 23 species we have information on the trophy but no indication of the source of the information. Methods considered scientifically valid are the presence of zooxanthellae in the digestive gland as evidence of an anthozoan diet and of

chloroplasts in the case of an algal diet, the presence of secondary metabolites from sponges/other heterobranchs or remnants of the food itself such as pieces of sponge or Entoprocta, and direct observation of feeding behaviour in the field or in the aquarium. Of the 52 species mentioned above, the data on the food preferences of only 22 of them can be said to be scientifically proven. For the rest, trophic data are mostly based on field observations/photographs of the species in the vicinity of presumed prey.

For the same Conero species, all the information on the substrata on which they were observed in the Conero was taken from the dataset and supplemented with the photographs published in Betti, 2021: in this way it was possible to calculate the correspondence between substrate and prey for those species for which both pieces of information were available *(Table 5)*.

In the end, only 38% of the species showed a correspondence between diet and substrate. If we exclude the species for which we don't have trophic information, the percentage rises to 51%.

Another finding from the research on food preferences is that species within the same superfamily tend to have a similar diet.

Conero species	Substrata	Food preferences	Correspondence
Aeolidiella alderi	BC; MS; S; SWT; R	ANT	0
Aeolidiella sanguinea	BC; CobPeb	ANT	0
Berghia coerulescens	HYD; SWT	ANT	0
Berghia verrucicornis	BC; HYD; MS; SWT; R	ANT	0
Calmella cavolini	AS; BC; HYD; MS; RCS; SWT	HYD	1
Cratena peregrina	BC; CR; CRB; HYD; MS; SP; SWT; URC	HYD	1
Edmundsella pedata	BC; HYD; MS;RCS; SWT	HYD	1
Facelina annulicornis	BC; CobPeb; SWT	HYD	0
Facelina auriculata	S; SWT	HYD	0
Facelina bostoniensis	HYD; MS; SWT	nn	nn
Facelina dubia	HYD	nn	nn
Facelina rubrovittata	HYD; MS; RCS; SWT	HYD	1
Facelina vicina	nn	nn	nn
Favorinus branchialis	BC; Br; RCS; SWT	Eg	0
Flabellina affinis	CobPeb; CRB; HYD; MS; SWT	HYD	1
Nemesignis banyulensis	BC; HYD; SWT	Br; HYD; ANT	1
Paraflabellina ischitana	HYD; MS; SWT	HYD	1
Spurilla neapolitana	BC; CobPeb; MS; RCS; S; SWT	ANT	0
Akera bullata	SWT; R	SG; SWT	1
Aplysia fasciata	SWT	SWT	1
Aplysia punctata	SWT; RCS	SWT	1
Bursatella leachii	CobPeb; R	S; SWT; Bac	0
Armina tigrina	ANT; BC; S; SWT; R	nn	nn
Felimare gasconi	SP; SWT	nn	nn
Felimare picta	BC; MS; SP; SWT	SP	1
Felimare tricolor	BC; SWT	SP	0
Felimare villafranca	BC; CobPeb; MS; S; SP; SWT	SP	1
Felimida krohni	CobPeb; HYD; MS; SP; SWT; R; RCS	SP	1
Felimida luteorosea	BC; HYD; MS; S; SP; SWT	SP	1
Felimida purpurea	BC; HYD; MS; SP; SWT; RCS	SP	1
Doto cervicenigra	SWT	HYD	0
Doto coronata	HYD; MS; SWT	HYD	1
Tethys fimbria	S; R	CR; EC; F; MOL	0
Baptodoris cinnabarina	SWT	SP	0
Discodoris rosi	BC; MS; S; SP; SWT	nn	nn
Discodoris stellifera	SWT	SP	0
Doris bertheloti	CobPeb; MS; SWT	SP	0
Doris ocelligera	SWT	nn	nn
Doris pseudoargus	BC; SWT	SP	0
Jorunna tomentosa	MS; SP; R; RCS	SP	1
Paradoris indecora	HYD; MS; SP; SWT; RCS	SP	1
Platydoris argo	BC; CobPeb; MS; R	SP	0
Rostanga rubra	Br; SP; SWT	nn	nn
Thordisa azmanii	MS; S; SWT; R	nn	nn
Thordisa filix	MS; SWT	nn	nn
Amphorina andra	SWT	HYD	0
Amphorina farrani	HYD; MS; SWT	HYD	1
Amphorina linensis	BC; SWT	nn	nn
Calma glaucoides	nn	nn	nn
Catriona aurantia	HYD	nn	nn
Coryphella lineata	BC; HYD; MS	HYD	1
Cuthona sp.	SWT	CN	0
Eubranchus tricolor	BC; HYD; MS; SWT	HYD	1
Eubranchus vittatus	nn	HYD	nn
Tergipes tergipes	MS; SWT	HYD	0
Trinchesia caerulea	BC; HYD; MS; SWT	HYD	1
Trinchesia cuanensis	SWT	HYD	0
Trinchesia diljuvia	nn	nn	nn
Trinchesia genovae	nn	HYD	nn
Trinchesia morrowae	BC; HYD	HYD	1
Haloa japonica	S	nn	nn
Haminoea navicula	S; SWT; R	S; SWT; Carc	2
Idaliadoris depressa	Br	nn	nn
Idaliadoris neapolitana	Br; SWT	Br	1
Okenia elegans	HYD; RCS; SWT	AS	0
Okenia mediterranea	BC; MS; SWT	ANT	0
Pelagella castanea	AS; SWT	T	1
Trapania lineata	Br; SP; RCS	Ent	0
Trapania maculata	SP	Ent	0
Philine quadripartita	CobPeb; S; SWT	MOL; POL	0
Philinopsis depicta	S; SWT	Het	0
Dendrodoris limbata	CobPeb; R	nn	nn
Calliopaea bellula	HYD	Eg	nn
Elysia viridis	FL; R; RCS; SWT	SWT	1
Ercolania viridis	RCS; SWT	SWT	1
Limapontia capitata	nn	SWT	nn
Placida dendritica	RCS; SWT	SWT	1
Placida viridis	nn	SWT	nn
Thuridilla hopei	BC; MS; SP; SWT; RCS	SWT	1
Berthella perforata	BC; HYD; MS; RCS; SWT	SP; T	0
Berthellina edwardsii	AS+SP; BC; SWT; R	SP	1
Pleurehdera stellata	MS; R	SP or T	0
Pleurobranchaea meckeli	S; SWT	SP; POL; NEM; ANT; HYD; CR; Het; Carc	0
Pleurobranchus membranaceus	S; SWT	AS	0
Limacia clavigera	CobPeb; SWT	Br	0
Palio nothus	nn	Br	nn
Polycera quadrilineata	Br; HYD; MS; SWT	Br	1
Antiopella cristata	BC; Br; HYD; MS; S; SP; SWT; R; RCS	Br	1
Candiella cincta	ANT; BIV; MS; SWT	ANT	1
Candiella manicata	MS	ANT	0
Marionia blainvillea	ANT; MS	ANT	1
Tylodina perversa	BC; MS; SP; SWT	SP; Cy	1

	Total	%	% (nn excluded)
Correspondences	36 (of which 8)	38% (9%)	**51% (11%)**
NO correspondences	33	35%	**46%**
nn	23	24%	-

Table 5. Comparison between Conero substrata, from occurrence records and pictures in Betti 2021, and food preferences in the Mediterranean Sea from literature for the Conero species. The darker horizontal lines delineate different superfamilies. The codes in red represent the food sources verified using appropriate analyses. The correspondence number indicates how many of the food sources match the substrata (red lettering is used for the match with certified food preferences). The percentages of correspondence are calculated.

The following codes are used: ANT = Anthozoans; AS = Ascidians; AS+SP = Ascydians+sponges; Bac = Bacteria; BC = Rock covered by encrusting organisms/anellids; BIV = Bivalves; Br = Bryozoans; Carc = Carcasses; CN = Cnidarians; CobPeb = Cobbles or pebbles; CR = Crustaceans; CRB = Crab; Cy = Cyanobacteria; EC = Echinoderms; Eg = Eggs; Ent = Entoprocta; F = Fishes; FL = Floating; Het = Heterobranchia; HYD = Hydroids; MOL = Molluscs; MS = Mussel shells; NEM = Nematodes; POL = Polychaetes; R = Rock; RCS = Rock covered by seaweeds; SG = Seagrasses; SWT = Seaweeds/Turf; S = Sediment; SP = Sponges; T = Tunicates; URC = Sea urchin.

Analysis of substrate preferences

From the records reported for the Mediterranean, it was possible to compare the substrate preferences recorded for the Conero with those for the rest of the Mediterranean *(Figure 17)*.

