Diatoms prefer strangers: non-indigenous crayfish host completely different epizoic algal diatom communities from sympatric native species

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Diatoms prefer strangers: non-indigenous crayfish host completely different epizoic algal diatom communities from sympatric native species

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Abstract

Despite their wide distribution and ecological importance, almost no information is available about the role of freshwater crayfish as basibiont for epizoic algae. Moreover, studies on epizoic freshwater diatoms have been largely neglected. In this study, we examined the occurrence of diatoms on two sympatric species, the native white-clawed crayfish *Austropotamobius* *pallipes* and the non-indigenous signal crayfish *Pacifastacus leniusculus*, coexisting in the same stream in NW Italy. We detected that signal crayfish showed a far more productive epizoic algal community than white-clawed crayfish. Microscopical analysis confirmed that non-indigenous crayfish hosted rich and diversified diatom communities while virtually no diatom was found on the native. After analyzing different hypotheses, we suggested that this significant difference can be the result of diverse crayfish behavioral habits. Because of the lack of studies investigating epibiontic diatoms on freshwater crayfish, we performed a detailed characterization on the epizoic flora, including comparisons with natural epilithic communities. The exponential diffusion of non-indigenous freshwater crayfish is a subject of greatest interest. Increasing our knowledge on their role as basibionts is definitely necessary to better understand their ecological role, also considering their influence on primary producer community and their role as vectors of algal species of concern, such as *Didymosphenia geminata*.

AQ1

Keywords

Austropotamobius pallipes
Bacillariophyceae
Epibiosis
Freshwater crayfish
Pacifastacus leniusculus

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s10530-018-1728-x) contains supplementary material, which is available to authorized users.

Introduction

Epibiosis (i.e., from the Greek ‘living on’) is the non-parasitic association of organisms, called epibionts, living attached to the body surface of other organisms, called basiobionts (Wahl 2009). When the epibiont lives on the surface of animals is also named epizoan. Several possible benefits come from the adoption of the epizoic habit, such as (1) an increased accessibility to light, minerals and nutrients, deriving from the host activity and metabolism; (2) an improved protection against grazing; (3) a more elevated and dynamic position, which reduces exposure to resuspended sediments, and (4) an evident advantage for dispersion (Francoeur et al. 2002; Romagnoli et al. 2007; Totti et al. 2011). On the other hand, the association between an epizoan and its host can have very different and also contrasting effects on this latter: epibiotic biofilm creates a sort of second skin on the basibionts that influences the interactions with the environment through several processes, i.e. changes in host’s surface physical and chemical properties, reduction in irradiation, modification of gases diffusion, nutrient uptake, camouflage and protection to desiccation but also functionality and movement impairment (Patil and Anil 2000; Wahl et al. 2012; Majewska et al. 2015).

Epizoic species and communities are heterogeneously distributed among hosts, with patterns that are currently still little known (Patoka et al. 2016). Occurrence of an epizoic species on a specific basibiont seems to depend on many factors, such as habitat (Reiss et al. 2003), environmental conditions (Wahl and Olaf 1999), season (Cabral et al. 2010), but also body surface characteristics (Wahl 2009), fitness and ontogenetic stage of the host (Dougherty and Russell 2005).

In this context, epizoic algae, and in particular diatoms, are probably more common and diffuse than previously supposed. At present, most studies on epizoic diatoms have been carried out in marine environments (Winemiller and Winsborough 1990; Robinson et al. 2016). The first description of a marine epibiont diatom dates back to 1892, when Giesbrecht observed stalked diatoms on an Adriatic copepod. Since then, diatoms have been found in the epibiotic communities attaching a multitude of different groups, such as Porifera (McClintock et al. 2005), Hydrozoa (Romagnoli et al. 2007), Bryozoa (Dahms et al. 2004), Gastropoda (D’Alelio et al. 2011), Bivalvia (Cerrano et al. 2006), Crustacea such as caridean shrimps (Bauer 1975), spider crabs (Madkour et al. 2012) and many others. In marine environments, the presence of epizoic diatoms was detected not only on sessile, benthic or generally scarcely mobile organisms, but also on large nekton representatives, such as turtles (Majewska et al. 2015), whales (Denys 1997) and even on the feathers of penguins (Randall and Randall 1984) and murres (Croll and Holmes 1982).

