



# Predator–prey interactions as key drivers for the invasion success of a potentially neurotoxic sea slug

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**Abstract** Only a fraction of the species that are introduced succeed in the non-native environment, while others fail to survive, reproduce and/or disperse. Understanding the causes underlying each outcome is key to designing better early detection, prevention and management programs, and to deepen our knowledge of the invasion process. Here, we examine whether predator–prey interactions favor the invasion of a potentially neurotoxic mollusk in the South Western Atlantic (SWA). The grey side-gilled sea slug

*Pleurobranchaea maculata* was recently detected in the SWA, where it has spread rapidly along the coast. In this work, we examine two hypotheses that may have driven the invasion success of *P. maculata*: (1) it has a high dietary plasticity, able to exploit resources in a variety of habitats within the invaded range and simultaneously (2) it lacks native predators that can control its abundance or spread. First, we identified the prey sources of *P. maculata* through experimental trials; then we compared its diet composition between different sites and seasons; and finally we experimentally assessed the effect of native potential predators. We found that diet composition is broad and varies in time and space, probably in relation to prey

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availability. Additionally, we observed that local predators do not recognize *P. maculata* as a prey. Probably, mechanisms underlying predator avoidance are related to the presence of chemical defenses that can be novel in the invaded environment. We propose that predator–prey interactions are key drivers favoring the establishment of non-native *P. maculata* via high levels of plasticity to exploit resources, the absence of biotic resistance and the increased availability of food associated with artificial substrates such as ports are probable mechanisms underlying the invasion success of this marine gastropod.

**Keywords** Invasive species · Invasion success · Predator–prey ecology · *Pleurobranchaea maculata* · Dietary plasticity · Biotic resistance · Resource availability · Novel weapons

## Introduction

Biological invasions represent a major environmental threat in the Anthropocene, recognized as one of the top five direct drivers of change in nature that have accelerated during the past 50 years (Vitousek et al. 1997; Orensanz et al. 2002; IPBES 2019; Schwindt et al. 2020). Yet, while some non-native species produce severe ecological or economic impacts, or threaten human welfare (Pyšek and Richardson 2010; Albins and Hixon 2013; Bortolus et al. 2015; Pejchar and Mooney 2009), many fail to establish after introduction (Zenni and Nuñez 2013). Predicting whether a species will succeed in the invasion or not, is the key to creating better early detection, prevention and management programs (van Kleunen et al. 2010). Understanding what causes these different potential outcomes has promoted countless research efforts (Kolar and Lodge 2001), and several hypotheses arose as a consequence (Simberloff and Von Holle 1999; Mack et al. 2000; Catford et al. 2009; Simberloff et al. 2013; Jeschke 2014; Enders et al. 2020). Despite some of these hypotheses having received more empirical support than others (Jeschke et al. 2012), a general and robust explanation for invasion success is still lacking. Some of the causes rely on the methodological difficulties confronted by invasion ecology (Heger et al. 2013; Gribben and Byers 2020). For example, while some of these hypotheses can be tested

within the invasive range only (i.e. *biotic resistance*, *plasticity*, *novel weapons*, *invasional meltdown*, etc.), others require comparisons between the traits and processes between the invasive and the native range simultaneously (i.e. *enemy release hypothesis*, *evolution of increased competitive ability*, etc.) (Hierro et al. 2005; Gribben and Byers 2020). To overcome these limitations, several authors have attempted to synthesize these hypotheses into more comprehensive frameworks (Barney and Whitlow 2008; Catford et al. 2009; Gurevitch et al. 2011; Heger et al. 2013; Saul et al. 2013; Jeschke 2014; Enders et al. 2020), to contextualize and hierarchize them and achieve a better understanding on the causes favoring the invasion process.

Although there are some differences among these comprehensive frameworks, the tendency is to group hypotheses that refer to similar processes in a group or cluster of hypotheses (Catford et al. 2009; Enders et al. 2020). One of these groups addresses how biotic interactions with native species affect the invader's success. Here, for example, the *biotic resistance hypothesis* proposes that the predators and competitors may limit the establishment of non-native species through predation and competition (Kremer and da Rocha 2016; Giachetti et al. 2019). Another group of hypotheses rely on the intrinsic traits of the non-native species as an explanation to its success (Enders et al. 2020). In this cluster, the *plasticity hypothesis* argues that successful invasive species are more plastic than native or non-invasive introduced species (Richards et al. 2006). This hypothesis predicts that the invasive species will succeed if is better able to maintain fitness in unfavorable conditions, to increase fitness in favorable conditions, or both. Another trait-based hypothesis is the *novel weapons hypothesis*, which posits that non-native species release or harbour allelopathic chemicals that have a negative effect on non-adapted native competitors or predators (Callaway and Ridenour 2004). For instance, the non-native nudibranch *Lamprohaminoea* (= *Haminoea*) *cyano-marginata* (Heller & T. E. Thompson, 1983) (Crocetta et al. 2017), produces brominated tetrahydropyran, a toxin that repels generalist potential predators like fish and crustaceans (Polner et al. 1989; Mollo et al. 2008). In animals, novel weapons can also consist of physical structures or behavioral responses that can also favor them as non-native predators, whenever local prey do not recognize them as a threat (Guiden et al. 2019), or

if they provide a competitive advantage over native predators (Albins 2013; Albins and Hixon 2013; Rojas-Vélez et al. 2019). A third cluster of hypotheses rely on the availability of resources to account for the invaders success (Enders et al. 2020). In this group, the *increased resource availability hypothesis* argues that invasiveness is favored if there is an increase in the levels of resources as a result of natural fluctuations or induced by anthropogenic disturbance (Sher and Hyatt 1999; Davis et al. 2000). For example, tunicate invasion has been promoted by fluctuating nutrient loads and an increase in substrate availability in several ports in the Gulf of Saint Lawrence (Locke et al. 2007). Ports are major hotspots for marine biological invasions (Hewitt et al. 2004; Schwindt et al. 2014) and provide an increased availability of settlement space through the presence of breakwaters, pilings and other structures, which enhances the success of invasive species (Holloway and Connell 2002; Glasby et al. 2007; Airoidi and Bulleri 2011).

In practice, almost every non-native species faces both restrictive and favorable conditions and interactions when introduced in a novel environment. Whether they succeed or fail in the invaded range greatly depends on the overall outcome of these factors. Thus, the mechanisms underpinning the invasion success of a species are a matter of great interest in invasion ecology and environmental conservation. The grey side-gilled sea slug *Pleurobranchaea maculata* (Quoy & Gaimard, 1832) is a generalist predator and scavenger which feeds on a variety of soft-bodied marine invertebrates and algae (Ottaway 1977; Willan 1984; Bökenhans et al. 2019) and it represents a potential food source for local pre-existent predators. It is native to New Zealand and South Eastern Australia and was recently introduced in the South Western Atlantic (SWA) (Farías et al. 2015, 2016). This species is the first opisthobranch known to accumulate tetrodotoxin (McNabb et al. 2010), and the first species in which this toxin was reported in the SWA (Farías et al. 2019), thus probably constituting a *novel weapon* in this area. This highly potent neurotoxin inhibits the propagation of action potentials in both muscular and nervous tissues through the blocking of sodium channels (Narahashi 2001). Recently, neurotoxins of *P. maculata* were responsible for a series of dog poisonings within the native range (McNabb et al. 2010) and possibly also in the invaded range (Papalardo and Battini 2019),

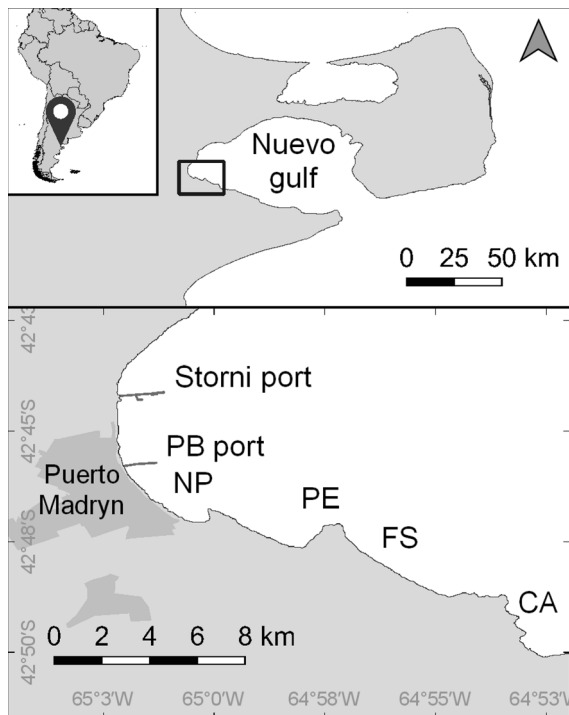
posing a threat to human welfare. In less than six years, it has become extremely abundant in some areas of the SWA, reaching densities of almost 5 ind m<sup>-2</sup> (Battini 2020) that represent a ten-fold increase over the highest abundances reported in the native range (Taylor et al. 2011). Additionally, it has spread along the coastline for more than 2000 km (Farías et al. 2016), and has the potential to spread even further (Battini et al. 2019).