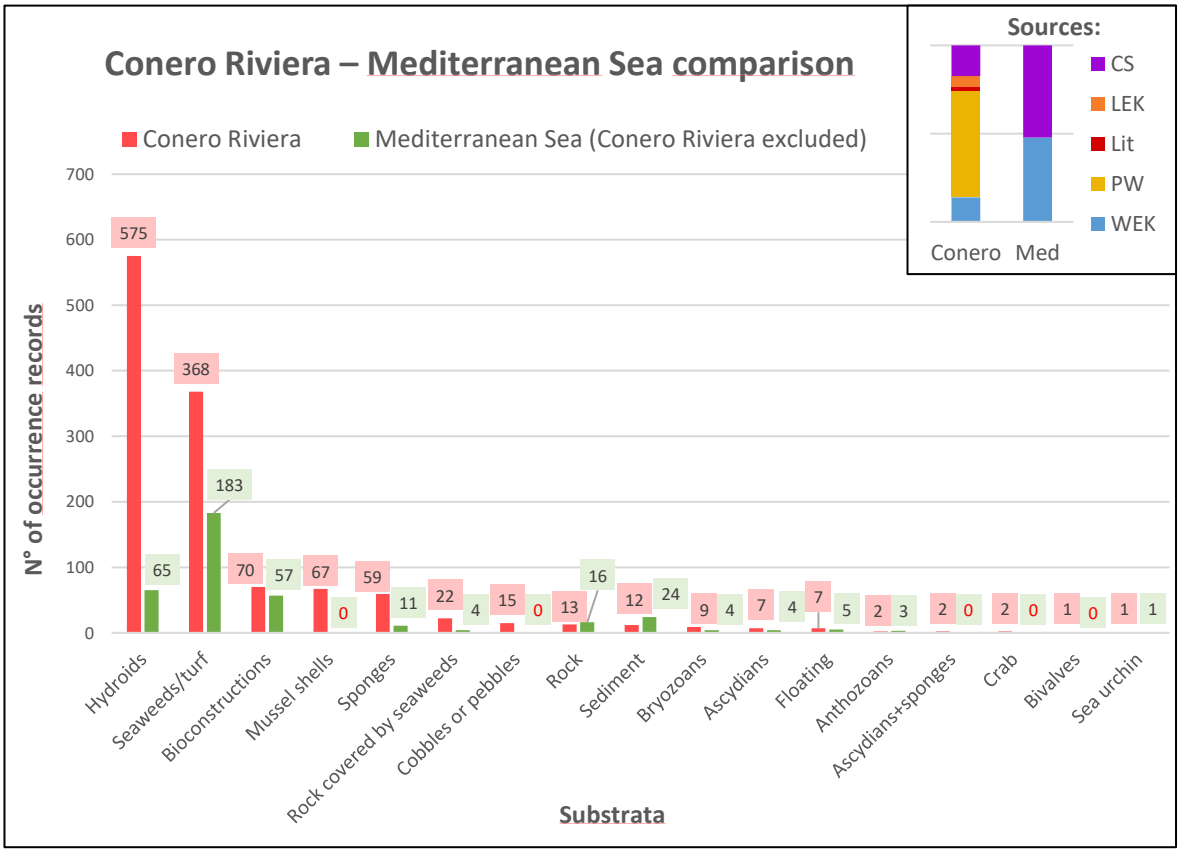


Figure 17. Comparison between the number of records for each substrate in the Conero Riviera and in the Mediterranean Sea (Conero Riviera excluded). The hystogram above shows the contribution of each source. The substrata with 0 records are written in red.

Although we have considerably more data for the Conero, where all the present work was carried out, the trend is similar, with hydroids, seaweeds/turf and bioconstructions being the most common substrata in both areas. There are five substrata (mussel shells, cobbles or pebbles, ascidians+sponges, crab and

bivalves) that are represented in the Conero but not in the rest of the Mediterranean. It should be noted that in 264 of the 641 Mediterranean records, the substrate was not specified.

The five most represented substrata in the Conero (≥ 50 records) were used to make a comparison with the Mediterranean in terms of the most abundant superfamilies on each substrate *(Figure 18)*. It was not possible to make a comparison for substrate mussel shells as no records were collected in the Mediterranean.

The most abundant superfamily coincides between the Conero and the Mediterranean for 2 of the 5 substrata considered (Aeolidioidea for hydroids and seaweeds/turf, which are also the most frequent substrata), whereas for bioconstructions and sponges the most abundant superfamilies are Chromodoridoidea for the Conero and Aeolidioidea and Doridoidea for the Mediterranean. On mussel shells, the most abundant superfamily is again the Aeolidioidea.

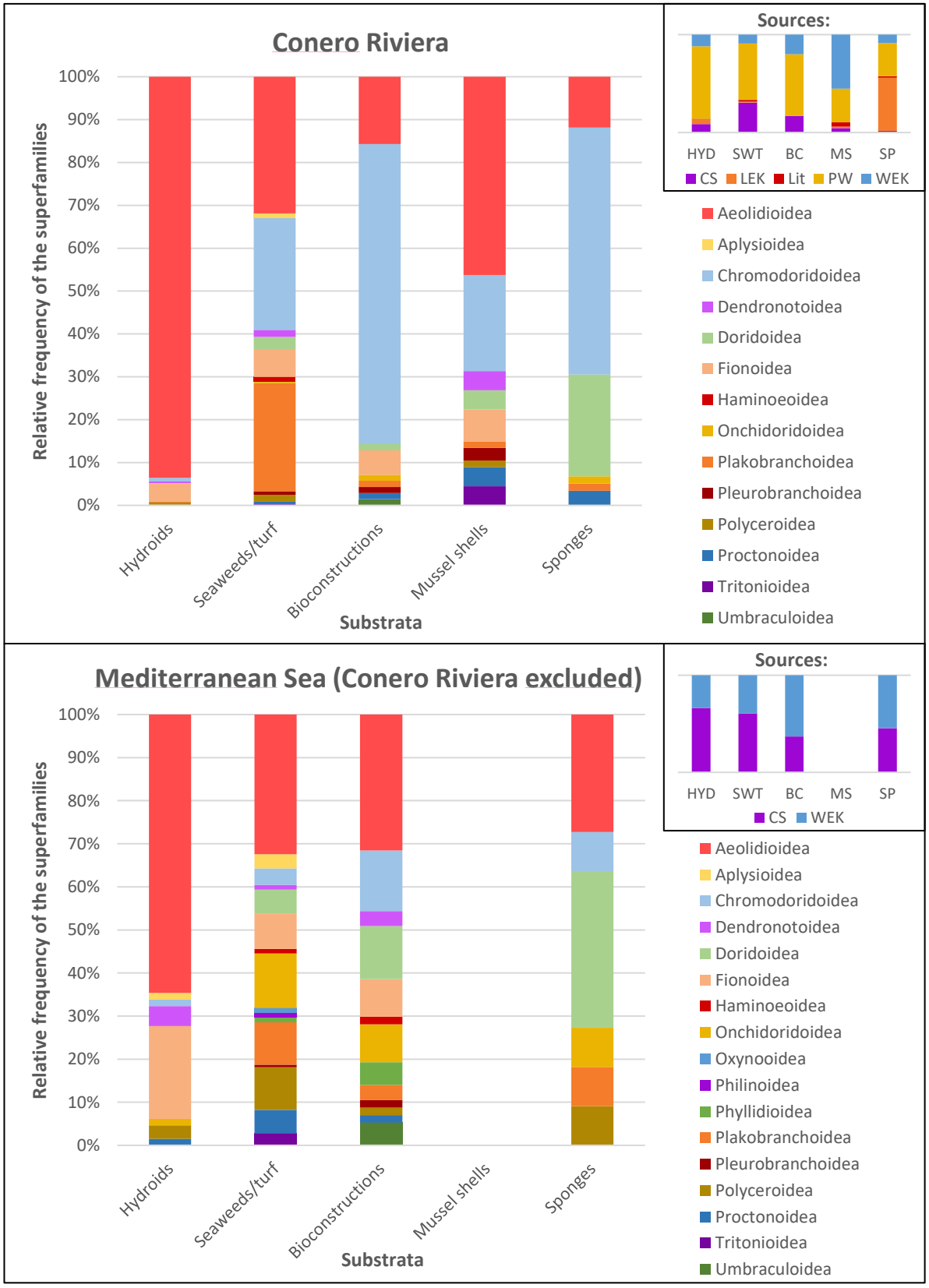


Figure 18. Comparison between the relative frequency of the superfamilies for different substrata in the Conero Riviera and in the Mediterranean Sea (Conero Riviera excluded). The small hystograms represent the contribution of each source. The columns are in order from the most to the less abundant substrate for the Conero Riviera.

In addition to the comparison of substrata, a further comparison was made between the Conero and the Mediterranean by considering only the most abundant species found in the Conero (≥ 50 records), i.e. in order of abundance: *Cratena peregrina* (Gmelin, 1791), *Flabellina affinis* (Gmelin, 1791), *Edmundsella pedata* (Montagu, 1816), *Felimida luteorosea* (Rapp, 1827), *Elysia viridis* (Montagu, 1804), *Paraflabellina ischitana* (Hirano & T. E. Thompson, 1990) and *Felimare villafranca* (Risso, 1818). For these species, an analysis was made of the substrata where they were found *(Figure 19)*.

The graph shows that there is more substrate differentiation in the Conero than in the rest of the Mediterranean for the same species. The dominant substrata for most species are hydroids and seaweeds/turf, both in the Conero and in the Mediterranean. Significant fractions of bioconstructions and sediment are observed in the Mediterranean, whereas they are almost absent in the Conero.

The sources in the Mediterranean are exclusively CS and WEK, in contrast to the Conero, where present work (the majority) and LEK are added to these two.

