NOT ALL NUDIBRANCHS ARE CARNIVOROUS: TROPHIC ECOLOGY OF *POLYCERELLA EMERTONI* IN THE EBRO DELTA

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ABSTRACT

Nudibranchs have always been regarded as a group of specialized predators. In Alfacs Bay (Ebro Delta), the cryptic nudibranch *Polycerella emertoni* is found associated with the pseudo-indigenous bryozoan *Amathia verticillata*, which has been thought to be its target prey. Here, we assess the trophic ecology of *P. emertoni* using a combination of methods, including stable isotope analyses coupled with Bayesian mixing models, examination of stomach contents, and video recording of feeding activities. A morphological analysis of the radula was also conducted to explore a possible relationship with feeding behavior. Finally, we investigated the seasonal abundance of both species throughout an entire year in order to assess the nature and strength of their association. Contrary to current belief, our results show that *P. emertoni* is a micro-herbivore that feeds on the periphyton covering *A. verticillata* (up to 99% of the diet, according to mixing models). In particular, a diverse diatom community was observed on *A. verticillata*, and several of these taxa were found within the stomach contents of *P. emertoni*. Our results evidence a distinctive radular formula and morphology compared to other species and suggest the presence of multiple feeding strategies in nudibranchs. The high seasonal association observed between *P. emertoni* and *A. verticillata* may be attributed to mimicking habitat features as a mechanism to avoid predation. The possible effects of periphyton removal in the survival of detached fragments of *A. verticillata* may have implications for the dispersal of fouling species and functioning of ecosystems.

Key words: diatoms · gut contents · herbivory · mimicry · nudibranch · stable isotopes · trophic ecology · bryozoan

1. INTRODUCTION

All nudibranchs are considered carnivorous (Clark 1975, Wagner et al. 2009) and to be amongst the most specialized predators in benthic marine ecosystems (Megina & Cervera 2003). Nudibranchs are frequently associated with sessile benthic species such as sponges, hydrozoans or bryozoans that they have become adapted to consume as slow-moving predators (Todd 1981). For this reason, nudibranchs have traditionally been organized into four major feeding categories: sponge-grazers, bryozoan-grazers, hydroid-grazers, and a 'miscellaneous' group that include other animals in their diets such as other nudibranchs and tunicates (Todd 1981). Knowledge on the trophic ecology of nudibranchs is mostly based on casual observational records whereas experimental and quantitative information is scarce and has largely been overlooked (Chadwick & Thorpe 1981, Megina & Cervera 2003). This is partly due to their small size, but mostly due to their scarcity (Todd 1981), compared to other common benthic invertebrate predators such as decapods and as asteroideans. For instance, stomach contents and food-assays have only been described for larger species of nudibranchs that measure more than 10 mm, like *Platydoris argo*, *Roboastra europaea* and *Phyllodesmium poindimiei*, in which manipulation and direct observation is easier (Megina et al. 2002, Wagner et al. 2009). Also, several observations of nudibranchs feeding on bryozoan and sponge substrates have been reported (Franz & Clark 1972, Harvell 1984). However, to our knowledge, no study has yet used stable isotope analysis for a long-term dietary assessment in nudibranchs. Furthermore, although nudibranchs are supposedly highly specialized predators, there is very little information about adaptations of the radular morphology to their feeding strategy (Nybakken & McDonald 1981). Therefore, several general perceptions about the trophic ecology and biology of the group require a major re-evaluation (Todd et al. 2001).

Polycerella emertoni is a small nudibranch originally described from the Atlantic coast of North America (Verrill 1880). Many authors have suggested an amphi-Atlantic distribution of the species, including the Mediterranean Sea (García-Gómez & Bobo 1986, Moro et al. 2017). Reported locations include areas of the Western Atlantic region such as Brazil (Marcus 1957), Cuba (Espinosa et al. 2015) and Venezuela (Gutiérrez et al. 2015); and locations in the Eastern Atlantic region, including the South-West of Spain (García-Gómez & Bobo 1986), the Azores islands (Amat & Tempera 2009), and the Canary Islands (Moro et al. 2017). In the Mediterranean Sea, it was first described in 1946 in the Fusaro coastal lagoon (Italy) (Schmekel 1965), and later reported in Malta (Sammut & Perrone 1998), Greece (Koutsoubas 2000), Tunisia (Antit et al. 2011), and Spain (Camps & Prado 2018). In the Atlantic Ocean *P. emertoni* has been observed associated with *Zostera* (Verrill 1880), filamentous algae (Verrill 1880), hydroids (Chambers 1934), and soft-bodied bryozoans such as *Amathia distans* (Marcus 1957) or *Amathia gracilis* (Franz & Clark 1972 as *Bowerbankia gracilis*). However, in the Mediterranean Sea it has only been observed associated with the widespread bryozoan *Amathia verticillata* (Camps & Prado 2018), which prompted Zenetos et al. (2004) to propose *P. emertoni* as an invasive species in the Mediterranean. Yet, although the putative alien status of *A. verticillata* has been debated in the recent literature (Galil & Gevili 2014), there are no molecular studies to confirm this issue and in the meantime, the species should be regarded of unknown origin (Floerl et al. 2009). Similarly for *P. emertoni*, the number of verified observations is considerably higher in the Mediterranean than in the Western Atlantic coast, so in the absence of molecular data to support its alien status, the species is considered as cryptogenic in this paper.

Amathia verticillata is a soft bodied, stoloniferous ctenostomate capable of forming large branching colonies. The species is widely distributed in temperate and tropical waters of the Western Atlantic, where it thrives in natural habitats such as seagrass meadows, mangroves, oyster reefs and rocky-shores (Galil & Gevili 2014). However, it was first described in human-modified Mediterranean environments (delle Chiaje 1822) such as harbors and marinas, where it causes undesired fouling (Rizgalla et al. 2019). According to Micael et al. (2018), *A. verticillata* is a seasonal species; its growth declines considerably once temperature drops below 18°C (in laboratory conditions), and it moves into a senescent cryptic phase in autumn and winter (Zabala 1986). This species can produce new colonies that are capable of surviving the cold-water period and settle on a variety of natural and artificial substrates until environmental conditions become favorable again (McKinney 1983, Robinson 2004).

Franz & Clark (1972) described *P. emertoni* as a specialized bryozoan-grazer, based on its strong association with *A. verticillata* in the Mediterranean Sea. However, no feeding observations, manipulative experiments, or biochemical assessments have been conducted to support this idea, which is also questionable given the records of *P. emertoni* on other substrates (Verrill 1880, Chambers 1934, Marcus 1957, Franz & Clark 1972). Overall, there is a lack of understanding about the relationship between the seasonal abundance of *P. emertoni* and that of its potential prey.

The aim of this study was to investigate the trophic ecology of *P. emertoni.* Our initial hypothesis, supported by all work on nudibranchs conducted previously, was that that *P. emertoni* is a predator and eats the bryozoan (*A. verticillata*) on which it occurs in the Ebro Delta. However, in the course of testing this hypothesis using stable isotope analysis we found a major discrepancy between the δ^{13}C signature of the nudibranch and of its host. This led us to investigate a second hypothesis, that *P. emertoni* does not feed on the bryozoan but on its microalgal biofilm, which is available in large quantities. To this end, we conducted video-recorded feeding activities and we conducted further stable isotope analyses coupled with Bayesian mixing models and stomach content analysis, to evaluate the diet of *P. emertoni* at different time scales. In addition, we conducted a morphological assessment of the radula of *P. emertoni* and compared it with available information from other species to evaluate possible differences in their trophic ecology. A second was to understand the association between *P. emertoni* and the seasonal availability of *A. verticillata*. For this, we performed seasonal estimates of the abundance of both *P. emertoni* and *A. verticillata* aimed at investigating the nature and the strength of the relationship between them.

2. MATERIALS AND METHODS

2.1. Study site

Alfacs Bay (40°36′N, 0°43’E) and Fangar Bay (40°47′N, 0°46′E) are semi-confined estuarine areas located respectively on the southern and northern hemi-deltas of the Ebro River (Catalonia, NW Mediterranean) (Fig. 1). Both bays receive recurrent discharges of freshwater rich in nutrients and organic matter from rice fields. Furthermore, they are subject to strong seasonal salinity gradients, particularly Fangar Bay, due to its smaller size. The southern shore of Alfacs Bay (the “Banya” sandspit) was included in the Ebro Delta Natural Park in 1986 and belongs to the Natura 2000 network of the European Union, because of habitats containing the seagrass *Cymodocea nodosa* and the presence of a large population of the fan mussel *Pinna nobilis* (Prado et al. 2020).

2.2. Morpho-anatomical examination and video recording of feeding habits

The external morphology of specimens was examined under the dissecting binocular microscope (Olympus SZX10). The buccal mass was extracted and soaked in 10 % potassium hydroxide (KOH) to remove surrounding tissue. The radula and jaws were then rinsed in distilled water. These structures were finally mounted on aluminium stubs with carbon adhesive discs, sputter-coated with gold in a Fisons Instrument SC 510, and examined using a Zeiss DSM 940A scanning electron microscope (SEM) at 15 kV at the Centres Científics i Tecnològics de la Universitat de Barcelona (CCiTUB). We also recorded nudibranch feeding habits with a camera (Nikon DS-Ri1) coupled to a dissecting microscope (Nikon SMZ1500). Trials were repeated during 1-2 hours until a reasonably good quality and illustrative movie was obtained.

2.3. Stable isotopes ratios ($\delta^{13}C$ and $\delta^{15}N$) and elemental contents

In a first examination of individuals of *P. emertoni* and *A. verticillata* collected in August 2018 in Alfacs Bay, a large deviation of 2.3 ‰ between the $\delta^{13}C$ signature of the consumer and the supposed diet (*A. verticillata*) was measured. This result was discordant with the general consideration that consumers have negligible values of ^{13}C fractionation (i.e. ~0 ‰) compared to their prey (Post 2002), and pointed to the possibility of an alternative diet. Hence, in August 2019 samples of *P. emertoni* were collected together with samples of *A. verticillata* and the associated microalgal biofilm. The collection of the biofilm was conducted by gently shaking the bryozoan colony apart within a zip plastic bag filled with seawater, and then filtering the detached material through a 100 µm mesh net to remove small broken bryozoan fragments. The remaining material was filtered again through 1 µm mesh net in order to retain the diatom fraction (size ranges from 2 µm up to several millimeters, although only a few species are larger than 200 µm; see Round et al. 1990). This process was conducted five times, and the associated bryozoan fragments were also kept for stable isotope analysis. For *P. emertoni*, whole individuals (N = 100) found on the bryozoan colony fragments were pooled in groups of 20 to obtain enough material for five replicates.

