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Relation Between Population Density and Body Size in Stream Communities

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The existence of a general relation between population density and body size in animal assemblages has been debated because of known biases and ambiguities in the published data and data handling. Using new comprehensive data sets from two geographically separated stream communities that encompass 448 and 260 invertebrate taxa with a wide range of body sizes, we show that an inverse proportionality between density and body size is a consistent feature in these communities. The scaling across taxa is not statistically different between the two systems, indicating a convergent pattern of communities. Variation in the regression slope among different taxonomic groups indicates that these communities are not governed universally by a single ecological or energetic rule.

Body size influences an organism's energetic requirements, its potential resource exploitation, and its susceptibility to predation. Dimensional analysis of the relation between population density (D) and body size (mass, W) for some published data yielded linear relations on logarithmic scales ($\log D = \alpha + \beta \log W$), where the slope β is around -0.75 when the ordinary least squares (OLS) regression is used (1–3) or is close to -1 when the reduced major axis (RMA) regression is used (4–7). However, several studies have shown that density-body size relations take a peaked or polygonal pattern with intermediate-sized species having the highest density, resulting in a nonsignificant or weak regression with a shallow slope (8, 9). These contrasting results are derived from data collected through different sampling procedures and are subjected to different regression methods (8–10). It has also been argued that data compiled from the literature result in "constructed" density versus body size relations of assemblages that may be greatly affected by sampling bias against small and rare species, which are usually not well represented in ecological studies (10). Underestimation of the densities of rarer species is likely to result in a shallower slope and a less significant, more scattered relation (11). Furthermore,

analyses taking this approach mainly involved terrestrial assemblages, with a bias toward taxonomically related species. Few studies have considered aspects of scaling across many taxonomic groups in an ecosystem (12, 13).

We used data from two geographically separate communities of benthic stream invertebrates to assess the generality of density–body size relation in stream systems. The data encompassed species of wide ranges of taxonomy and body size and allowed us to

achieve a high taxonomic resolution. Also, population densities of all the species in an assemblage were estimated with reference to the same habitat area.

We sampled riffle/pool sections of the gravel streams Oberer Seebach in Austria and Afon Mynach in Wales (14). The two streams were similar in mean annual water discharge and fractal dimension of habitat, but different in grain-size composition (15). Population density and body size (16) of the species included in the analysis were evaluated for each of the two streams. 448 and 260 invertebrate species occurred in the streambed sediments of the Oberer Seebach and Afon Mynach, respectively (17, 18).

In both communities, abundance declined in a broad band with increasing body mass without showing a peaked pattern (Fig. 1). Body weight explained a significant amount of variation observed in the population density of both communities [$F(1,446) = 380.42$, $F(1,258) = 269.70$; $P < 0.001$]. As well as the OLS regression, we used the OLS-bisector regression (OLS_{BIS}) to estimate the relation between population density and body mass (19, 20). The OLS_{BIS} regression gave a slope of -1.03 for both streams, which was not significantly different from -1 but differed from -0.8 (Table 1). In contrast, the slopes of the OLS regression were significantly different from -1 but not from -0.75 (Table 1) and did

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Table 1. Regression slopes for the relation between body size (μg of dry mass) and population density (individuals per m²) in benthic invertebrate communities of the streams Oberer Seebach and Afon Mynach. n, number of species; r^2, variance explained by the correlation of body size with densities of all species (AS), detritivorous species (D), and predatory/omnivorous species (P/O), respectively; b_{OLS}, ordinary least squares regression [OLS(y|x)] slope; and b_{BIS}, slope of the ordinary least squares–bisector regression (OLS_{BIS}) (19), separately calculated across all species, detritivorous, and predatory/omnivorous species in the community. Bootstrap confidence limits (95%) are given in parentheses for b of OLS and OLS_{BIS}.

