Characterizing the niches of two very similar \textit{Nitzschia} species and implications for ecological assessment

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The distributions of \textit{Nitzschia inconspicua} Grunow and \textit{N. soratensis} E. Morales & Viz in response to environmental variables were compared using a dataset composed of samples from 46 locations in UK streams and rivers; they were also investigated using laboratory experiments. There were only three samples in which both species occurred with a relative abundance > 1\%. \textit{Nitzschia soratensis} preferred water of lower alkalinity, conductivity, reactive phosphorus and total oxidized nitrogen. The different preferences for conductivity of the two species were consistent with the results of the laboratory experiments, although no difference in response to phosphorus was observed in these trials. Interpretation of field results was confounded by correlations among environmental variables. However, taken together with the laboratory experiments, the data suggest that \textit{N. inconspicua} and \textit{N. soratensis} are separated primarily by local geology and habitat rather than by human pressures. Discrepancies between the results from laboratory experiments and observed distributions in the field underline the need for caution in using the latter alone for characterizing the realized niche of species for ecological assessment.

\textbf{Keywords:} alkalinity, ecological assessment, ecophysiology, indicator species, \textit{Nitzschia}, phosphorus, salinity

\section*{Introduction}

Widespread use of the taxonomic composition of diatoms for ecological assessment relies on the association between a species and a particular set of ecological variables. Analysis of an assemblage, therefore, allows the condition of a particular water body to be inferred (Kelly 2013). Recent advances in diatom taxonomy have revealed greater diversity than hitherto expected, along with evidence suggesting that many apparently well-established species are, in fact, complexes composed of several closely related taxa (Krammer 2002, Mann et al. 2008, Vanormelingen et al. 2013). This, in turn, has led to suggestions that improved taxonomic understanding should lead to better ecological discrimination (e.g., Jüttner et al. 2013). However, this has rarely been subjected to critical examination. Recent work to unravel the taxonomy of small \textit{Nitzschia} species (Trobajo et al. 2013) provides an opportunity to investigate the habitat requirements of two of these species, which, in turn, raises some more general issues about the potential benefits of fine-scale taxonomy for ecological assessment.

One implication of Trobajo et al. (2013) is a recognition that the entity described as ‘\textit{Nitzschia inconspicua} Grunow 1862’ in Krammer & Lange-Bertalot (1988), which is very common in streams in north-west Europe and beyond, consists of at least two separate species (Figs 1–8): \textit{N. inconspicua sensu stricto} and \textit{N. soratensis} E. Morales & Vis (2007). There is, however, no automatic benefit to ecological assessment from this conclusion. Re-examination of populations originally identified as \textit{N. inconspicua} alongside environmental data should allow us to determine the extent to which these two species have distinct habitat preferences. Closely related species often have similar ecological requirements (Rimet & Bouchez 2012) and factors responsible for speciation may include biogeography and local geology, as well as distinct preferences for ecological conditions associated with different levels of human pressure. \textit{Nitzschia inconspicua} and \textit{N. soratensis}, despite being morphologically very similar, are in fact not particularly closely related (Rovira 2013). They do, nonetheless, present an interesting opportunity to evaluate the role that natural habitat variation (e.g., catchment geology, which, in turn, influences factors such as alkalinity and conductivity) and human pressure arising from nutrient and organic enrichment play on a pair of species which have often been regarded as a single taxonomic entity.

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Materials and methods

Field data

An initial search of samples in which *N. inconspicua* sensu lato had been recorded at ≥ 4% was performed using the database of Bowburn Consultancy. This comprises several thousand sites, mostly from rivers distributed throughout the UK, which had been analysed as part of national monitoring programmes. Methods of sample collection and preparation are summarized in Kelly et al. (2008). Slides of 46 samples thus identified were re-analysed by light microscopy (LM), and the proportions of *N. inconspicua* sensu stricto and *N. soratensis* were determined in each of them (differentiating characteristics of these two taxa are shown in Figs 1–8). Corresponding environmental data were obtained from the Environment Agency’s national database. These values are averages of monthly samples taken throughout the year when the sample was collected; ‘reactive-P’ refers to the standard UK Environment Agency analytical method for ‘orthophosphate’ (Mackereth et al. 1978), which is performed on unfiltered water samples. Not all variables were available for all samples.