Interestingly, epizoic diatoms have been almost neglected in freshwater environments, where studies on algal epibiosis rarely mention diatoms (Burkholder 1996; Azam et al. 2016). Most species of snails (such as *Physa* spp. and *Helisoma* spp.) generally lack visible growth of algae on their shells, although they may host diatoms (Abbott and Bergey 2007). Freshwater diatoms have also been found on freshwater turtles (Wetzel et al. 2012), rotifers (Wujek 2006), mayfly nymphs (Wujek 2013), and copepods (Winemiller and Winsborough 1990).

Among crustaceans, for their dimensions, movement and hard exoskeletons, decapods ideally represent optimal hosts for diatoms and other microalgae.

Oddly, marine decapods are a well-known surface for epibiont algae settlement (McGaw 2006) while, despite their widespread distribution, there are only anecdotal, scattered and indirect records of epizoic algae on freshwater decapods (i.e., Tuchman 1996; Ďuriš et al. 2006; Fuelling et al. 2012).

In recent years, the presence of invasive species in freshwater ecosystems has grown enormously throughout the world (Havel et al. 2015; Fenoglio et al. 2016). In this context, the introduction of non-indigenous crayfish in European inland systems represents an interesting research subject (Gherardi et al. 2011). In Northern Italy, in addition to the presence of the non-indigenous *Procambarus clarkii* (Girard 1852) and *Faxonius limosus* (Rafinesque 1817) (family Cambaridae), recent studies reported the sudden and numerically significant expansion of the signal crayfish *Pacifastacus leniusculus* (Dana 1852) (family Astacidae) in NW Italy (Bo et al. 2016). The spread of this cold water species, well adapted to life in the low order, mountainous streams, is cause for particular concern because this Astacidae is invading areas in which still resists the native species, the white clawed crayfish *Austropotamobius pallipes* (Lereboullet 1858).

Aim of this study was to analyze the diatom epizoic community of two sympatric freshwater crayfish, one native and one non-indigenous, both in terms of chlorophyll-a content and taxonomic composition. We also characterised epilithic diatom communities of the area and we explored possible correlations between epizoic community composition and basibionts characteristics, considering in addition the possible role of crayfish as vectors of noteworthy diatom taxa.

Materials and methods

The study was conducted in a single reach of the Valla stream, NW Italy (44°27′50.23″N–8°20′59.87E, 388 m a.s.l.). In the examined station, this stream is a typical third-order Apennine lotic environment, 4.0–4.5 m wide, with a mean depth of 30–40 cm, coarse substrate and riparian vegetation composed mainly of *Salix* spp., *Populus* spp. and *Robinia pseudoacacia*. As far as we know, this is the only site in Italy in which the two species (the native white-clawed crayfish *A. pallipes* and the non-indigenous signal crayfish, *P. leniusculus*) are sympatric. In general, such sites are in general extremely rare, as the massive presence of the signal crayfish very often leads to the complete disappearance of *A. pallipes*. To avoid any possible influence of time and season, all samples were realized in the same day. We chose the late summer as sampling period, as suggested by Nowicki et al. (2008) for *A. pallipes* at these latitudes. On 13 September 2016, crayfish were collected in a 1.0 km reach of the Valla stream. The method of capture, hand picking, allowed all crayfish to remain intact. Epizoic analysis were performed on ten *A. pallipes* and ten *P. leniusculus*. All crayfish were sexed and total length (from the tip of the rostrum to the posterior edge of the telson) was measured using a 0.1 mm precision caliper. On the surface of collected specimens, the amount of benthic chlorophyll-a of the three main autotrophic groups composing the biofilm (i.e., diatoms, cyanobacteria and green algae) was detected by means of a Pulse Amplitude Modulated (PAM) fluorimeter, the BenthoTorch®, developed by BBE Moldaenke GmbH (Schwentinental, Germany). BenthoTorch® is a Pulse Amplitude Modulated (PAM) fluorimeter emitting light pulses at three different wavelengths (470, 525 and 610 nm), recording the response of cyanobacteria, diatoms and green algae at 690 nm wavelength (Kahlert and McKie 2014). On each crayfish, we measured three replicates of benthic chlorophyll-a on three dorsal points of the carapace. Moreover, epizoic samples were collected by brushing the whole dorsal surface of collected crayfish with a toothbrush. In order to characterize the flora colonizing the stretch, epilithic diatoms were also collected from 10 cobbles randomly selected in the main flow, following the standard procedure (European Committee for Standardization 2003) and producing one composite sample. Epizoic and epilithic diatom samples were preserved in ethanol immediately after sampling (final concentration ethanol 60%). Diatom treatment and slide preparation were performed following the standard procedure (European Committee for Standardization 2003). Samples were digested with hydrogen peroxide (30%) and HCl (1 N) in order to obtain a clean suspension of diatom frustules. Permanent slides were mounted with Naphrax® and observed under light microscope (Leitz Diaplan) with a 100× oil immersion objective. Diatom identification was based on several diatom floras and monographies, as well as on numerous taxonomic papers (Krammer and Lange-Bertalot 1986, 1988, 1991a, b; Lange-Bertalot and Metzeltin 1996; Krammer 1997a, b; Reichardt 1999; Lange-Bertalot 2001; Krammer 2002, 2003; Werum and Lange-Bertalot 2004; Blanco et al. 2010; Hofmann et al. 2011; Bey and Ector 2013; Falasco et al. 2013; Ector et al. 2015). The detected taxa were then classified according to moisture preferences (van Dam et al. 1994), ecological guilds (Passy 2007), life forms (Rimet and Bouchez 2012) and conservation status (Lange-Bertalot and Steindorf 1996). For each slide, at least 400 diatom valves were identified and counted in order to produce an inventory. Finally, a last screening of the whole slide was performed in order to detect rare species. We used a Kruskal–Wallis test to detect differences in chlorophyll-a between crayfish exoskeletons and a PERMANOVA to test differences between diatom communities on cobbles and crayfish.

