Corrections

ECOLOGY. For the article “Continental diatom biodiversity in stream benthos declines as more nutrients become limiting,” by Sophia I. Passy, which appeared in issue 28, July 15, 2008, of *Proc Natl Acad Sci USA* (105:9663–9667; first published July 3, 2008; 10.1073/pnas.0802542105), the author notes two printer’s errors. In the Abstract, line 4, the phrase “I tested whether niche dimensionality and, with this species, richness scale positively with NLR in running waters” should instead read: “I tested whether niche dimensionality and, with this species, richness scale positively with NLR in running waters.” In addition, in Table 1, the column 6 and 7 headings NH4--N and NO3--N should instead appear as NH4-N and NO3-N, respectively. The corrected table appears below.

<table>
<thead>
<tr>
<th>Table 1. Nutrient concentrations in milligrams per liter (iron in (\mu g/\text{liter})) measured in the NAWQA study streams across the three habitats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Habitat</strong></td>
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<tr>
<td>---------------</td>
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<tr>
<td><strong>All</strong></td>
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<tr>
<td>RTH, N = 1,189</td>
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<td>DTH, N = 759</td>
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<tr>
<td>Phytoplankton, N = 330</td>
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</tbody>
</table>

BC, basic cations; Pdiss, dissolved phosphorus. Concentrations below the given thresholds were determined to be limiting to eutrophic diatoms based on regression tree models (Table S1) or *literature data (47). N*, number of observations.

PHYSIOLOGY. For the article “TASK channel deletion in mice causes primary hyperaldosteronism,” by Lucinda A. Davies, Changlong Hu, Nick A. Guagliardo, Neil Sen, Xiangdong Chen, Edmund M. Talley, Robert M. Carey, Douglas A. Bayliss, and Paula Q. Barrett, which appeared in issue 6, February 12, 2008, of *Proc Natl Acad Sci USA* (105:2203–2208; first published February 4, 2008; 10.1073/pnas.0712000105), the authors note that on page 2204, right column, line 18, the statement “a recent study reports sex differences in effects of TASK-1 subunit deletion on adrenal development and zonation; males appear normal, and females show marked developmental abnormalities (25)” is misreferenced and should instead cite ref. 48. The added reference appears below.


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BIOPHYSICS. For the article “Shifting transition states in the unfolding of a large ankyrin repeat protein,” by Nicolas D. Werbeck, Pamela J. E. Rowling, Vasuki R. Chellamuthu, and Laura S. Itzhaki, which appeared in issue 29, July 22, 2008, of *Proc Natl Acad Sci USA* (105:9982–9987; first published July 16, 2008; 10.1073/pnas.0705300105), the authors note that, due to a printer’s error, on page 9985, left column, 3 lines from the bottom, “The data are consistent with a model in which TS2 has repeats 2–6 folded (as well as repeats 7–12 folded) and TS has only repeats 5 and 6 folded (as well as repeats 7–12 folded)” should instead read: “The data are consistent with a model in which TS1 has repeats 2–6 folded (as well as repeats 7–12 folded) and TS2 has only repeats 5 and 6 folded (as well as repeats 7–12 folded).”

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Continental diatom biodiversity in stream benthos declines as more nutrients become limiting

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Biodiversity of both terrestrial ecosystems and lacustrine phytoplankton increases with niche dimensionality, which can be determined by the number of limiting resources (NLR) in the environment. In the present continental study, I tested whether niche dimensionality and, with this species, richness scale positively with NLR in running waters. Diatom richness in 2,426 benthic and 383 planktonic communities from 760 and 127 distinct localities, respectively, was examined as a function of NLR, including basic cations, silica, iron, ammonia, nitrate, and dissolved phosphorus. The patterns found in the two communities were opposite: as more resources became limiting, diatom richness declined in the benthos but increased in the phytoplankton. The divergence of benthic from both planktonic and terrestrial communities is attributed to the complex spatial organization of the benthos, generating strong internal resource gradients. Differential stress tolerance among benthic diatoms allows substantial overgrowth, which greatly reduces nutrient transport to the biofilm base and can be supported only by high ambient resource levels. Therefore, niche dimensionality in the benthos increases with the number of resources at high supply. These findings provide a mechanistic explanation of the well documented phenomenon of increased species richness after fertilization in freshwater as opposed to terrestrial ecosystems. Clearly, however, new theoretical approaches, retaining resource availability as an environmental constraint but incorporating a trade-off between tolerance and spatial positioning, are necessary to address coexistence in one of the major producer communities in streams, the algae.