In this work, we aim to disentangle the predator–prey interactions involving the non-native *P. maculata* in the SWA to address whether they favor or reduce the probabilities for its invasion success. We hypothesize that *P. maculata* is a predator with high dietary plasticity, able to exploit resources in habitats with different characteristics within the invaded range and it has no predators that can control its abundance or spread. Specifically, our objectives are (1) to identify the diet composition of *P. maculata* in different types of habitats within the SWA and (2) to determine the effect of local potential predators (fish, octopus and crabs) on the survival of *P. maculata*. We discuss the results within the framework of hypotheses related to the intrinsic characteristics of this species (plasticity, novel weapons), the biotic interactions entangled in the invaded region (biotic resistance), and the availability of resources in different types of habitats.

## Materials and methods

### Study area

The study was conducted in the Almirante Storni port of Puerto Madryn (42°44'15" S, 65°01'44" W) and adjacent areas, which are located in the Nuevo gulf, in the Argentine Patagonia (Fig. 1). The port is a natural coastal port with a mean tidal range of 4.13 m ([www.hidro.gob.ar](http://www.hidro.gob.ar)) and a maximum depth of 9 m in the study site. The area has a mean annual sea surface temperature of 14.3 °C, ranging from a minimum of 8 °C in winter (July–August) and a maximum of 21 °C in summer (January–March), and a relatively constant salinity that varies between 33.5 and 33.9‰ (Rivas and Ripa 1989). Together with the Piedra Buena port, they constitute the port area of Puerto Madryn, which represents the most active port along the Argentine Patagonia (Schwindt et al. 2014).



**Fig. 1** Map of the study area showing the location of the two main ports of Puerto Madryn, the Storni and Piedra Buena (PB) ports and other localities mentioned in the text: NP: Nuevo park, PE: Punta Este, FS: Folías shipwreck and CA: Cerro Avanzado point

#### Diet composition of *Pleurobranchaea maculata*

##### *Aquarium experiments*

In order to identify potential prey sources of *P. maculata*, we conducted an experiment using ten different species as potential prey. These species (Table 1) were selected from the local species pool considering the diet reported in specific literature (Ottaway 1977; Willan 1984; Bökenhans et al. 2019). We manually collected potential prey and predators by SCUBA diving from the Storni port and transported to the CCT CONICET-CENPAT Experimental Aquarium Service (SAE for its Spanish spelling) in insulated containers, within 2 h from collection, where we housed them in 100 L plastic containers. We replaced approximately 30% of the total volume with filtrated water every 2–3 days to ensure good water quality. We regularly measured salinity (® VEE GEE A366ATC Refractometer) and nitrite levels using specific tests (® Sera GmbH). We acclimatized all the

animals to the aquarium for at least a week prior to the experiments, and we fed each *P. maculata* with a dead mussel every 1–2 days prior to the starvation period. We chose dead mussels [*Perumytilus purpuratus* (Lamarck, 1819)] as food sources for the sea slugs during the acclimatization period because of various reasons: (a) mussels are recognized food sources in the field (Taylor et al. 2015; Bökenhans et al. 2019) and were readily eaten in preliminary trials; (b) as most potential live prey were selected as experimental prey, we intentionally wanted to offer dead food during this period to minimize confounding effects with the experiment and (c) other dead food sources such as fish or squid rapidly deteriorated the water quality during preliminary trials, while mussels were relatively ‘clean’ and required minimum water exchange. To normalize the satiation levels of the sea slugs prior to the experiment, acclimatization was followed by a starvation period that lasted 4 days. Based on preliminary tests, this period was sufficient to guarantee that the animals would not be satiated during the experiments (Fig. 8 in Supplementary material).

The experimental setup consisted of plastic 100 L rectangular containers, which were divided into three similar compartments using a plastic mesh with 2 mm<sup>2</sup> holes to ensure water and oxygen exchange between compartments. We randomly assigned the treatments to each compartment, following a randomized block design. Treatments were as follows: prey-predator treatment (PPT), consisting of a single individual of *P. maculata* and a single individual (or colony fragment) of the potential prey species, prey control (PYC), consisting of a single individual (or a colony fragment similar in size to PPT) of the potential prey species and predator control (PDC), consisting of a single *P. maculata* and a dead mussel similar to those offered during the acclimatization period. The rationale for the latter control was that if the predators continued to feed on the same food sources that were offered during the acclimatization period, this indicated that they were healthy and hungry during the experimental period. We performed at least 10 independent replicates for each potential prey species. At the end of the experiments, which lasted 48 hs, we determined whether each prey had been consumed or not (see Supplementary for details on consumption criteria for each species). We compared the frequency of consumption between PDC and PPT through Fisher exact tests. Trials in which the prey in PYC died were not

**Table 1** Species used as potential prey in the aquarium experiments, showing the number of replicates for each species, the percentage of replicates in which the food offer was consumed in the predator control (PDC) and the predator–

prey trial (PPT), the significance test (Fisher exact test) comparing these consumption rates, and collection codes for vouchers added to the IBIOMAR-CONICET Collection (CNP-INV)

Species	N	Consumption (%)		p-value	Collection code
		PDC	PPT		
<b>CNIDARIA</b>					
<b>Hydrozoa</b>					
* <i>Ectopleura crocea</i> (Agassiz, 1862)	10	100	90	1.000	CNP-INV 3100
<b>Anthozoa</b>					
<i>Parabunodactis imperfecta</i> Zamponi & Acuña, 1992	11	100	100	1.000	CNP-INV 3815
<i>Actinothoe lobata</i> (Carlgren, 1899)	15	94	43.8	0.006	CNP-INV 3812
<b>MOLLUSCA</b>					
<b>Gastropoda</b>					
<i>Chaetopleura isabellei</i> (d'Orbigny, 1841)	10	100	0	< 0.0001	CNP-INV 3848
<i>Doris fontainii</i> d'Orbigny, 1837	12	73	0	0.001	CNP-INV 3076
* <i>Pleurobranchaea maculata</i> (Quoy & Gaimard, 1832)	10	100	66.7	0.206	CNP-INV 3081
<b>CHORDATA</b>					
<b>Asciidiacea</b>					
* <i>Diplosoma listerianum</i> (Milne Edwards, 1841)	10	100	50	0.032	CNP-INV 3028
<i>Aplidium variabile</i> (Herdman, 1886)	12	75	0	< 0.0001	CNP-INV 3024
* <i>Ciona robusta</i> Hoshino & Tokioka, 1967	10	80	0	0.001	CNP-INV 3029
* <i>Asciidiella aspersa</i> (Müller, 1776)	10	100	0	< 0.0001	CNP-INV 3034

Non-native species are marked with an \*. Note that a significant difference implies that the prey was consumed in a different (less) proportion than the control food (dead mussels)

considered in the analyses. After the experiments, we placed a voucher of every prey species in the IBIOMAR invertebrate collection (CNP-INV) in order to ensure taxonomic reliability and experimental repeatability (Table 1).

*Stomach content analysis*

In order to determine the diet composition and account for seasonal and spatial variation, we collected specimens of *P. maculata* in winter (June–August) and summer (January–March) from the port and from a natural rocky reef area near Punta Este (Fig. 1). Approximately 50 individuals (minimum of 45) were collected manually in each site and season through SCUBA diving. To minimize food digestion, immediately after collection we fixed the individuals in formalin 10% in seawater for 48 hs and preserved them in 70% ethanol until dissection.