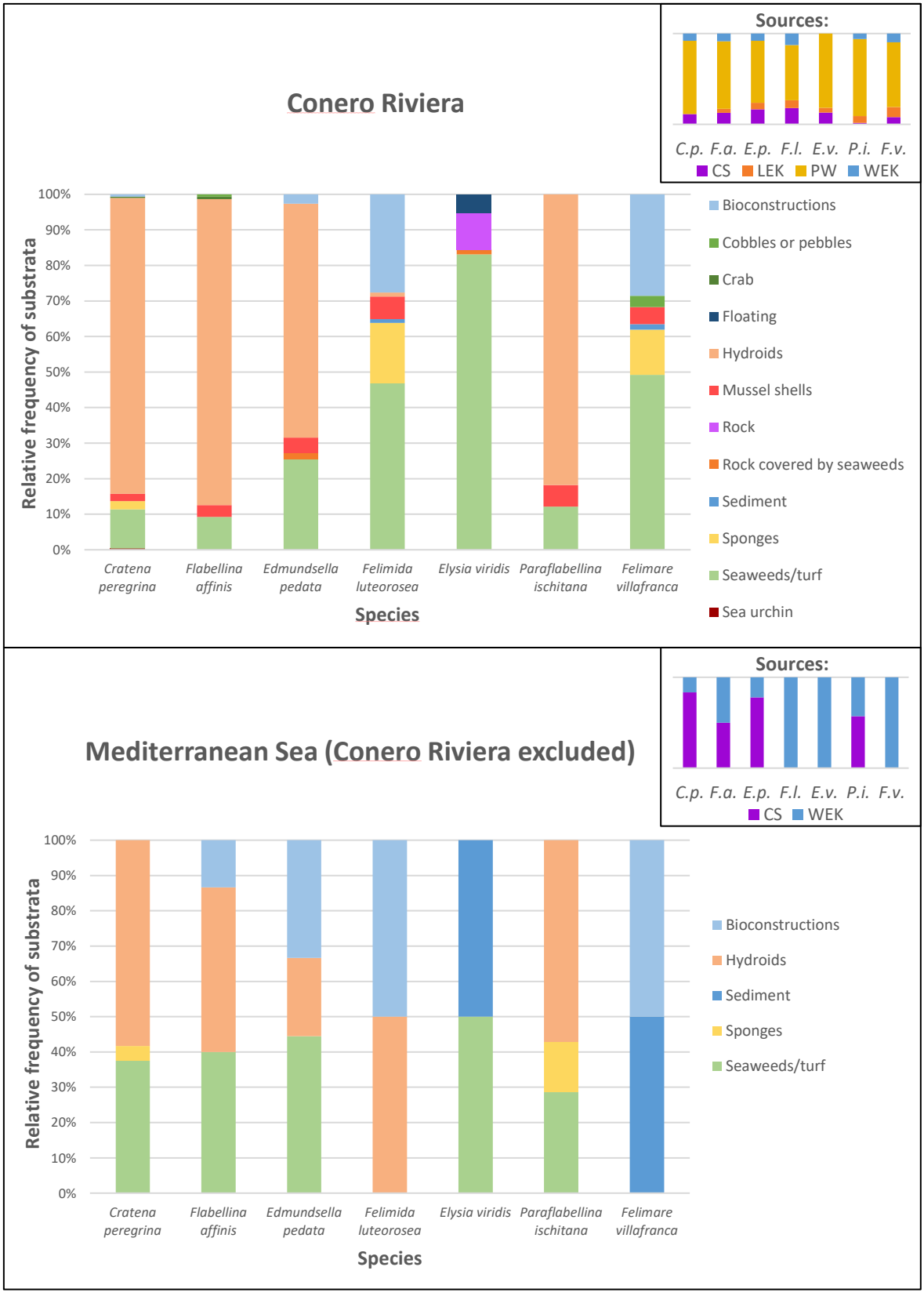


Figure 19. Comparison between the relative frequency of the substrata for different species in the Conero Riviera and in the Mediterranean Sea (Conero Riviera excluded). The small hystograms represent the contribution of each source.

Comparison between natural and artificial substrata species

For both the Conero and the Mediterranean, an analysis of the type of substrate was carried out in order to observe whether heterobranchs showed a preference for natural or artificial substrata *(Figure 20)*.

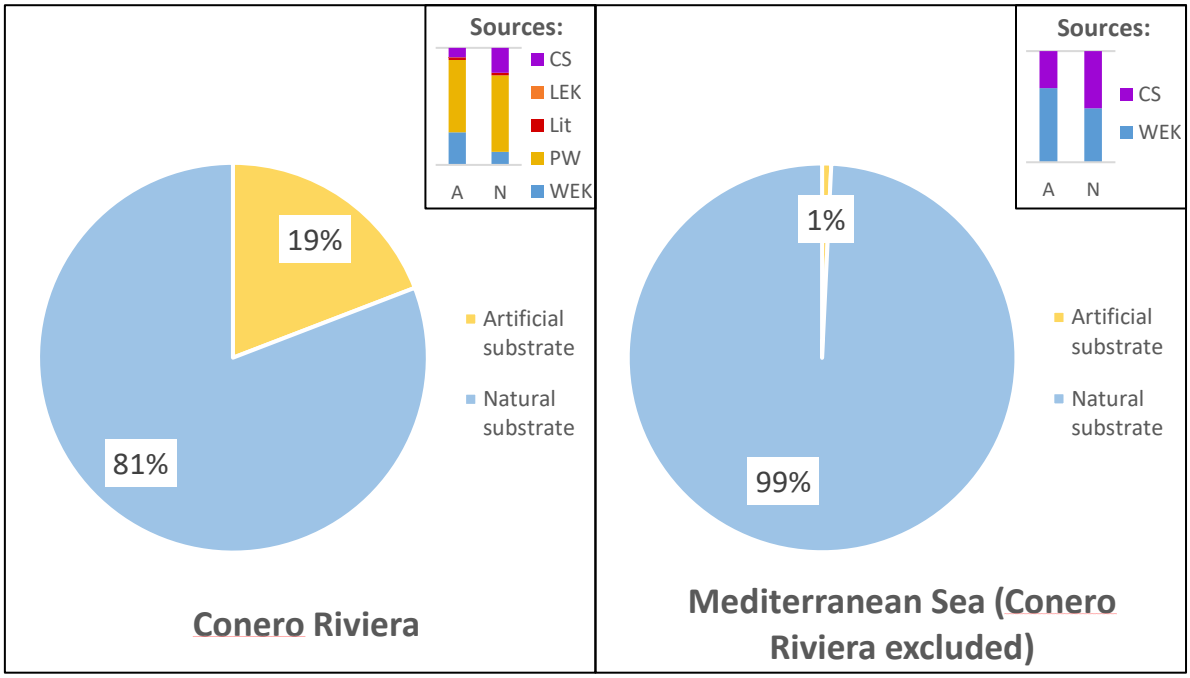


Figure 20. Comparison between the nature of substrata in the records of the Conero Riviera and in the Mediterranean Sea (Conero Riviera excluded). The relative frequency is represented. The following codes are used: "N" for natural substrata; "A" for artificial substrata. The hystograms above show the contribution of each source.

In addition, a further comparison was made to see if the preference of heterobranchs for a particular type of substrate was related to the superfamily to which they belonged *(Figure 21)*.

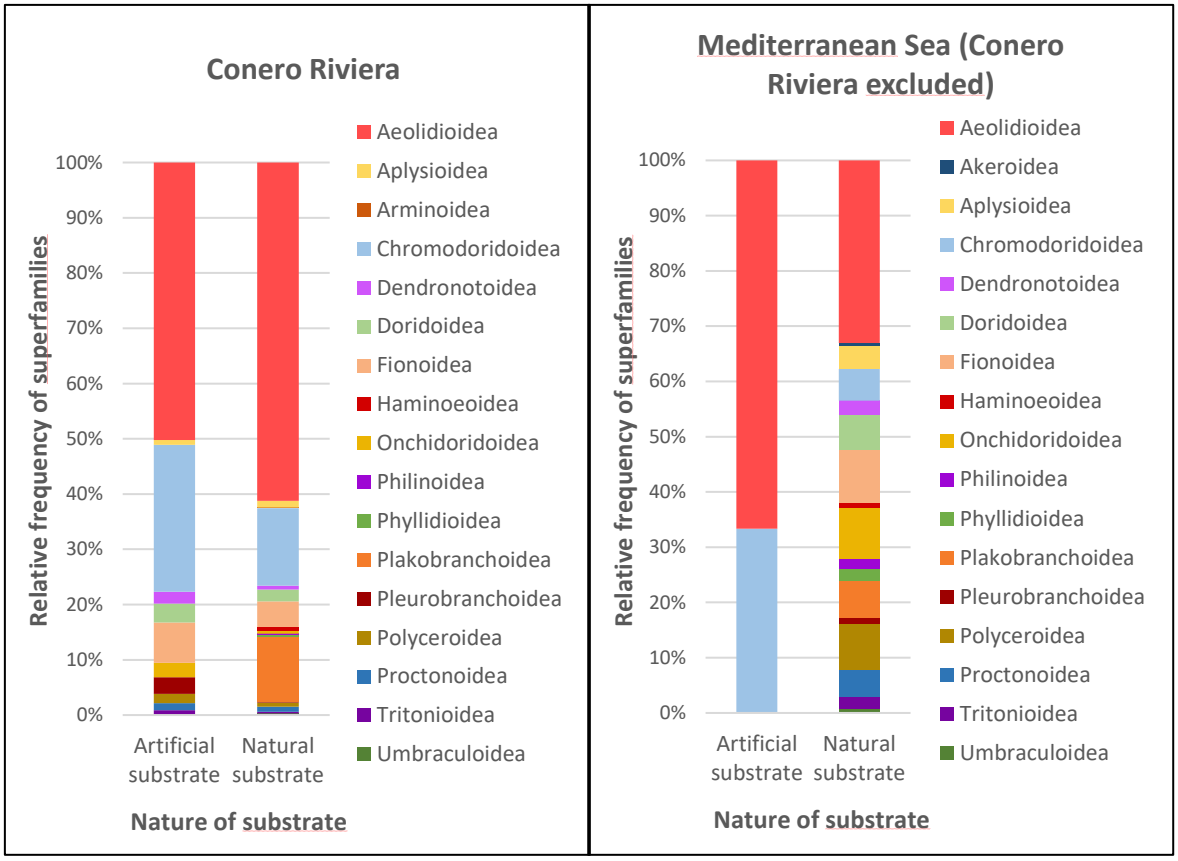


Figure 21. Comparison between the relative frequency of superfamilies depending on the nature of substrata in the Conero Riviera and in the Mediterranean Sea.