Methods

Three stream habitats were sampled quantitatively for algae, including richest targeted habitats (RTHs) with hard substrates and faster currents, depositional targeted habitats (DTHs) with soft sediments and slower currents, and water column (for phytoplankton). NLR was defined as the number of nutrients, including basic cations, iron, silica, ammonia, nitrate, and dissolved phosphorus (Table 1) that were available at concentrations limiting the growth of eutrophic species (see Materials and Methods for details). Diatom richness in the benthos steadily declined with the increase in NLR by about two species per doubling NLR. Notably, when the variability in species richness was compared by averaging per NLR, the regressions of mean species richness against NLR produced highly significant parameters, statistically equivalent to the parameters given in Fig. 1, but explained 90–95% of the variance across the three habitats (Fig. S2).

Results and Discussion

Three stream habitats were sampled quantitatively for algae, including richest targeted habitats (RTHs) with hard substrates and faster currents, depositional targeted habitats (DTHs) with soft sediments and slower currents, and water column (for phytoplankton). NLR was defined as the number of nutrients, including basic cations, iron, silica, ammonia, nitrate, and dissolved phosphorus (Table 1) that were available at concentrations limiting the growth of eutrophic species (see Materials and Methods for details). Diatom richness in the benthos steadily declined with the increase in NLR by about two species per doubling NLR. Notably, when the variability in species richness was compared by averaging per NLR, the regressions of mean species richness against NLR produced highly significant parameters, statistically equivalent to the parameters given in Fig. 1, but explained 90–95% of the variance across the three habitats (Fig. S2).

Author contributions: S.I.P. designed research, analyzed data, and wrote the paper. The author declares no conflict of interest. This article is a PNAS Direct Submission.

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The habitat specificity of diatom richness behavior along the gradient of NLR can be explained with the differences in spatial organization between the two communities, that is, algal biofilms maintain 3D structure, whereas the well mixed phytoplankton represents a 2D community. The complex three-dimensionality of the benthos is implicated in the striking deviations of its richness–NLR relationship from the postulates of niche theory, tested exclusively with 2D communities (2, 4–6). More specifically, diatoms exhibit well documented nutrient preferences (14) and many species, referred to as eutrophic henceforth, require high nutrient concentrations for growth and reproduction. Other species, for example, oligotrophic and indifferent, referred to as noneutrophic henceforth, are well adapted to low nutrient levels. Nutrient preferences indicate the tolerance to nutrient limitation, that is, eutrophic species are sensitive, but noneutrophic species are tolerant. Many tolerant species, such as the most widely spread diatom *Achnanthidium minutissimum* (Kützing) Czarnecki, are small and, because of this, maintain larger populations and broader regional distributions than larger species (15). Collectively, these attributes make them early colonizers even in nutrient-enriched systems, where, owing to their high tolerance to low resource levels, they can sustain viable populations even in extreme overgrowth by later colonizers and concomitant resource depletion within the biofilm (16). Although tolerant species like *A. minutissimum* can persist in comparatively high numbers throughout succession (17), a preference for unoccupied substrates restricts their distribution to the understory (18, 19).

The significantly negative response of diatom richness to NLR in the benthos reflects the incapability of resources at low supply to create gradients of sufficient length to sustain a 3D structure comprising both noneutrophic (tolerant) and eutrophic (sensitive) species. Instead, resources at low supply (nutrients or light) support only tolerant species (generally of smaller growth habit), which assemble into thin biofilms, consisting of dense understory and sparse (if any) overstory components (18, 20, 21). High resource supply, on the other hand, triggers a succession that culminates in the establishment of a thick multilayered benthic mat, composed of understory and well developed overstory flora of tolerant and sensitive species, respectively (Fig. 2). Nutrient manipulation experiments report up to a 10-fold increase in algal biomass with N and P additions and >20-fold increase with C addition [reviewed in Borchardt (22)]. Fertilization increases the abundance of overstory species (18, 21, 23) and the overall richness in the biofilm across all benthic substrates, that is, rocks, plants, and soft sediments (24). However, observations of depressed reproduction and population density and higher mortality within the algal biofilm (11, 16, 25) suggest that coexistence is achieved through the combined effect of interspecific competition and tolerance. Therefore, high ambient resource levels generate longer gradients of these resources within the 3D community, sustaining a greater number of species that repre-
sent a broader spectrum of stress tolerance. Consequently, niche dimension in the benthos is determined by the number of resources at high supply. These observations disagree with a basic premise of both classical and stochastic niche theory that a superior competitor for a resource always wins in competition for this resource and with a basic prediction that niche dimensionality correlates positively with NLR (2, 10). To explain this contradiction, the mechanisms behind algal coexistence are evaluated.