In the laboratory, we carefully dried the animals by using paper towels, and weighed them to estimate the whole wet weight (WW). We dissected the individuals through a dorsal incision of the mantle and removed the entire gut, from the esophagus to the distal intestine to weigh it and estimate the full stomach weight (FSW). Then we removed the stomach content and stored it in 70% ethanol for subsequent content analysis, and weighed the empty gut to estimate the empty stomach weight (ESW). We assessed the feeding intensity through the repletion index (RI), which measures the relative amount of food in that each individual had in the stomach at the time of collection and was calculated as follows:

$$RI = \frac{(FSW - ESW)}{WW - (FSW - ESW)}$$

We identified all the taxa present in the stomachs at the lowest possible level under a stereomicroscope (Leica S6D Greenough) using taxonomic literature

(Lichtschein de Bastida and Bastida 1980; Monniot et al. 1991; Blake and Ruff 2007; Galea et al. 2007; Häussermann and Försterra 2009; Rocha et al. 2012; Boraso de Zaiexo 2013; Roig-Juñent et al. 2014; Forcelli and Narosky 2015; Genzano et al. 2017; Schwindt et al. 2018), prioritizing local or regional literature unless it was unavailable, and consulting the CNP-INV invertebrate collection when necessary. In order to assess dietary variations due to spatial and temporal food availability, we calculated the frequency of occurrence (FO) of each prey item for each site and season. To evaluate if the number of stomachs was sufficient to describe the diversity of the diet of *P. maculata* in each site and season, we performed randomized cumulative prey curves for each site and season using package ‘vegan’ (Oksanen et al. 2019) in R (R Core Team 2020). We considered the number was sufficient when the curve reached a slope of 0.1 or lower, which represents that 10 extra stomachs are needed to add an extra species to the prey list. However, we also tested whether the accumulation curve reached an asymptote comparing the slope for the endpoints of the curve to an horizontal line using a Student’s *t*-test (Bizzarro et al. 2007).

Finally, to analyze possible differences in *P. maculata* size among sites and seasons, we compared the BW among sites and seasons using linear models fitted by generalized least squares through package ‘nlme’ (Pinheiro et al. 2018) in R. Given that preliminary analyses suggested that residuals were heteroscedastic, we applied a variance structure through the VarFixed parameter of the function ‘gls’. To evaluate the differences in RI among sites and seasons, we performed generalized linear models using beta distribution with package ‘betareg’ (Zeileis et al. 2018) in R. To evaluate the differences in diet composition among sites and seasons, we performed a multiple correspondence analysis (MCA) using ‘ade4’ package (Dray et al. 2020) in R. We performed all the analyses using the R v3.5.2 software (R Core Team 2020), and significance level for all statistical tests was set at an  $\alpha = 0.05$  (Zar 2010).

#### Effect of local potential predators

We reviewed the scientific literature on predators of ‘opisthobranch’ sea slugs and identified the main groups for which there is a record of predation on a sea slug species (Table 2). We selected as potential

predator species that simultaneously (1) belong to one of these major groups, (2) are present in the study area and (3) are sufficiently abundant so that it was possible to work with them in aquarium or field experiments. Therefore, we selected two decapod species [*Ovalipes trimaculatus* (De Haan, 1833) and *Carcinus maenas* (Linnaeus, 1758)], one cephalopod [*Octopus tehuetchus* (d’Orbigny, 1834)] and four species of fish [*Sebastes oculatus* Valenciennes, 1833, *Pinguipes brasilianus* Cuvier, 1829, *Acanthis-tius patachonicus* (Jenyns, 1840) and *Pseudopercis semifasciata* (Cuvier, 1829)] (Table 3).

#### Aquarium experiments

We evaluated predator–prey interactions between *P. maculata* and crabs or octopus in the aquarium (*O. trimaculatus*, *C. maenas* and *O. tehuetchus*). We manually collected the crabs in Cerro Avanzado point or Punta Este (Fig. 1) through SCUBA diving, and octopuses in the Storni port using artisanal traps made from PVC tubes. We acclimatized the crabs and octopus to the experimental conditions for a minimum of one week, during which we fed them with live mussels (*Mytilus* spp.) and crabs (small *C. maenas*), respectively. Then, we starved them for a 4–5 day period before initiating the trials, which lasted 24 h. We performed the trials in rectangular plastic containers similar to those used in the previous section.

Each trial consisted of a single specimen of predator, and four individuals of *P. maculata* offered as prey. At the end of each trial, we recorded if at least one sea slug was consumed and the general condition of the predator (i.e. if it maintained normal movements across the container, if it reacted to mechanical stimulation, if there was any sign of injury or limb loss in the case of crabs, etc.). To control for predator satiation, another predator specimen was simultaneously offered four individuals of the same prey species used during the acclimatization period, in an independent container. In the case of the octopus *O. tehuetchus*, we controlled for predator satiation using the same predator individual that was used in an initial trial with *P. maculata*, instead of using independent individuals for the two types of food sources. Then, every octopus was offered a small *C. maenas* similar to that used during the acclimatization period and we recorded whether it was consumed or not after another 24 h following the trial with *P. maculata*. This was

**Table 2** Review of the species or groups of species that have been reported preying upon a sea slug (Mollusca: Heterobranchia)

Predator	Prey	References
<b>PLATYHELMINTHES</b>		
<i>Paraplanocera oligoglana</i> (Shmarda, 1859)	<i>Aplysia juliana</i> (Quoy & Gaimard, 1832)	Kirkendale (2006)
<i>Paraplanocera oligoglana</i> (Shmarda, 1859)	<i>Mariaglaja inornata</i> (Baba, 1949)	Kirkendale (2006)
<b>NEMERTEA</b>		
Unidentified nemertean	<i>Aplysia parvula</i> (Mörch, 1863)	Koh (2005a)
<i>Lineus fuscoviridis</i> Takakura, 1898	<i>Atagema intecta</i> (Kelaart, 1859)	Koh (2006)
<b>MOLLUSCA</b>		
<b>Gastropoda</b>		
<i>Gymnodoris ceylonica</i> (Kelaart, 1858)	<i>Stylochelius striata</i> (Quoy & Gaimard, 1832)	Johnson (1999a), Bonnet (2006)
<i>Philinopsis speciosa</i> Pease, 1860	<i>Stylochelius striata</i> (Quoy & Gaimard, 1832)	Roberts (2005)
<i>Navanax inermis</i> (J. G. Cooper, 1862)	<i>Aplysia californica</i> J. C. Cooper, 1863	Wright (2006)
<i>Gymnodoris striata</i> (Eliot, 1908)	<i>Plakobranchus ocellatus</i> van Hasselt, 1824	Johnson (1999b)
<i>Gymnodoris citrina</i> (Bergh, 1877)	<i>Gymnodoris citrina</i> (Bergh, 1877)	Johnson (2000)
<i>Tyrannodoris luteolineata</i> (Baba, 1936)	<i>Nembrotha kubaryana</i> Bergh, 1877	Anderson (2006), Gudgeon (2006)
<i>Tyrannodoris luteolineata</i> (Baba, 1936)	<i>Nembrotha aurea</i> Pola, Cervera & Gosliner, 2008	Ogden (2008)
<i>Gymnodoris impudica</i> (Bergh, 1905)	<i>Chromodoris lineolata</i> (van Hasselt, 1824)	Toh (2008)
<i>Gymnodoris impudica</i> (Bergh, 1905)	<i>Chromodoris westraliensis</i> (O'Donoghue, 1924)	Anderson (2008)
<i>Californiconus californicus</i> (Reeve, 1844)	<i>Triopha catalinae</i> (Cooper, 1863)	Valdés et al. (2013)
<b>Cephalopoda</b>		
<i>Octopus insularis</i> Leite & Haimovici, 2008	Unidentified Aplysiidae	Rosas-Luis et al. (2019)
<b>ARTHROPODA</b>		
<b>Decapoda</b>		
Unidentified crab	<i>Aplysia juliana</i> (Quoy & Gaimard, 1832)	Koh (2005b)
<i>Calcinus tubularis</i> (Linnaeus, 1767)	<i>Thuridilla hopei</i> (Vérany, 1853)	Horst (2009)
<i>Caphyra yookadai</i> Sakai, 1933	<i>Tritoniopsis elegans</i> (Audouin, 1826)	Kuroe and Mada (2007)
<i>Necora puber</i> (Linnaeus, 1767)	<i>Spurilla neapolitana</i> (Delle Chiaje, 1841)	Silva (2008)
<i>Metacarcinus magister</i> (Dana, 1852)	<i>Aplysiopsis enteromorphae</i> (Eliot, 1905)	Trowbridge (1994)
<i>Hemigrapsus oregonensis</i> (Dana, 1851)	<i>Aplysiopsis enteromorphae</i> (Eliot, 1905)	Trowbridge (1994)
<i>Sagaminopteron nigropunctatum</i> Carlson & Hoff, 1973	<i>Lambrachaeus ramifer</i> Alcock, 1895	Anker and Ivanov (2020)
<i>Mexichromis mariei</i> (Crosse, 1872)	<i>Lambrachaeus ramifer</i> Alcock, 1895	Anker and Ivanov (2020)
<b>ASTEROIDEA</b>		
<i>Solaster</i> sp.	<i>Cadlina</i> cf. <i>luteomarginata</i>	Hildering and Miller (2007)
<i>Coscinasterias muricata</i> Verrill, 1867	<i>Doris cameroni</i> (Allan, 1947)	Chuk (2007)
<b>Pycnogonida</b>		
Unidentified Pycnogonida	<i>Okenia virginiae</i> (Gosliner, 2004)	Arango and Brodie (2003)
<i>Anoplodactylus californicus</i> Hall, 1912	<i>Dondice occidentalis</i> (Engel, 1925)	Piel (1991)
<i>Anoplodactylus evansi</i> Clark, 1963	<i>Aplysia parvula</i> Mörch, 1863	Rogers et al. (2001)
<b>ACTINOPTERYGII</b>		
<i>Caulolatilus microps</i> Goode & Bean, 1878	Tectipleura (various species)	Bielsa and Labisky (1987)
<i>Sebastes carnatus</i> (Jordan & Gilbert, 1880)	Unidentified Heterobranchia	Loury (2011)
<i>Gadus macrocephalus</i> Tilesius, 1810	Unidentified Nudibranchia	Yang and Nelson (1999)