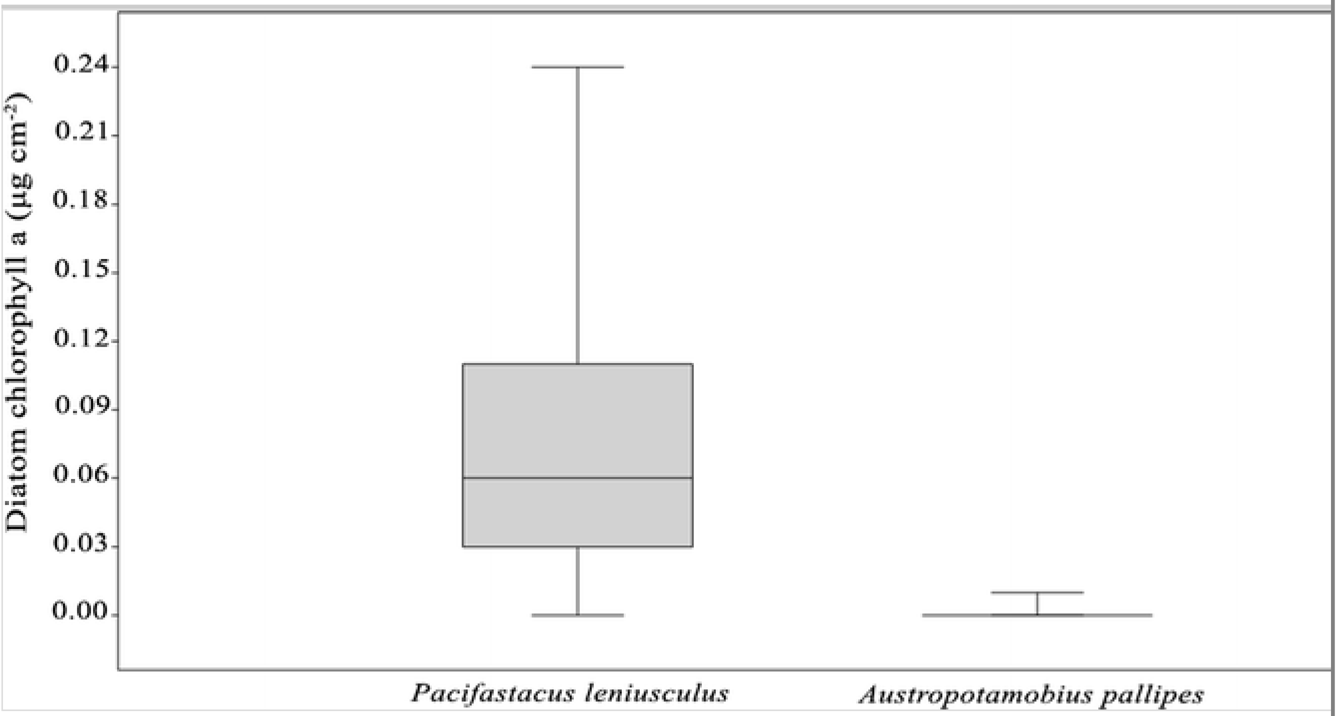
Results

Chlorophyll-a measurements on crayfish

We collected and examined 10 individuals (5 females and 5 males) of *P. leniusculus* and 10 individuals (7 females and 3 males) of *A. pallipes*. As expected, non-indigenous crayfish resulted to be on average longer (10.9 ± 1.07 cm; total body length) than native ones (7.2 ± 1.51 cm). By means of BenthoTorch measurements, we detected a significant difference in the diatom chlorophyll-a between the two crayfish species (Fig. 1—Kruskal–Wallis H = 14.3, $p < 0.001$).

Fig. 1

Mean diatom chlorophyll-a on the two sympatric crayfish species (± SD)



Mean diatom chlorophyll-a on *P. leniusculus* was 0.08 µg cm^{-2} (± 0.06 SD). No diatoms were detected on *A. pallipes* (with the exception of one, single replicate-0.001 µg cm^{-2}).

Diatom communities on cobbles

First, we characterized the diatom flora growing on cobbles, in order to achieve a baseline knowledge of the species present in our study site. The complete list of recorded taxa is shown as Supplementary Material. Epilithic diatom community was well diversified (Shannon index = 3.63) and, in total, 79 species belonging to 26 genera were identified (Table 1). Following the classification concerning moisture preferences (van Dam et al. 1994), 41% of the species were classified as “MOIST 3” (e.g. mainly occurring in waterbodies, also rather regularly on wet and moist places). Part of the community (ca. 5%) was classified as “MOIST 2” (mainly occurring in waterbodies, sometimes on wet places), while less than 2% was strictly aquatic and 52% of the community was not classifiable (Fig. 2).

