

NOTE

A case of close interspecific interactions between diatoms: selective attachment on a benthic motile species

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ABSTRACT: Although diatoms are extensively used as bioindicators, positive interactions between species have rarely been investigated. In this study, the attachment of diatoms of the genus *Amphora* on benthic motile diatoms is described. Analysis of a river biofilm sample showed that the attached diatoms clearly select large sigmoid *Nitzschia* hosts. Examination of a regional database suggests that the attachment of *Amphora copulata* may be common in rivers, and that the preferred host is *Nitzschia sigmoidea*.

KEY WORDS: Amphora · Nitzschia · Bacillariophyta · Bioindicators · Niche

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The extensive use of diatoms as bioindicators of water quality assumes that the composition of diatom communities is largely driven by abiotic environmental filtering (Lobo et al. 2016). However, neutral processes and especially biotic interactions may also considerably shape microalgal communities (Bottin et al. 2016). The most studied interactions among diatom species are those mediated by competition for light and nutrients (Titman 1976). Cases of positive interactions between species have rarely been documented (e.g. Vanelslander et al. 2009). Epiphytism generally implies a large difference in size between the epiphytes and their host. Diatoms are well known epiphytes on filamentous algae and aquatic macrophytes; however, the attachment of a diatom on another diatom is less common and has been rarely reported. In this study, we present the selective attachment of a small freshwater diatom species on another benthic motile species. This interaction was qualitatively described by Round & Lee (1989) from observations on glasses placed on top of sediment

surfaces. Here, we provide a quantitative analysis from a local to a regional scale.

A biofilm sample was scraped in the Arc river (43° 30' 41.7" N, 5° 28' 57.2" E), France, on the flank of a large rock near the river bank. The fresh sample was observed under a light microscope. Diatoms were the most abundant microalgal group. Amongst a large amount of filaments of *Melosira varians*, many long and often sigmoid diatoms belonging to the genus *Nitzschia* were seen covered with a variable number of small diatoms (Fig. 1). The attached diatoms exhibited a cymbal shape when located on the edge of the host valve and a biconvex outline when in the middle of the host valve. These observations typically correspond to the valve and girdle views of diatoms belonging to the genus *Amphora*. Nevertheless, it is difficult to identify species from fresh material (Cox 1996) and a sample digestion with boiling H₂O₂ (1 h) was necessary to remove organic matter and observe the specific ornamentation of the siliceous frustules.