Data set	n	r^2	b_{OLS}	b_{BIS}
		Oberer Seebach		
AS	448	0.460	−0.702† (−0.773, −0.631)	−1.032* (−1.091, −0.974)
D	362	0.425	−0.702† (−0.787, −0.618)	−1.070* (−1.139, −1.002)
P/O	86	0.586	−0.721† (−0.851, −0.591)	−0.940* (−1.053, −0.827)
		Afon Mynach		
AS	260	0.508	−0.733† (−0.821, −0.645)	−1.025* (−1.099, −0.951)
D	210	0.450	−0.691† (−0.796, −0.587)	−1.028* (−1.118, −0.939)
P/O	50	0.614	−0.794† (−0.977, −0.611)	−1.002* (−1.152, −0.852)

*OLS and OLS_{BIS} b values significantly departing from −0.75 and −0.8, respectively.   †OLS and OLS_{BIS} regression coefficients significantly departing from −1.0 (t tests, $P < 0.05$).

Fig.1. Linear regressions of log population density on log body size of benthic invertebrate species found in two stream communities. Bisector regressions (OLS_{BIS}) are fitted over 448 species of the Oberer Seebach and over 260 species of the Afon Mynach. The regression equations are as follows: Seebach, log density $= 2.26 - 1.03$ log DM (where DM is dry mass); Mynach, log density $= 2.15 - 1.03$ log DM. Symbols represent log-transformed mean densities and body mass for invertebrate species belonging to 13 different taxonomic groups as defined in the key.
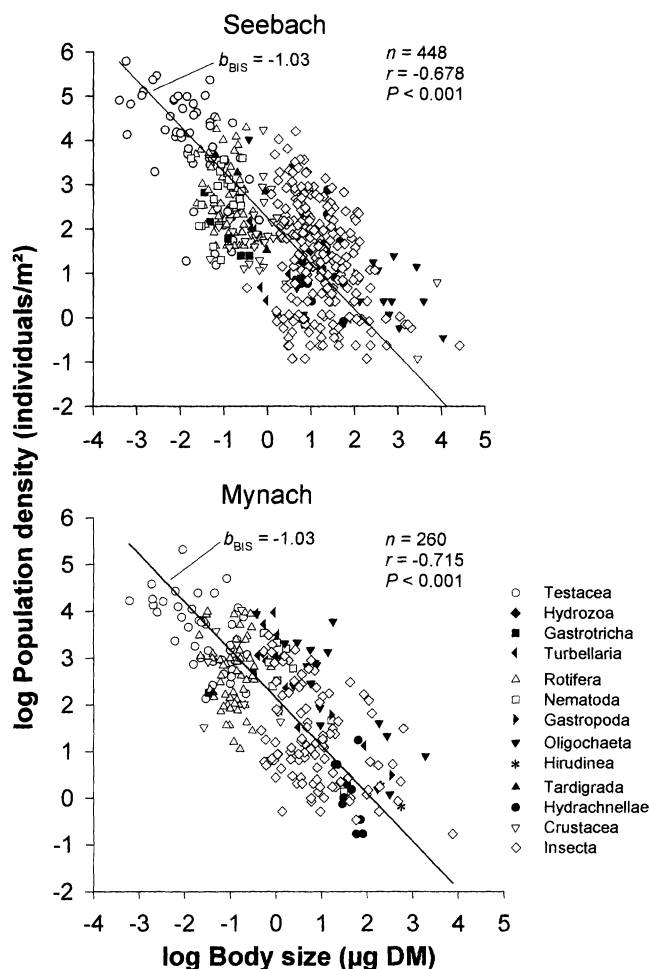