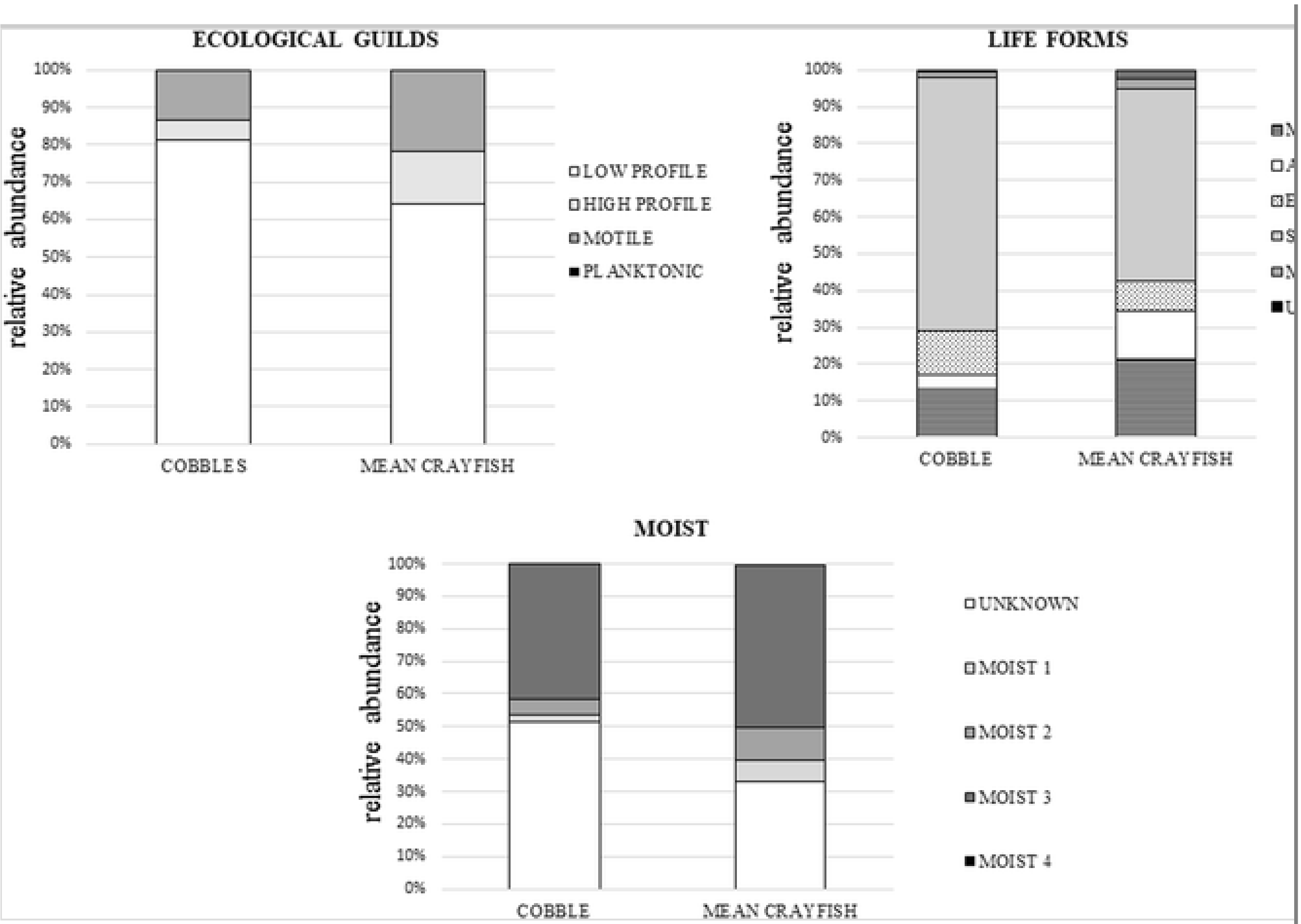
Table 1

Number of genera and species recorded on cobbles and *Pacifastacus leniusculus* on the whole slide. Shannon diversity index and evenness calculated basing on the identification of at least 400 valves

	n species	**n genera**	**Shannon index**	**Eveness**
	Whole slide		**On 400 individuals**	
Cobbles	79	26	3.63	0.70
Crayfish				
Specimen #				
1	68	26	4.33	0.76
2	76	26	3.54	0.68
3	82	34	3.98	0.70
4	91	35	4.57	0.78
5	77	30	3.62	0.69
6	75	28	4.18	0.73
7	93	33	3.78	0.66
8	56	23	3.79	0.69
9	76	32	4.51	0.79
10	71	28	4.65	0.81

Fig. 2

Epilithic and epizoic diatom community composition in terms of ecological guilds, life forms and moisture preferences. MOIST 1 = never or very rarely occurring outside water bodies; MOIST 2 = mainly occurring in water bodies, sometimes on wet places; MOIST 3 = mainly occurring in water bodies, also rather regularly on wet and moist places; MOIST 4 = mainly occurring on wet and moist or temporarily dry places



AQ2

Following the classification proposed by Passy (2007) and Rimet and Bouchez (2012), 81% of the epilithic community was represented by the *low profile* guild, e.g. taxa which are sensible to nutrient enrichment but resistant to physical disturbance. About 14% was composed by *motile* guild (e.g. fast moving taxa mainly belonging to the genera *Navicula*, *Nitzschia* and *Surirella*); while the remaining 5% of the community belonged to the *high profile* guild (e.g. species adapted to slow flows and high nutrient content). Community 3D structure seemed well developed due to the presence of adnate (4%), erected (12%) and stalked (69%) taxa. Erected diatoms included species mainly belonging to the genera*, Diatoma*, *Encyonopsis* and *Fragilaria*, while stalked were mainly represented by the genera *Achnanthidium, Cymbella* and *Gomphonema*. In detail, the most abundant species (> 2% of relative abundance) were (in order of abundance) *Achnanthidium atomoides*, *A. lineare, A. minutissimum*, *A. pyrenaicum, Amphora pediculus*, *Denticula tenuis, Encyonopsis minuta*, *E. subminuta, Navicula cryptotenella, Nitzschia lacuum*. Species belonging to the genus *Achnanthidium,* such as *A. minutissimum* and *A. pyrenaicum,* dominated in the sample, representing almost 65% of the community. Concerning the conservation status, about 56% of the community was classified as “not endangered”, while 13% as “endangered” (*A. lineare* and *A. gracillimum*), 31% was not classified (Lange-Bertalot and Steindorf 1996). Particularly interesting was the record of *Didymosphenia geminata*, recently recognized as a global invasive species, able to produce important blooms under optimal environmental conditions.