All samples were dried separately at 60 ºC during 24 h, and then ground to fine homogeneous powder in a ceramic mortar. Samples were analyzed with a Flash 112 IRMS delta C series EA Thermo Finningan mass spectrometer connected to an elemental analyzer for the determination of C and N contents (at the isotopic ratio mass spectrometry facility in CCiTUB). Isotope ratios in samples were calculated from linear calibration curves constructed with standard reference materials of known composition and a blank correction. The difference in isotopic composition between the sample and reference materials was determined by:

$$\delta\ (‰) = (R_{sample} / R_{standard} - 1) \times 1000$$

where R_{sample} is the isotopic ratio in the sample, R_{standard} is that in the standard reference material, and δ (‰) is the difference in isotopic composition of the sample relative to that of the reference (Vienna Peedee Belemnite and atmospheric nitrogen for carbon and nitrogen, respectively). The reproducibility of the stable isotope measurements was ~0.1‰.

2.4. Stable isotope mixing models

The MixSiar Bayesian mixing model was used to identify the long term biomass contributions of food resources to the diet of *P. emertoni*. This model was outlined by Moore & Semmens (2008) and incorporates uncertainty and prior information into stable isotope mixing models. MixSiar v.1.0.4 uses stable isotope signatures with their standard error (SE) and tissue-diet discrimination factor input variables to estimate the probability distributions (5th, 25th, 50th, 75th, and 95th percentiles) of each food item to the mixture and accounts for uncertainty associated with multiple sources. The estimated median contribution (i.e., the 50% percentile) for each food source is usually given for comparative purposes. All the stable isotope data of the samples from Alfacs Bay in August 2019 were pooled and input into the MixSiar Bayesan mixing model. For $\delta^{15}N$, we fed the model with the 3.40 ± 0.18‰ (mean ± SE) fractionation value indicated for consumers by Zanden & Rasmussen (2001), whereas for $\delta^{13}C$, we assumed the commonly accepted view of no fractionation (Post 2002). Since these fractionation rates constitute a theoretical approximation, additional runs were conducted using 3.4 ± 1‰ values for $\delta^{15}N$ and 0 ± 0.5‰ values for $\delta^{13}C$ in order to assess the importance of possible deviations.

2.5. Stomach contents and biofilm community analysis

For molecular biology, the *P. emertoni* specimens collected from Alfacs Bay in October 2019 were preserved individually in Eppendorf tubes of 0.20 mL with 0.10 mL of ultrapure water, and stored at -80ºC until further processing. We analyzed the stomach content of N = 15 nudibranchs. To do this, we used an adapted protocol from Trobajo & Mann (2019) to digest remains of organic matter in the samples and investigate the diatom community within the stomach content of the nudibranchs. Briefly, nudibranchs were placed one by one on clean cover slips. Once the coverslips were fully dry, they were placed on a ceramic hotplate within a fume cabinet. When the temperature reached 90ºC a drop of HNO_3 (65-70 %) was added to each coverslip and allowed to evaporate for 2-3 min. This operation was repeated several times until there was no visible colored residue. Coverslips were mounted with Naphrax (Brunel Microscopes, Chippenham) for light microscopy (Nikon Eclipse 90i) or attached to stubs for SEM examination.

For evaluation of the periphyton covering the bryozoan, samples from the same mats of *A. verticillata* where nudibranchs were found were processed as previously indicated for isotopic analyses and then subjected to the same acid digestion steps mentioned above. In addition, some bryozoan samples (from August 2019) were also prepared for SEM observation. Diatom were identified to species wherever possible using mainly Witkowski et al. (2000) and Álvarez-Blanco and Blanco (2014).

2.6. Seasonal abundance of *Amathia verticillata* and *Polycerella emertoni*

In Alfacs Bay, the monthly biomass (g dry weight [DW] m^{-2}) of the bryozoan *A. verticillata* (August 2018 to August 2019) was estimated using random 5 × 5 m quadrats (N = 5) deployed in three different random sites at depths ranging from 60 to 80 cm (site A: 40°36'N, 0°43'E; site B: 40°36'N, 0°43'E; site C: 40°37'N, 0°44'E, see Fig. 1). The bryozoan colonies within each quadrat were collected within plastic bags and transported to the laboratory for further processing. All samples were dried separately at 60ºC until they reached constant weight (24 h), then weighed (0.01 g accuracy). Surface seawater temperature (ºC), salinity (psu), oxygen (mg L^{-1}) and pH were recorded monthly at the three sampling sites by using a multiparameter YSI 556 MPS instrument.

The abundance of *P. emertoni* individuals on the bryozoan *A. verticillata* (No. of individuals per g wet weight [WW]$^{-1}$) and its egg masses (No. of egg masses per g wet weight [WW]$^{-1}$) were estimated every month in which the bryozoan was found. Specifically, random samples of free-floating colonies of *A. verticillata* were collected and preserved within ice-coolers to prevent death by overheating. Once in the laboratory, a total of 30 subsamples were inspected for the presence of individuals and egg masses of *P. emertoni* under a dissecting binocular microscope (Nikon SMZ1500).

Then, subsamples were blotted dry for approx. 3 min and weighed to the nearest 0.001 g. In addition, the entire biomass of the bryozoan was surveyed for additional individuals, which tended to detach and accumulate on the edges of the container, where they could easily be collected. All individuals found were kept in absolute ethanol for later studies.

In Fangar Bay, *A. verticillata* was collected on October 2018 and July 2019, at approx. 40 cm depth. In this bay, only the abundance of *P. emertoni* and its egg masses were assessed due to logistic difficulties in reaching the area. Sample collection and processing was conducted as in Alfacs Bay.

2.7. Statistical analyses

Abundance of Amathia verticillata and Polycerella emertoni. Patterns in the abundance of *A. verticillata* (g dry weight [DW] m^{-2}) among sites (random factor, three levels) and months (fixed factor, thirteen levels) were investigated with a one-way repeated measure ANOVA. Multiple regression analysis was used to establish the relationship between the abundance of *A. verticillata* and environmental variables. Similarly, differences in the abundance of *P. emertoni* (individuals g WW^{-1} of *A. verticillata*) and their egg masses across the months of presence (fixed factor, three levels) and between bays (fixed factor, two levels) were investigated with a Student's *t* test for independent samples.

Stable isotopes and elemental contents. Differences in the δ^{13}C and δ^{15}N composition and in the elemental contents of *P. emertoni* and *A. verticillata* in Alfacs Bay between years (2018 vs. 2019) were tested with a two-tailed unpaired *t* test with Welch's correction. While stable isotope signatures and elemental contents of potential food items in August 2019 were analyzed with a one-way ANOVA.

For all parametric analyses, homogeneity of variance and normality assumptions were tested by Cochran's test and the Kolmogorov-Smirnov distribution-fitting test of the residuals, respectively. The critical level of significance was fixed at $p < 0.05$. However, in some instances the assumptions were not met by transformation, and the level of significance was fixed at $p < 0.01$ to minimize the possibility of making a Type II error. Student-Newman-Keuls (SNK) post hoc comparisons were used when necessary to identify significant differences in the interaction between sites and months. All analyses were performed using the software package STATISTICA v.13 (StatSoft, Inc., USA).

3. RESULTS

3.1. Morpho-anatomical features and video recording of feeding habits

Examined individuals of *P. emertoni* featured a small (maximum length 5-6 mm, typically 3-4 mm), translucent body with bright yellowish tones and small scattered dark brown and/or green spots (Fig. 2a-d). The small size and color patterns camouflage this species very effectively on the bryozoan where it lives. Egg masses were approx. 1.8 mm length, and over 100 eggs were found within detached mats of *A. verticillata* (Fig. 2e).

The jaws of the animals were broadly rounded with prominent anterior wings, and smooth masticatory borders. The radula was completely translucent, elongate, and narrow (Fig. 3a,e), had a radular formula 28-30 × 2.1.0.1.2, and lacked any rachidian teeth (Fig. 3c,e,f). The lateral tooth is ca. 2 times larger than the marginal ones and had an elongated and narrow base that widened in its upper area where it had two hook-shaped cusps, the upper one being wider and more developed (Fig. 3b,c,d). The two marginal teeth had a broad rectangular base and a pointed cusp (Fig. 3b,c,e,f).

Results obtained from the video recording of the nudibranch feeding habits showed an individual of *P. emertoni* repeatedly sucking a kenozooid of *Amathia verticillata*, without visible loss of, or damage to, the bryozoan tissue (see S1).

3.2. Stable isotopes (δ^{13}C and δ^{15}N) and elemental contents

δ^{15}N values of *P. emertoni* in Alfacs Bay were significantly higher (df = 5.27, t-value = 18.64, $p < 0.05$) in 2018 than in 2019 (12.59 ± 0.03 and 11.02 ± 0.07 ‰, respectively), and δ^{13}C values also differed (df = 4.67, t-value = 0.77, $p < 0.05$) between years (-17.40 ± 0.03 and -16.61 ± 0.13 ‰, respectively) (Fig. 4). In contrast, for elemental contents, no significant differences were observed between 2018 and 2019 (%C: 40-01 ± 1.22 % and 39.03 ± 0.35 %; %N: 12.01 ± 0.02 % and 11.16 ± 0.01 %, respectively for each year).

For *A. verticillata*, δ^{15}N values in Alfacs Bay were not significantly different between 2018 and 2019 (δ^{15}N: 7.88 ± 0.04 ‰ and 7.96 ± 0.09 ‰, respectively), but δ^{13}C values did differ (df = 6.70, t-value = 19.77, $p < 0.05$) (-15.11 ± 0.09 ‰ and -18.60 ± 0.14 ‰, respectively). For elemental contents, no significant differences between years were observed (%C: 13.25 ± 0.39 % and 12.2 ± 0.46 %; %N: 2.72 ± 0.06 % and 2.76 ± 0.11 %).