**Table 2** continued

Predator	Prey	References
Tetraodontidae	<i>Stylocheilus striatus</i> (Quoy & Gaimard, 1832)	Roberts (2005)
Labridae	<i>Aplysia</i> spp.	Rogers (2001)
<i>Hypsypops rubicundus</i> (Girard, 1854)	<i>Peltodoris nobilis</i> (MacFarland, 1905)	Harris (2006)
Pomacentridae	<i>Phidiana lasrucensis</i> Bertsch & Ferreira, 1974	Hermosillo (2002)
<i>Oligocottus maculosus</i> (Girard, 1856)	<i>Aplysiopsis enteromorphae</i> (Eliot, 1905)	Trowbridge (1994)

done in order to minimize the amount of replicates considering a) animal availability and b) ethical considerations related to the use of octopus in experimental assays (Moltschaniwskyj et al. 2007). The rationale was similar to that explained in the “Aquarium experiments” for the diet composition section, as we intended to assess whether the predators (octopuses) were healthy and hungry when offered *P. maculata* as a prey. In case the octopus did not feed upon *P. maculata*, we are confident that the additional 24 h starvation period (5–6 days) did not significantly alter the predatory behavior of the octopus with the control food. Alternatively, in the case the octopus would have eaten *P. maculata*, then we could assume that the predators were hungry and there was no need to offer a control prey. We performed at least 10 replicates for each predator species (Table 3) and compared the frequency of consumption of control food with the frequency of consumption of *P. maculata* through Fisher exact tests.

### Field experiments

Due to the sensitivity of local fish to manipulation and aquarium maintenance, we evaluated their predatory response through a field experiment. Trials consisted in offering to each potential predator, a specimen of *P. maculata* or a similarly sized control food (piece of raw meat) through SCUBA diving using a long (~ 40 cm) handled homemade plastic spoon (Fig. 12 in the Supplementary material). For each fish, we recorded whether the sea slug was consumed (C), rejected (R), or avoided (A). While both R and A

implied that the sea slugs were not consumed, the difference between the two behavior categories was that R included an initial exploratory manipulation of the prey followed by an active rejection (fish swallowed the sea slug and then spat it out), while in A there was no initial exploratory manipulation, and the predator avoided the prey after a careful visual inspection. We compared the frequency of consumption events between prey types for each predator species using Fisher exact tests (see Supplementary material for further details). The individuals of *P. maculata* were collected shortly before the trials in the same sites where these were performed to avoid the translocation of specimens. Even though the selected species (*A. patachonicus*, *S. oculatus*, *P. semifasciata* and *P. brasiliensis*) are among the most abundant benthic-feeding fish species within the Nuevo gulf (Irigoyen et al. 2013), we performed the trials in sites where these species are both (a) very common and (b) confident to the presence of divers. The sites we selected (Folías shipwreck and the Nuevo park; Fig. 1) are recognized diving points in the area and thus are frequently visited by recreational SCUBA divers. Also, these fishes are highly inactive during most of the time, so trials were performed during spring and summer, when warmer sea temperature favors their feeding activity (Beltramino et al. 2019). These two factors maximized the probabilities of the fish accepting the food offered from the diver, at the risk of overestimating the predatory behavior of the fishes. We performed all statistical analyses using R v3.5.2 (R Core Team 2020), and significance level for all statistical tests was set at  $\alpha = 0.05$  (Zar 2010).



**Table 3** Species used as potential predators in aquarium (A) and field (F) trials, amount of replicates performed for each species (N), average and range size of predators and prey and percentage of control food consumed by each predator. The last column indicates if there was consumption upon the sea slugs based on a Fisher exact test comparing the control food and experimental (sea slug) consumption percentages. The latter

was zero for every predator, so it is not indicated in another column. Non-native species are marked with an \*. In the case of fish, replicate numbers are indicated for control-treatment food items. Sizes correspond to carapace width<sup>†</sup>, body length<sup>††</sup> and dorsal mantle length<sup>†††</sup> (all in mm). nd: no data. \*\*Caution is advised when reading this significance test because replicates were not independent between treatments (see main text)

Species	Trial	N	Control food	Predator size (range) in mm	Prey size (range)		Control prey consumption (%)	Consumption (significance)
					Control	Treatment		
<b>ARTHROPODA</b>								
<b>Decapoda</b>								
* <i>Carcinus maenas</i> (Linnaeus, 1758)	A	14	Mussels	60.6 (45–74) <sup>†</sup>	33.2 (13.6–47.5) <sup>††</sup>	79.1 (20–95) <sup>††</sup>	41	NO ( $p < 0.001$ )
<i>Ovalipes trimaculatus</i> (De Haan, 1833)	A	10	Mussels	82.3 (51–106) <sup>†</sup>	35.9 (16.6–48.9) <sup>††</sup>	77.7 (42–105) <sup>††</sup>	55	NO ( $p < 0.001$ )
<b>MOLLUSCA</b>								
<b>Cephalopoda</b>								
<i>Octopus tehuelchus</i> (d’Orbigny, 1834)	A	11	Crabs	41.0 (34.8–45.9) <sup>†††</sup>	28.6 (25–34) <sup>†</sup>	64.3 (34–77) <sup>††</sup>	81	NO ( $p < 0.001$ )**
<b>CHORDATA</b>								
<b>Actinopterygii</b>								
<i>Sebastes oculatus</i> Valenciennes, 1833	F	5–5	Raw meat	nd	nd		100	NO ( $p = 0.008$ )
<i>Pinguipes brasiliensis</i> Cuvier, 1829	F	4–3	Raw meat	nd	nd		100	NO ( $p = 0.029$ )
<i>Acanthistius patachonicus</i> (Jenyns, 1840)	F	13–13	Raw meat	nd	nd		100	NO ( $p < 0.001$ )
<i>Pseudorpercis semifasciata</i> (Cuvier, 1829)	F	18–17	Raw meat	nd	nd		89	NO ( $p < 0.001$ )

## Results

### Diet of *Pleurobranchaea maculata*

#### Aquarium experiments

The aquarium experiments revealed that *P. maculata* preyed upon a minimum of five of the species present in the Nuevo gulf, belonging to all the groups we selected based on the literature: sea anemones, hydrozoans, colonial ascidians and sea slugs. However, we found that within these groups, there are

species-specific differences in the frequency and intensity of predation. For example, among sea slugs, smaller individuals of *P. maculata* were predated but there was no predation on the native sea slug *Doris fontainii* d’Orbigny, 1837 (Table 1). In the case of colonial ascidians, we found that none of the species was consumed in a frequency that matched the control food, although in the case of the non-native *Diplosoma listerianum*, colonies were consumed in 50% of the replicates, while for the native *Aplidium variabile* no consumption was observed (Table 1). In the case of sea anemones, we found differences in body surface

consumed between both species. Predation intensity was significantly higher ( $p < 0.001$ ) for *Parabundodactis imperfecta* Zamponi & Acuña, 1992 than for the acontiate *Actinothoe lobata* (Carlgren, 1899) (Fig. 11a in Supplementary material). In agreement with this observation, we noticed some degree of aversive behavior of *P. maculata* while eating *A. lobata*, which we did not register with *P. imperfecta* (Fig. 10 in Supplementary material). Generally, this behavior correlated with the extrusion of acontia by *A. lobata*, structures that are absent in *P. imperfecta*.