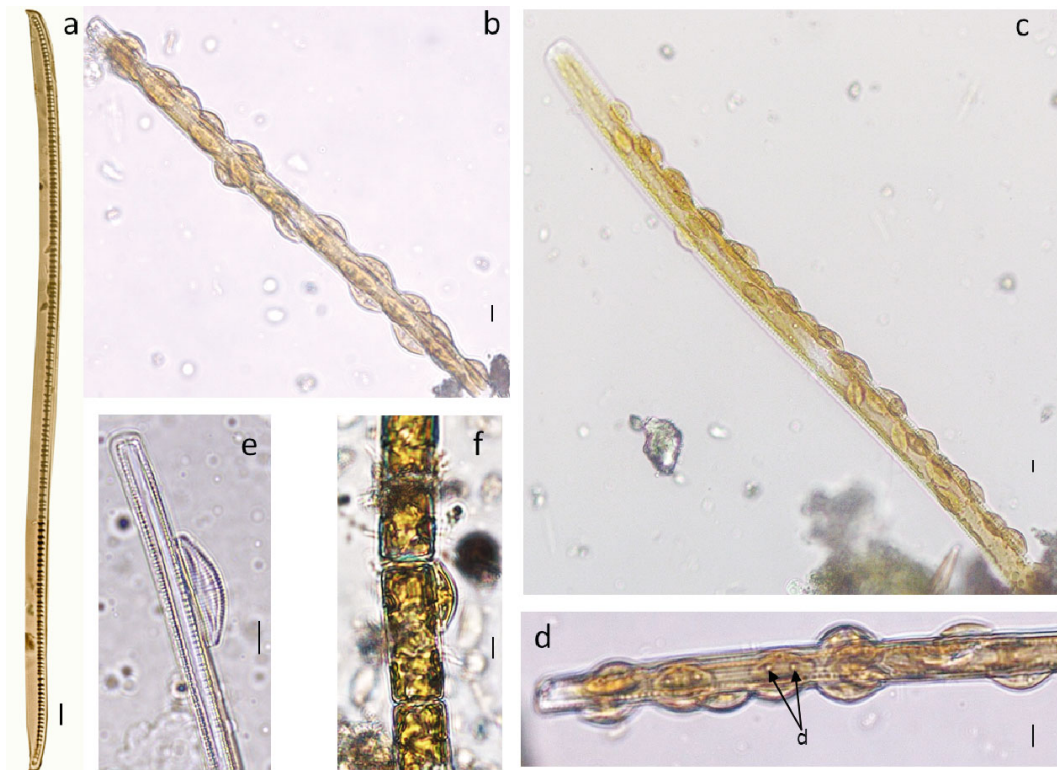


Fig. 1. Light microscope photographs. (a) A frustule of *Nitzschia sigmoidea*; (b–d) a cell of *N. sigmoidea* covered with *Amphora* sp. The 2 conspicuous droplets (arrows, d) are characteristics of *Amphora* and can be seen in some attached cells in (d). (e) Frustules of *N. sigmoidea* and *A. copulata* probably accidentally re-associated after sample H_2O_2 treatment. (f) A single cell of *A. copulata* on a filament of *Melosira varians*. Scale bars = 10 μm

The attached diatoms and their host were separated during the treatment.

The most abundant *Amphora* species (4.8%) and the most likely to correspond to the high number of attached diatoms was *A. copulata* (Kützing) Schoeman & Archibald 1986 (ACOP) (Table 1). However, the attached diatoms may also belong to the less abundant *Amphora* species identified in the sample, such as *A. pediculus* (1%) or *A. indistincta* (0.2%).

The long sigmoid *Nitzschia* could be either *N. sigmoidea* or *N. vermicularis*, which had similar relative abundance in the sample. These species of *Nitzschia* are able to move on a substrate in curved directions. The *Nitzschia* observed in the river sample moved at a speed of about $20 \mu m s^{-1}$ which did not seem reduced when attached diatoms were present (see the video in Supplement 1 at www.int-res.com/articles/suppl/a080p055_supp/).

After observation of the live material, the sample was fixed with dilute Lugol to stop cell movement before counting. There was no visible effect of fixation on cell associations. In order to assess the host specificity of the attached diatoms, 5 groups of abundant and large diatom forms were considered as

potential hosts for ACOP (Table 2). The cells from each group, with and without attached diatoms, were counted under a light microscope at 100 \times magnification (Table 2). About two-thirds of the long *Nitzschia* had attached diatoms. However, no epiphyte was seen on more than 100 observed individuals of the genus *Gyrosigma*, which have several common characteristics with the *Nitzschia* species that were observed in the sample: large size, sigmoid shape and similar motility. There were only 5 cases (3%) of attachment on filaments of *Melosira varians*, and in each case only 1 cell of ACOP was visible on the filament (Fig. 1). No attachment was observed on any large *Navicula* species nor on the other genera of large diatoms represented in the river sample. These results demonstrated a clear host selection.

The significance of attachment of ACOP at a larger spatial scale was assessed through the analysis of co-occurrences in a recently published regional database of species incidence and environmental conditions among 196 sites in western France (Bottin et al. 2016). ACOP was present in 44% of the samples in the regional database and in 86% of the samples in which *N. sigmoidea* occurred. We focused on the potential

Table 1. Taxa present in the H₂O₂-treated epilithic sample with estimation of their relative abundance (total abundance = 501)