Comparison between exposed and hidden species

A similar analysis to that carried out for substrate type was performed to calculate the percentage of records of individuals found in hidden or exposed locations *(Figure 22)*, and consequently the most common superfamilies found according to exposure *(Figure 23)*. The hidden species with ≥ 3 records are highlighted.

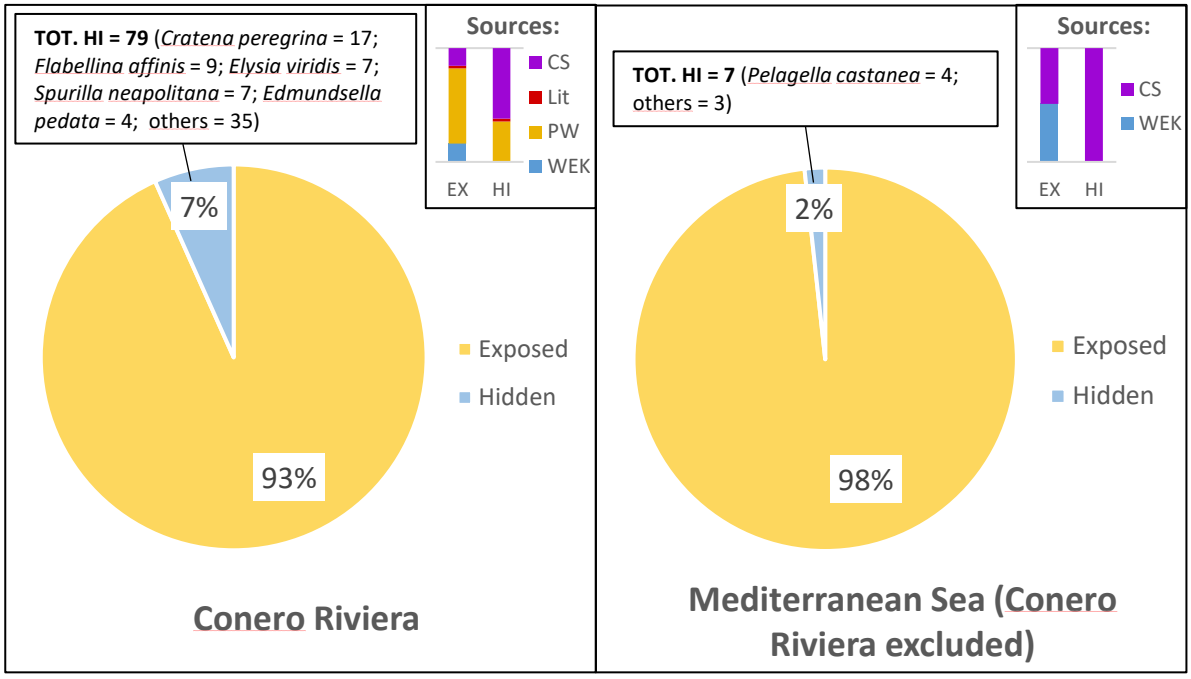


Figure 23. Comparison between the exposure in the records of the Conero Riviera and in the Mediterranean Sea (Conero Riviera excluded). The relative frequency is represented. The following codes are used: "EX" for exposed individuals; "HI" for hidden individuals. The species to which the hidden individuals belong is highlighted. The hystograms above show the contribution of each source.

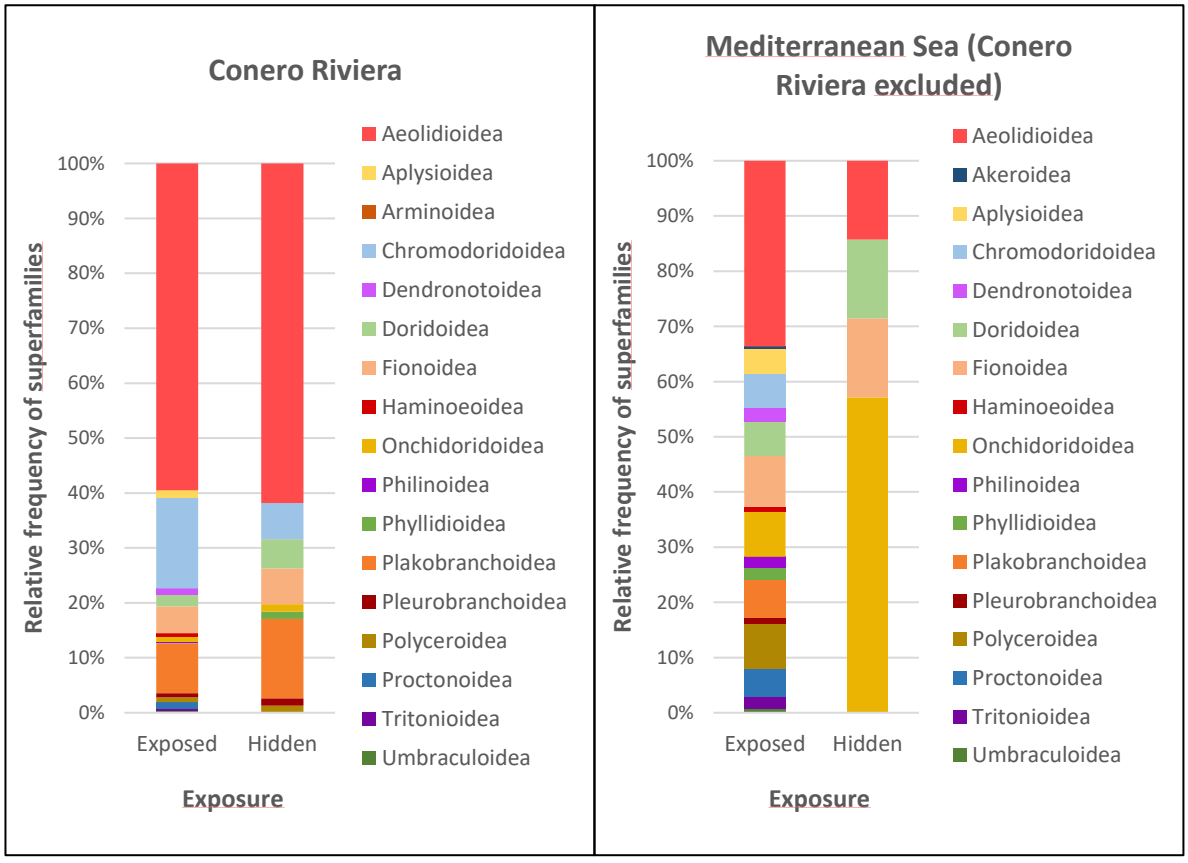


Figure 22. Comparison between the relative frequency of superfamilies depending on the exposure in the Conero Riviera and in the Mediterranean Sea.

4. DISCUSSION

Prior to this work, two studies were conducted on the heterobranch molluscs of the Conero Riviera (Betti, 2021, and Riccardi et al., 2022, this last focusing on the intertidal environment of rocky tide pools), which reported 75 species (72 from the study by Betti, 2021 and 3 sighted for the first time by Riccardi et al., 2022). The integration of this literature data with direct observation and data from LEK and citizen science, has led to the implementation of this list, which now includes 92 species (17 more than previously recorded).

Moreover, it has been confirmed that the use of alternative sources (citizen science, LEK and WEK) can play an important role in increasing scientific knowledge on benthic invertebrates (Di Camillo et al., 2018; Krželj et al., 2020), contributing with 670 records (44% of the dataset).

The data collection work carried out here therefore greatly enriches the ecological and behavioural knowledge of the Conero species.

4.1 Substrata of heterobranchs from the Conero Riviera

For the Conero Riviera, heterobranch records were collected for 15 sites. However, not all of these provide relevant information, as some have less than

10 records. Furthermore, for 3 of them, the few data available come only from CS, in particular from records provided by volunteers without pictures (a source that cannot be scientifically verified). The present work covers 10 of the 15 sites, 6 of which are also the sites with the most records. In particular, the top 3 sites with the most records are generally well frequented by local dive centres who tend to bring their tourists there: Relitto M/N Nicole, Secca dell'Ospedale and Due Sorelle.

It is interesting to note that in fourth place is Passetto, particularly the tide pools environment, with 160 records, of which less than half are present work: in fact, the accessibility of the intertidal environment favours citizen science activities.

The analysis of substrata in relation to sites shows that the most observed for most sites is hydroids, followed by seaweeds/turf. This may indicate that these are the preferred food sources of the local heterobranch species (Adiwijaya et al., 2021). The analysis of the most abundant superfamilies in relation to substrate may further support this hypothesis, showing that approximately the same superfamilies are always found on each substrate.