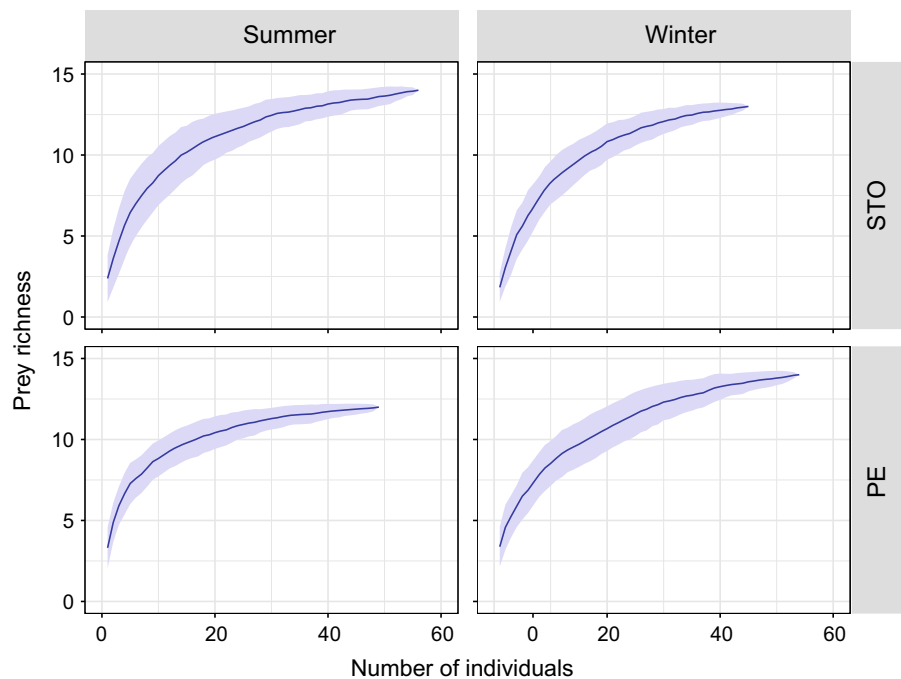
### Stomach content

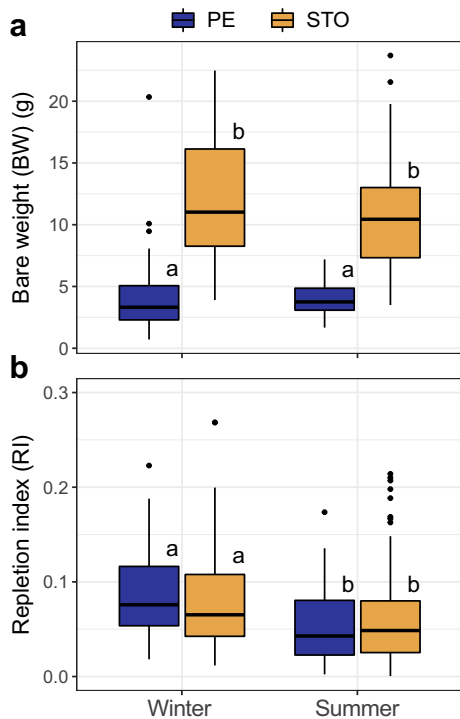
The cumulative prey curves show that the number of analyzed stomachs was sufficient to reach a slope of less than 0.1 in both sites and seasons (Fig. 2). However, the slope was significantly different to zero in all cases (Storni port, summer:  $t = 13.45$ ,  $p < 0.001$ , winter:  $t = 16.33$ ,  $p < 0.001$ ; Punta Este, summer:  $t = 10.33$ ,  $p = 0.002$ , winter:  $t = 18.33$ ,  $p < 0.001$ ), indicating that diet composition is even richer than we observed. With a similar number of samples, the composition of the diet almost did not vary between sites and seasons, except for the natural rocky reef in summer, where the diet was slightly less diverse (Fig. 2). Sea slugs were larger in the port, as

indicated by the BW of *P. maculata*, which was higher than in the natural rocky reef ( $F = 130.91$ ,  $p < 0.001$ ), but the relative consumption rate, as evidenced by the RI, did not differ between both sites ( $\chi^2 = 0.22$ ,  $p = 0.638$ , Fig. 3). Among seasons, we found no differences in BW ( $F = 3.16$ ,  $p = 0.077$ ), but the RI was lower in summer than in winter in both sites ( $\chi^2 = 20.64$ ,  $p < 0.001$ , Fig. 3).

We found 31 taxa in the stomach content of *P. maculata* (Table 4; Fig. 4), including algae (9 taxa), invertebrates (21 taxa) and vertebrates (1 taxa). The MCA showed clear differences in terms of diet composition between sites, and less clear differences between seasons. The latter were associated with the presence of some specific taxa that were more frequent during the summer than in winter, such as copepods and peracarids (see the summer samples grouped in the bottom left corner in Fig. 5). In terms of the different taxa, diet in the port area was characterized by the high frequency of unidentified invertebrates (mainly large soft bodied invertebrates), bivalves and sea anemones while the diet in the natural rocky reef was characterized by the higher abundance of Rhodophyta, the non-native colonial ascidian *Diplosoma listerianum*, Ochrophyta [mainly *Dictyota dichotoma* (Hudson) J.V.Lamouroux] and terebellid palps (Fig. 6). Overall, terebellid palps were the most

**Fig. 2** Prey accumulation curve as a function of the number of analyzed stomachs (number of individuals) for *Pleurobranchaea maculata* from the Storni port (STO) and the natural rocky reef (PE) for both seasons (summer and winter). The line represents the mean and the shaded area the standard deviation of the accumulation curve based on random permutations of the data





**Fig. 3** Whole wet weight and repletion index of *Pleurobranchaea maculata* collected in the port area (STO) and the natural rocky reef (PE) during winter and summer. Similar letters indicate no significant differences between groups at an  $\alpha = 0.05$ . The lower and upper hinges represent the first and third quartiles, the whiskers represent values within 1.5 times the interquartile range from the upper and lower hinges, and higher or smaller are indicated with black dots

abundant items in both sites and seasons, followed by red algae, the non-native colonial ascidian *D. listerianum*, unidentified invertebrates, *D. dichotoma* and bivalves (Fig. 6), mainly *Aulacomya atra* and *Mytilus* spp. (based on the foot size and being them the most common bivalves in the study area). Unidentified invertebrates were mainly large prey, such as anthozoans and bivalves, which were rarely consumed as a whole; instead, their body surface was gradually scrapped (see previous section) and therefore the material was highly disintegrated and difficult to identify. Some prey items showed a strong seasonal pattern, being more frequent in summer (*D. listerianum*, peracarids and copepods) or in winter (gastropods and nemertean). Others showed a clear spatial pattern, being more frequent in the port area (e.g. bivalves) or in the natural rocky reef (e.g. Ochrophyta), while others showed both, such as Rhodophyta, that were more frequent in winter and

in the natural rocky reef; nematodes, that were more frequent during the summer only in the natural rocky reef, or hydrozoans, which showed a great seasonal effect only in the port (Fig. 6).

### Predators of *Pleurobranchaea maculata*

#### Aquarium experiments

None of the selected potential predators consumed *P. maculata* under the experimental conditions, but they did consume the control prey items (Table 3). These differences were significant when comparing the frequency of consumption of at least one prey specimen (Fisher exact test for *C. maenas*:  $p < 0.001$ ; for *O. trimaculatus*:  $p < 0.001$ ; *O. tehueltchus*:  $p < 0.001$ ). In the case of both crab species, we frequently observed that the predators approached the slugs and softly grabbed them with their claws in an exploratory attitude that lasted a few seconds, and in any of these events, the slugs were not injured in any visible way. All individuals, both predators and prey, were in a good condition after every trial and there was no mortality recorded for any predator species.

#### Field experiments

Fish did not consume *P. maculata* (Table 3), even though the conditions of the experiment maximized the probabilities of consumption. Regardless of the species, most of the fish swallowed the slugs when we offered them in the field, but immediately spat them out and avoided them thereafter, causing no apparent harm or injury to the slug. Instead, when we offered the control food to the predators, almost all the individuals ate it voraciously (Fig. 7). On some occasions, fish immediately entered a crevice after swallowing the food items, so we were unable to observe whether they ultimately consumed or rejected the prey. These cases were not considered in the analyses. In both sites where these trials were performed, naturally occurring individuals of *P. maculata* were numerous and within the reach of the fish. However, we did not see them attack these slugs either during the course of the experiment.

