



## Diatoms as indicators: The influences of experimental nitrogen enrichment on diatom assemblages in sub-Arctic streams

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### ABSTRACT

Indices using diatoms are widely used to assess water quality, but are usually constructed from field correlations and not tested through rigorous experimentation. We tested experimentally the performance of the Sørensen and the Shannon indices, and the trophic diatom index (TDI). Nitrogen was naturally limiting in the eight remote sub-Arctic streams used and we measured the effects of experimental nitrogen enrichment on diatom assemblages. Diatom densities increased significantly in the enriched reaches but there was no significant difference in invertebrate density between control and treatment reaches. Grazing effects were thus controlled for. Diversity within streams (Shannon index) was significantly reduced by nutrient addition but the Sørensen index did not change. The trophic diatom index (TDI), which is presumed to reflect nutrient concentration, was not influenced by nutrient addition and generally the values were low in both control and treatment reaches. Densities of the diatom genera *Achnanthes* and *Gomphonema* increased significantly with enrichment while those of *Nitzschia* and *Fragilaria* decreased significantly. Less abundant diatom species, which collectively constituted around 40% in relative abundance in the control reaches, were around 15–18% in treatment reaches. Growth forms were altered by the nutrients. Diatoms attached by mucilage pads were more abundant in treated reaches compared with control reaches. Motile diatoms became scarcer. The size of diatom species was unaffected by nutrient enrichment. This study showed that it is important to test experimentally indices that are developed for particular habitats before using them elsewhere.

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### 1. Introduction

Diatoms often dominate the algal communities of streams and rivers (van den Hoek et al., 1995; Allan, 2006); their communities are dynamic and diverse, and their ecology is relatively well understood. They are claimed to be sensitive towards many environmental factors such as ionic content, pH, dissolved organic matter and nutrients, and therefore have been used to monitor streams and rivers in many regions (Potapova and Charles, 2007; Kelly et al., 2008a,b; Stevenson et al., 2008). Furthermore, diatoms have been found to be better correlated with chemical measures of water quality than other algae (Kelly et al., 2008a,b).

Studies on the effects of nutrient enrichment on stream algae have suggested that with increasing phosphorus, diatom species richness declines (Peterson et al., 1985) and algal biomass increases

(Bothwell, 1989). Pringle (1990) showed that addition of inorganic and organic phosphorus increased numbers of motile diatoms, mainly *Nitzschia* (Hass.) and *Navicula* (Bory), on sandy substrates and *Cocconeis placentula* (Ehrenb.) and *Achnanthes minutissima* (Kütz.) on glass slides. Sometimes, however, nitrogen rather than phosphorus may be the key limiting nutrient (Moss et al., 2012). Gudmundsdottir et al. (2011b) manipulated nitrogen in eight Icelandic streams, varying in temperature. They found out that biofilm, chlorophyll *a* and bryophyte (*Fontinalis antipyretica* Hedw.) biomass increased with increased nitrogen. With this and other evidence, and the general acceptance that stream diatom communities reflect nutrient status, various diatom-based indicators and indices have been developed (Kelly et al., 1995), whilst more widely used diversity indices, for example the Shannon's index and the Sørensen's index (Magurran, 2004), can be readily applied to diatom communities. Diatom-based indices have been the leading tools in Europe for biomonitoring, and the European indices have also been applied outside Europe. Comparison among indices using field data, however, suggests that different indices do not always give similar results (Besse-Lototskaya et al., 2011).

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One of the requirements of the European Water Framework Directive (European Union, 2000) is the use of benthic algal communities for the assessment of ecological status of flowing waters and many member states have turned to specific diatom indices for this (Kelly et al., 1998). The trophic diatom index (TDI), which uses a scoring system based on indicator species (Kelly and Whitton, 1995), is used to assess eutrophication in several states. The index is based on links among phosphorus or nitrate concentrations and diatom communities, derived from field correlations (e.g. Borhardt, 1996; Potapova et al., 2004; Kelly et al., 2008a), but has not been tested experimentally in a rigorous way. There are many factors acting simultaneously with phosphorus, not least combined nitrogen, grazing and flow effects that could confound such correlations. Most least-impacted freshwaters are probably co-limited by both phosphorus and nitrogen (Moss et al., 2012), and even when either is limiting, concentrations of the other are generally correlated. Phosphorus is conventionally considered to be the limiting nutrient on primary producers in freshwaters but nitrogen may be limiting, especially where human settlement has developed or where phosphorus is abundant naturally in the soils e.g. in neo-volcanic Icelandic bedrock (Ritter, 2007).

All indices depend on the species composition of communities. There are disadvantages to this in that species substitution is common in many communities as a result of natural fluctuations and random effects. There is a possibility, however, that changes in traits, such as mode of attachment and size, may give more accurate reflection of change. Daufresne et al. (2009) found that that size, in a range of organisms, is reduced with temperature, but trait analysis has not been used as much as taxonomy in the assessment of anthropogenic impact. A system of streams in Iceland has given us the opportunity, not only to make an experimental test of how effective various indices of diatom community composition are in reflecting change in nutrient loading, but also to assess the use of traits as well as taxonomic indices.