When the two potential diets (biofilm of microalgae and *A. verticillata*) and the consumer (*P. emertoni*) were investigated together for Alfacs Bay in 2019, results showed the presence of significant effects for both stable isotope signatures and elemental contents (Table 1, Fig. 4). The δ^{13}C signature of the nudibranch *P. emertoni* (-16.61 ± 0.12 ‰) was very similar to that of the biofilm (-16.76 ± 0.06 ‰), whereas that of *A. verticillata* was significantly lower (-18.60 ± 0.15 ‰) (Fig. 4). The δ^{15}N signature was significantly higher in *P. emertoni* (11.02 ± 0.07 ‰), followed by the biofilm (9.89 ± 0.32 ‰) and *A. verticillata* (7.96 ± 0.09 ‰) (Table 1, Fig.4). For elemental contents, %C was higher in *P. emertoni* (39.03 ± 0.35 %), but not significantly different between *A. verticillata* and the biofilm (12.20 ± 0.46 % and 13.06 ± 0.27 %, respectively) (Table 1). Elemental N was significantly different between organisms, with higher values in *P. emertoni* (11.16 ± 0.10 %), followed by *A. verticillata* (2.76 ± 0.11 %), and in the biofilm (1.67 ± 0.03 %) (Table 1).

3.3. Stable isotope mixing models

Results for the 50 % percentile showed that the main component of the *P. emertoni* diet in Alfacs Bay was the biofilm of microalgae growing on the bryozoan (99.90 %) with only a minor component of *A. verticillata* (0.10 %). Changes of ± 1 ‰ in the value of $\Delta^{15}N$ did not cause any change in the outcome of the result, whereas changes of ± 0.5 ‰ in the value of $\Delta^{13}C$ resulted in a variability of 81 to 100 % contribution (respectively for -0.5, and + 0.5 rates) of periphyton to the nudibranch diet.

3.4. Stomach contents and biofilm community analysis

Diatom cells were found in 12 of the 15 individuals of *P. emertoni* whose stomach contents were examined (8 of the 11 examined in LM and 4 out of 4 by SEM). The diatom species and their abundance seemed to vary among individuals (see Table. 2, Fig. 5h-n). The two digested samples of the biofilm growing on the bryozoan *A. verticillata* showed a rich and highly diverse community of benthic diatoms (Fig. 5a-g, 6), some of the most common being *Nitzschia* cf *incognita*, *Navicula* cf *normaloides*, *N.* cf *salinicola*, *Halamphora coffeaeformis* group, *Hyalosynedra* cf *hyalina*, *Cocconeis* spp, *Mastogloia* cf *lanceolata*, *M.* cf *cuneata*, *Seminavis* spp., *Grammatophora* spp. and *Brachysira* spp..

Determining potential selectivity of *P. emertoni* for particular diatom species or guilds was beyond the scope of this study. However, the approach we used could easily be adapted to look for dietary preferences.

3.5. Seasonal abundance of *A. verticillata* and *P. emertoni*

Seasonal temperatures in Alfacs Bay ranged between 13.7 and 30.8 ºC, with an average of 20 ± 0.8 ºC, and this was the most influential variable affecting the growth of *A. verticillata* (Fig. 7). There was significant temporal variability in bryozoan abundance in Alfacs Bay across sampling months and years, with higher occurrence from July to August at water temperatures from 27 to 30.8 ºC (Table 3, Fig. 8). Spatial variability among study sites was not significant, and neither was the Time × Site interaction (Table 3).

Results from multiple regression analysis showed a significant correlation between *A. verticillata* abundance and water temperature ($r^2 = 0.58$, df = 1, $F = 23.17$, $p < 0.05$); whereas oxygen, salinity and pH did not have a significant effect.

Student's *t* test showed significant differences in the abundance of *P. emertoni* and its eggs masses across months and bays (Table 2). Both individuals and egg masses were dominant in Alfacs Bay during the summer period, whereas abundances in Fangar Bay peaked in October (Fig. 9, Table 4).

DISCUSSION

This study provides the first record of herbivorous behavior in the order Nudibranchia by evidencing feeding on periphyton by the cryptic nudibranch *P.*

emertoni. This evidence was achieved using an array of techniques including video recording, stable isotopes ratios, and stomach contents. Our video recording showed an individual of *Polycerella emertoni* repeatedly sucking a kenozooid of *Amathia verticillata* but without actual loss of bryozoan tissue or damage to it. This suggests *P. emertoni* is feeding on the biofilm covering the external branching structure of the bryozoan. According to the Bayesian stable isotope mixing model used, this biofilm may constitute up to 99 % of the diet of *P. emertoni*, although contributions ranging from ca. 80 to 100 % are possible depending on variations in the fractionation rates (3.4 ± 1 ‰ for ^{15}N and 0 ± 0.5 ‰ in ^{13}C). The δ^{13}C signature of *P. emertoni* (-16.61 ‰) was very similar to that of the biofilm (-16.76 ‰), as expected for diet sources under the hypothesis of null ^{13}C fractionation (Post 2002). This biofilm was present in large abundance on the surface of *A. verticillata*, and was mostly composed of a diverse array of diatom species which were also found in the stomach contents of most of the individuals of *P. emertoni* examined (e.g. *Nitzschia* cf *incognita*, *Navicula* cf *normaloides, N.* cf *salinicola* or *Hyalosynedra* cf *hyalina*). An important next question to answer is whether *P. emertoni* exhibits any selectivity in its feeding across the three-dimensional structure of the biofilm, including from mainly attached, sometimes chain-forming species to several solitary and mobile ones.

Although this study provides the first and only evidence of herbivorous behavior in nudibranchs, there are some other orders of Opisthobranchia whose species are characterized by different feeding behavior, including herbivory. For instance, species from the order Cephalaspidea show the greatest diversity of feeding strategies, ranging from herbivory to active predation (Kohn 1983). In the order Sacoglossa, most of species described are strict herbivores (Raven et al. 2001), but some in the genera *Olea* and *Calliopaea* are indicated to be specialized egg-predators (Coelho et al. 2006, Filho et al. 2019).

The existence of a possible association between radula morphology and food type was investigated by Nybakken and McDonald (1981) in an array of nudibranch species feeding on different types of prey, such as bryozoans, cnidarians, and tunicates. Unfortunately, their description was limited to the number of teeth per row and the full radular formula was not provided. The only species known to be similar to *P. emertoni* in their radula morphology are *Triopha catalinae, Limacia cockerelli* (MacFarland, 1905, Nybakken & McDonald 1981 as *Laila cockerelli*) and *Antiopella barbarensis,* which supposedly display a food preference for hard bryozoan colonies, which is not consistent with the diet of *P. emertoni*. Nybakken and McDonald (1981) noticed that the teeth of *P. emertoni* have a relatively narrow base compared to other species of nudibranchs with a similar number of teeth per row (such as *T. catalinae*), suggesting that this character might also be important for determining diet type. On the other hand, *P. emertoni* displays a radular morphology and formula (28-30 x 2.1.0.1.2) like those in some species of similar size and behavior in the genus *Okenia* (family Goniodorididae). For instance, *O. zoobotryon*, presumably a specialist consumer of *A. verticillata,* has a quite similar radular formula of 25 × 1.1.0.1.1 (Pola 2014). *O. polycerelloides* (29 × 1.1.0.1.1 in Sales et al. 2019), *O. problematica* (10-12 × 1.1.0.1.1 in Pola et al. 2019), *O. evelinae* (28 × 1.1.0.1.1 in Marcus 1957) are also comparable to *O. zoobotryon*, which could mean that they have a similar diet. The radula of *P. emertoni* and these *Okenia* species is also long and narrow, with few teeth in each row; the lateral teeth have 1-2 cusps and denticulation on their inner face and smaller marginal teeth. Other investigated species in the Polyceridae family, such as *Polycerella hedgpethi* (10-11 × 3-4.2.0.2.3-4 in Miller 2001) and *P. glandulosa* (28-40 × 3.1-2.0.1-2.3 in Behrens

1988), also display radular formulas somewhat different to both *P. emertoni* and to the previously mentioned *Okenia* species. It is also worth noting that the radula of *P. emertoni* also differs from most other radulae since it has no denticles at the end of the teeth, although the functionality of these denticles is unclear. Despite these differences in the number and shape of teeth, the radulae of these small nudibranchs are generally very similar in structure: long, narrow, and with at least one well-developed lateral tooth with at least one hook-shaped cusp, which may reflect an array of specialized feeding strategies, including the micro-herbivory condition of *P. emertoni*. However, this potential relationship is not straightforward and needs to be investigated further, including the proper determination of dietary items with analytical techniques similar to those used in the present study.

The abundance of *P. emertoni* was strongly seasonal in the study areas of the Ebro Delta. The numbers of both individuals and egg masses peak between July and October, depending on the bay, at water temperatures between 22 and 27ºC. Seasonality has already been reported in some nudibranch species (Chambers 1934), and it is possibly related to their high thermal sensitivity (Clark 1975). For instance, Lambert (1990) reported large summer peaks in the nudibranch community (including *Doto coronata*, *Dendronotus frondosus* and *Eubranchus exiguous*) associated with colonies of the hydrozoan *Obelia geniculata* in Cape Neddick (York, ME). Another possible explanation of nudibranch seasonality is high specificity for their dietary resources (Aerts 1994). In the Mediterranean, *P. emertoni* has been consistently observed associated with the pseudo-indigenous bryozoan *A. verticillata* (Camps & Prado 2018), suggesting that the bryozoan provides a unique resource that is not readily available elsewhere. Yet, *P. emertoni* from the Atlantic has been observed in other habitats such as on filamentous algae (Verrill 1880), indicating that *A. verticillata* is not itself the food source but other resources associated with those substrates. O*kenia zoobotryon*, which is taxonomically close to *P. emertoni*, is also primarily found associated with *A. verticillata*. However, small numbers of adults have also been observed on the macroalga *Gracilaria* spp. and on other bryozoans, such as *Amathia maxima* (Winston, 1982, Robinson 2004 as *Bowerbankia maxima*), thus also raising questions concerning the diet of this species. It remains to be determined whether these filamentous algae and bryozoans also possess a diatom epiphyton and whether *Okenia* feeds on it.