**Table 4** Frequency of occurrence of each prey item found in the stomach content of *P. maculata* in summer and winter in the port (STO) or the natural rocky reef (PE). Non-native species are marked with an \*

Taxon	STO		PE	
	Summer	Winter	Summer	Winter
<b>OCHROPHYTA</b>				
* <i>Dictyota dichotoma</i> (Hudson) J.V. Lamouroux, 1809	0.018	0.041	0.216	0.527
* <i>Undaria pinnatifida</i> (Harvey) Suringar, 1873	0	0	0.020	0
<b>RHODOPHYTA</b>				
* <i>Lomentaria clavellosa</i> (Lightfoot ex Turner) Gaillon, 1828	0.088	0	0.039	0.055
* <i>Anotrichium furcellatum</i> (J. Agardh) Baldock, 1976	0.053	0.162	0.588	0.055
Rhodomelaceae	0.105	0.054	0.176	0.582
<i>Ceramium</i> sp.	0.035	0.081	0.157	0.400
<i>Heterosiphonia</i> sp.	0	0.014	0.039	0.091
<i>Stylonema</i> sp./ <i>Erythrotrichia</i> sp.	0	0	0	0.018
<b>CHLOROPHYTA</b>				
<i>Ulva</i> sp.	0.018	0	0	0.055
<b>CNIDARIA</b>				
<b>Hydrozoa</b>				
<i>Obelia</i> sp.	0	0.122	0.020	0.018
<i>Plumularia</i> sp.	0	0	0.020	0.055
<b>Anthozoa</b> (unidentified)	0.088	0.135	0.020	0.018
<b>NEMATODA</b> (unidentified)	0.088	0.068	0.294	0.073
<b>NEMERTEA</b> (unidentified)	0	0.054	0	0.018
<b>ANNELIDA</b>				
<b>Polychaeta</b>				
Syllidae	0.053	0	0.078	0.018
Phyllodocidae	0	0	0.078	0.018
Cirratulidae	0.053	0	0	0
Maldanidae	0.018	0	0	0
Terebellidae	0.018	0	0.039	0
Palps (Terebellidae-Cirratulidae)	0.228	0.297	0.784	0.945
Unidentified Polychaeta	0	0	0.020	0.036
<b>BRYOZOA</b>				
<b>Gymnolaemata</b>				
* <i>Bugulina flabellata</i> (Thompson in Gray, 1848)	0.018	0	0	0
<b>MOLLUSCA</b>				
<b>Bivalvia</b> (unidentified)	0.368	0.230	0	0
<b>Gastropoda</b>				
* <i>Pleurobranchaea maculata</i> (Quoy & Gaimard, 1832)	0	0.041	0	0.018
Aeolidiidae	0	0	0	0.018
<b>CRUSTACEA</b>				
<b>Copepoda</b> (unidentified)	0.158	0	0.059	0.036
<b>Peracarida</b>				
Amphipoda	0.035	0	0.039	0
Isopoda	0	0.014	0	0
<b>Malacostraca</b>				
<i>Pachycheles chubutensis</i> Boschi, 1963	0.018	0	0	0

**Table 4** continued

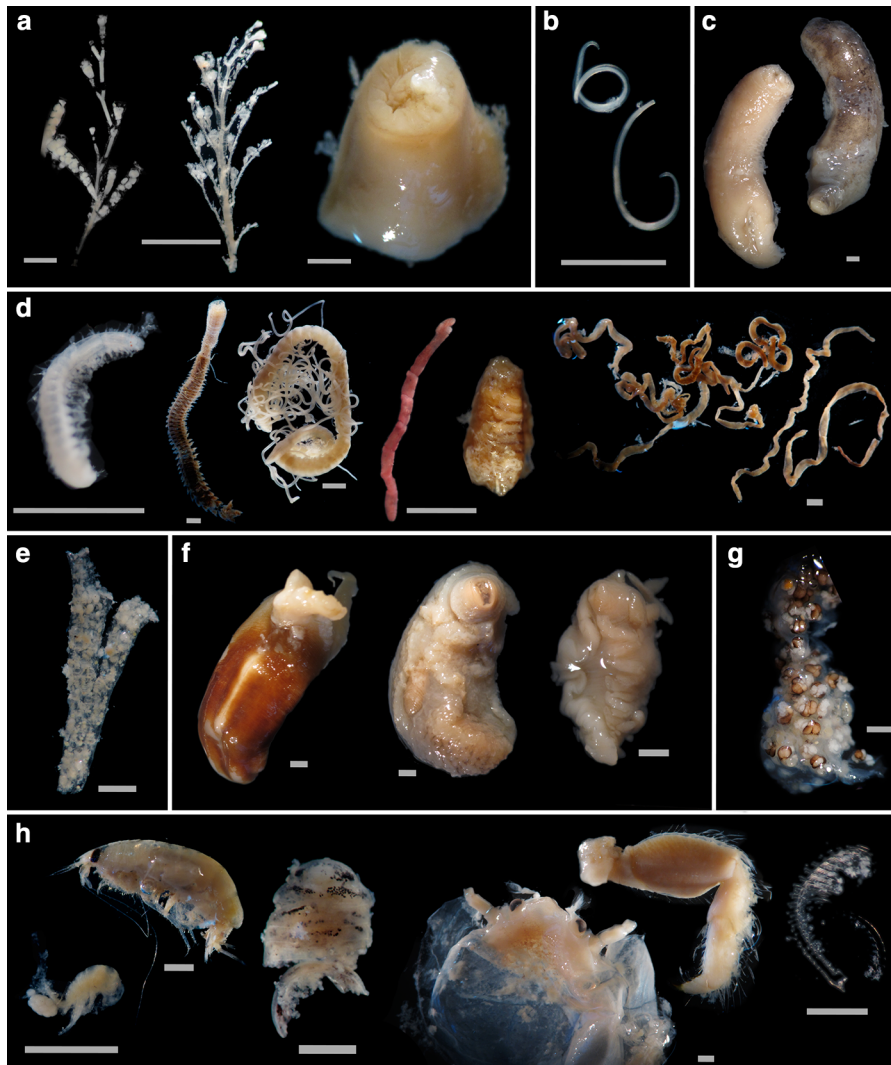
Taxon	STO		PE	
	Summer	Winter	Summer	Winter
<b>Cirripedia</b> (unidentified)	0	0.014	0	0
<b>CHORDATA</b>				
<b>Ascidacea</b>				
* <i>Diplosoma listerianum</i> (Milne Edwards, 1841)	0.456	0.176	0.686	0.345
<b>Actinopterygii</b>				
Fish larvae	0	0	0	0.018
<b>Unidentified material</b>				
Algae	0.088	0.027	0.157	0.091
Invertebrates	0.526	0.473	0.216	0.255

## Discussion

Our results support the hypotheses that (a) the non-native sea slug *Pleurobranchaea maculata* is a highly opportunistic predator with a high dietary plasticity including more than 29 taxa of marine invertebrates and algae, and (b) that it lacks predators in the South Western Atlantic (SWA). We found that diet composition varied both geographically and temporally, probably reflecting differences in the availability of prey, interspecific competition with other opportunistic predators, or both. The ability to exploit feeding resources in spatially and temporally heterogeneous environments greatly aligns with the *plasticity* hypothesis (Richards et al. 2006). In addition, our experimental and field observations firmly indicate that the effect that predators can have on *P. maculata* is limited within the invaded range, as the most abundant potential predators showed an active rejection of *P. maculata* (but see recent findings by Battini and Bravo 2020). These results suggest that there is an absence of *biotic resistance* against *P. maculata* within the SWA, although further evidence is needed to support the hypothesis that predation avoidance is mediated by the presence of neurotoxins that are novel to the environment (i.e. *novel weapons hypothesis*).

Supporting the hypothesis of a high dietary plasticity, we found some prey items to be unexpected based on prior and subsequent observations on *P. maculata*. First, some preys were protected by a hard structure, such as bivalves, barnacles and small crabs,

and prior evidence indicated that *P. maculata* would eat only soft-bodied invertebrates (Willan 1984). The relatively high frequency in which some of them were found, especially bivalves, suggests a scavenger habit of *P. maculata*, supporting the observations made by Bökenhans et al. (2019). Second, the non-native colonial tunicate *Diplosoma listerianum* was among the most frequent components of the diet of *P. maculata* in the field, but consumption in the aquarium was relatively low. As *D. listerianum* is one of the dominant colonial ascidians in the field (Battini 2020), this result suggests that although it is not a preferred item such as sea anemones, it may be frequently consumed due to its higher availability in relation to other prey items. Although a poor experimental consumption can be attributable to the low nutritional value of ascidians compared to other benthic invertebrates (Wacasey and Atkinson 1987), this study provides valuable information to future experimental trials focusing on diet selection and preference. Other very frequent items, such as terebellid palps and algae, appear as very important items in the diet of *P. maculata* in the natural rocky reef, but not in the port area. Terebellid palps were very rarely associated with whole polychaetes, suggesting that palps are either digested much slower than the rest of the body, or are torn off from their bodies during browsing predation (Woodin 1982). The latter alternative appears to be most probable considering the sedentary habits of terebellids, which live inside tubes in the sediment or between other organisms, exposing their palps only to