The most abundant superfamily is undoubtedly the Aeolidioidea, which was also the one with the most species in the Conero. The Chromodoridoidea, although not among the most species-rich superfamilies, still represent a good fraction of the records for several substrata, just after the Aeolidioidea.

In terms of substrata, the comparison between the Passetto tide pools and the rest of the Conero shows that the prevalence of hydroids, generally observed in almost all the sites, is not found in the intertidal environment, where there is a prevalence of seaweeds/turf: this may be due to the fact that the tide pools environment has extreme conditions (Huggett & Griffiths, 1986), perhaps less tolerant for the growth of more complex organisms such as hydroids, bivalves, sponges or encrusting organisms in general. This is reflected in the fact that the dominant substrata in the pools environment after seaweeds are precisely "non-living" substrata (rock, floating heterobranchs, sediment). The consequence is a reduced substrate diversity in the tide pools.

The analysis of sources shows that the data for tide pools come exclusively from citizen science and present work, highlighting the paucity of specific studies on this topic in the literature. Even when considering citizen science, tide pools have a higher percentage of data than all other sites combined: this reiterates the importance of site accessibility for volunteer participation in data collection and monitoring projects (Jacob, 2008).

With regard to the comparison of natural and artificial substrate species, it is important to note that most of the sites examined had exclusively natural substrata. In fact, none of the records correspond to heterobranchs found directly on artificial substrate, but the secondary substrate was taken into

account (i.e. the nature of the surrounding habitat). The sites with a predominance of artificial substrate are Relitto M/N Nicole, Due Sorelle (due to the presence of the Nicole and Potho wrecks) and La Spiaggiola (characterised by a breakwater reef perpendicular to the coast).

A large proportion (57%) of the Conero species for which we have information on the nature of the substrate were found exclusively on natural substrata: this is probably due to the fact that, as mentioned above, few sites have artificial substrata. However, the percentage of species found exclusively on artificial substrate is a good 13%, while 31% of species were sighted on both types. Although the species exclusive to artificial substrate are grouped in a few superfamilies (5 out of 17), no pattern correlating superfamily-substrate nature can be found. This lack of correlation is even more evident when we consider that the natural substrate-exclusive species are evenly distributed among the superfamilies.

The literature has shown how the type of substrate influences the benthic assemblages, which is also reflected in the associated communities: this is due to differences related to habitat complexity, wave exposure, age of the substrate, dispersal potential of propagules and larvae, substrate inclination and orientation (Sedano Vera et al., 2019), which generally lead to lower biodiversity on artificial substrata (Airoldi & Bulleri, 2011). This lower

biodiversity is also due to the dominance of fouling species, which tend to monopolize the available space and thus reduce the amount of substrate available for heterobranchs (Siddik et al., 2019).

In this case, however, not many conclusions can be drawn as it must be taken into account that 983 records were collected on natural substrata, compared to 233 on artificial substrata (4:1 ratio): it is possible that a more targeted data collection, perhaps with an equal number of samples on both types of substrate, could give clearer results.

4.2 Differences and similarities

The search for information on the trophy of heterobranchs in the literature showed that there are gaps in the ecological knowledge of the Conero heterobranchs. In fact, the trophy of 19% of the species on the checklist has not been recorded throughout the Mediterranean. For the remaining species, trophic information is available, but for about 1/3 of these the source is unknown, so it cannot be concluded that the information is scientifically valid. Even where the source is known, it is not always based on a valid scientific method: overall, only 22 out of 92 species (24%) can be said to have a diet that is known with certainty and scientifically proven.

These data on trophy were compared with the substrate of the records for the Conero Riviera: in practice, the substrata corresponding to the dietary preferences extrapolated from the literature were counted and this number was converted into correspondence. Only for 51% of the species for which it was possible to make the calculation (some did not have both data) a match was found, always with a value of 1 (except for the species *Haminoea navicula* (da Costa, 1778), the only one with a value of 2). However, if we only consider species with a verified food source, this percentage drops dramatically to a value of 11%. Furthermore, it is possible that the substrate observed in the pictures from WEK was chosen by the photographer which moved the animal to have a better background.

An analysis of the substrata photographed together with the nudibranch, shows that the most common are sessile organisms, among which hydroids, seaweeds/turf and bioconstructions are recorded both for the Conero area and the Mediterranean Sea. At least hydroids and seaweeds/turf may represent food sources for the considered molluscs.

Mussel shells are not represented as a substrate in the rest of the Mediterranean, although they are one of the substrata with the most records for the Conero. In fact, *Mytilus galloprovincialis* Lamarck, 1819 forms mussel beds that constitute a very important biocenotic component in the Riviera, hosting a rich

community of associated organisms, including heterobranchs (Cerrano et al., 2014). However, as the data collection was different between the Conero and the rest of the Mediterranean (the sources considered are not the same), it would be wrong to conclude that this is the cause of the difference.

For the rest of the Mediterranean, there are substrata with 0 records, which are instead represented in the Conero: this is probably because the present work focused on the latter, leading to a gap between the number of records in the two areas (1536 against 641).

For the comparison between the superfamilies, only the substrata with more than 50 records in the Conero (hydroids, seaweeds/turf, bioconstructions, mussel shells and sponges) were considered. As far as hydroids are concerned, the most common superfamilies for both areas are Aeolidioidea and Fionoidea: the comparison with their feeding preferences seems to confirm that hydroids are among the favourite prey of both superfamilies. The Aeolidioidea also seem to be the most abundant superfamily on seaweeds/turf (both for the Conero and the Mediterranean), mussel shells and bioconstructions (only for the Mediterranean), although this is not directly reflected in their dietary preferences. The superfamily Chromodoridoidea in the Conero represents an important fraction on bioconstructions and sponges: this is consistent with their based on Porifera. Doridoidea were also abundant on sponges in both areas,

again consistent with their trophy. Only in the case of the Conero Plakobranchoidea represent a significant percentage on seaweeds/turf, which can always be explained by a diet based on algae. In the case of the Mediterranean, there seems to be a greater diversity of superfamilies in almost all the substrata represented, which makes the dominance of some over others less evident. However, it cannot be concluded that this is due to a difference in biodiversity between the two areas, since the absence of some superfamilies in the Conero representation is due to a lack of substrate data and not to an actual absence of records for these species.

The generalization by superfamily was made for the sake of simplicity, as it was observed in *Table 5* that species within the same superfamily have almost similar diets. A more specific analysis, considering species instead of superfamilies, was carried out only for those with more than 50 records in the Conero Riviera. The results on the substrata of these species observed in the Conero are mostly in line with their food preferences: there is a strong preponderance of hydroids for the Aeolidioidea, i.e. *Cratena peregrina* (Gmelin, 1791), *Flabellina affinis* (Gmelin, 1791), *Edmundsella pedata* (Montagu, 1816) and *Paraflabellina ischitana* (Hirano & T. E. Thompson, 1990), seaweeds/turf for *Elysia viridis* (Montagu, 1804) (Plakobranchoidea) and a good percentage of bioconstructions and sponges for the

Chromodoridoidea *Felimida luteorosea* (Rapp, 1827) and *Felimare villafranca* (Risso, 1818), although for the latter the prevalence is seaweeds/turf. For the rest of the Mediterranean, these results seem to be fairly confirmed, although the dominance of one substrate over another is less obvious, probably due to the much smaller number of records.

A comparison between natural and artificial substrate shows that the Conero has a much higher percentage of records on artificial substrate than the rest of the Mediterranean (19% vs. 1%). However, this result cannot be taken as an indication of a greater preference of heterobranchs for artificial substrate in one area over the other, as this could be due to the fact that the sites considered do not have enough of this type of substrate. In general, however, a dominant percentage of records found on natural substrate is observed for both areas.

As far as the superfamilies of the Conero are concerned, almost the same taxa are observed on the two types of substrate: the only difference that is quite obvious is the proportion of Plakobranchoidea, which is rather pronounced on the natural substrate and absent on the artificial one. However, as the number of records for the two types of substrate is very different, no hypotheses can be made to justify this difference. In the rest of the Mediterranean, the natural substrate has approximately the same taxa as the Riviera del Conero, only more evenly distributed, while the number of superfamilies for the artificial substrate

is reduced to 2: not enough records were collected on the artificial substrate to allow a meaningful comparison between substrata of different types. However, it should be noted that these two superfamilies are the two most represented on the artificial substrate of the Conero Riviera.

The analysis carried out to highlight any preference of heterobranchs for exposed rather than hidden substrate showed a percentage of >90% for exposed substrate for both the Conero and the rest of the Mediterranean. This does not necessarily mean that heterobranchs do not tend to hide, as it must be considered that if they are hidden, they are also more difficult to find and therefore to record. The species with the highest number of hidden records on the Conero are also the species with the highest number of records overall; therefore, they may be the easiest to find precisely because they are more abundant. This does not mean, then, that the most frequently found hidden species are necessarily those that prefer this type of lifestyle. As far as the Mediterranean is concerned, the number of hidden records is much smaller, with only the species *Pelagella castanea* (Alder & Hancock, 1845) having more than 3 records.

The comparison between the superfamilies for the Conero shows a greater diversity on the exposed type substrate, as might be expected from the difference in the number of records: however, this difference is rather slight.

The same cannot be said for the rest of the Mediterranean, where only four taxa were found on the hidden substrate. With regard to the exposed individuals, however, approximately the same superfamilies were found as in the Conero, but they were distributed more evenly.