An alternative plausible explanation for the high degree of specificity between cryptic nudibranch species and their habitats could be related to their capacity to mimic particular substrates. Mimicry has been widely observed in many marine invertebrates in relation to predation, anti-predation, warning or reproduction (Randall 2005). In nudibranchs, the loss of the shell as a defensive mechanism has been compensated for by adaptations to prevent being detected, such as homochromy (Faulkner & Ghiselin 1983), but all currently known cryptic nudibranchs are thought to feed on their habitat. In fact, many marine invertebrates camouflage themselves as their habitat but feed on other species, so they do not necessarily incorporate coloration patterns from their diet. For instance, the pink shrimp *Pontonides unciger*, which lives firmly attached to the coral *Cirripathes* spp., provides a remarkable example of commensal mimicry by adopting the shape and color of the polyps (Tazioli et al. 2006) while feeding on zooplankton (Terrana et al. 2019). Another similar example is the crab *Xenocarcinus tuberculatus*, which displays the same varied colorations of its black coral host (Tazioli et al. 2006), despite its diet being based on detritus and zooplankton (Gosliner et al. 1996). Also, some species of caprellids can change the color of their exoskeleton depending on the substrate where they shelter and may exhibit a significant affinity for particular bryozoans (Keith 1971), although their diet is mainly based on detritus, tiny crustaceans and diatoms (Guerra-García et al. 2015).

The abundance of the bryozoan *A. verticillata* showed the same marked seasonality as *P. emertoni*, as large mats were developed during the summer and disappeared progressively in September-October (Micael et al. 2018). According to McKinney (1983) and Robinson (2004), *A. verticillata* is capable of establishing new colonies from small fragments of the stolon that are filled with a yolk-like substance and constitute resting structures persisting over the winter period (Jebram 1973). In spring, when temperature and food conditions are favorable, fragments may settle on a variety of natural and artificial substrates and grow asexually (McKinney 1983, Robinson 2004). Small-sized nudibranchs, taking advantage of the presence of their highly seasonal food, exhibit very rapid growth and can grow, mature sexually, reproduce, and lay eggs from a few days to a few weeks after the larval metamorphosis. These nudibranchs are known as opportunists or r-strategists; they do not usually maintain stable populations, and they suffer large fluctuations in the abundance of individuals over short periods of time. There are usually several generations in a single year. The larvae of benthic invertebrates may remain in the plankton for periods ranging from hours to months before settling and metamorphosing into their juvenile form (Hadfield & Paul 2001). If the larvae of *P. emertoni* are capable of surviving long periods in the plankton (in the order of months), the summer reappearance of *A. verticillata* may provide the specific biological cues required to initiate settlement as indicated for other species of nudibranchs (Hadfield & Paul 2001), but more studies are needed to confirm this idea.

Overall, the findings of our study question the general paradigm that all nudibranch are carnivores and point to the need for a reassessment of the trophic ecology of the many species in this order, particularly for those that have similar behavior and size to those of *P. emertoni*. At least a new trophic category of biofilm-grazer needs to be considered alongside the four ecological categories traditionally recognized (Todd 1981). Until more nudibranch species are studied using a similar approach to that applied in the present work, the full implications of our findings for food web/nutrient transfers will not be clear. Nonetheless, our study provides evidence of a previously neglected route for periphyton carbon and other components (such as silicon, which is metabolized and accumulated mostly by diatoms) to enter higher trophic levels during the summer period when the species become seasonally abundant. Animals take up silicon in their diet and incorporate it into connective tissues and bones, where it plays a physiological role in the calcification process (Harst & Obreza 2010). Hence, grazing of diatoms by *P. emertoni* may provide a pathway for silicon transfer to secondary consumers throughout the trophic food-web. In the particular case of Opisthobranchia, Carefoot and Pennings (2003) also found that heavier silicon-rich food was readily eaten by sea hares, and significantly reduced the time spent swimming, which has possible implications for habitat fidelity in these species. In turn, removal of periphyton may also have positive effects on the settlement of bryozoan larvae (see for instance Dahms et al. 2004) adjacent to conspecific adults, as well as enhance the survival of small detached fragments of *A. verticillata*, thus favoring its dispersal and introduction into new areas.

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1. LITERATURE CITED

Aerts LAM (1994) Seasonal distribution of nudibranchs in the southern Delta area, SW Netherlands. J Molluscan Stud 60(2):129-139

Amat JN, Tempera F (2009) *Zoobotryon verticillatum* delle Chiaje, 1822 (Bryozoa), a new occurrence in the archipelago of the Azores (North-Eastern Atlantic). Mar Pollut Bull 58(5) :761-764

Antit M, Gofas S, Salas C, Azzouna A (2011) One hundred years after *Pinctada*: an update on alien Mollusca in Tunisia. Mediterr Mar Sci 12(1):53-73

Álvarez-Blanco I, Blanco S (2014) Benthic diatoms from Mediterranean coasts. Acta Bot Hung 56:3-4

Camps J, Prado P (2018) *Polycerella emertoni* associated to *Amathia verticillata* in the Ebro Delta, NE Spain (Western Mediterranean). p.675. In: Yokes M, Andreou V, Bakiu R, Bonanomi S and others (2018) New Mediterranean Biodiversity Records (November 2018). Mediterr Mar Sci 19:673-689

Carefoot TH, Pennings C (2003) Influence of proximal stimuli on swimming in the sea hare *Aplysia brasiliana*. J Exp Mar Biol Ecol 288(2):223-237

Chadwick SR, Thorpe JP (1981) An investigation of some aspects of bryozoan predation by dorid nudibranchs (Mollusca: Opisthobranchia). In: Larwood GP, Nielsen C (eds) Recent and Fossil Bryozoa. 5th International conference on bryozoan. Olsen & Olsen, Durham, p 51-58

Chambers LA (1934) Studies on the organs of reproduction in the nudibranchiate mollusks, with special reference to *Embletonia fuscata* GOULD. Bull Am Mus Nat Hist 66:599-641

Clark KB (1975) Nudibranch life cycles in the Northwest Atlantic and their relationship to the ecology of fouling communities. Helgoländer Meeresunters 27:28-69

Coelho R, Malaquias M, Calado G (2006) *Calliopaea bellula* feeding upon egg-masses of *Haminoea orbignyana*: oophagy among opisthobranch molluscs. J Mar Biol Assoc UK 86:423-424

Dahms HU, Dobrestov S, Qian PY (2004). The effect of bacterial and diatom biofilms on the settlement of the bryozoan *Buguleta neritina*. J Exp Mar Biol Ecol 313(1):191-209

Delle Chiaje S (1822) Memorie sulla storia e anatomia degli animali senza vertebre del Regno di Napoli. Vol. 1. Dalla stamperia de '*Fratelli Fernandes*', Napoli.

Espinosa J, Ortea J, Caballer M, Moro L (2005) Moluscos marinos de la península de Guanahacabibes, Pinar del Río, Cuba, con la descripción de nuevos taxones. Avicennia 18:1-84

Faulkner DJ, Ghiselin MT (1982) Chemical defense and evolutionary ecology of dorid nudibranchs and some other opisthobranch gastropods. Mar Ecol Prog Ser 13:295-301

Filho HG, Paulay G, Krug PJ (2019) Eggs sunny-side up: A new species of *Olea*, an unusual oophagous sea slug (Gastropoda: Heterobranchia: Sacoglossa), from the western Atlantic. Zootaxa 4614(3):541-565

Floerl O, Inglis GJ, Gordon DP (2009) Patterns of taxonomic diversity and relatedness among native and non-indigenous bryozoans. Diversity Distrib 15:438-449

Franz DR, Clark KB (1972) A discussion of the systematics, reproductive biology, and zoogeography of *Polycerella emertoni* and related species (Gastropod: Nudibranchia). Veliger 14(3):265-270

Galil B, Gevili R (2014) *Zoobotryon verticillatum* (Bryozoa: Ctenostomatida: Vesiculariidae), a new occurence on the Mediterranean coast of Israel. Mar Biodivers Rec 7

García-Gómez JC, Bobo A (1986) Un nuevo doridáceo para el litoral ibérico: *Polycerella emertoni* A. E. Verrill (1880) (Gastropoda: Nudibranchia). Boll Malacol 22(1-4):49-56

Guerra-García JM, Ros M, Baeza-Rojano E (2015) Seasonal fluctuations and dietary analysis of fouling caprellids (Crustacea: Amphipoda) from marinas of southern Spain. Mar Biol Res 11(7):703-715

Gosliner TM, Behrens DW, Williams GC (1996) Coral reef animals of the indo-pacific. Animal life from Africa to Hawaii exclusive of the vertebrates. Sea Challengers Inc, Monterey, CA (USA)

Gutiérrez MC, Ortea J, Rivero N, Tucker GC, Malaquias MAE, Narciso S (2015) The opisthobranch gastropods (Mollusca: Heterobranchia) from Venezuela: an annotated and illustrated inventory of species. Zootaxa 4034(2):201-256

Hadfield MG, Paul VJ (2001) Natural chemical cues for settlement and metamorphosis of marine-invertebrate larvae. In: McClintock JB, Baker BL (eds) Marine Chemical Ecology. CRC Press, Boca Raton, p 431-461

Harst M, Obreza A (2010) The role of silicon compounds in living organism. Farm Vestn 61(1):37-41

Harvell D (1984) Why nudibranchs are partial predators: Intracolonial variation in bryozoan palatability. Ecology 65(3):716-724

Jebram D (1973) Stolonen-Entwicklung und Systematik bei den Bryozoan Ctenostomata. Z Zool Syst Evolut 11:1-48

Keith DE (1971) Substrate selection in caprellid amphipods of Southern California, with emphasis on *Caprella californica* Stimpson and *Caprella equilibra* Say (Amphipoda). Pac Sci 25:387-394

Kohn AJ (1983) Feeding biology of gastropods. In: Saleuddin ASM, Wilbur KM (eds) The Mollusca. Academic Press, London, p 1-63

Koutsoubas D, Arvanitidis C, Dounas C, Drumond L (2000) Community structure and dynamics of the molluscan fauna in a Mediterranean lagoon (Gialova Lagoon, SW Greece). Belg J Bot 130(1):135-142

Lambert WJ (1990) Population ecology and feeding biology of nudibranchs in colonies of the hydroid *Obelia geniculata.* PhD dissertation, University of New Hampshire, Durham

Mckinney FK (1983) Asexual colony multiplication by fragmentation: an important mode of genet longevity in the Carboniferous bryozoan Archimedes. Paleobiology 9:35-43