For laboratory experiments, *N. inconspicua* was isolated from benthic diatom samples collected from the lower Ebro River and its tributaries (Catalonia, Spain), whereas *N. soratensis* was obtained from Houselop Beck (County Durham, UK). Several clones of each species were established by micropipetting single cells into Petri dishes containing Woods Hole MBL medium (Nichols 1973); this medium was also used for maintaining the stock cultures. The PO₃⁻⁴-P preferences of the two species were assessed by inoculating three clones of each into MBL media modified by changing the PO₃⁻⁴-P concentrations to give a series of four treatments containing 1.63 (unmodified MBL medium), 0.163, 0.033 and 0.016 mg L⁻¹ PO₃⁻⁴-P. The salinity preferences of *N. soratensis* were studied by growing clones under different salinity conditions (see Table 1), which were obtained by using different percentages of MBL and f/2 (McLachlan 1973) media; f/2 medium was prepared with filtered natural seawater (35 parts per thousand, ppt) from Alfacs Bay (W Mediterranean Sea). The effects of salinity on the growth of *N. inconspicua* had already been assessed by Rovira (2013).

Cells of each clone (see Tables 3 and 4) were grown in illuminated cabinets: (1) at 21°C under a light intensity of c.40 μ mol photons m⁻² s⁻¹ and a light/dark cycle of 16:8 h for the phosphorus experiment, and (2) at 20°C under a light intensity of c. 75 μ mol photons m⁻² s⁻¹ and a light/dark cycle of 12:12 h for the salinity experiment. Growth was assessed visually (in two replicates for each clone and treatment) by inspection under an inverted microscope (Leica DMI3000), when the clones had just entered the stationary phase (two weeks).

Differences in ecological preferences between the two species were tested using a weighted *t*-test (Pasak 2013).
when the alkalinity exceeds 100 mg L\(^{-1}\) (Fig. 9a; Table 1), being rarely found in abundance of lower alkalinity than \(N\). inconspicua. The second species was rare (13 samples had both species although in most cases the \(N\). inconspicua contained a significantly higher percentage). Re-analysis of the 46 field samples indicated that 35 contained \(N\). inconspicua, whereas 23 contained \(N\). soratensis; 13 samples had both species although in most cases the second species was rare (<1% of total valve count). Only three samples contained both species at a relative abundance of >1%.

\(N\). soratensis had a statistically significant preference for water of lower alkalinity than \(N\). inconspicua (Fig. 9a; Table 1), being rarely found in abundance when the alkalinity exceeds 100 mg L\(^{-1}\) CaCO\(_3\), whereas \(N\). inconspicua is only found in abundance when alkalinity exceeds 150 mg L\(^{-1}\) CaCO\(_3\). \(N\). soratensis was also significantly associated with water of lower conductivity and slightly lower pH than \(N\). inconspicua (Fig. 9b, c). Statistically significant preferences of \(N\). soratensis for lower P and total oxidized N concentrations were also observed (Fig. 9d, f; Table 1), but no significant differences were recorded for ammonia-N or biochemical oxygen demand (BOD) (Fig. 9e, f; Table 1).

However, analysis of environmental preferences from the field samples is complicated by correlations among variables within the dataset (Table 2). In particular, there are strong associations between alkalinity and conductivity, as well as among variables representing organic and inorganic pollution (Table 2). Although there is no significant linear correlation between alkalinity and reactive-P, high values of reactive-P are all associated with sites with high alkalinity (Supplementary Material Fig. 1) and there is, furthermore, a significant correlation between reactive-P and conductivity (Table 2), which makes it difficult to untangle causality from the many correlations from the field data alone.

| Variable                  | \(N\). inconspicua | \(N\). soratensis | \(t\)-value | \(p\)-value |
|---------------------------|--------------------|------------------|------------|------------|
| Alkalinity (mg L\(^{-1}\) CaCO\(_3\)) | 32                | 197.2            | 81.6       | 12.44      | < 0.001    |
| Ammonia-N (mg L\(^{-1}\)) | 37                | 0.266            | 0.216      | 0.877      | N.S.       |
| BOD (mg L\(^{-1}\)O\(_2\)) | 37                | 2.30             | 2.54       | 1.055      | N.S.       |
| Conductivity (µS cm\(^{-1}\)) | 20               | 988              | 383        | 18.47      | < 0.001    |
| pH                        | 36                | 8.08             | 7.78       | 6.78       | < 0.001    |
| Reactive-P (mg L\(^{-1}\)) | 37                | 1.020            | 0.326      | 5.52       | < 0.001    |
| Total oxidized N (mg L\(^{-1}\)) | 36              | 10.08            | 4.42       | 6.86       | < 0.001    |

Notes: Environmental preferences are calculated from the annual mean value of the variable in question at each site; not all variables were available for all sites. \(N\) refers to the number of values used for each comparison. N.S.: not significant.