Taxon	Relative abundance (%)
<i>Melosira varians</i> Agardh	48.1
<i>Gyrosigma sciotense</i> (Sullivan et Wormley) Cleve	9.0
<i>Amphora copulata</i> (Kützing) Schoeman & Archibald	4.8
<i>Navicula lanceolata</i> (Agardh) Ehrenberg	4.8
<i>Nitzschia recta</i> Hantzsch in Rabenhorst	4.4
<i>Nitzschia sociabilis</i> Hustedt	4.2
<i>Nitzschia dissipata</i> (Kützing) Grunow ssp. <i>dissipata</i>	2.6
<i>Navicula tripunctata</i> (O. F. Müller) Bory	2.6
<i>Nitzschia dubia</i> W. M. Smith	2.0
<i>Diatoma vulgare</i> Bory	1.4
<i>Navicula gregaria</i> Donkin	1.4
<i>Amphora pediculus</i> (Kützing) Grunow	1.0
<i>Nitzschia vermicularis</i> (Kützing) Hantzsch in Rabenhorst	1.0
<i>Surirella brebissonii</i> Krammer & Lange-Bertalot var. <i>brebissonii</i>	1.0
<i>Nitzschia sigmoidea</i> (Nitzsch) W. Smith	0.8
<i>Nitzschia wuellerstorffii</i> Lange-Bertalot	0.8
<i>Frustulia vulgare</i> (Thwaites) De Toni	0.6
<i>Gyrosigma attenuatum</i> (Kützing) Rabenhorst	0.6
<i>Navicula cryptotenella</i> Lange-Bertalot	0.6
<i>Nitzschia heufleriana</i> Grunow	0.6
<i>Nitzschia thermaloides</i> Hustedt	0.6
<i>Encyonema prostratum</i> (Berkeley) Kützing	0.4
<i>Navicula reichardtiana</i> Lange-Bertalot var. <i>reichardtiana</i>	0.4
<i>Tryblionella apiculata</i> Gregory	0.4
<i>Amphora indistincta</i> Levkov	0.2
<i>Cocconeis euglyptoides</i> (Geitler) Lange-Bertalot	0.2
<i>Caloneis lancetula</i> (Schulz) Lange-Bertalot & Witkowski	0.2
<i>Cocconeis pediculus</i> Ehrenberg	0.2
<i>Cymatopleura solea</i> (Brebisson in Breb. & Godey) W. Smith var. <i>solea</i>	0.2
<i>Diatoma vulgare</i> Bory (abnormal form)	0.2
<i>Fallacia helensis</i> (Schulz) D.G. Mann	0.2
<i>Fallacia pygmaea</i> ssp. <i>subpygmaea</i> Lange-Bertalot Cavacini Tagliaventi & al	0.2
<i>Navicula antonii</i> Lange-Bertalot	0.2
<i>Nitzschia capitellata</i> Hustedt in A. Schmidt & al	0.2
<i>Nitzschia dubia</i> W.M. Smith (abnormal form)	0.2
<i>Nitzschia angustata</i> (W. Smith) Grunow	0.2
<i>Nitzschia intermedia</i> Hantzsch ex Cleve & Grunow	0.2
<i>Navicula kotschyi</i> Grunow	0.2
<i>Nitzschia linearis</i> (Agardh) W.M. Smith var. <i>linearis</i>	0.2
<i>Nitzschia paleacea</i> (Grunow) Grunow in van Heurck	0.2
<i>Nitzschia palea</i> (Kützing) W. Smith var. <i>palea</i>	0.2
<i>Navicula radiosa</i> Kützing	0.2
<i>Navicula rostellata</i> Kützing	0.2
<i>Navicula simulata</i> Manguin	0.2
<i>Navicula veneta</i> Kützing	0.2
<i>Nitzschia linearis</i> (Agardh) W. M. Smith var. <i>tenuis</i> (W. Smith) Grunow in Cleve	0.2
<i>Nitzschia supralitoreae</i> Lange-Bertalot	0.2
<i>Rhoicosphenia abbreviata</i> (C. Agardh) Lange-Bertalot	0.2
<i>Reimeria uniseriata</i> Sala Guerrero & Ferrario	0.2
<i>Surirella amphioxys</i> W. Smith	0.2
<i>Surirella ovalis</i> Brébisson	0.2
<i>Stephanocostis chantaicus</i> Genkal & Kuzmin	0.2
<i>Tryblionella debilis</i> Arnott ex O'Meara	0.2
<i>Ulnaria ulna</i> (Nitzsch) Compère	0.2