The trade-offs hypothesized to underlie species diversity in spatially heterogeneous habitats include competitive ability for a resource vs. (i) tolerance to a physical factor, (ii) competitive ability for another resource, (iii) colonization ability, (iv) susceptibility to herbivory, and (v) mortality (9). These trade-offs, however, cannot explain diatom richness in the heterogeneous benthic environment for the following reasons. Although diatoms compete for nutrients and light, differential equilibrial resource levels (R*), that is, competitive abilities, are insufficient to determine the competitive outcome, because in a 3D community later recruits (generally with higher R*) are the first to intercept and sequester the incoming resources. The remaining unutilized resources diffuse down the benthic mat to the understory species (with lower R*). Consumption by the overstory substantially and significantly reduces the resources reaching the understory, including micronutrients (26), carbon, ammonia, phosphate, nitrate, and silica (27–30), and light (16). Admittedly, algae growing on noninert substrates such as plants or sediments may procure nutrients from these substrates, but this does not necessarily diminish their uptake from the water column (29).

Additionally, internal nutrient recycling may partially alleviate the progressive resource depletion within the mat (31), but it can only sustain periphyton biomass (27), whereas nutrient input from the water column to interstitial spaces is necessary for positive algal growth rates (30). Algal 3D growth toward the source of resources (water column) allows fewer trade-off opportunities in comparison with 2D plant communities, where some species may maximize their root system for more efficient water and nutrient uptake, whereas others can invest in higher aboveground growth for better light harvesting. In addition, diatoms are overwhelmingly clonal completing their individual lives with cell division, that is, reproduction, which makes theories that successfully explain coexistence of sessile multicellular organisms through differential competition, colonization, and mortality (32, 33), inapplicable. Finally, diatoms exist and therefore disperse as adult individuals, whereas in plant communities, the vulnerable juveniles colonize new habitats but suffer prolonged mortality in the process of growing to maturity and competing for resources with the established adults (10).

The species richness of microscopic diatoms can both increase both the cause and the consequence of the number and length of the gradients within the biofilm, which are ultimately set by the resources’ ambient levels. High ambient levels result in long gradients allowing the coexistence of stress-tolerant superior competitors (with low R*), but with unfavorable spatial positioning (biofilm base), and stress-sensitive inferior competitors (with high R*), but with beneficial location (toward the biofilm surface). Notably, the benefits of this spatial arrangement are reversed under grazing pressure (34). However, an inherently spatially and temporally discontinuous herbivory resulting from a grazer distribution that is strongly dependent on sediment type and season can leave a number of benthic patches intact. Nutrient limitation in the water column, on the other hand, is comparatively spatially and temporally continuous and all patches are subject to the same ambient levels.

The present observations provide a mechanistic explanation for the well established disparity between freshwater and terrestrial systems, where fertilization induces positive and negative changes in richness, respectively (35). The model presented in Fig. 2, however, need not be restricted to stream communities. Its predictions should be tested and potentially extended to other communities, whose functioning depends on species 3D coexistence. For example, in lacustrine phytoplankton, eutrophication increases species richness (36) and alters the spatial organization of the benthic mat in a manner identical to the patterns described here for streams (37). Similarly to stream diatoms, the coexistence of encrusting and turf-forming macroalgae as a two-story community in marine subtidal systems is driven by species’ abilities to tolerate vs. overgrow (13). Nevertheless, further research within the present framework is necessary considering that in neither system is the role of NLR well understood, and in the absence of a steady water-mixing force, the effects of nutrient limitation can be much more severe than in streams, where current reduces, to an extent, the nutrient depletion around cells in the benthos and stimulates algal metabolism (38).

The 3D organization of benthic algae clearly distinguishes them from their planktonic counterparts, occupying a well mixed environment, where nutrient uptake is comparatively spatially independent. Apart from turbulent water motion and circulation, which homogenize the planktonic environment, the nutrient gradients in the plankton are further disrupted by the sinking of the algae (39). These fundamental differences between benthic and planktonic habitats in terms of number and length of the resource gradients have been implicated in the profound disparity between the two communities in taxonomic and allo- metric organization and functioning (40–42). Therefore, lacking the complex gradient structure of the benthos, the phytoplankton was expected to respond to nutrient enrichment like a 2D community. Indeed, coexistence in lake phytoplankton, maintained by competition trade-offs, was shown to scale positively with NLR (4, 5). Stream phytoplankton, however, exhibited the expected species richness increase only at the highest NLR (Fig. 1). The lack of richness decline at lower NLR may be a function of benthic immigration, which can be considerable in rivers (43) and can thus offset competitive exclusion. Nonetheless, the exponential richness response to NLR in the phytoplankton dataset (Table 1) may have prevented the capturing of the entire richness spectrum at higher NLR and the few species-rich communities, encountered at this NLR range, may have unduly influenced the richness-
NLR relationship, which was modeled with an exponential rather than a more gradual (e.g., linear) fit.