Laboratory experiments

\(N\). soratensis thrived at salinities of c. 0.26 and 1.48 ppt (Table 3). At higher salinities, growth was significantly reduced and cells died at salinities of 14 ppt and above, indicating the stenohaline freshwater character of this species, in contrast to the brackish–marine/euryhaline preference of \(N\). inconspicua (Rovira 2013).

Both \(N\). inconspicua and \(N\). soratensis grew well over all the phosphorus concentrations tested (1.63, 0.163, 0.033 and 0.016 mg L\(^{-1}\)), with no or very little change in the growth response (Table 4), suggesting the indifference of these two species to phosphorus content.

Discussion

The assumption that improved taxonomic understanding leads to better ecological discrimination is widely stated (e.g., Jüttner et al. 2013), yet rarely tested. There is, for example, detailed information concerning phylogenetic structure within the \(N\). palea complex (Kützing) W. Smith (Trojano et al. 2009, 2010, Rimet et al. 2014) but, as yet, no information about the accompanying ecological differences. An earlier study did demonstrate differences in ecological preferences of demes of \(S\). pupula (Kützing) Mereschkowsky in lakes along a eutrophication gradient (Pouilléková et al. 2008), although, in retrospect, it paid insufficient attention to differences in the types of lakes, including both low alkalinity lochs (e.g., Loch Venachar) and high alkalinity lakes (e.g., Malham Tarn). Although this was a useful contribution to a wider study of cryptic speciation within the \(S\). pupula complex (e.g., Evans et al. 2007, Vanormelingen et al. 2013), understanding the consequences for ecological assessment are not straightforward because part of the apparent variation is due to underlying geological influences rather than to differing intensities of human pressure. This is important because the objective of ecological assessment is not just to describe patterns, but also to predict the consequences of potentially expensive remedial activities. The present...
Fig 9. Distribution of *N. inconspicua* (●) and *N. soratensis* (○) in relation to environmental variables.

The study goes a step further than Poulicková et al. (2008), by attempting to separate the effects of geological ‘type’ (assumed to be more-or-less constant for any water body) from the effects of human pressure.

The combination of field and experimental data reported here and by Rovira (2013) suggests that *N. inconspicua* and *N. soratensis* are not only phylogenetically distantly related, but also have different physiological requirements and ecology, as suggested by Trobajo et al. (2013). Trobajo et al. (2013) described the ecological preferences of *N. inconspicua* as ‘brackish–marine/euryhaline’ and those of *N. soratensis* as ‘strictly freshwater’. Our data confirm that *N. soratensis* is restricted by its physiology to freshwater, and that in saline or brackish environments only *N. inconspicua* will be found. However, it is important to remember that *N. inconspicua*, being euryhaline, can also occur in freshwaters, constituting ≥ 10% of all diatoms at inland hard water sites in our dataset that are free from known saline influences. Although the two species were found together in almost a third of the 46 samples of UK rivers, one was usually present in low numbers, suggesting that the species have distinct niches.
with contrasting distributions along alkalinity and conductivity gradients (Fig. 9a, b). However, understanding the realized niche of these two species from field data alone is difficult or impossible because several important variables are correlated in our dataset. Only laboratory experiments, in which the effects of individual factors can be isolated, allow progress in separating causality from correlation.

Our experimental data indicate that *N. soratensis*, although a freshwater species, can grow well at salinities of at least 1.48 ppt, which is equivalent (following Hart et al. 1991) to a conductivity of 2180 µS cm$^{-1}$, well above the field values at which *N. soratensis* seems to disappear from the community. Similarly, although field data might suggest that *N. inconspicua* prefers higher phosphorus concentrations, the laboratory-based experiment suggests that both species may be indifferent to phosphorus, at least to concentrations between 0.016 and 1.63 mg L$^{-1}$.