hosts observed in the Arc river sample: *N. sigmoidea* (in 11% of total samples), *N. vermicularis* (7%), and *M. varians* (78%). *Cymatopleura solea* (13%) was also considered due to its presence in the river sample and

the database, its large size, and because it presents fibulae on its frustule which may be a criterion of host selection by ACOP (Round & Lee 1989). Two hypotheses were tested. The first assumes that there are no

Table 2. Number of diatom forms with and without attached diatoms identified in the fresh biofilm sample (2 microscope slides were completely screened). The forms were assumed large enough to be potential hosts. In brackets: taxa representing each form identified after sample treatment with boiling H₂O₂

Potential host diatom forms	With attached diatoms	Without attached diatoms
Long <i>Nitzschia</i> (>100 µm, <i>N. sigmoidea</i> , <i>N. vermicularis</i> , ...)	39	20
<i>Gyrosigma</i> (<i>G. sciotense</i> and <i>G. attenuatum</i>)	0	105
Filaments of <i>Melosira varians</i>	5 ^a	149
Large <i>Navicula</i> (<i>N. tripunctata</i> , <i>N. lanceolata</i> , <i>N. capitatoradiata</i> , ...)	0	94
Others (>50 µm, <i>Cymatopleura</i> , <i>Surirella</i> , <i>Nitzschia</i> , <i>Diatoma</i> , ...)	0	48

^aIn each case, only 1 cell was attached on the filament

more co-occurrences of ACOP with its potential host in the database than there may be by chance if the occurrences of ACOP were distributed randomly among sites. Therefore, the exact probability of obtaining at least as many co-occurrences with ACOP as observed in the database was calculated from the number of possible combinations (Table 3, see also Supplement 2 at www.int-res.com/articles/suppl/a080p055_supp/). Also, random permutations (n = 9999) of incidence data of ACOP across sites were carried out and the observed number of co-occurrences with each potential host species was compared with the distribution of simulated co-occurrences by permutations (Fig. 2). The co-occurrences of ACOP with *N. sigmoidea* and *C. solea* were significant (p < 0.05) whereas they were not significant with the 2 other species. Thus, there is no sign in the database of an association of ACOP with *N. vermicularis* or *M. varians*. This suggests that among the 2 *Nitzschia* species, *N. sigmoidea* may be a preferred host for ACOP and that attachment on *M. varians* is probably rare.

The second hypothesis is that the occurrences of ACOP are only driven by abiotic environmental factors and are not favored by the presence of a potential host species (*N. sigmoidea* or *C. solea*). Therefore, as with permutations, new patterns of occurrences of ACOP among sites were simulated, this time taking into account the species' ecological preferences. Following Bottin et al. (2016), random forests were used (R package 'randomForest'; Liaw & Wiener 2002) to predict the occurrence of ACOP from the 16 abiotic environmental parameters describing each sample of the database (elevation, slope, dis-

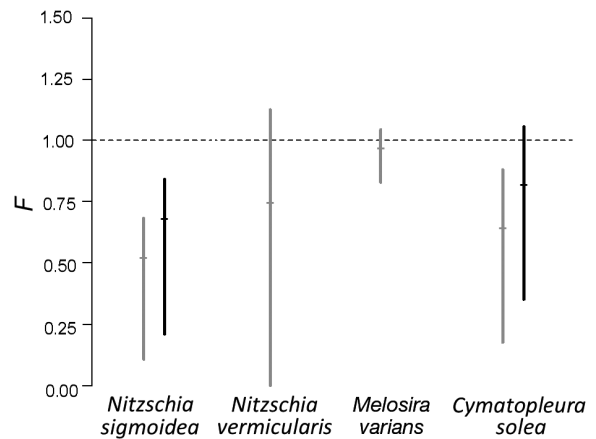


Fig. 2. Distribution of the *F*-ratio of observed and simulated co-occurrences of *Amphora copulata* with 4 potential host species. Vertical segments: lower 95% of the distribution; dashes: median. Grey lines: results of permutations (n = 9999) of *A. copulata*'s occurrences among sites; black lines: simulations of *A. copulata*'s occurrences using an environmental model (random forest) taking into account physico-chemical conditions at each site