These comparisons based on nature of substrate/exposure unfortunately do not have sufficient data to establish a possible preference of superfamilies, and more generally of heterobranchs, for one type of substrate over another. Nevertheless, the data collected in this work could form the basis for future studies in this regard.

5. CONCLUSION

With a total of 2177 records, collected using 5 different valid sources, this work contributes to the enrichment of the currently scarce knowledge of the Heterobranchia of the Conero Riviera and the rest of the Mediterranean.

It also highlights the importance of volunteers' awareness and participation in data collection programmes, all facilitated by technological advances. Therefore, it could be of interest to inform an increasing number of citizens about the importance of their contribution to the advancement of scientific knowledge. In this context, the dataset constructed in this study could provide guidelines for a more targeted data collection: in fact, only by collecting a large amount of data on a broad scale, the habit of heterobranchs to have a small home range can be demonstrated with greater certainty.

In order to collect more and more records, all sources should be considered, including "emerging" ones (Social Media such as Instagram or Tik Tok, which have become increasingly popular in recent years).

The collection of a huge number of records could also be used to demonstrate that the small home range is strictly related to the trophy. Since information on the trophy of heterobranchs is often published without a source and without being correlated by scientific analyses to prove the veracity of the information, further studies should be carried out to fill these gaps that currently exist in the

literature: in order to confirm with scientific accuracy the food preferences of the various species, it would be necessary to proceed, for example, with analyses of stomach contents or radula typology, or through observation of feeding behaviour in aquaria.

In conclusion, in a context such as the present one, studies like this one are therefore crucial to increase the efficiency of data collection and the understanding of the heterobranchs-prey relationship: only once a good level of knowledge has been achieved, could one consider using heterobranchs as bioindicators to monitor sessile species in response to the increasingly evident climate change.

6. REFERENCES

Aboul-Ela, I. A., 1959. On the Food of Nudibranchs. *Biological Bulletin,* 117(3), pp. 439-442.

Adiwijaya, C., Bengen, D. G. & Zamani, N. P., 2021. Coral reefs substrate composition influence on nudibranch diversity. *IOP Conference Series: Earth and Environmental Science,* 771(1), p. 012009.

Aikem, R. B., 2003. Some aspects of the life history of an intertidal population of the nudibranch Dendronotus frondosus (Ascanius, 1774) (Opisthobranchia: Dendronotoidea) in the Bay of Fundy. *The Veliger,* 46(2), pp. 169-175.

Albano, P. G. et al., 2021. Native biodiversity collapse in the eastern Mediterranean. *Proceedings of the Royal Society B,* 288(1942), p. 20202469.

Albouy, C. et al., 2012. Combining projected changes in species richness and composition reveals climate change impacts on coastal Mediterranean fish assemblages. *Global Change Biology,* 18(10), pp. 2995-3003.

Allf, B. C. et al., 2022. Citizen Science as an Ecosystem of Engagement: Implications for Learning and Broadening Participation. *BioScience,* 72(7), pp. 651-663.

Anadón, J. D., Giménez, A., Ballestar, R. & Pérez, I., 2009. Evaluation of local ecological knowledge as a method for collecting extensive data on animal abundance. *Conservation biology,* 23(3), pp. 617-625.

Andrimida, A. & Hermawan, R., 2020. Assessing cryptic marine fauna diversity as underwater macrophotography (UMP) objects in Sempu Strait, Indonesia. *E3S Web of Conferences,* Volume 153, p. 01001.

Archambault, J. M., Cope, W. G. & Kwak, T. J., 2018. Chasing a changing climate: Reproductive and dispersal traits predict how sessile species respond to global warming. *Diversity and Distributions,* 24(7), pp. 880-891.

Arey, L. B., 1918. The multiple sensory activities of the so-called rhinophore of nudibranchs. *American Journal of Physiology-Legacy Content,* 46(5), pp. 526-532.

Aringoli, D. et al., 2014. Il ruolo della gravità nell'evoluzione geomorfologica di un'area di falesia: il caso del Monte Conero (Mare Adriatico, Italia centrale). *Studi costieri,* Volume 22, pp. 19-32.

Badreddine, A., Bitar, G. & Sanamyan, N., 2022. First Record of Four Species of Sea Anemones (Cnidaria: Actiniaria) For Lebanese Coast, Eastern Mediterranean Sea. *Journal of Fisheries and Livestock Production,* 10(3), p. 333.

Becerro, M. A., Turon., X., Uriz, M. J. & Templado, J., 2003. Can a sponge feeder be a herbivore? Tylodina perversa (Gastropoda) feeding on Aplysina aerophoba (Demospongiae). *Biological Journal of the Linnean Society,* 78(4), pp. 429-438.

Belmonte, T., Alvim, J., Padula, V. & Muricy, G., 2015. Spongivory by nudibranchs on the coast of Rio de Janeiro state, southeastern Brazil. *Spixiana,* 38(2), p. 187.

Benham, C. F., 2017. Aligning public participation with local environmental knowledge in complex marine social-ecological systems. *Marine Policy,* Volume 82, pp. 16-24.

Berglund, A., 1990. Sequential hermaphroditism and the size-advantage hypothesis: an experimental test. *Animal Behaviour,* 39(3), pp. 426-433.

Betti, F., 2010. *La Fauna Marina della Riviera del Conero.* Imola: Editrice La Mandragora.

Betti, F., 2021. *Il regno dei nudibranchi. Guida ai molluschi eterobranchi della Riviera del Conero.* Imola: Editrice La Mandragora.

Bianchi, C. N. et al., 2019. Consequences of the marine climate and ecosystem shift of the 1980-90s on the Ligurian Sea biodiversity (NW Mediterranean). *The European Zoological Journal, 86(1), ,* pp. 458-487.

Biondi, E., Bagella, S., Casavecchia, S. & Pinzi, M., 2000. *Piano di gestione naturalistica del Parco Naturale del Conero. Indagini e normativa,* Regione Marche, Parco Naturale del Conero: s.n.

Bouchet, P. et al., 2017. Revised Classification, Nomenclator and Typification of Gastropod and Monoplacophoran Families. *Malacologia,* 61(1-2), pp. 1-526.

Bowen-Jones, E. & Entwistle, A., 2002. Identifying appropriate flagship species: the importance of culture and local contexts. *Oryx,* 36(2), pp. 189-195.

Canessa, M. et al., 2021. Rocky substrate affects benthic heterobranch assemblages and prey/predator relationships. *Estuarine, Coastal and Shelf Science,* 261(1), p. 107568.

Chan, H. Y. et al., 2022. The effect of environmental factors on spatial-temporal variation of heterobranch sea slug community in northern Taiwan. *Frontiers in Marine Science,* Volume 9, p. 1042961.

Chevaldonné, P. & Lejeusne, C., 2003. Regional warming-induced species shift in north-west Mediterranean marine caves. *Ecology Letters,* 6(4), pp. 371-379.

Coll, M. et al., s.d. The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PloS one,* 5(8), p. e11842.

Comitato Promotore per il Referendum sull'istituzione dell'Area Marina Protetta Costa del Conero, 2021. *L'Area Marina Protetta Costa del Conero – Note informative,* s.l.: s.n.

Cooper, C. B. et al., 2021. Inclusion in citizen science: The conundrum of rebranding. *Science,* 372(6549), pp. 1386-1388.

Crocetta, F. et al., 2017. Local ecological knowledge versus published literature: a review of non-indigenous Mollusca in Greek marine waters. *Aquatic Invasions,* 12(4), p. 415–434.

Cyrne, R. et al., 2018. Nudibranchs out of water: long-term temporal variations in the abundance of two Dendrodoris species under emersion. *Helgoland Marine Research,* Volume 72, pp. 1-10.

Damien, M. & Tougeron, K., 2019. Prey–predator phenological mismatch under climate change. *Current opinion in insect science,* Volume 35, pp. 60-68.

Dharmawan, I. G. W. D. et al., 2021. Illuminating species diversity of nudibranch in Indonesian coral reef ecosystem using molecular identification. *IOP Conference Series: Earth and Environmental Science,* Volume 944, p. 012033.

Di Camillo, C. G. et al., 2010. The benthic assemblage of Conero promontory: a model for the study of seasonal cycles in the North Adriatic Sea. *Biologia Marina Mediterranea,* 17(1), p. 112.

Di Camillo, C. G. & Cerrano, 2015. Mass mortality events in the NW Adriatic Sea: phase shift from slow-to fast-growing organisms. *PloS one,* 10(5), p. e0126689.

Di Camillo, C. G. et al., 2012. Temporal variations in growth and reproduction of Tedania anhelans and Chondrosia reniformis in the North Adriatic Sea. *Hydrobiologia,* Volume 687, pp. 299-313.

Di Camillo, C. G. et al., 2018. Building a baseline for habitat-forming corals by a multi-source approach, including Web Ecological Knowledge. *Biodiversity and Conservation,* Volume 27, pp. 1257-1276.

Dietz, J. M., Dietz, L. A. & Nagagata, E. Y., 1994. The effective use of flagship species for conservation of biodiversity: the example of lion tamarins in Brazil. *Creative conservation: interactive management of wild and captive animals,* pp. 32-49.

Doney, S. C. et al., 2012. Climate change impacts on marine ecosystems. *Annual review of marine science,* Volume 4, pp. 11-37.

Edmunds, M., 1991. Does warning coloration occur in nudibranchs?. *Malacologia,* Volume 32, pp. 241-255.