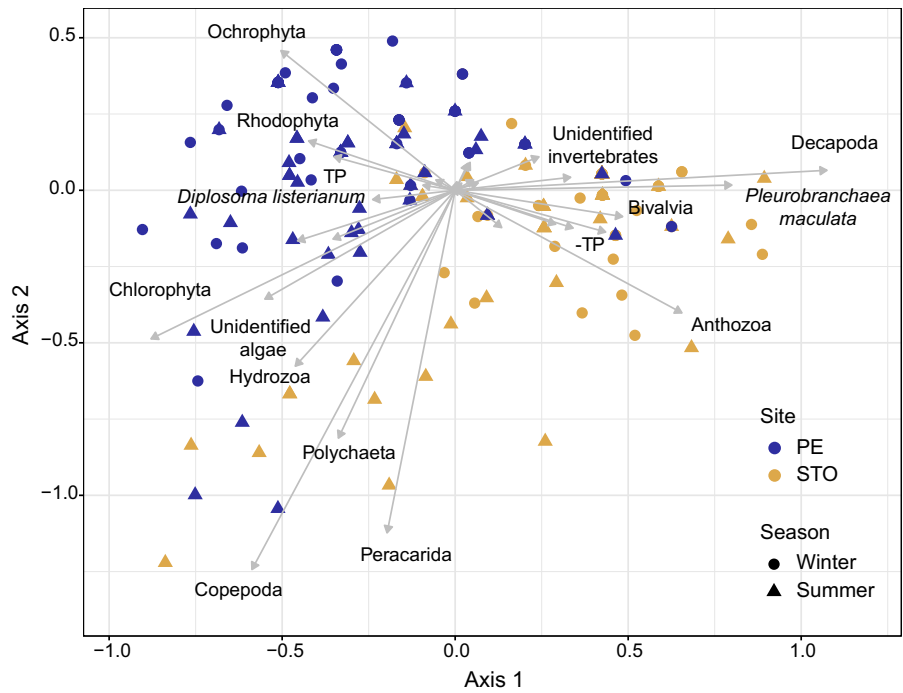
**Fig. 4** Photographs of the main taxa found in the stomach content of *Pleurobranchaea maculata* grouped by phylum: **a** Cnidaria, **b** Nematoda, **c** Nemertea, **d** Polychaeta, **e** Bryozoa, **f** Mollusca, **g** Chordata and **h** Arthropoda. Scale bar = 1 mm

capture food (detritus). In natural rocky reefs, terebellids are relatively common (Rechimont et al. 2013) and live in tubes inside the substrate, from where palps emerge radially (Dales 1955). Sub-lethal browsing predation, which affects anterior ends of benthic infaunal species, and its impact on prey population dynamics has been thoroughly described for spionid polychaetes (de Vlas 1979; Zajac 1985, 1995), but it is not so commonly described for terebellids, especially in the SWA. Further study is needed to address the impact of sub-lethal predation of *P. maculata* on native populations of these terebellids. Overall, these observations support the *plasticity hypothesis*,

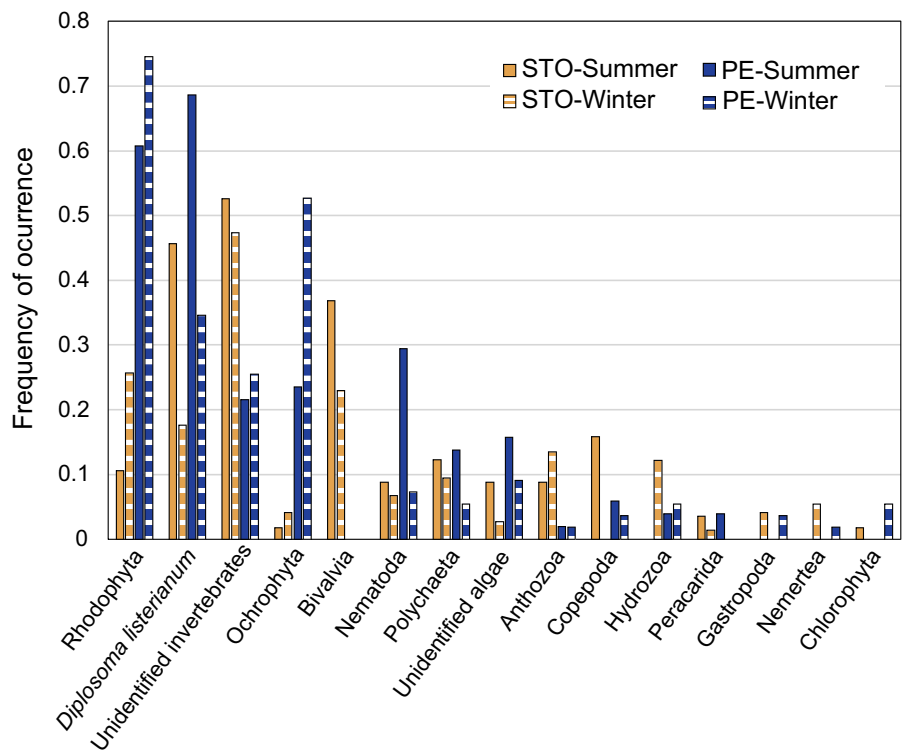
suggesting that *P. maculata* is able to exploit resources even in novel unfavorable environments (Richards et al. 2006).

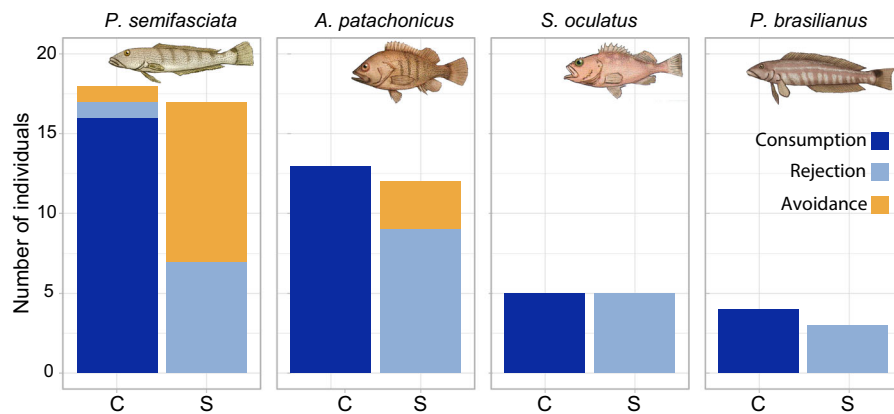
Artificial structures such as pilings and pontoons favor the establishment of marine sessile species by creating novel habitats for benthic and epibenthic communities (Connell 2001; Glasby et al. 2007; Dafforn et al. 2012; Dafforn 2017). In the port area, the abundance of bivalves and anthozoans is very high, while these taxa are scarcer and scattered in natural rocky reefs (Rechimont et al. 2013; Epherra 2016). We found that diet composition was consistently richer in both food items in the port area than in the natural

**Fig. 5** Multiple correspondence analysis (MCA) for the stomach content of *Pleurobranchaea maculata* individuals from the port area (STO) and the natural rocky reef (PE) at both seasons (summer and winter). Labels are shown only for the taxa that most accounted for the ordination due to their presence or absence (–) are shown in the biplot. TP: terebellid palps



**Fig. 6** Frequency of occurrence of the main prey items in stomach content of *Pleurobranchaea maculata* collected in summer and winter in the port area (STO) and the natural rocky reef (PE)





**Fig. 7** Number of fish per species that consumed, rejected (swallowed and final rejection) or avoided (not swollen despite careful inspection) when offered control food (C) or a specimen of *Pleurobranchaea maculata* (S) as potential prey in the field

rocky reefs. Bivalves and anthozoans have a larger nutritional value than *D. listerianum* and terebellid palps (Bell et al. 2003), which were the most frequent food items in the natural rocky reef. It is likely that the pilings of the port represent important feeding areas to *P. maculata*, which would also explain why there is a higher abundance of these slugs on the pilings compared to the surrounding bottom (Battini 2020). Also, it is possible that the differences in food properties between sites accounted for the dissimilarity in the size (weight) of the slugs, although an alternative explanation may be that they reflect ontogenetic shifts in the diet of *P. maculata*. However, a careful inspection of the frequency of each type of prey item per size class did not reveal a pattern consistent with the latter alternative. Whether the increase in body mass results in an enhanced reproductive success or not remains to be tested, but our results suggest that the increment in the supply of resources provided by the artificial structures in the Nuevo gulf might have favored the establishment of *P. maculata*. These observations resemble those of Bökenhans et al. (2019), who found anthozoans and bivalves in stomachs of slugs collected in a shipwreck, but not in the surrounding natural rocky reef. These evidences suggest that artificial structures indirectly favor *P. maculata* through the provision of more condensed and nutritionally richer food sources, which aligns with the *increased resource availability hypothesis* (Sher and Hyatt 1999; Davis et al. 2000). Furthermore, it may indicate that *P. maculata* can increase its fitness in favorable novel environments, in

agreement with the *plasticity hypothesis* (Richards et al. 2006).