Diatom communities on crayfish

The results detected by means of BenthoTorch on the native *A. pallipes* (no diatom chlorophyll-a) were confirmed through slide analyses at light microscope, in which the number of diatom valves resulted not significant to perform species inventories. Regarding the diatom communities on the non-indigenous *P. leniusculus,* the number of identified species was high (mean: 76.5 ± 10.74 SD/specimen), so as the number of genera (mean: 29.5 ± 3.95 SD/specimen; Table 1). Shannon biodiversity index ranged from 3.54 to 4.65 (Table 1).

To determine statistically whether diatom composition differs among substrates, we performed a PERMANOVA. Despite this analysis did not reveal significant differences between control and crayfish samples ($F_{2,8} = 1.70$, $p = 0.094$), this result should be interpreted with caution. The low number of samples as well as the unbalanced sampling design could potentially lead to low statistical power to detect control versus crayfish differences in diatom communities. Concerning moisture preferences, we detected a greater presence of taxa classified as “MOIST 3” (50%) and “MOIST 4” (0.6%; e.g. mainly occurring in wet and moist or temporarily dry places) in comparison with epilithic community (Fig. 2). Concerning ecological guilds, it was possible to observe a decrease of species belonging to the *low profile* class (from 81% on cobbles to 64%, in mean, on crayfish). On the contrary, we observed an increase in the *motile* and *high profile* guilds, representing on crayfish 22 and 14% respectively of the communities. *A. minutissimum* and *A. pyrenaicum* were still the most abundant species, whereas we detected a lower percentage of *A. atomoides, A. lineare, Denticula tenuis*, *Encyonopsis subminuta* and *Nitzschia lacuum* on crayfish. These taxa were mainly substituted by *Amphora pediculus*, *Denticula kuetzingii, Navicula cryptotenella, Nitzschia fonticola* and *N. tabellaria.* Concerning life forms, it was possible to notice an increase of the adnate taxa on crayfish in comparison to cobbles. On the contrary, erected and stalked diatoms showed lower abundances on crayfish than on cobbles.

Concerning the conservation status, in mean, communities on crayfish were mainly composed of “not endangered” (ca. 67%) or “not classified” (24%) taxa. About 9% of the communities were composed of species classified as threatened with extinction at different levels. Among these, *Achnanthidium gracillimum* (classified as “endangered”) and *Nitzschia tabellaria* (classified as “declining”) were the most abundant. *Aneumastus stroesei*, a European species nowadays classified as declining due to the loss of its typical habitat (calcareous oligo-mesotrophic lakes), was often found in crayfish samples, while only rarely on cobbles. Moreover, this was the first record of *Gomphosphenia holmquistii* in North-West Italy. This species was collected for the first time in Alaska (Foged 1968). After this record, the species was detected also in Germany (Hofmann et al. 2011; Bak et al. 2012), in France (Bey and Ector 2013; Ector et al. 2015), in South–East Poland (Noga et al. 2016) and more recently in North–East Italy (Cantonati et al. 2016). Records of *G. holmquistii* are still very rare and its autecology is currently under definition. However, *G. holmquistii* seems to prefer low water temperatures, medium–low conductivity and alkaline-neutral pH. This species did not present particular preferences in terms of geological composition and was found both on carbonate springs (Cantonati et al. 2016) and not-calcareous rivers (Noga et al. 2016). Apart from rivers and springs, this species was also recorded on a wet wall of an artificially illuminated corridor inside a dam (Noga et al. 2016). Therefore, it is possible to suppose that this is not a strictly aquatic taxon. In this context, we found a higher percentage of aerophilous taxa on crayfish than cobbles. Indeed, *G. holmquistii* and *Geissleria acceptata* and *Diadesmis contenta* (all classified as “MOIST 4”) were exclusive of samples collected from crayfish. Moreover, we observed higher abundances of mucous forming species, mainly belonging to the genus *Encyonema*, on crayfish than cobbles. This genus is known to cope with desiccation, being able to live in mucilage colonies.