Faulkner, D. J. & Ghiselin, M., 1983. Chemical defense and evolutionary ecology of dorid nudibranchs and some other opisthobranch gastropods. *Marine Ecology Progress Series,* 13(2-3), pp. 295-301.

Firth, L. B. et al., 2014. Biodiversity in intertidal rock pools: Informing engineering criteria for artificial habitat enhancement in the built environment. *Marine Environmental Research,* Volume 102, pp. 122-130.

Furfaro, G., Chimienti, G. & Mariottini, P., 2020. DNA barcoding unveiling rare species: the case of Pruvotfolia pselliotes (Labbé, 1923) (Mollusca: Gastropoda: Nudibranchia) in the Mediterranean Sea. *The European Zoological Journal,* 87(1), pp. 459-462.

Furfaro, G. & Mariottini, P., 2020. A new Dondice Marcus Er. 1958 (Gastropoda: Nudibranchia) from the Mediterranean Sea reveals interesting insights into the phylogenetic history of a group of Facelinidae taxa. *Zootaxa,* 4731(1), pp. 1-22.

Furfaro, G. & Trainito, E., 2017. A new species from the Mediterranean Sea and North-Eastern Atlantic Ocean: Knoutsodonta pictoni n. sp. (Gastropoda Heterobranchia Nudibranchia). *Biodiversity Journal,* 8(2), pp. 725-738.

Furfaro, G. et al., 2022. Mediterranean matters: Revision of the family Onchidorididae (Mollusca, Nudibranchia) with the description of a new genus and a new species. *Diversity,* 15(1), p. 38.

Gadgil, M., Olsson, P., Berkes, F. & Folke, C., 2003. Exploring the role of local ecological knowledge in ecosystem management: three case studies. *Navigating social-ecological systems: building resilience for complexity and change,* pp. 189-209.

Geissler, C., Davidson, A. & Niesenbaum, R. A., 2023. The influence of climate warming on flowering phenology in relation to historical annual and seasonal temperatures and plant functional traits. *PeerJ,* Volume 11, p. e15188.

Goddard, J. H., Gosliner, T. M. & Pearse, J. S., 2011. Impacts associated with the recent range shift of the aeolid nudibranch Phidiana hiltoni (Mollusca, Opisthobranchia) in California. *Marine Biology,* 158(5), pp. 1095-1109.

Gonor, J. J., 1961. Observation on the biology of Lobiger serradifalci, a shelled sacoglossan opisthobranch from the Mediterranean. *Vie et Milieu,* 12(3), pp. 381-403.

Goodheart, J. A. et al., 2017. Prey preference follows phylogeny: evolutionary dietary patterns within the marine gastropod group Cladobranchia (Gastropoda: Heterobranchia: Nudibranchia). *BMC Evolutionary Biology,* Volume 17, pp. 1-14.

Gosliner, T. M., 2001. Aposematic coloration and mimicry in opisthobranch mollusks: new phylogenetic and experimental data. *Bollettino Malacologico,* 37(5-8), pp. 163-170.

Grande, C., Templado, J., Cervera, J. L. & Zardoya, R., 2004. Molecular phylogeny of euthyneura (Mollusca: Gastropoda). *Molecular biology and evolution,* 21(2), pp. 303-313.

Greenwood, P. G. & Mariscal, R. N., 1984. The utilization of cnidarian nematocysts by aeolid nudibranchs: Nematocyst maintenance and release in Spurilla. *Tissue and Cell,* 16(5), pp. 719-730.

Haber, M. et al., 2010. Coloration and defense in the nudibranch gastropod Hypselodoris fontandraui. *Biological Bulletin,* 218(2), pp. 181-188.

Hadfield, M. G., 1963. The Biology of Nudibranch Larvae. *Oikos,* 14(1), pp. 85-95.

Haszprunar, G., 1985. The Heterobranchia–a new concept of the phylogeny of the higher Gastropoda. *Journal of Zoological Systematics and Evolutionary Research,* 23(1), pp. 15-37.

Havenhand, J. N. & Todd, C. D., 1989. Reproductive Effort of the Nudibranch Molluscs Adalaria proxima (Alder & Hancock) and Onchidoris muricata (Muller): An Evaluation of Techniques. *Functional Ecology,* 3(2), pp. 153-163.

Heller, J., 1993. Hermaphroditism in molluscs. *Biological Journal of the Linnean Society,* 48(1), pp. 19-42.

Hoegh-Guldberg, O. & Bruno, J. F., 2010. The impact of climate change on the world's marine ecosystems. *Science,* 328(5985), pp. 1523-1528.

Huggett, J. & Griffiths, C. L., 1986. Some relationships between elevation, physico-chemical variables and biota of intertidal rock pools. *Marine Ecology Progress Series,* 29(2), pp. 189-197.

Irving, A. D. et al., 2009. Light, sediment, temperature, and the early life-history of the habitat-forming alga Cystoseira barbata. *Marine Biology,* Volume 156, pp. 1223-1231.

Jacob, D., 2008. *A Citizen Science Program for the Center for Alaskan Coastal Studies,* s.l.: s.n.

Jensen, K. R., 1999. Copulatory behaviour in three shelled and five non-shelled sacoglossans (Mollusca, Opisthobranchia), with a discussion of the phylogenetic significance of copulatory behaviour. *Ophelia,* 51(12), pp. 93-106.

Krželj, M., Cerrano, C. & Di Camillo, C. G., 2020. Enhancing Diversity Knowledge through Marine Citizen Science and Social Platforms: The Case of Hermodice carunculata (Annelida, Polychaeta). *Diversity,* 12(8), p. 311.

Lambert, W. J., 2013. Population biology of an intertidal dorid nudibranch (Onchidoris muricata) in the Southern Gulf of Maine, USA: changes in phenology due to an invasive prey?. *American Malacological Bulletin,* 31(1), pp. 17-23.

Lederhendler, I. I., Barnes, E. S. & Alkon, D. L., 1980. Complex responses to light of the nudibranch Hermissenda crassicornis (Gastropoda: Opisthobranchia). *Behavioral and Neural Biology,* 28(2), pp. 218-230.

Lewin, R. A., 1970. Toxin secretion and tail autotomy by irritated Oxynoe panamensis (Opisthobranchiata; Sacoglossa). *Pacific Science,* 24(3), pp. 356-358.

Mackenstedt, U. & Märkel, K., 2001. Radular structure and function. In: *The biology of terrestrial Molluscs.* New York: CABI Publishing, p. 558.

Marie, B. et al., 2013. The shell-forming proteome of Lottia gigantea reveals both deep conservations and lineage specific novelties. *Federation of European Biochemical Societies (FEBS) Journal,* Volume 280, pp. 214-232.

Martin, R., 2003. Management of nematocysts in the alimentary tract and in cnidosacs of the aeolid nudibranch gastropod Cratena peregrina. *Marine Biology,* Volume 143, pp. 533-541.

Masini, L. et al., 2007. Research and characterization of pathogenic vibrios from bathing water along the Conero Riviera (Central Italy). *Water research,* 41(18), pp. 4031-4040.

Mazziotti, C., Tisselli, M. & Doneddu, M., 2014. Segnalazione di Pleurobranchaea meckeli (de Blainville, 1825) per le coste emiliano-romagnole. *Notiziario S.I.M.,* Volume 32, pp. 1-3.

Mendonça, V. et al., 2018. What's in a tide pool? Just as much food web network complexity as in large open ecosystems. *PloS one,* 13(7), p. e0200066.

Miller, J. A. & Byrne, M., 2000. Ceratal autotomy and regeneration in the aeolid nudibranch Phidiana crassicornis and the role of predators. *Invertebrate Biology,* 119(2), pp. 167-176.

Moussa, M. et al., 2024. Insight on thermal stress response of demosponge Chondrosia reniformis (Nardo, 1847). *Science of The Total Environment,* Volume 913, p. 169648.

M, T. & M., T., 2007. *Le grotte del Passetto. Storia ambientale e cultura materiale della marina di Ancona.* s.l.:Affinità elettive.

Murphy, B. F. & Hadfield, M. G., 1997. Chemoreception in the nudibranch gastropod Phestilla sibogae. *Comparative Biochemistry and Physiology Part A: Physiology,* 118(3), pp. 727-735.

Newcomb, J. M. et al., 2014. Circadian rhythms of crawling and swimming in the nudibranch mollusc Melibe leonina. *Biological Bulletin,* 227(3), pp. 263-273.

Nimbs, M. J. et al., 2016. Southern range extensions for twelve heterobranch sea slugs (Gastropoda: Heterobranchia) on the eastern coast of Australia. *Marine Biodiversity Records,* Volume 9, pp. 1-12.

Nimbs, M. J. & Smith, S. D., 2016. Welcome strangers: Southern range extensions for seven heterobranch sea slugs (Mollusca: Gastropoda) on the subtropical east Australian coast, a climate change hot spot. *Regional Studies in Marine Science,* Volume 8, pp. 27-32.

Nimbs, M. J. & Smith, S. D., 2018. Beyond Capricornia: tropical sea slugs (Gastropoda, Heterobranchia) extend their distributions into the Tasman Sea. *Diversity,* 10(3), p. 99.

Nimbs, M. J., Willan, R. C. & Smith, S. D., 2015. Range extensions for heterobranch sea slugs (formerly opisthobranch) belonging to the families Diaphanidae, Plakobranchidae and Facelinidae on the eastern coast of Australia. *Marine Biodiversity Records,* 8(76), pp. 1-6.