Marcus E (1957) On Opisthobranchia from Brazil (2). Zool J Linn Soc 43:390-486

Megina C, Cervera JL (2003) Diet, prey selection and cannibalism in the hunter opisthobranch *Roboastra europaea*. J Mar Biol Assoc UK 83(3):489-495

Megina C, Carballo JL, Cervera JL, García-Gómez JC (2002) The diet of *Platydoris argo* (Gastropoda: Nudibranchia) and the dietary specialization of sponge eating dorids. J Molluscan Stud 68(2):173-189

Micael J, Gillon A, Jardim N, Rodrigues P, Costa AC (2018) Sexual reproduction in the invasive bryozoan *Amathia verticillata* (Ctenostomatida: Vesiculariidae). J Coast Conserv 22(2):305-314

Moore JW, Semmens BX (2008) Incorporating uncertainty and prior information into stable isotope mixing models. Ecol Lett 11:470-480

Moro L, Ocaña O, Ortea JA (2017) Sobre la distribución de *Polycerella emertoni* Verrill, 1880, (Mollusca: Nudibranchia: Polyceridae) en las costas atlánticas de África continental, con observaciones sobre el género *Amathia* Lamoroux, 1812 (Bryozoo: Versicularioidea), su alimento, en las islas Canarias. Vieraea 45:83-88

Nybakken J, McDonald G (1981) Feeding mechanisms of west American nudibranchs feeding on bryozoan, cnidaria and ascidiacea, with special respect to the radula. Malacalogia 20(2):439-449

Pola M (2014) The identity of *Okenia zoobotryon* (Smallwood, 1910) (Nudibranchia: Goniodorididae): Redescription and proposed designation of a neotype. Am Malacol Bull 33(1):72-77

Pola M, Paz-Sedano S, Macali A, Minchin D, Marchini A and others (2019) What is really out there? Review of the genus *Okenia* Menke, 1830 (Nudibranchia: Goniodorididae) in the Mediterranean Sea with description of two new species. PloS ONE 14(5):e0215037

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83(3):703-718

Prado P, Andree KB, Trigos S, Carrasco N, Caiola N and others (2020) Breeding, planktonic and settlement factors shape recruitment patterns of one of the last remaining major population of *Pinna nobilis* within Spanish waters. Hydrobiologia 847(3):771-786.

Randall JE (2005) A review of mimicry in marine fishes. Zool Stud 44:229-326

Raven JA, Walker DI, Jensen KR, Handley LL, Scrimgeour, CM and others (2001) What fraction of the organic carbon in sacoglossans is obtained from photosynthesis by kleptoplastids? An investigation using the natural abundance of stable carbon isotopes. Mar Biol 138(3):537-545

Rizgalla J, Shinn AP, Crocetta F (2019) New records of alien and cryptogenic marine bryozoan, mollusc, and tunicate species in Libya. Bioinvasions Rec 8(3):590-597

Robinson NM (2004) Interactions between the nudibranch *Okenia zoobotryon* and its bryozoan prey *Zoobotryon verticillatum*. MSc Thesis, University of Central Florida, Orlando, Florida

Round FE, Crawford RM, Mann DG (1990) Diatoms: biology and morphology of the genera. Cambridge University Press, Cambridge, United Kingdom

Sales L, Migotto AE, Baroni S, Cunha CM (2019) Taxonomic reassessment and redescription of *Okenia polycerelloides* (Ortea & Bouchet, 1983) (Nudibranchia: Goniodorididae) based on morphological and molecular data. Mar Biodivers 49:2351-2368

Sammut CR, Perrone AS (1998) A preliminary check-list of Opisthobranchia (Mollusca, Gastropoda) from the Maltese Islands. Basteria 62:221-240

Schmekel L (1965) Die Gattung *Polycerella* Verrill im Mittelmeer (Gastr. Ophistobranchia). Pubbl Staz Zool Napoli 34:226-234

Tazioli S, Bo M, Boyer M, Rotinsulu H, Bevestrello G (2007) Ecological observations of some common antipatharian corals in the marine park of Bunaken (North Sulawesi, Indonesia). Zool Stud 46(2):227-241

Terrana L, Lepoint G, Eeckhaut I (2019) Assessing trophic relationship between shallow-water black corals (Antipatharia) and their symbionts using stable isotopes. Belg J Zool 149(1):107-121

Todd CD, Walter J, Daviee J (2001) Some perspectives on the biology and ecology of nudibranch molluscs: generalisations and variations on the theme that prove the rule. Boll Malacol 37:105-120

Todd CD (1981) The ecology of nudibranch molluscs. Oceanogr Mar Biol Ann Rev 19:141-234

Trobajo R, Mann DG (2019) A rapid cleaning method for diatoms. Diatom Res 34:115-124

Verrill AE (1880) Notice of recent additions to the marine invertebrate of the northeastern coast of America, with descriptions of new genera and species and critical remarks on others. Part II- Mollusca, with notes on Annelida, Echinodermata, etc, collected by the United States Fish Commission. Proc U S Natl Mus 3:356-409

Wagner D, Kahng SE, Toonen RJ (2009) Observations of the life history and feeding ecology of a specialized nudibranch predator (*Phyllodesmium poindimiei*), with implications for biocontrol of an invasive octocoral (*Carijoariisei*) in Hawaii. J Exp Mar Biol Ecol 372(1-2):64-74

Witkowski A, Lange-Bertalot H, Metzeltin D (2000) Diatom flora of marine coasts I. Iconogr Diatomol 7. A.R.G. Gantner Verlag K.G. Ruggell.

Zabala M (1986) Fauna dels Briozous dels Països Catalans. PhD disseration. University of Barcelona, Spain

Zanden MJV, Rasmussen JB (2001) Variation in δ15N and δ13C trophic fractionation: implications for aquatic food web studies. Limnol Oceanogr 46(8):2061-2066

Zenetos A, Gofas S, Russo G, Templado J (2004) CIESM atlas of exotic species in the Mediterranean. Vol. 3. Molluscs. In: Briand F (ed) CIESM Publishers, Monaco

FIGURES

Fig 1. Map of Europe, showing the location of the Ebro Delta. In detail, the Ebro Delta with the two bays, Alfacs and Fangar Bay (Catalonia, NW Mediterranean), indicating the three sampling sites (A, B, C).

Fig 2. *Polycerella emertoni*: a) and b) dorsal view; c) copulation between two individuals; d) lateral view; f) egg mass attached with the bryozoan branches

Fig 3. Scanning electron micrographs of the radula of *P. emertoni;* a) Radular bulb; b) marginal and lateral teeth; c) radular membrane and teeth in lateral view; d) detail of the lateral teeth; e) detail of the anterior teeth of the radula; f) marginal teeth. Scale bars = 10 µm, except in b and f that is 1 µm.

Fig 4. Stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) of the *P. emertoni* from Alfacs in 2018 and 2019 (PEA-18 and PEA-19, respectively), also of possible food items including *A. verticillata* (AVA-18 and AVA-19, respectively), and the biofilm of microalgae growing on the bryozoan (BF). Error bars are SE.

Fig 5. Light microscope photographs of diatom species: a-g) from biofilm on *Amathia verticillata*; h-n) from stomach contents of *Polycerella emertoni* individuals. a and h) *Nitzschia* cf *incognita*; b and i) *Navicula* cf *normaloides*; c, d and g) *Mastogloia* cf *lanceolata*; e) *Grammatophora* sp and *Halamphora* sp. (top cell); f) *Hyalosynedra* cf *hyalina* (left) and *Hyalosira* sp. (right); g and n) *Cocconeis* cf *scutellum*; k) *Brachysira* cf *estoniarum*; l) *Hyalosynedra* cf *hyalina*; m) cf S*tenoneis*. Scale bar (in m) = 10µm.

Fig 6. Scanning electron photographs of the biofilm on *Amathia verticillata*: a and b) overview and detail of attached diatom cells and organic matrix; c) high abundance of appressed *Cocconeis* cells with a single *Nitzschia* species (center cell); d) slim, rectangular cells of *Hyalosira* (arrows) between polyps on *A. verticillata*. Scale bars = 10 µm, except in d that is 100 µm.

Fig 7. Time series (August 2018 to August 2019) of environmental data in the three study sites (A, B and C) in Alfacs Bay, Catalonia; a) Temperature (ºC); b) Salinity (psu); c) Oxygen (mg L^{-1}); d) pH. Error bars are SE. The bar on the X axis indicated change of year.

Fig 8. Boxplots of abundance of *A. verticillata* (g dry weight [DW] m^{-2}) (5-95 percentile) per month (August 2018 to August 2019) at the three study sites (A, B and C) in Alfacs Bay, Catalonia. Error bars are SE. The bar on the X axis indicated change of year.

Fig 9. Abundance of *P. emertoni* and its egg masses per g WW^{-1} of *A. verticillata* in Alfacs and Fangar Bays in the months when present. Error bars are SE.