Finally, our results support the hypothesis that *P. maculata* lacks predators in the southern SWA, along which it is currently expanding its distribution (Battini et al. 2019). Experimentally, we found that the most abundant potential predators including crabs, fish and octopus, do not predate on *P. maculata*. Local predators can greatly determine the outcome of an invasion following the introduction of a novel potential prey (Rogers et al. 2016; Yorisue et al. 2019) through biotic resistance. In the Nuevo gulf area, the absence of predators (i.e. biotic resistance) could certainly be a fundamental driver for the invasion success of *P. maculata*. Our results suggest that the only ‘predators’ that this species has in the southern SWA are larger conspecifics that prey on small individuals. Cannibalistic habits represent intraspecific forms of predator–prey interactions that can lead to the self-regulation of population levels (Fox 1975; Moksnes 2004). However, we found that successful cannibalism occurred in a very low frequency and only when other types of prey were unavailable, suggesting that it is an opportunistic behavior rather than an important self-regulation process. Overall, in this work we found support for the absence of biotic resistance as an explanation for the invasion success of *P. maculata*. As far as we know, no predation events upon this species (or genus) have been reported elsewhere, although there is no clear evidence that these do not occur in the native range. Addressing this issue would add very valuable information to better understand the causes driving the invasion success of



*P. maculata* in the SWA, as well as it would provide key information to predict the outcomes of further introductions and conceive potential management strategies (Gribben and Byers 2020).

The evidence found in this study indicates that the mechanisms mediating predator avoidance are related to the presence or secretion of unpalatable substances from the mantle, and not to other types of behavioral or morphological defenses. Prey specimens of *P. maculata* were rejected before consumption, usually preceded by an initial harmless prey manipulation. Then, the possibility of defenses based on toxicity following ingestion (Caro and Ruxton 2019), which we initially proposed as the mechanism underlying predator avoidance, was unsupported. The presence of neurotoxins appears as one of the most interesting aspects of this species as a potential prey, as they represent *novel weapons* in the SWA (Farías et al. 2019) that could threaten naïve native predators (Llewelyn et al. 2014). The adaptive value of neurotoxins as an antipredator defense was discussed by Williams (2010), who suggested that the function of these toxins could be partially inferred by its distribution in tissues. In *P. maculata*, an antipredator function for TTX was proposed as it is contained in basement membrane layer and bound cells of the epidermis (Salvitti et al. 2015), and there is evidence that some predatory fish can sense neurotoxins through gustatory receptors (Yamamori et al. 1988), which can ultimately lead to prey rejection (Itoi et al. 2014). Blue-ringed octopuses (*Hapaloclaena* spp.) also harbor neurotoxins in the skin and there is no evidence that it is secreted (Williams 2010). In these octopuses, the adaptive value of TTX probably relates to toxicity following ingestion, which is consistent with the presence of blue aposematic rings that indicate the presence of TTX through visual stimuli (Guilford and Cuthill 1991; Williams 2010). In contrast, there are no signs of aposematism in *P. maculata*, which has a cryptic rather than a conspicuous appearance. In this species, there is no evidence that TTX can be secreted upon any kind of stimuli, although traces of the toxin were found in the mucus of some individuals (Khor et al. 2013). However, neurotoxins are not the unique chemical defense in *P. maculata*. The absence of a protective shell in many Heterobranchia has promoted the development of a diverse range of defense strategies (Behrens et al. 2005). Among them, the acidification of the mantle through dorsal glands was

described in other pleurobranchids, as well as in other unrelated Heterobranchia (Thompson 1969; Wägele et al. 2006; Avila et al. 2018). Indeed, the mucus secreted by *P. maculata* is extremely acidic, with pH values ranging between one and two (Salvitti et al. 2015). Interestingly, *P. maculata* is not the only species in the study area with such a level of mantle acidification. We found that the native pleurobranchid, *Berthella patagonica* (d'Orbigny, 1835) and the native dorid *Geitodoris patagonica* Odhner, 1926 had very similar pH values (Muniain 2001; Battini 2020). Hence, we could not determine whether neurotoxins or mantle acidification were responsible for producing the response we observed in fish, crabs and octopuses, although prey rejection was probably driven by either of these chemical defenses. A more detailed assessment of the chemical ecology of *P. maculata* would provide more precise evidences of the mechanisms underlying predator avoidance, and if its invasion success can be explained, at least in part, by the *novel weapons hypothesis*.

Invasion success rarely relies on a single factor (Barney and Whitlow 2008; Catford et al. 2009; Gurevitch et al. 2011; Heger et al. 2013; Saul et al. 2013; Enders et al. 2020). Instead, it depends simultaneously on various drivers and on how they combine to favor or hinder the invasion process. In this work, we provide evidence that supports that the dietary plasticity and efficient anti-predator strategies are traits that can greatly increase the invasive potential of *P. maculata*. We suggest that these traits can also determine the fate of introduced gastropods besides other intrinsic morphological or reproductive characteristics (Martel et al. 2004). The majority of sea slugs appear to have few predators (Valdés et al. 2013), but many species have a very specialized diet (Wägele 1989; Trowbridge 1991; Barnes and Bullough 1996), so these traits appear as relatively rare within the group. Indeed, selective dietary requirements were suggested as limiting factors to the introduction and spread of some sea slugs such as the grazer *Syphonota geographica* (A. Adams and Reeve, 1850). This species could only establish in the Mediterranean Sea after the introduction of its preferred seaweed *Halophila stipulacea* (Forssk.) Asch. (Mollo et al. 2008). However, some of the most widespread sea slugs like *Polycera hedgpethi* Er. Marcus, 1964 (Keppel et al. 2012), *Cuthona* (= *Trinchesia*) *perca* (Er. Marcus, 1958) (Martynov et al. 2007) or *Tenellia*

*adspersa* (Nordmann, 1845) (Dhanya et al. 2017) have apparently been favored by their dietary specialization. Their specialization in other widespread non-native prey species such as the hydrozoan *Ectopleura crocea* (Agassiz, 1862), the anemone *Diadumene lineata* (Verrill, 1869) or the bryozoan *Bugula neritina* (Linnaeus, 1758) (Keppel et al. 2012; Fofonoff et al. 2018) probably aided their transportation along with their prey as part of the hull biofouling of ships many decades and centuries ago. However, to the best of our knowledge, no general invasion patterns have been studied for sea slugs at a global scale (Roll et al. 2009).

Even though predator–prey interactions can directly determine the fate of the invasion process (Rogers et al. 2016; Dick et al. 2017; Fincham et al. 2019; Yorisue et al. 2019), other numerous drivers such as the propagule pressure, environmental favorability, competition, parasitism and reproductive ecology also play key roles. Some of these issues have already been investigated for *P. maculata* (Battini et al. 2019), while others need further study. For example, there is a need to assess how intrinsic characteristics related to the reproductive ecology influence the success in the establishment of *P. maculata* in the non-native range. Probably, the high reproductive potential (Gibson 2003) also facilitates the invasion success. In addition, further study is needed to evaluate how the trophic interactions of this species could affect the structure and function of native ecosystems (Bökenhans et al. 2019, this study). Finally, there is a need to understand the dynamics of propagule pressure and dispersal mechanisms of *P. maculata* along the SWA, which will not only provide information related on the invasion history of the species, but also a clearer perspective on how to prevent and manage the introduction and spread of other invasive species.

In conclusion, our work supports the hypotheses that *P. maculata* is an opportunistic predator with a high dietary plasticity that reflect an ability to exploit resources in different types of habitats. Further study is needed to determine to what extent this predation has an ecological impact on native benthic communities. Additionally, a closer examination into geographical and seasonal patterns in the distribution of species with similar trophic characteristics, such as sea urchins and crabs, may contribute to understanding to what degree the trophic niches of these species are partitioned, and how that may have contributed to

the invasion success of *P. maculata*. Finally, we found that *P. maculata* lacks predators in the SWA that can control the abundance and spread of this species (but see Battini and Bravo 2020). Thus, our study suggests that predator–prey interactions and dietary plasticity appear as key drivers for the invasion success of *P. maculata* in Patagonia, and they are likely to favor the establishment of this invasive sea slug in other regions. Our study also suggests that combined dietary plasticity and efficient anti-predator strategies are traits that can determine the outcome of this introduced sea slug and probably other marine non-native species worldwide.

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