Our hypotheses were first that the diatom cell density per unit area would increase with increased limiting-nutrient concentration in the streams. Secondly, we hypothesized that diversity, expressed by the Shannon's index (Magurran, 2004) would fall with increased nutrient concentration. We also expected overlap between species (i.e. similarity, calculated according to Sørensen's index) to increase with nutrient enrichment (Magurran, 2004). When nutrients are abundant, fast-growing diatom species are likely to benefit whilst many slow-growing species are likely to become rarer, and may even disappear from the streams, resulting in lower diversity. Thirdly, we hypothesized that the trophic diatom index (TDI) (Kelly et al., 1998) would reflect the experimental nutrient enrichment in the streams (Kelly et al., 2008a) with higher TDI (more tolerant species observed) with nutrient enrichment. The index is calibrated against phosphorus and nitrate concentration in streams and rivers, but it is widely assumed to reflect nutrient pressures in general. Lastly we hypothesized that nutrient enrichment would change the trait characteristics (size, nature of attachment and motility) of the diatom community and that this approach might then be valuable in indicator studies. We expected to find increased numbers of motile species where nutrients were abundant (because such unattached species are vulnerable to washout but might be able to build up populations able to compensate for such losses if increased nutrients support increased growth rates), and increased numbers of smaller diatoms with nutrient enrichment, owing to the advantages of their potentially shorter life cycles, when resources are not limited (Moss, 1973). The experimental streams varied in conductivity, temperature and invertebrate community (see Gudmundsdottir et al., 2011a) but our design included control and treatment reaches within each of these short and uniform streams so that other variables were controlled for.

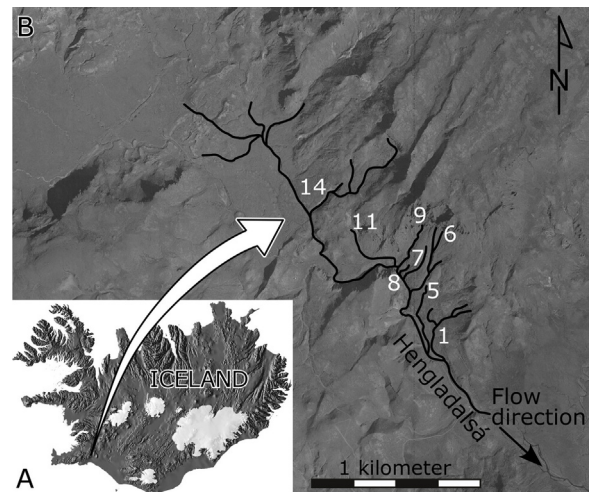


Fig. 1. Map of Iceland (a) and the eight selected streams (b) within Hengill geothermal area.

## 2. Methods

### 2.1. Study area

Nutrient enrichment experiments were carried out in the Hengill geothermal area in SW Iceland (N 64°03'; W 21°18'; 350–420 m a.s.l.) (Fig. 1), an active volcano covering 173 km<sup>2</sup>. Numerous small streams (rheocrenes) emerge, close to one another, from the hillsides and run along a flat valley floor on hyaloclastite bedrock to join the River Hengladalsá. The compact study area (Gudmundsdottir et al., 2011a,b) minimizes confounding factors and it is rare for such controlled experiments to be carried out on several streams within a very small area such as this. Friberg et al. (2009), using nutrient diffusion substrates, found that the stream algal communities were nitrogen-limited. The mean air temperature in July is 8–10 °C and the annual precipitation is 1870–3080 mm (Björnsson, 2003; Crochet et al., 2007). Vegetation in the area is mainly monocotyledonous tundra and because shrubs are sparse (Gudjonsson and Egilsson, 2006), little or no allochthonous particulate organic matter enters the streams (Friberg et al., 2009; Woodward et al., 2010).

Eight small streams were used, each 30–75 m long with no detectable longitudinal trends in chemistry or biological communities. Their annual mean temperature ranged from 4 to 24 °C (Friberg et al., 2009). Their conductivity was between 96 and 497  $\mu\text{Scm}^{-1}$  at 25 °C and pH 7.92–8.42, whilst nutrient concentrations were much more uniform (TP: 0.008–0.029 mg L<sup>-1</sup>, TN: <0.1–0.19 mg L<sup>-1</sup>) (Gudmundsdottir et al., 2011b). The main invertebrate functional feeding groups were scrapers and filter-collectors. *Simulium vittatum* (Zettersteth) and the gastropod *Radix peregra* (Müller) were dominant in the warmer streams, whilst chironomids were dominant in the colder streams. Chironomids comprised 16 of 35 invertebrate taxa in the streams (Friberg et al., 2009). Brown trout (*Salmo trutta* L.) was the only fish species observed.

### 2.2. Experimental design

Each of the eight streams (Fig. 1) was divided into an upper control reach (15–25 m), and lower treatment reach (15–25 m) with 5-m buffer zones between reaches. The study area is not settled by humans and there is no agriculture, resulting in near pristine reference (in the definition of the Water Framework Directive (2000)) sites to compare as controls with the treated reaches. Finding reference sites is difficult or impossible in most parts of Europe because

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