Fig. 2. Linear regressions of log population density on log body size for invertebrate species. (**A**) Species common to both streams ($n = 105$) and (**B**) those restricted to either Seebach ($n = 343$ species) or Mynach ($n = 155$ species). The OLS_{BIS} regression equations are as follows: (A) Mynach, log density $= 2.21 - 1.04$ log DM and Seebach, log density $= 2.55 - 1.02$ log DM; (B) Mynach, log density $= 2.10 - 1.01$ log DM and Seebach, log density $= 2.16 - 1.01$ log DM. Symbols represent log-transformed mean densities and body mass for species found in the gravel streams Afon Mynach (●) and Oberer Seebach (○).
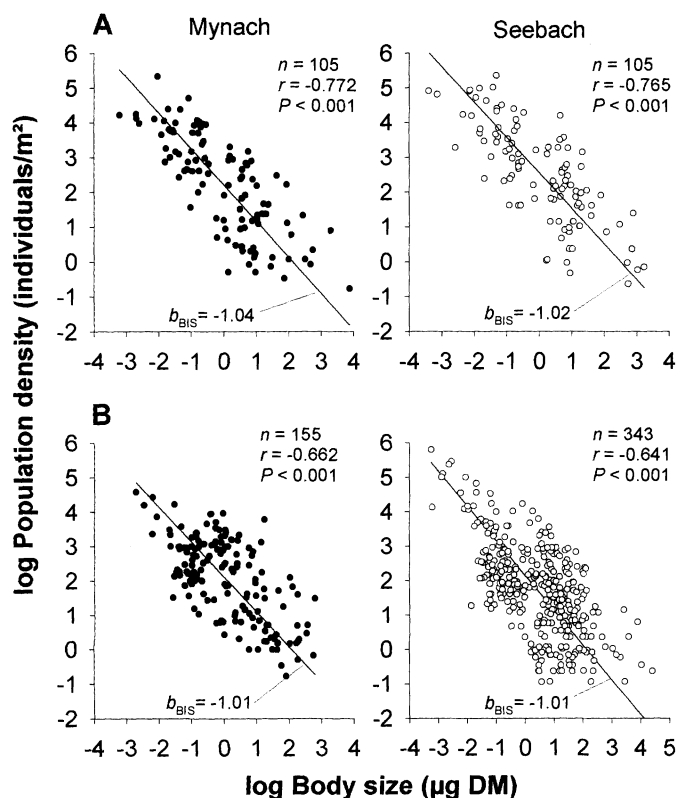
not differ significantly between streams (*21*). The observed negative proportionality cannot be attributed to the hypothesis of a declining number of species with body size (*22*), as the largest number of species apparently occurred in the intermediate range of body size in both communities (Fig. 1).

Even where a density–body size relation characterizes a community, different trophic levels may have different patterns of scaling. Therefore, we separated detritivores and predators (including omnivores) and subjected them to the same test (Table 1). The scaling of population density with body size was not significantly different between the feeding guilds in the two streams (*23*). However, the OLS_{BIS} regression demonstrates that detritivore density declined more rapidly with increasing body mass than did predator density. In contrast, this trend was not shown by the OLS regression (Table 1).

Invariant community-wide patterns may or may not arise from the presence of a set of species common to geographically separate communities. 17.4% of all species were found in both streams, and they show a similar population density to body mass scaling (Fig. 2A) but with different regression intercepts [analysis of covariance (ANCOVA), $F(1,207) = 7.05$; $P = 0.009$]. Furthermore, those species found only in one of the streams also generated similar density–body size relations in the two streams (Fig. 2B) (ANCOVA, $P > 0.1$).

We also tested whether within-taxon relations were similar to the observed across-taxa relations (Table 2). The OLS and OLS_{BIS} slopes of the eight most species-rich taxonomic groups are both positive and negative and fall randomly around a mean of -0.66 (± 0.03 SE) and -1.28 (± 0.02 SE) in the Seebach and a mean of -0.63 (± 0.08 SE) and -1.10 (± 0.30 SE) in the Mynach. This suggests that no single ecological or energetic rule can account for the patterns in different taxonomic groups.

A general principle of benthic community structure can only be found if similarity between assemblages can be unambiguously established. In this respect, our study clearly shows that thoroughly censused stream communities with a wide range of taxa and body sizes have an inverse proportionality between density and body size, which does not arise from a decreasing number of species in larger body size classes. Moreover, as the slope of the density-size regression varied widely among taxonomic groups in this study, the pattern cannot be explained by a single ecological model such as the "energy equivalence rule" (*24*). In the streams we studied, food and space are unlikely to be limited (*25, 26*); organisms feed on a wide

Table 2. Regression slopes for the relation between body size (μg of dry mass) and population density (individuals per m^2) of benthic invertebrate assemblages in the streams Oberer Seebach (SB) and Afon Mynach (MY). n, number of species; r^2, variance explained by the correlation of body size with species in the stream assemblages; b_{OLS}, ordinary least squares; and b_{BIS}, bisector regression slope separately calculated through all species of abundant taxonomic groups in SB and MY. Bootstrap confidence limits (95%) are given in parentheses for b of OLS and OLS$_{BIS}$.