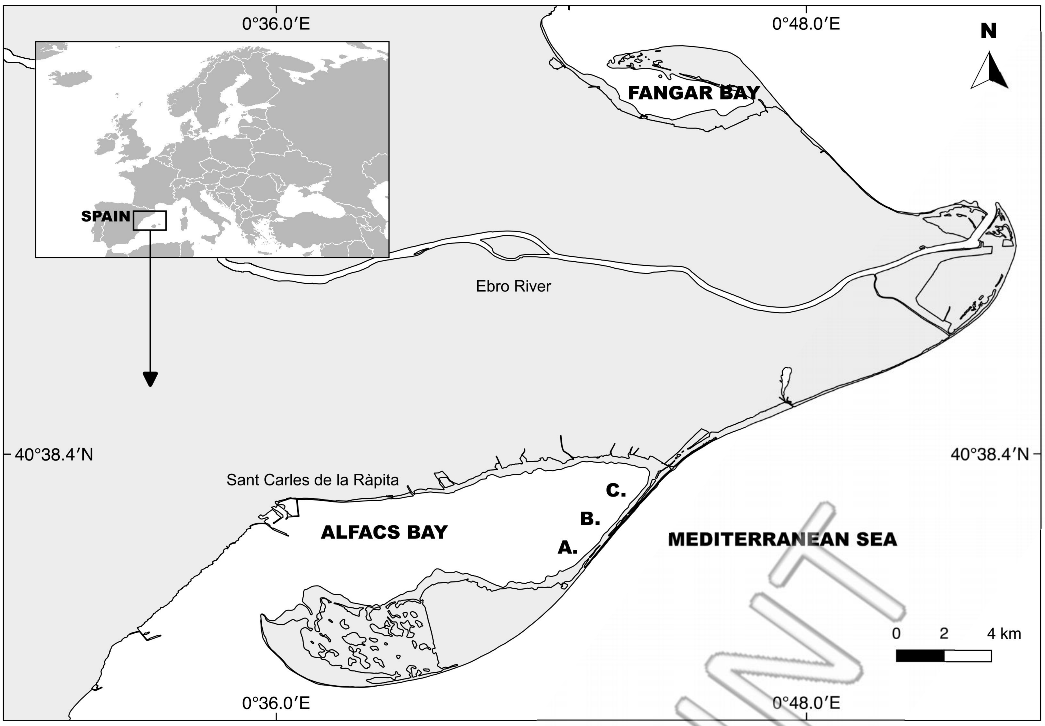
TABLES

Table 1. One-way ANOVA for differences in stable isotopic signatures and elemental contents among food items (*A. verticillata*: AVA, and biofilm of microalgae growing on the bryozoan: BF) and the nudibranch (*P. emertoni*: PE) of Alfacs Bay in August 2019: a) δ^{13}C; b) δ^{15}N; c) % C; c) % N. Statistically significant results are indicated in bold**.**

Table 2. Stomach contents of the 11 *P. emertoni* examined under light microscopy (LM) and 4 *P. emertoni* seen with scanning electron microscopy (SEM). Diatom density was assessed visually and in a qualitative way (++++ = very high; +++ = high; ++ = moderate, + = low; 0 = diatom cells not observed). Voucher slides and SEM stubs are held in IRTA center of Sant Carles de la Ràpita.

Table 3. One-way repeated measures ANOVA results for differences in the abundance of *A. verticillata* across sites (A, B and C) and between months: 2018 (Aug-18, Sep, Oct, Nov and Dec) and 2019 (Jan, Feb, Mar, Jun, Jul, Aug-19). Abundance data were log(x) transformed. Statistically significant results are indicated in bold. *p < 0.01 (see Material and Methods section)

Table 4. Student's *t*-test results for differences in the abundance of a) *P. emertoni* and b) eggs masses of *P. emertoni* between bays (Alfacs (A) and Fangar (F)) and at the different months when presence was observed: 2018 (Jul, Aug-18 and Oct) and 2019 (Aug-19, Jul and Oct). Statistically significant results are indicated in bold.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SPAIN
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Fig. 1