Finally, we found *Didymosphenia geminata* in all the epizoic samples.

Discussion

At present, a vast and increasing number of studies are addressed to different aspects of invasive freshwater crayfish biology and ecology (Holdich et al. 2014; Vaeßen and Hollert 2015; Souty-Grosset et al. 2016), but remarkably there are almost no information about their possible role as basibionts.

It is known that crayfish exoskeleton can host several epibionts, but most studies (1) are focused only on native species, (2) pay attention almost exclusively on zooepibionts, such as ciliates, rotifers, annelid worms, flatworms, and other crustaceans (see Edgerton et al. 2002). For example, one of the rare studies dedicated to epibiosis in invasive crayfish reported the presence of zebra mussels, *Argulus* egg masses and bryozoan colonies on the exoskeleton of *Orconectes limosus* in Czech freshwaters (Ďuriš et al. 2006). Another study reported the occurrence of a non-indigenous, American entocytherid ostracod on *Procambarus clarki* in the Iberian Peninsula, suggesting the latter has become an ‘invaded invader’ (Aguilar-Alberola et al. 2012). Moreover, Cuéllar et al. (2002) found Mastigophora, Ciliophora, Turbellaria, Rotifera, Annelida, Acarina and Ostracoda colonising the exoskeleton of *P. leniusculus* in a Spanish crayfish farm.

Strangely, no information about the role of invasive crayfish as basibionts for algae is at present virtually available. For this reason, the most interesting finding of this study is that freshwater crayfish not only can host epizoic algae, but that different species of crayfish can host extremely different algal communities. We detected that algal communities showed significantly higher levels of chlorophyll-a on the non-indigenous *P. leniusculus* rather than on the native *A. pallipes*. In particular, diatoms appear to colonize abundantly the first, with a total of 183 species, while they are virtually absent in the second.

It is likely that this evident diversity can be related to some differences between the two basibiont crayfish species. Here are some considerations and hypotheses on which biological and ecological characteristics may be of greater importance in this field. First of all, undoubtedly surface structure represents an important character in selecting and shaping diatom community. For this reason, a first hypothesis could be that differences in the epibiont communities can be related to differences in the substratum (i.e. crayfish exoskeleton) structure. For example, a study concerning marine gastropod shell colonization demonstrated that diatoms preferentially colonize highly organized surfaces with low fractal dimension values and regular landscapes: the more organized is a shell surface, the richer and more diverse is the diatom community (D’Alelio et al. 2011). In our study we did not analyse structural differences between exoskeletons of these two astacids, but bibliographic data allow us to assume that this probably is not the main reason for the differences we found. Indeed, Souty-Grosset et al. (2006) reported that *A. pallipes* shows a greater roughness and therefore a greater complexity of the exoskeleton than *P. leniusculus*, so if this was the main reason then algal colonization should be the opposite of what we found.

AQ3

Another interesting point is related to molting. Crustaceans, being ecdysozoa, grow through a series of molts, in which the external cuticle or exoskeleton is completely replaced, with the temporary (because movable epibionts such as temnocephalids and branchiobdellids are able to return on the crayfish immediately) loss of all associated epibionts. In freshwater crayfish, temperature is the main factor regulating the ecdysis, so in the same stream reach the number of mute is roughly the same for all sympatric species (also if molt frequency can vary with age). The fact that *P. leniusculus* is on average larger is not related to a greater number of molts but to a greater growth between one mute and the other (Souty-Grosset et al. 2006).

After these considerations, our main hypothesis it that differences in diatom colonization can be the result of different behavioural activities of the two sympatric crayfish species. In many months of field samplings (pers. obs.), *P. leniusculus* was the only crayfish we saw in activity during the day while we never observed any native specimen moving on the substrate in the sunlight hours. In fact, it is well known that *A. pallipes* displays mostly nocturnal/crepuscular activity and spends diurnal hours in refuges under rocks and masses, hidden under aquatic roots or in bank-side cavities (Robinson et al. 2000; Barbaresi and Gherardi 2001; Ghia et al. 2009). Using radio-telemetry and mark-recapture methods, Robinson et al. (2000) discovered for example that the greatest part of movements of this species occurred in the dark hours, with 90.4% of activity taking place between 21:00 and 06:00. On the contrary, we observed *P. leniusculus* in activity during daylight hours in all sampling occasions, and this pattern is confirmed by other authors (Lewis 2002). In fact, a comparison between activity of non-indigenous and native species reported that diurnal movements of *P. leniusculus* constitute 33% of its total activity and was much higher than those of some European species, such as *Astacus astacus* (12%) and *Pontastacus leptodactylus* (16%) (Lozan 2000). Another study reports that peaks of activity of this non-indigenous crayfish started since 16:00 in the afternoon, while more than 50 crayfish can be active per 100 m^2 (Abrahamsson 1983).