Data set	Stream	n	r^2	b_{OLS}	b_{BIS}
Testacea	SB	54	0.386	−0.979* (−1.124, −0.833)	−1.507* (−1.660, −1.354)
	MY	42	0.294	−0.621† (−0.754, −0.489)	−1.120* (−1.246, −0.994)
Turbellaria	SB	11	0.530	−1.008 (−1.998, −0.018)	−1.364 (−2.361, −0.367)
	MY	15	0.529	−1.056 (−1.560, −0.552)	−1.428 (−2.083, −0.772)
Rotifera	SB	58	0.065	−0.619† (−0.923, −0.316)	−1.592*† (−2.165, −1.020)
	MY	52	0.005	−0.158*† (−0.501, 0.185)	−1.135 (−2.322, 0.053)
Nematoda	SB	22	0.434	−0.955 (−1.206, −0.705)	−1.409*† (−1.575, −1.243)
	MY	10	0.002	0.075 (−1.030, 1.181)	1.044* (−0.972, 3.060)
Oligochaeta	SB	21	0.315	−0.507† (−0.771, −0.244)	−0.917 (−1.035, −0.799)
	MY	18	0.655	−0.982* (−1.144, −0.819)	−1.208*† (−1.352, −1.064)
Hydracarina	SB	19	0.009	−0.204† (−0.814, 0.406)	−1.174 (−2.358, 0.011)
	MY	10	0.233	−1.482 (−2.590, −0.380)	−2.543 (−4.295, −0.791)
Crustacea	SB	33	0.229	−0.401*† (−0.681, −0.120)	−0.870 (−1.649, −0.092)
	MY	7	0.038	−0.331 (−1.307, 0.646)	−1.231 (−2.752, 0.314)
Insecta	SB	219	0.118	−0.607*† (−0.689, −0.526)	−1.435*† (−1.512, −1.359)
	MY	99	0.164	−0.509*† (−0.603, −0.416)	−1.174*† (−1.235, −1.113)

*b values significantly departing from −0.75 and −0.8, respectively. †b values significantly departing from −1.0 (t tests, $P < 0.05$).

range of particle and prey sizes and are found both at the sediment surface and in the hyporheic interstitial zone, using the habitat in a three-dimensional way (27). Although species composition differs between the two geographically separated streams, the scaling of population density with body size is not different. Therefore, the density–body size relation might be controlled by factors that are similar in the two streams, such as water discharge regimes and physical complexity (fractal D) of the habitat (28–30).