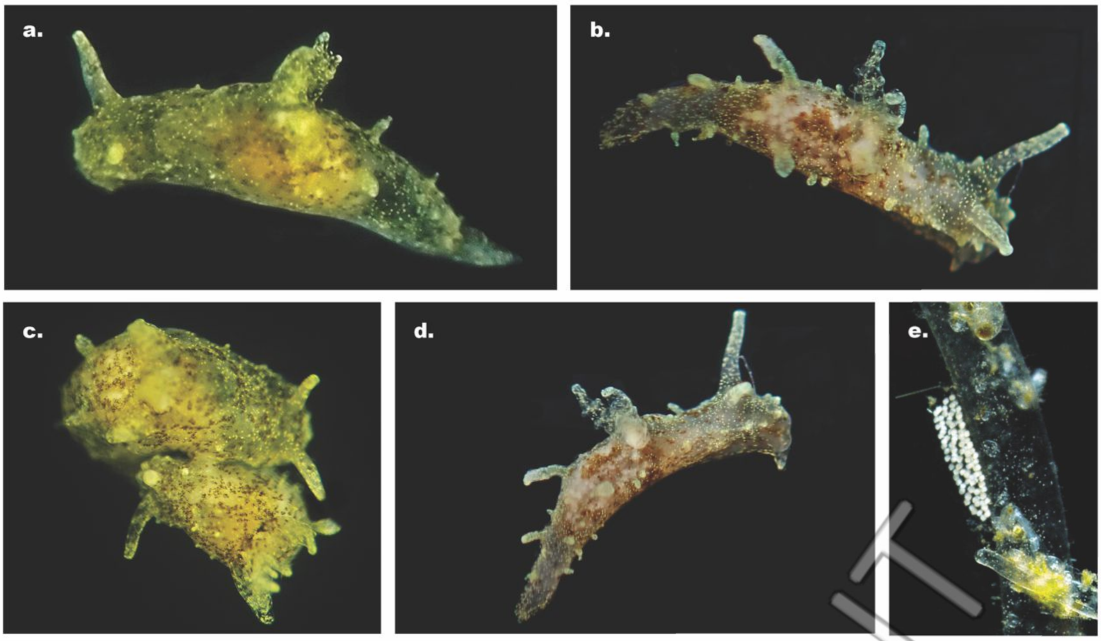

Fig. 2

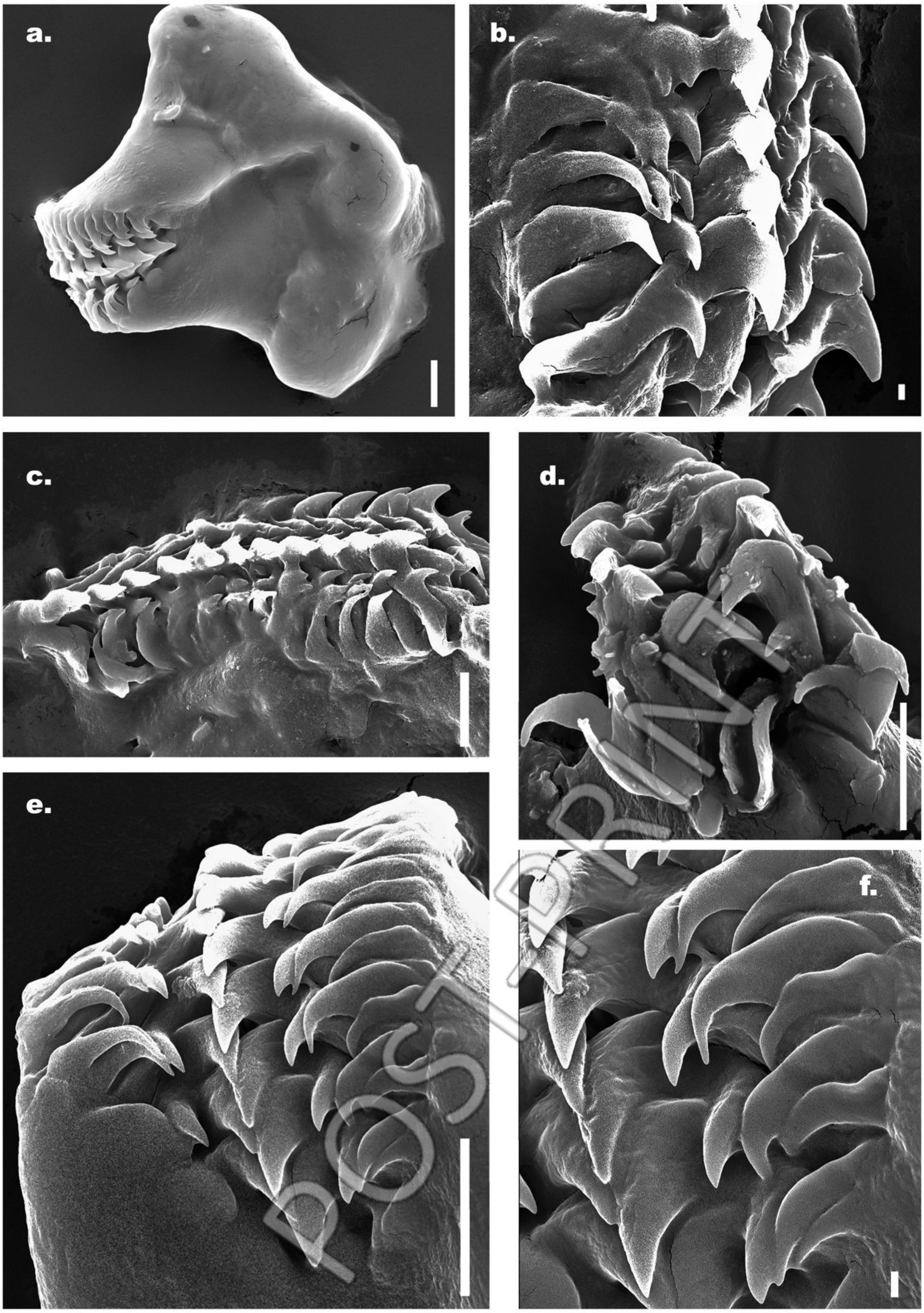

Fig. 3

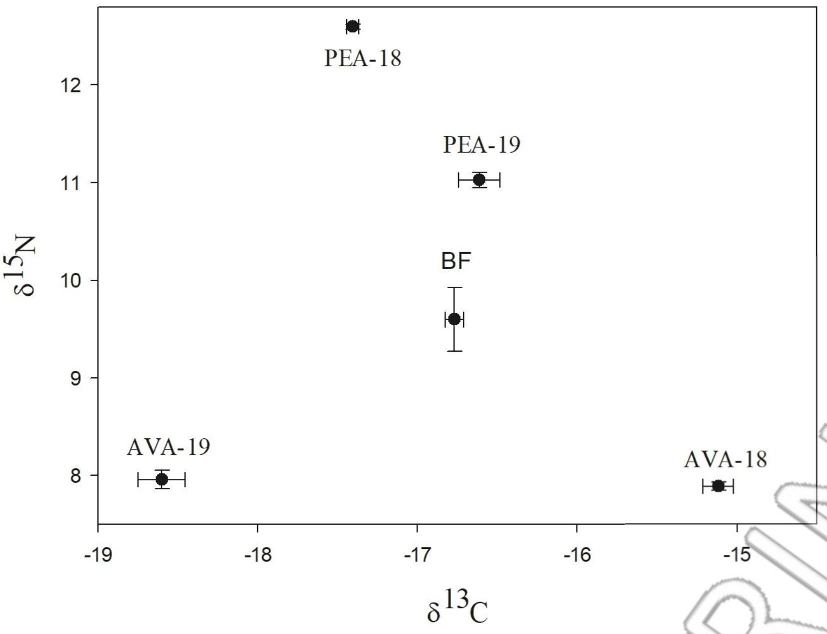

Fig. 4

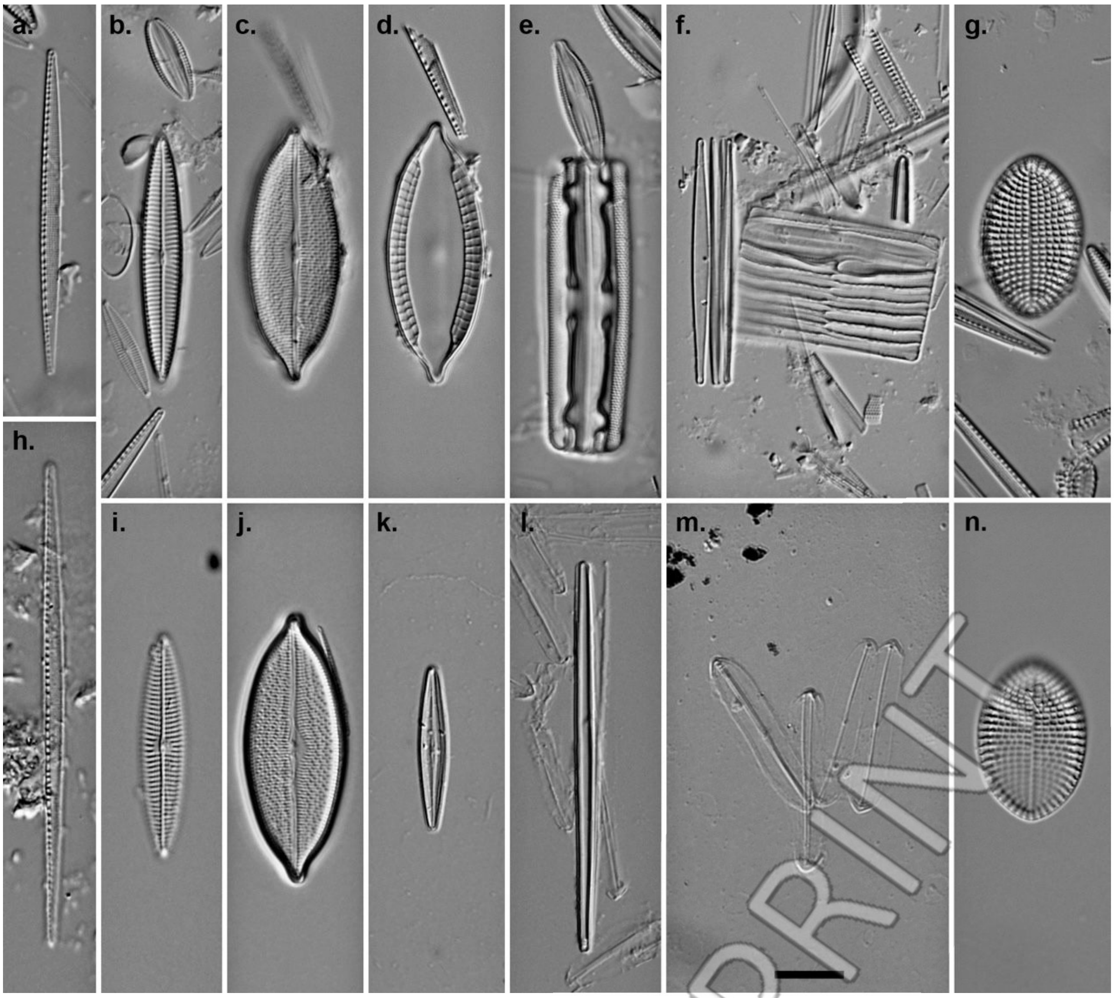

Fig. 5

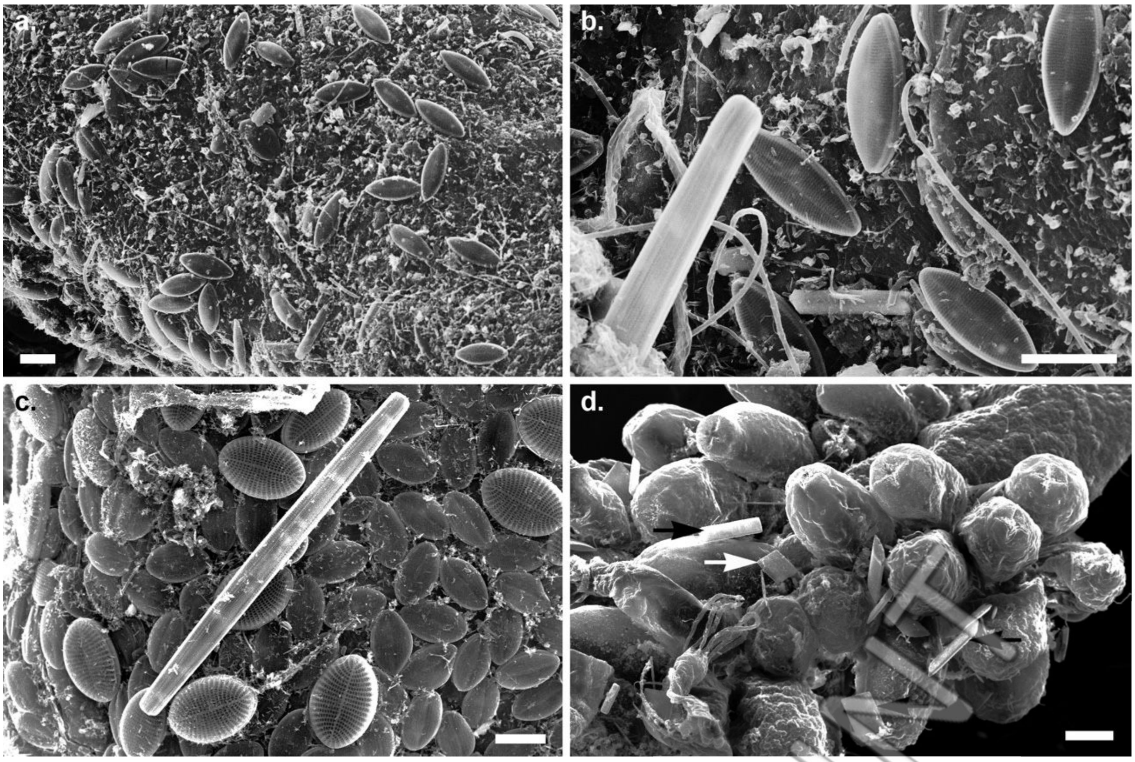

Fig. 6

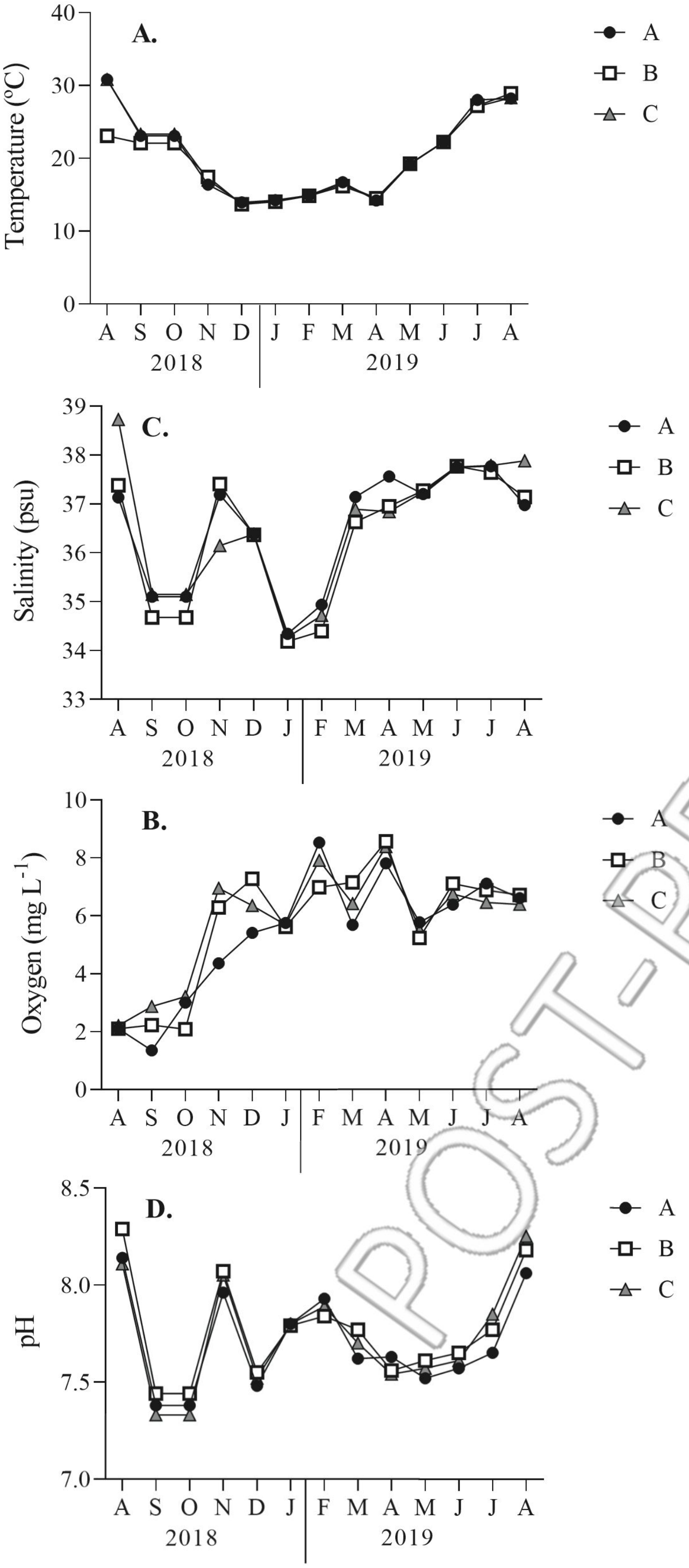
A.
Temperature (°C)
40
30
20
10
0
A S O N D J F M A M J J A
2018
2019
A
B
C
C.
Salinity (psu)
39
38
37
36
35
34
33
B.
Oxygen (mg L^{-1})
10
8
6
4
2
0
D.
pH
8.5
8.0
7.5
7.0