This continental investigation on stream diatoms, which not only respond to, but also engineer their environment, demonstrates that resource supply represents a major constraint on diatom coexistence because species have adapted to it via an unprecedented trade-off between tolerance and spatial position, the latter being determined by growth habit and/or colonization time (Fig. 2). Considering that the entire stream ecosystem, including zooplankton, macroinvertebrates, and fish, is founded on the community of algal producers, it is critical to develop theoretical approaches and implement management practices to address and preserve diatom biodiversity in streams.

Materials and Methods

The NAWQA Dataset. This dataset contains 2,809 quantitative algal samples collected from 803 distinct localities across three stream habitat types (Fig. 51): richest targeted habitats (RTHs), depositional targeted habitats (DTHs), and water column (http://water.usgs.gov/nawqa/protocols/OFR02–150/OFR02–150.pdf). RTHs maintain the taxonomically richest community growing on coarse-grained substrates, woody snags, or macrophytes. DTH diatoms are found in organically rich or sandy depositional areas along the stream margins. Phytoplankton is a community of algae suspended in the water column. Between May 1993 and August 2004, RTHs and DTHs were sampled from a defined depth (usually 0 to 5 cm and 1.5 m, respectively), whereas phytoplankton was collected from a defined volume of stream water in 127 localities. Water chemistry data for the month of algal collection were available for 1,484 RTH, 942 DTH, and 383 phytoplankton samples. If a particular locality was sampled more than once per month, the average nutrient values were taken for that month, but the algal samples were retained as separate data points to preserve the variability in species data. Nutrients, including nitrogen, phosphorus, calcium, magnesium (basic cations), silica, iron, ammonia, nitrate, and dissolved phosphorus, were measured by the U.S. Geological Survey according to national standards (44). The nutrients in each habitat (Table 1) were categorized as limiting to eutrophic species if their concentrations were below a predetermined threshold value (defined below) vs. unlimited, with concentrations equal to or higher than the threshold. NLR in a sample represents the sum of all nutrients with concentrations lower than the threshold.

Specialized phycology labs carried out the diatom identification in permanent mounts prepared from acid-digested samples. Diatom richness was calculated as the number of species in 300 cell counts. More information on the habitats, sampling, and laboratory techniques is given in Passy and Legrand (41). Diatom richness was regressed against NLR in the three studied habitats by using a curve-fitting program (45). Selection of the best models was based on good fit, high R², and significance of all parameters. Linear equations in the two benthic communities and an exponential equation in the phytoplankton matched these criteria.

Eastern New York State Dataset. To determine thresholds of nutrient limitation, I used a dataset of diatom relative abundance along pronounced and generally nutrient-dependent nutrient gradients in streams of the basins of the Delaware River, the Croton River, and the Hudson River, as well as from the Catskills and the Adirondacks. During the 1999 and 2000 summer low-flow conditions, 71 distinct stream localities were sampled for benthic algae and water chemistry, including basic cations, silica, ammonia, nitrate, and dissolved phosphorus [details on the area and sampling and laboratory techniques are given in Passy and Bode (46)] In each sample, 300 acid-cleaned diatom frustules were identified and the percent relative abundance of all eutrophic species (ES%) was calculated. All species with preference for nutrient enrichment, that is, mesotrophic to hypereutrophic, according to Van Dam et al. (14), were included in ES% to ensure that this metric was sensitive to any amount of enrichment. The range of ES% was between 0 and 93% with a median of 58%, indicating that some streams had very low nutrient concentrations, completely limiting the growth of eutrophic species, but others were heavily fertilized and dominated by eutrophic species, although small numbers of noneutrophic species were still able to persist. Regression tree models of ES% against each nutrient split the samples into two more-or-less homogeneous groups at a threshold value of the predictor and explained between 13 and 54% of the variance in ES% (Table 1). The first group contained samples with nutrients lower than the threshold and was generally dominated by noneutrophic species (mean ES% = 28–44). The second group encompassed samples with nutrients equal to or higher than the threshold and was dominated by eutrophic species (mean ES% = 62–80). The threshold values from these analyses estimated the onset of nutrient limitation for eutrophic species and were used to define limiting nutrients in the NAWQA dataset. Iron was not measured in this survey and its threshold was derived from Schelske (47), who showed that phytoplankton primary productivity was limited at iron concentrations <20 µg/liter. This value is consistent with the iron concentrations reported for oligotrophic stream reaches (24).

ACKNOWLEDGMENTS. I thank Rick Bell for providing the environmental NAWQA dataset and for helping with it, Janie Moore for creating the maps, and two anonymous reviewers and J. Brown for insightful suggestions.