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14. Quantitative samples were taken of the streambed sediments in the Afon Mynach, a tributary of the River Dee in North Wales [52°57′N, 3°38′W; 260 m above sea level (a.s.l.)], and in the Oberer Seebach in Lower Austria (47°51′N, 5°04′E; 615 m a.s.l.). Benthic samples were collected in riffle/pool areas with modified Hess-samplers (mesh net, 50 μm) to a sediment depth of 10 cm using three different surface areas (100, 200, and 300 cm^2). Freeze-core samples with
prior electropositioning were obtained from sediment layers down to 40 cm (30, 32). The sampling schemes were designed to collect all species and individuals ≥50 μm in proportion to their occurrence and to cover the range of ecological conditions (e.g., variations of flow and habitat structure) possibly experienced by the populations with equal probability. Data used in the analysis are mean values calculated for a standardized surface area (1 m^2) of 311 samples collected from the Oberer Seebach and 316 samples from the Afon Mynach. Benthic samples were collected from the Oberer Seebach on a weekly interval between October 1991 and 1992 and on a monthly interval between March 1993 and November 1994 (27, 33). In the Mynach samples were collected on a bimonthly interval from November 1993 through November 1994 and seasonally from July 1996 to September 1997. Sixty-four and 80 sediment samples were collected in the Mynach and Seebach, respectively, for granulometric analyses.
15. Both gravel streams were circumneutral (pH ≈ 7), and during the sampling period, mean annual discharge was 0.86 (±0.06 SE) m^3/s in Oberer Seebach and 1.02 (±0.12 SE) m^3/s in Afon Mynach. The particle-void interface of vertical and horizontal sections of the streambed sediments is characterized by a mean fractal dimension (based on the Minkowski and Kolmogorov method) of $D = 1.67$ (±95% confidence level, 0.10) in the Seebach and $D = 1.58$ (±95% confidence level, 0.11) in the Afon Mynach (30). Grain-size composition differed significantly between both streams [analysis of variance (ANOVA), $F(1,142) = 49.02$; $P < 0.001$] with a median grain size of 16.5 mm in the Seebach and 9.1 mm in the Mynach.
16. All large invertebrates (≥300 μm) were counted under a stereo microscope at ×25 to ×50 magnification. The abundances of smaller organisms were assessed by splitting each sample into 10 subsamples of 1 ml each and then counting all individuals found in 1-ml graticule cells. Species identification procedures were as described in (27). Depending on abundances, the body length, width, and height of at least 300 individuals of each species were measured to the nearest 5 μm with an eyepiece micrometer and an image analysis system. Body mass (dry weight) of species that vary greatly in size between larval stages (insects) was obtained from logarithmic regressions of total body length (measured in mm) versus weight (milligrams) of individuals. Large organisms were weighed individually, whereas smaller ones (<1 mm) were weighed in groups of 5 to 50 individuals using a Mettler ME 22-Microbalance (Greifensee, Switzerland). Regressions were based on 15 to 100 individuals per species collected during the sampling period. If fewer than 15 individuals of a species were found, mean individual dry weight was used. If body mass could not be directly measured, it was either estimated by interpolation of known body weights or obtained from other sources. Mean individual body weight of rotifer species was estimated from logarithmic length-weight regressions of data from Duncan (34). Mean body weight of shelled amoeba species was obtained by the method outlined in Schönborn (35).
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19. To compare our data with published results, we used OLS ($y|x$) regressions to model the relation between weight and density. However, to establish the underlying relation between x and y for comparison with theory, methods that treat x and y values symmetrically are more appropriate (36). One such structural relation method that was used for density versus body size relations is the RMA (5, 6). A shortcoming of the RMA method is that the amplitude of the RMA regression depends only on population dispersion and is independent of population correlation. Though a least-squares procedure, it does not measure relations between variables (36). On the basis of simulation studies, the OLS$_{BIS}$, which is the line that bisects the angle formed by the OLS($y|x$) and its inverse [OLS($x|y$)], has a smaller variance about its

theoretical fit than other least squares regressions (37). Therefore, we conducted regression analyses on the basis of both the OLS(y|x) and OLS$_{BIS}$ methods.

20. From the data of Peters [appendix IIIb in (2)], we estimated the hypothetical energy use OLS$_{BIS}$ slope of aquatic invertebrates to be 0.79 (median value; $n = 17$). Thus, abundance would have to scale with mass to the -0.8 power for energy equivalence to hold if we assume the slope of OLS$_{BIS}$ of log body mass against log metabolic rate to be 0.8.

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38. We thank G. Bretschko from the Biological Station Lunz, Austria, for his hospitality and scientific contributions and two anonymous referees for helpful comments and advice. Funded by NERC grant GR3/09844 to M.T.