Fig. 7

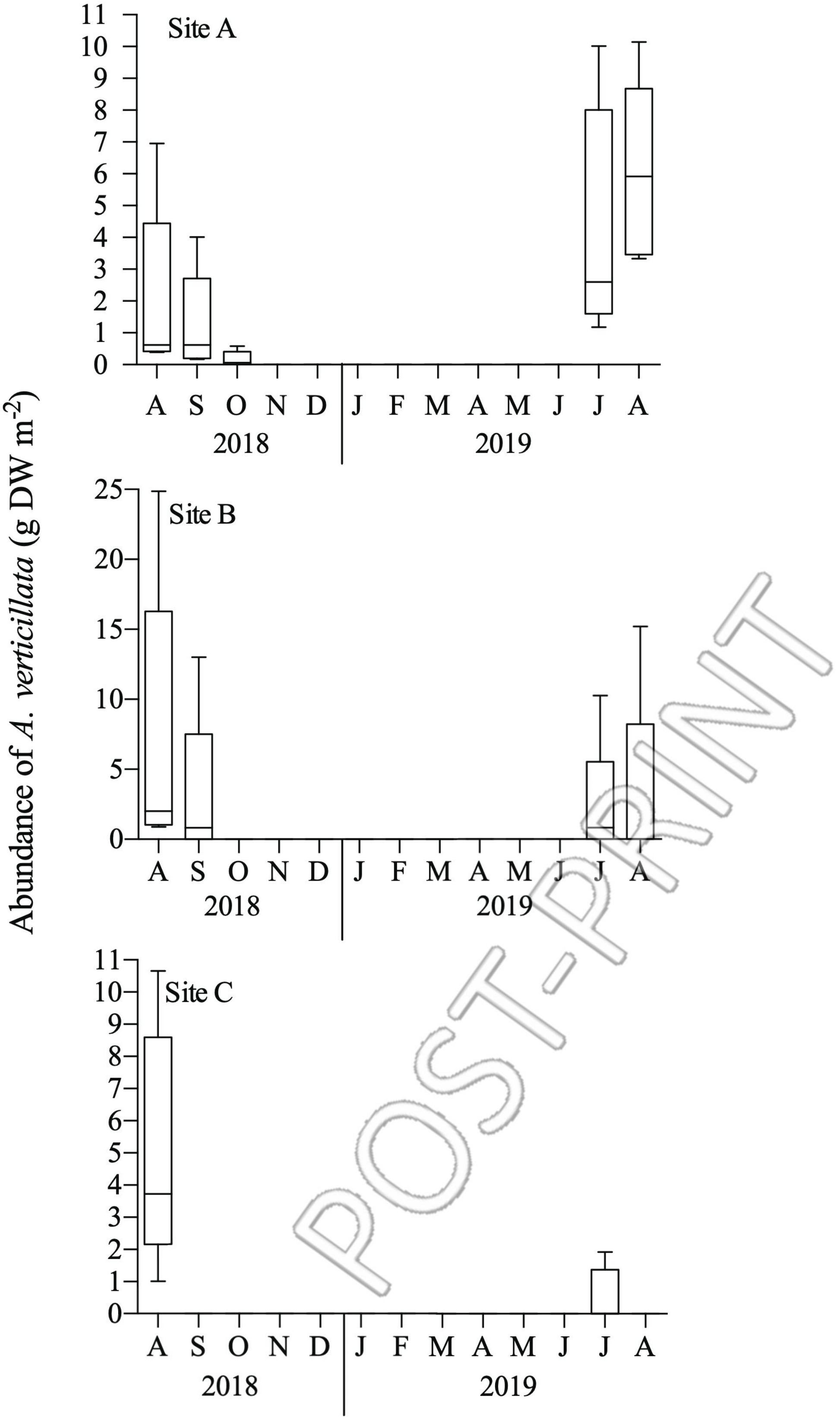
Abundance of A. verticillata (g DW m-2)
Site A
11
10
9
8
7
6
5
4
3
2
1
0
A S O N D J F M A M J J A
2018
2019
Site B
25
20
15
10
5
0
A S O N D J F M A M J J A
2018
2019
Site C
11
10
9
8
7
6
5
4
3
2
1
0
A S O N D J F M A M J J A
2018
2019

Fig. 8

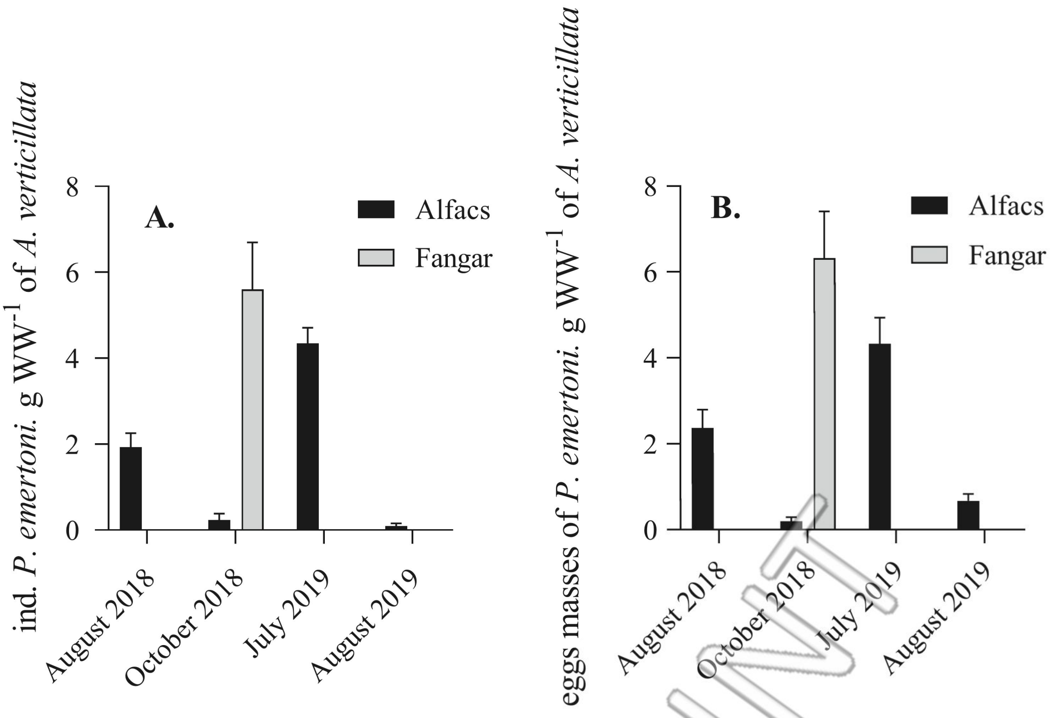
A.
ind. P. emertoni. g WW-1 of A. verticillata
8
6
4
2
0
Alfacs
Fangar
August 2018
October 2018
July 2019
August 2019
B.
eggs masses of P. emertoni. g WW-1 of A. verticillata
8
6
4
2
0
Alfacs
Fangar
August 2018
October 2018
July 2019
August 2019

Fig. 9

Table 1.

ANOVAs				
a) $\delta^{13}C$	df	MS	*F*	*p*
Species	2	6.11	87.44	**<0.001**
Error	12	0.07		
SNK	PE=BF>AVA			
b) $\delta^{15}N$	df	MS	*F*	*p*
Species	2	11.77	58.11	**<0.001**
Error	12	0.20		
SNK	PE>BF>AVA			
c) %C	df	MS	*F*	*p*
Species	2	6.11	1726	**<0.001**
Error	12	0.67		
SNK	PE>AVA=BF			
d) %N	df	MS	*F*	*p*
Species	2	134.30	3630	**<0.001**
Error	12	0.04		
SNK	PE>AVA>BF			

Table 2.

Individual	Voucher slide or stub	Diatom species observed	Diatom density
1	LMA	Lots of cells of cf *Stenoneis*; some cells of *Hyalosynedra* cf *hyalina*; some *Mastogloia* spp.	++++
2	LM1	Some cells of cf *Stenoneis*, *Navicula* sp. *salinicola*, *Hyalosynedra* cf *laevis* and *Mastogloia* cf *inaequalis*	+++
3	LM2	*Mastogloia* sp.	+
4	LM3	Some cells of *Striatella unipunctata*; *Navicula* cf *salinicola*, cf *Stenoneis*, round fragilarioid, *Proschkinia* sp.	+++
5	LM4	-	0
6	LM5	Some cells of *Cocconeis* cf *scutellum*; *Navicula* cf *normaloides*; *Mastogloia* cf *lanceolata*	+++
7	LM6	-	0
8	LM7	-	0
9	LM8	Few cells of *Mastogloia* cf *lanceolata*	++
10	LM9	Some cells of *Tabularia* cf *fasciculata*; *Brachysira estonarium* and cf *Stenoneis*	++
11	LM10	Some cells of *Nitzschia* cf *incognita*, *Navicula* cf *salinicola* and *Halamphora coffeaeformis* group	++
12-15	SEM1-4	Some cells of *Nitzschia* cf *incognita*, *Navicula* cf *salinicola*, *N.* cf *normaloides*, *Halamphora coffeaeformis* group, *Cocconeis* spp, *Mastogloia* cf *lanceolata, M.* cf *cuneata, Hyalosynedra* cf *hyalina, Striatella unipunctata, Diploneis* sp.	Not applicable

Table 3.

RM-ANOVA				
	df	MS	*F*	*p*
Site= S	2	11.92	1.45	0.27
Error	12	8.22		
Month= Mo	12	34.78	5.78	**<0.001**
Mo x S	24	10.30	1.71	0.02*
Error	144	6.01		
SNK (Mo)	Aug-18= Sep-18= Jul-19 =Aug= 19 > other months			

Table 4.

Student's *t* test			
a) Individuals of *P. emertoni*	df	t-value	*p*
Oct (A) vs. Oct (F)	58	-4.83	**<0.001**
Jul (A) vs. Jul (F)	58	11.70	**<0.001**
Aug-18 (A) vs. Aug-19 (A)	58	5.47	**<0.001**
b) Eggs masses of *P. emertoni*	df	t-value	*p*
Oct (A) vs. Oct (F)	58	-5.62	**<0.001**
Jul (A) vs. Jul (F)	58	7.16	**<0.001**
Aug-18 (A) vs. Aug-19 (A)	58	3.75	**<0.001**