Hence, together, current field and laboratory data point to conductivity and/or alkalinity as the main factor(s) separating these two species in streams. However, this too needs to be confirmed in the laboratory, for example, by pH-drift experiments to investigate how alkalinity might affect the growth of the two species via their abilities to take up different forms of inorganic carbon, as in the eco-physiological studies of desmids and chrysophytes performed by Spijkerman et al. (2005) and Maberly et al. (2009), respectively. If different inorganic carbon uptake mechanisms do indeed underlie the ecological distributions of *N. inconspicua* and *N. soratensis* in freshwaters, this would mean that they are separated primarily by catchment geology (stream type) rather than by nutrient pollution (human pressure). Although their optima for reactive-P differ (Table 1), both species thrive best when P concentrations are elevated while ammonia-N concentrations and BOD are relatively low.

What are the consequences of these results for ecological assessment? In practice, most studies prior to the publication of Hofmann et al. (2011) included *N. soratensis* with *N. inconspicua*. Hofmann et al. (2011) probably separated *N. soratensis* from *N. inconspicua*, but called it *N. abbreviata*, Hustedt in A. Schmidt et al. (Trobajo et al. 2012). However, because both *N. inconspicua* and *N. soratensis* prefer elevated P concentrations, this is unlikely to have serious consequences for the interpretation of ecological status in situations in which nutrients are the most likely pressure. However, there will also be situations in which pressures other than nutrients are significant. One of these is secondary salinization, a major issue globally in freshwater ecosystems (Millennium Ecosystem Assessment 2005, Cañedo-Argüelles et al. 2013). In such situations, *N. soratensis* might play a sentinel role because it would be predicted to disappear as salinity levels increased.
Table 4. Growth of *N. soratensis* and *N. inconspicua* strains after 15 days under different PO$_4^{3-}$ concentrations, assessed visually.

| Species       | Clone code 1 | Clone code 2 | Locality                  | Salinity preference | Replicate | 1.63 mg L$^{-1}$PO$_4^{3-}$ | 0.163 mg L$^{-1}$PO$_4^{3-}$ | 0.033 mg L$^{-1}$PO$_4^{3-}$ | 0.016 mg L$^{-1}$PO$_4^{3-}$ |
|---------------|--------------|--------------|----------------------------|---------------------|-----------|-----------------------------|-------------------------------|-------------------------------|-------------------------------|
| *N. soratensis* | NitMKC       | NS_5         | Houselop Beck, County Durham, UK | Stenohaline         | R1        | +++                         | +++(+)                       | +++                           | +++                           |
|               |              |              |                            |                     | R2        | +++                         | +++(+)                       | +++                           | +++                           |
| *N. soratensis* | DM1008MK     | NS_1         | Houselop Beck, County Durham, UK | Stenohaline         | R1        | +++                         | +++                           | +++                           | +++                           |
|               |              |              |                            |                     | R2        | +++                         | +++                           | +++                           | +++                           |
| *N. inconspicua* | L50          | G2_5         | Set River, Catalonia, Spain | Euryhaline          | R1        | +++                         | +++                           | +++                           | +++                           |
|               |              |              |                            |                     | R2        | +++                         | +++                           | +++                           | +++                           |
| *N. inconspicua* | L53          | G3_1         | Set River, Catalonia, Spain | Euryhaline          | R1        | +++                         | +++                           | +++                           | +++                           |
|               |              |              |                            |                     | R2        | +++                         | +++                           | +++                           | +++                           |
| *N. inconspicua* | L55          | G1_3         | Ebro River, Catalonia, Spain | Euryhaline          | R1        | +++                         | +++                           | +++                           | +++                           |
|               |              |              |                            |                     | R2        | +++                         | +++                           | +++                           | +++                           |

Notes: R1, R2: Growth assessment of replicates 1 and 2, respectively; +++: very good growth; +++(+): good to very good growth; +++: good growth. Clone code 1 refers to the clone names in the voucher slides held at the Royal Botanic Garden Edinburgh. Clone code 2 corresponds to the codes in Rovira (2013), based on the clones’ *rbc* L or large-subunit (LSU) ribosomal DNA genotype.

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