Nybakken, J., 1978. Abundance, diversity and temporal variability in a California intertidal nudibranch assemblage. *Marine Biology,* Volume 45, pp. 129-146.

Osmani, L., 2010. *Database relazionali e applicazioni gis e webgis per la gestione, l'analisi e la comunicazione dei dati territoriali di un'area protetta. Il Parco Regionale del Conero come caso applicativo,* s.l.: s.n.

Paine, R. T., 1963. Food recognition and predation on opisthobranchs by Navanax inermis (Gastropoda, Opisthobranchia). *The Veliger,* Volume 6, pp. 1-9.

Palumbi, S. R. et al., 2009. Managing for ocean biodiversity to sustain marine ecosystem services. *Frontiers in Ecology and the Environment,* 7(4), pp. 204-211.

Patenam, R., Dyke, A. & West, S., 2021. The Diversity of Participants in Environmental Citizen Science. *Citizen Science: Theory and Practice,* 6(1), pp. 1-16.

Ponder, W. F. & Lindberg, D. R., 1997. Towards a phylogeny of gastropod molluscs: an analysis using morphological characters. *Zoological Journal of the Linnean Society,* 119(2), pp. 83-265.

Ponder, W. F., Lindberg, D. R. & Ponder, J. M., 2020a. *Biology and Evolution of the Mollusca.* Boca Raton: CRC Press.

Ponder, W. F., Lindberg, D. R. & Ponder, J. M., 2020b. *Biology and Evolution of the Mollusca.* Boca Raton: CRC Press.

Proksch, P., 1994. Defensive roles for secondary metabolites from marine sponges and sponge-feeding nudibranchs. *Toxicon,* 32(6), pp. 639-655.

Pruvot-Fol, A., 1954. *Faune de France Vol.58: Mollusques opisthobranches.* Paris: P. Lechevalier.

Rankin, J. J., 1979. A freshwater shell-less mollusc from the Caribbean: structure, biotics, and contribution to a new understanding of the Acochlidioidea. *Life Sciences Contributions Series,* Volume 116, pp. 1-123.

Riccardi, A., Colletti, A., Virgili, R. & Cerrano, C., 2022. Diversity and behavior of sea slugs (Heterobranchia) in the rocky tide pools of Conero Riviera (western Adriatic Sea). *The European Zoological Journal,* 89(1), pp. 856-869.

Rinaldi, A., 2012. *Atlante della fauna e flora marina dell'Adriatico nord-occidentale.* Imola: Editrice La Mandragora.

Rindi, F. et al., 2020. Long-term changes in the benthic macroalgal flora of a coastal area affected by urban impacts (Conero Riviera, Mediterranean Sea). *Biodiversity and Conservation,* 29(7), pp. 2275-2295.

Riolo, F. & Betti, F., 2015. First record of Europe's smallest marine fish Lebetus guilleti (Gobiidae) in the Italian seas. *Marine Biodiversity Records,* Volume 8, p. e12.

Rogers, C. N., De Nys, R. & Steinberg, P. D., 2000. Predation on juvenile Aplysia parvula and other small anaspidean, ascoglossan and nudibranch gastropods by pycnogonids. *The Veliger,* 43(4), pp. 330-337.

Sarà, M., 1990. *Zoologia II - Parte speciale.* Bari: Cacucci Editore.

Schrödl, M., Jörger, K. M., Klussman-Kolb, B. & Wilson, N. G., 2011. Bye bye 'Opisthobranchia'! A review on the contribution of Mesopsammic sea slugs to euthyneuran systematics. *Thalassas: An International Journal of Marine Sciences,* 27(2), pp. 101-112.

Schrödl, M. & Neusser, T. P., 2010. Towards a phylogeny and evolution of Acochlidia (Mollusca: Gastropoda: Opisthobranchia). *Zoological Journal of the Linnean Society,* 158(1), pp. 124-154.

Sedano Vera, F. et al., 2019. Comparing sessile benthos on shallow artificial versus natural hard substrates in the Eastern Mediterranean Sea. *Mediterranean Marine Science,* 20(4), pp. 688-702.

Seltzer, C., 2019. Making Biodiversity Data Social, Shareable, and Scalable: Reflections on iNaturalist & citizen science. *Biodiversity Information Science and Standards,* Volume 3, p. e10197.

Siddik, A. A., Al-Sofyani, A. A., Ba-Akdah, M. A. & Satheesh, S., 2019. Invertebrate recruitment on artificial substrates in the Red Sea: role of substrate type and

orientation. *Journal of the Marine Biological Association of the United Kingdom,* 99(4).

Smale, D. A. et al., 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change,* 9(4), pp. 306-312.

Smith, S. D. A. & Davis, T. R., 2019. Slugging it out for science: volunteers provide valuable data on the diversity and distribution of heterobranch sea slugs. *Molluscan Research,* 39(3), pp. 214-223.

Smith, S. D. A. & Nimbs, M. J., 2022. Citizen Scientists Record Significant Range Extensions for Tropical Sea Slug Species in Subtropical Eastern Australia. *Diversity,* 14(4), p. 244.

Taraporevala, N. F., Lesoway, M. P., Goodheart, J. A. & Lyons, D. C., 2022. Precocious Sperm Exchange in the Simultaneously Hermaphroditic Nudibranch, Berghia stephanieae. *Integrative Organismal Biology,* 4(1), pp. 1-13.

Todd, C. D., Lambert, W. J. & Davies, J., 2001. Some perspectives on the biology and ecology of nudibranch molluscs: generalisations and variations on the theme that prove the rule. *Bollettino Malacologico,* 37(5-8), pp. 105-120.

Todd, C. D., Lambert, W. J. & Thorpe, J. P., 1998. The genetic structure of intertidal populations of two species of nudibranch molluscs with planktotrophic and pelagic lecithotrophic larval stages: are pelagic larvae “for” dispersal?. *Journal of Experimental Marine Biology and Ecology,* 228(1), pp. 1-28.

Todd, C. D., Lambert, W. J. & Thorpe, J. P., 1998. The genetic structure of intertidal populations of two species of nudibranch molluscs with planktotrophic and pelagic lecithotrophic larval stages: are pelagic larvae “for” dispersal?. *Journal of Experimental Marine Biology and Ecology,* 228(1), pp. 1-28.

Toffanin, L., 2021. *Caratterizzazione della macroflora algale lungo la Riviera del Conero,* s.l.: s.n.

Trainito, E. & Doneddu, M., 2014. *Nudibranchi del Mediterraneo.* II a cura di Milano: Il Castello.

Trainito, E., Doneddu, M. & Furfaro, G., 2022. Aliens in changing seascapes: a newly reported non-native sacoglossan (Mollusca, Heterobranchia) in the western Mediterranean Sea. *Check List,* 18(3).

Trowbridge, C. D., 1994. Defensive responses and palatability of specialist herbivores: predation on N.E. Pacific ascoglossan gastropods. *Marine Ecology Progress Series,* 105(1-2), pp. 61-70.

Tullrot, A., 1994. The Evolution of unpalatability and warning coloration in soft-bodied marine invertebrates. *Evolution,* 48(3), pp. 925-928.

Turchetti, M. & Tarsetti, M., 2007. *Le grotte del Passetto. Storia ambientale e cultura materiale della marina di Ancona.* Ancona: Affinità elettive.

UNEP/MAP-RAC/SPA, 2015. Adriatic Sea: Description of the ecology and identification of the areas that may deserve to be protected. In: *edited by Cebrian D. and Requena S., RAC/SPA.* Tunis(TN): s.n., p. 92.

Valdés, Á., Blanchard, L. & Marti, W., 2013. Caught naked: First report a nudibranch sea slug attacked by a cone snail. *American Malacological Bulletin,* 31(2), pp. 337-338.

Van der Meij, S. E. T. & Reijnen, B. T., 2012. First observations of attempted nudibranch predation by sea anemones. *Marine Biodiversity,* Volume 42, pp. 281-283.

Vohland, K. et al., 2021. *The science of citizen science.* Cham, CH: Springer Nature.

Vorster, A. P. A., Krishnan, H. C., Cirelli, C. & Lyons, L. C., 2014. Characterization of sleep in Aplysia californica. *Sleep,* 37(9), pp. 1453-1463.

Wägele, M. & Johnsen, G., 2001. Observations on the histology and photosynthetic performance of "solar-powered" opisthobranchs (Mollusca, Gastropoda, Opisthobranchia) containing symbiotic chloroplasts or zooxanthellae. *Organisms Diversity & Evolution,* 1(3), pp. 193-210.

Zenetos, A. et al., 2016. Adriatic 'opisthobranchs'(Gastropoda, Heterobranchia): shedding light on biodiversity issues. *Marine Ecology,* 37(6), pp. 1239-1255.

Zunino, S. et al., 2018. The ecology of the Mediterranean stony coral Cladocora caespitosa (Linnaeus, 1767) in the Gulf of Trieste (northern Adriatic Sea): a 30-year long story. *Marine Biology Research,* 14(3), pp. 307-320.

ACKNOWLEDGEMENTS

Special thanks go to Marco Giuliano and Vladimiro Sisani, who hosted us at their dive centres, to Ricardo Mendes and Gloria Vono, who contributed to the present work with their dives, and to Pierfrancesco Cardinale, Cristina Gioia Di Camillo, Julian Di Giandomenico, Giulia Furfaro, Francesco Martini, Luca Paoletti, Agnese Riccardi, Matteo Ricotti and Yann Toso, who provided useful information on their findings.