In the light of above considerations, we can assume that epizoic algae are favoured by the more diurnal behaviour of the signal crayfish, which allows them more growth and development. By contrast, the nocturnal habits of the native species, which spends the light hours sheltered under boulders or otherwise, drastically inhibits or even prevents the growth of photosynthetic epibionts. We did not find any branchiobdellids associated with these crayfish.

Some other interesting considerations can be made analysing the epibiont diatom communities on *P. leniusculus*. Firstly, diatom communities of both cobbles and crayfish reflected the good status of the stream reach, with a high presence of β-mesosaprobious and oligosaprobious taxa. Important dissimilarities were also found in terms of life forms, with erected and stalked diatoms more abundant on cobbles than on crayfish. This can be due to the fact that algal colonisations are characterised by clear successional patterns, in which communities at the beginning are mainly dominated by adnate species while older and well-structured communities should include all growth forms, all equally important in quantitative terms (Round et al. 1990). In this context, cobbles represent a more stable substrate for diatom colonization, because crayfish molts and movements can prevent the progressive community maturation. Our findings confirmed the study of D'Alelio et al. (2011), who observed the dominance of adnate species on gastropod shell surface, probably due to their stronger adhesion abilities. Concerning moisture, our data evidenced a slight increase in species considered aerophilous at different extent on crayfish, probably related to the displacement activities of these crustaceans. Finally, some considerations about *Didymosphenia geminata*, a stalked diatom originally described from cold oligotrophic and xenosaprobic waters of the North Europe. Over recent years, the numerous records of this species out of its original distributional area and ecological range has increased the interest of the scientific community. Indeed, under optimal environmental conditions (e.g. low nutrient content and conductivity, stable hydrological conditions, increased exposure to UV radiation) *D. geminata* produces important blooms, able to cover the whole riverbed. The macroscopic mats (several centimetres thick) covering the cobbles are mainly composed of the polysaccharidic stalks produced by the cells. The negative effects of the blooms on the freshwater ecosystem are significant at different level of the trophic chain and can have negative effects on human water exploitation. At present, it has been generally accepted that the spread of *D. geminata* is human-mediated (Kilroy and Unwin 2011), while no evidences on the role of animals in the diffusion of this species have been reported. Our results represent the first evidence that this species can use allochthonous crayfish to spread, opening interesting further research and reflection on invasive species management.

Conclusions

Except for a mention in an old paper (Chidester 1912), this is probably the first study investigating algal epibiosis on freshwater crayfish. Importantly, this study gave the opportunity to analyse for the first time epibiont diatom communities colonizing sympatric populations of non-indigenous and native crayfish. The progressive diffusion of non-indigenous freshwater crayfish is a subject of greatest interest in the current hydrobiological scenario. Increasing our knowledge on their role as basibionts is definitely necessary to better understand their ecological role also considering their influence on primary producer community and their role as hosts and vectors of extremely interesting algal species, such as *Didymosphenia geminata* and *Gomphosphenia holmquistii*.

Acknowledgements

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Electronic supplementary material

Below is the link to the electronic supplementary material.

Supplementary material 1 List of diatom taxa detected on cobbles and *Pacifastacus leniusculus*. Red List column refers to the classification concerning the conservation status proposed by Lange-Bertalot and Steindorf (1996): 1 = threatened with extinction. 2 = severely endangered. 3 = endangered. G = probably endangered. R = rare. V = decreasing. * = at present not considered threatened. ? = not threatened. · = common. Z = not listed. D = data scarce. The columns “cobbles” and “crayfish” displayed on which substrate the species was recorded (± included in the inventory; R = rare, observed only during the final screening). The last 11 columns show the percentage of relative abundance of each taxa in the samples. (XLSX 32 kb)

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