Table 3. Number of occurrences and co-occurrences with *Amphora copulata* of 4 potential host species among the 196 samples of a regional database (Bottin et al. 2016), and probability of randomly obtaining at least as many co-occurrences under the random and abiotic environmental-control hypothesis

Diatom species	Occurrences	Co-occurrences with <i>A. copulata</i>	Probability of at least as many co-occurrences	
			Random	Environmental
<i>Amphora copulata</i>	87	–	–	–
<i>Nitzschia sigmoidea</i>	22	19	0.00002 ^a	0.00248 ^c
<i>Nitzschia vermicularis</i>	13	8	0.15891 ^a	–
<i>Melosira varians</i>	153	70	0.2975 ^b	–
<i>Cymatopleura solea</i>	25	17	0.00995 ^a	0.15617 ^c

^aExact probability; ^bestimated from permutations (n = 9999); ^cestimated from random forests

tance to the source and water quality parameters). The presence of ACOP at the sites where a potential host was present was predicted from a random forest (500 trees) trained with observed occurrences of ACOP at the sites where the potential host did not occur ($R^2 = 0.34$ with *N. sigmoidea* and 0.25 with *C. solea*). The obtained pseudoprobabilities of presence (prediction scores) at all sites were used to simulate $n = 10\,000$ patterns of occurrence of ACOP by drawing for each site, n times in a Bernoulli law. They were also used to derive the probabilities of obtaining at least as many co-occurrences as observed in the database (Table 3, Supplement 2). Under this second hypothesis, the observed co-occurrences of ACOP with *C. solea* were not significant. However, the co-occurrences with *N. sigmoidea* were still significant ($p < 0.05$) and suggest that *N. sigmoidea* may facilitate the presence of ACOP by providing an adequate substrate for attachment. Nevertheless, the tests only showed that the data are consistent with a common attachment of ACOP on *N. sigmoidea* in rivers, but the extent of this phenomenon can only be demonstrated by microscopic observations. Moreover, the power of the tests is limited for several reasons. The main reason is the difference in mean abundance of ACOP and *N. sigmoidea* across sites which results from the size-abundance ecological relationship (White et al. 2007; 6:1 ratio in our sample), whereas the counting effort is usually constant among samples. Co-occurrences of the 2 species may be therefore underestimated. Thus, it cannot be concluded from the high proportion of sites where ACOP is present but *N. sigmoidea* is not ($39\% = [87 - 19] / [196 - 22]$; Table 3) that ACOP may have other hosts or other habitats.

This case of attachment is particularly interesting because it involves a large number of diatoms on the frustule of a single motile diatom (Fig. 1) which can move in spite of the numerous attached cells. Moreover, this attachment is selective and probably widespread as the co-occurrences of ACOP and *N. sigmoidea* in a regional database suggest. ACOP may benefit from this interaction because the host moves relatively quickly and can transport it to areas where light and nutrients are available (Cohn & Disparti 1994, Bondoc et al. 2016). This can be at the expense of *N. sigmoidea*, which may suffer higher competition for resources with attached cells, undergo stronger

drag and increased downstream drift, and be less mobile in dense biofilms. Ecophysiological studies on the association of the 2 species are needed to examine the possibility that this relationship may be an epiphytism, a parasitism or even a mutualism if benefits for *N. sigmoidea* could be demonstrated. More generally, there is a need to clarify positive interactions between species for a more accurate use of diatoms as bioindicators in neo- or paleolimnology. Indeed, if the presence of a species is determined more by biotic interactions than by water quality, this species will have a poor indicator value. Moreover, for paleo-environmental reconstructions, it makes a difference whether a species is epiphytic on macrophytes or on epilithic diatoms.

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