17 March 2000; accepted 22 June 2000

Prokaryotic Regulation of Epithelial Responses by Inhibition of IκB-α Ubiquitination

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Epithelia of the vertebrate intestinal tract characteristically maintain an inflammatory hyporesponsiveness toward the lumenal prokaryotic microflora. We report the identification of enteric organisms (nonvirulent *Salmonella* strains) whose direct interaction with model human epithelia attenuate synthesis of inflammatory effector molecules elicited by diverse proinflammatory stimuli. This immunosuppressive effect involves inhibition of the inhibitor κB/nuclear factor κB (IκB/NF-κB) pathway by blockade of IκB-α degradation, which prevents subsequent nuclear translocation of active NF-κB dimer. Although phosphorylation of IκB-α occurs, subsequent polyubiquitination necessary for regulated IκB-α degradation is completely abrogated. These data suggest that prokaryotic determinants could be responsible for the unique tolerance of the gastrointestinal mucosa to proinflammatory stimuli.

In humans, the mucosal lining of the intestine coexists in intimate contact with a diverse prokaryotic microflora. The intestinal epithelial cells have necessarily evolved mechanisms to prevent or limit activation of cellular immuno-inflammatory stress responses in this microbe- and antigen-rich environment (1). Immune and inflammatory responses in the gut and other immunocompetent tissues often involves the transcription factor NF-κB. This DNA binding protein is the transcriptional effector of an evolutionarily conserved regulatory pathway that is activated by a myriad of proinflammatory stimuli and is required for the de novo synthesis of numerous proinflammatory cytokines, chemokines, adhesion proteins, and other molecules critical for

normal immuno-inflammatory function (2). Proinflammatory stimuli activate NF-κB through tightly regulated phosphorylation, ubiquitination, and proteolysis of a physically associated class of inhibitor molecules, IκB's (3). IκB is inducibly phosphorylated by a multisubunit kinase complex (IκB kinase) and is subsequently ubiquitinated by a second multiprotein complex, recently isolated by several groups and designated E3-SCF$^{\beta\text{-TrCP}}$, with the β-TrCP subunit functioning as the IκB-specific ubiquitin ligase (E3) (4–7). Polyubiquitinated IκB is thus targeted for degradation by the 26S proteasome (8), allowing NF-κB to translocate to the nucleus, bind to its sequence recognition motif on target promoters, and activate transcription of effector genes, an example of which is the neutrophil chemokine interleukin-8 (IL-8). A variety of endogenous anti-inflammatory mediators, as well as clinically effective anti-inflammatory drugs, are known to exert their effects, at least in part, by blockade

of various aspects of the NF-κB signal transduction pathway (9); however, there has been no description of interference with the ubiquitination step.

Although the vast majority of enteric organisms do not elicit intestinal inflammation, it is becoming increasingly recognized that enteropathogens that cause acute inflammatory colitis do activate the NF-κB pathway, resulting in secretion of chemokines including IL-8 (10–12). We hypothesized that if proinflammatory enteric pathogens activate NF-κB and subsequent events, nonpathogenic microorganisms may be able to selectively attenuate this pathway as a mechanism of intestinal immune tolerance. We observed that colonization of the apical aspect of polarized T84 model epithelia with nonpathogenic *Salmonella* strains, both laboratory-derived and naturally occurring (*S. typhimurium* PhoP^c and *Salmonella pullorum*), attenuates basolateral IL-8 secretion characteristically elicited by apical infection with proinflammatory strains (wild-type *S. typhimurium* and Hil A mutant) (Fig. 1A) (13–15). Colonization with *S. typhimurium* PhoP^c and *S. pullorum* was also capable of attenuating the IL-8 secretion elicited by a spectrum of proinflammatory stimuli [tumor necrosis factor-α (TNF-α), the calcium mobilizing agent carbachol, and the phorbol ester PMA (phorbol 12-myristate 13-acetate)] (Fig. 1B). This anti-inflammatory effect could be eliminated by nondenaturing heat killing of the organisms and was not present in bacterial lysates or conditioned media (Fig. 1C), suggesting the phenomenon is mediated through direct interactions of the epithelium with viable bacteria. The anti-inflammatory effects were maximal after a precolonization period of 30 min, though detectable suppressive effects were observed with colonization 10 min after addition of proinflammatory agonist (16). Colonization of T84 model epithelia with the anti-inflammatory organisms showed no strain-specific effects on transepithelial resistance or short-circuit currents elicited with forskolin. These indices of normal

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